7th World Congress for Hair Research

ALOPECIA AREATA

P001

Hair follicle cycle and inflammatory infiltrate in alopecia areata at early stage

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Objective: To investigate the histopathological features including changing of hair follicle cycle and the inflammatory infiltrate of lymphocytes, eosinophils, and mast cells in alopecia areata (AA).

Method: Clinical, laboratory, and pathological features of 136 patients with AA were investigated.

Results: Follicular counts from biopsy specimens of the 136 patients showed largely decreased anagen/telogen and terminal-vellus ratios. ''Swarm-of-bees'' peribulbar lymphoid infiltration existed in 73.52% (100/136) of the patients, and those with it either had a shorter duration or had active hair loss. Eosinophils were present in 47.8% (63/136) of cases in all stages of AA. Patients with diffuse type were more likely to have eosinophilic infiltrate than those with other types of AA. Cell counts of mast cells and the activated mast cell in AA lesions were more abundant in the deeper peri-follicular than in the superficial peri-follicular area, but there was no difference between the peri-vascular deeper and superficial parts of the dermis. Superficial peri-vascular and deeper peri-follicular mast cell counts had a positive correlation with serum IgE level. Superficial peri-vascular mast cells and the activated mast cell in AA lesions were found to be more abundant in patients in the active phase ($P = 0.028$, 0.004). The patients who had a ''swarm-of-bees'' peribulbar lymphoid infiltration had more mast cell infiltration both in the superficial and deeper peri-vascular areas. The patients with higher peripheral IgE level, as well as those with pigment casts in the follicular tract, had more activated mast cells in the deeper peri-follicular area.

Conclusion: The presence of eosinophils, mast cells, and lymphocytes was found to be correlated with disease severity and decrease of follicular terminal–vellus ratio. Also, the peri-vascular infiltration of mast cells into the superficial dermis suggests that mast cells play an important role in the pathological process of AA.

P002

Exomic sequencing of immune-related genes reveals novel candidate variants associated with alopecia universalis

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Alopecia areata (AA) is a common autoimmune disorder mostly presented as round patches of hair loss and subclassified into alopecia totalis/alopecia universalis (AT/AU) based on the area of alopecia. Although AA is relatively common, only 5% of AA patients progress to AT/AU, which affect the whole scalp and whole body, respectively. To determine the genetic determinants of this orphan disease, we undertook whole-exome sequencing of 6 samples from AU patients, and 26 variants in immune-related genes were selected as candidates. When an additional 14 AU samples were genotyped for these candidates, 6 of them remained at the level of significance in comparison with 155 Asian controls ($P < 1.92 \times 10^{-3}$). Linkage disequilibrium was observed between some of the most significant SNPs, including rs41559420 of *HLA-DRB5* ($P < 0.001$, OR 44.57) and rs28362679 of *BTNL2* ($P < 0.001$, OR 30.21). While *BTNL2* was reported as a general susceptibility gene of AA previously, *HLA-DRB5* has not been implicated in AA. In addition, we found several genetic variants in novel genes (*HLA-DMB*, *TLR1*, and *PMS2*) and discovered an additional locus on *HLA-A*, a known susceptibility gene of AA. This study provides further evidence for the association of previously reported genes with AA and novel findings such as *HLA-DRB5*, which might represent a hidden culprit gene for AU.

P003

Association between *IL16* gene polymorphisms and susceptibility to alopecia areata in the Korean population

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Introduction: The pathogenesis of alopecia areata (AA) still remains unclear. However, several studies have indicated that AA is an organ-specific autoimmune disease and that T cells, as well as certain cytokines, such as IL-1β, IFN-γ, and TNF-α, may play an important role in its development. It is known that CD8+ T cells are the direct modulators of hair loss, while CD4+ cells play a classic ''helper'' role in AA onset. IL-16 plays a role in trafficking of several immune cells and may be a major chemotactic signal for CD4+ cells. The aim of this study was to investigate the significance of *IL16* gene polymorphisms in the susceptibility to AA and several phenotypes of AA.

Methods: We conducted a case-control association study of 228 AA patients and 270 matched healthy controls. Genotype frequencies of four single-nucleotide polymorphisms (SNPs) in *IL16* gene were studied. Statistical analyses were performed according to onset age, presence of family history, clinical subtypes of AA, and presence of nail involvement or body hair involvement.

Results: One SNP (rs17875491) of the *IL16* gene showed significant difference between the AA patients and control group. One SNP (rs11073001) of the *IL16* gene showed significant difference between the AA group with presence of family history and the AA group with absence of family history. Strong LD blocks were formed between rs17875486 and rs17875491, and between rs11073001 and rs1803275.

Conclusion: *IL16* gene polymorphisms may contribute to the increased susceptibility to AA in the Korean population, and *IL16* gene polymorphisms may be associated with presence of family history.

P004

Differences in comorbidity profiles between early-onset and late-onset alopecia areata patients: a retrospective study of 871 Korean patients

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Alopecia areata (AA) is an autoimmune disease that presents as patchy, nonscarring hair loss, affecting about 2% of the population. AA is thought to be frequently occurring in association with other autoimmune diseases such as thyroid disorders and atopic disease. Also, several epidemiologic studies have reported that onset before adolescence is more likely to be associated with atopic disease as a comorbid disorder and a poorer prognosis. To compare the comorbidity profiles of early-onset AA patients with late-onset AA patients, we divided 871 AA patients who visited the Department of Dermatology of Yonsei Wonju Christian Hospital in the last 10 years into two groups, on the basis of their onset ages. The early-onset AA group and late-onset group comprised patients below 13 years old and older than 13 years, respectively. On reviewing their histories of comorbid disorders and laboratory findings, past histories of hypertension, diabetes mellitus, and thyroid diseases were significantly higher in the late-onset group, and probabilities of abnormal hematologic tests, glucose, lipid profiles, and renal function tests were also significantly higher in the late-onset group. However, these results may be considered to be influenced by age. Interestingly, prevalence of atopic dermatitis, family history of AA, and positivity of antinuclear antibodies (ANA) were significantly higher in the early-onset group. As a result, it may be concluded that onset of AA before adolescence is significantly associated with higher prevalence of atopic dermatitis, higher family history of AA, and higher positivity of ANA.

P005

Non-scarring patch alopecia in patients with active systemic lupus erythematosus differs from that of alopecia areata

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Background: Clinical features of non-scarring patch alopecia in patients with active systematic lupus erythematosus (SLE) are similar to those of alopecia areata (AA).

Objectives: To identify the specific features of non-scarring patch alopecia in SLE and those of AA, in order to correctly diagnose and treat SLE patients presented as non-scarring patch alopecia primarily.

Methods: Data including clinical, dermatoscopic, histopathological, and laboratory data from patients with patch alopecia, including 21 active SLE patients and 21 AA patients, were retrospectively analyzed and compared.

Results: The alopecia patches commonly presented as incomplete alopecia in the SLE group, but complete alopecia in the AA group. Exclamation-mark hairs, black dots, broken hair, and yellow dots were more common in AA patients, while hair shaft hypopigmentation and thinning, angiotelectasis, peripilar sign, white dots, and honeycomb pigment pattern were more common in SLE patients. Interfollicular polymorphous vessels were the most common presentation of angiotelectasis in the alopecia region of SLE, while interfollicular arborizing vessels were observed more in the non-alopecia region of SLE and alopecia region of AA. After treatment, increase in vellus hair was the earliest feature that emerged in both groups, while hair shaft hypopigmentation was the earliest feature that disappeared in SLE patients, and broken hair in AA patients. The prevalence of ANA, dsDNA, AHA, AnuA, and decreased level of WBC was significantly higher in SLE patients than in AA patients. The distinct histopathological changes of the alopecia region helped in differential diagnosis. After treatment, no SLE patients had relapse of alopecia, while 41.7% of AA patients did.

Conclusion: Distinct clinical, dermoscopic, and histopathological features were found in the alopecia region of SLE patients, in comparison to that of AA patients. Serological autoantibody test is of value to confirm the differential diagnosis. Local angiotelectasis and vasculitis in vicinity to hair follicles may be involved in the pathogenesis of alopecia in SLE.

P006

Early stage of alopecia areata is associated with T-cell infiltration, Th1/Th2 balance shift and epithelial apoptosis in the upper dermis

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Objective: To explore the mechanisms underlying the early stage of AA, focusing on T-cell subpopulation and follicular apoptosis.

Materials and methods: Specimens were collected from 15 AA patients at early stage (⩽3 months, positive hair-pull test), 15 AA patients at late stage (>3 months, negative hair-pull test) by surgical dissection at the edge of the scalp lesion, and from the scalp of 13 normal controls. Double staining of T-cell markers and apoptosis cell markers (Annexin V and Caspase 3) was performed. Semi-quantitative RT–PCR assay was applied on both parts to detect mRNA expression of Th1, Th2 cytokines, hair follicle cycle signaling molecules and apoptosis-associated factors.

Result: In early-stage AA patients, there was more intense perivascular and perifollicular CD3+, CD4+, CD8+ T lymphocyte infiltration than in late-stage AA patients and normal controls in the upper dermis ($P < 0.05$), but not in deep layer. Moreover, Caspase 3 and Annexin V-positive cells were more in the upper perivascular and perifollicular areas ($P < 0.05$), but less in the deep perifollicular area ($P < 0.05$) compared to late stage.

In contrast to normal controls, elevated mRNA levels of Annexin V, Caspase 3 and FAS were observed in both the upper and deep layers of early-stage AA, with Annexin V and Caspase 3 also upregulated in the deep layers of late cases. In contrast to the normal controls, in early stage, an elevated mRNA expression of Th1 cytokines (IL-12, IFN-γ) and a lower mRNA expression of Th2 cytokine IL-10 in the upper layer were observed, whereas mRNA expression of IL-10 in the deep layer was similar to that in normal controls.

Conclusion: In the early stage of AA, Th1/Th2 balance shift, T-cell infiltration, and epithelial apoptosis in the upper dermis were more obvious than in the deep layer. Therefore, more functional study into inflammation in the superficial layer of the dermis is required in order to further discern its link with follicular regression in AA.

P007

Alopecia areata in the elderly: a 10-year retrospective study

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Aims: Alopecia areata (AA) is a well-known immune-mediated form of hair loss that usually occurs in the young adults. AA in the elderly is relatively rare; therefore, little data have been reported. The purpose of this study is to understand better the clinical characteristics of AA in the elderly.

Methods: We performed a 10-year retrospective study of AA in the elderly who visited our dermatologic clinic from January 2002 to December 2011. A clinical review of medical records and subsequent telephone interviews were performed by dermatologists. The age of onset, duration of disease, family history, past history, emotional stress before onset, AA type and severity, ophiasis, nail changes, gray hair, coexisting systemic and/or dermatologic diseases, therapeutic responses, and clinical courses were observed.

Results: Among 1761 patients who were newly diagnosed with AA, 61 patients (3.5%) were older than 60 years of age at first visit. The oldest patient was 90 years old and the mean age was 71. The duration from the recognition of initial hair loss to the time of the first visit was less than 6 months in 45.9% (28/61). There were 74.3% (26/35) of the patients with an extent of hair loss less than 50% of the scalp. There was no statistically significant correlation between extent of AA and graying ($P=0.679$). Our study also demonstrated favorable therapeutic response, which was found in 62.9% (22/35).

Conclusion: We observed various clinical characteristics of AA in the elderly. As a result, we assumed that AA in the elderly showed mild disease severity. Large-scale studies are required to validate our results.

P008

Diphencyprone (DPCP) in treatment of patchy alopecia areata (AA)

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Introduction: The effectiveness of DPCP in AA has been demonstrated in several studies with variable response rates (5–85%), which has led to considerable speculation surrounding its therapeutic value and efficacy.

Objectives: To assess the efficacy of DPCP in the treatment of AA.

Methodology: Forty-six subjects with mild-to-moderate AA having minimum two patches were sensitized with 2% DPCP. During each weekly visit, patients were treated with gradually higher concentrations of DPCP, which was applied to a single patch while another served as control. The patients' hair regrowth was graded as excellent (76–100%), good (51–75%), moderate (26–50%) and mild (<25%) at months 3 and 6.

Results: Of the 46 patients, 20 were less than 10 years of age. Extent of AA was <25% in 30 subjects and >25% in 16. Thirty-nine subjects were available for analysis at 3 months and 29 at 6 months (no of dropouts: 17). At the end of 3 months, 15 subjects had good to excellent response, 7 had mild to moderate, and 16 had no response. At the end of 6 months, of the 29 subjects, 16 had good to excellent response, 10 had mild to moderate, and 3 had no response, giving an efficacy rate (excellent + good) of 55% (16/29).

At 6 months, the response in 18 subjects with <25% AA was good to excellent in 9 and mild to moderate in 7; that in 11 subjects with >25% AA was good to excellent in 7 and mild to moderate in 3. On the control patch in 29 subjects, seven showed spontaneous hair growth. Common side effects were redness, itching, blisters, occipital lymphadenopathy, severe eczema, and hyperpigmentation.

Conclusion: DPCP is an effective treatment in patchy AA. A long period of therapy is needed and it will increase the percentage of responders.

P009

Efficacy and tolerability of medium-dose oral prednisolone pulse therapy over 4–8 weeks in alopecia areata-an update with 53 cases

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Alopecia areata (AA) is a common disease with limited effective therapeutic options, so identification of a stage-adapted, effective, safe, pragmatic, and time-saving therapy is still needed. A monocenter retrospective, clinical outcome study of oral medium-dose prednisolone pulse therapy was performed in 52 patients (53 treatments; mean age 43 years; 37 female, 15 male) with active AA (33 AA multilocularis, 19 AA of different type). A continuous, specific therapy scheme was defined over 4 weeks, starting with 50 mg prednisolone per day, descending to 10 mg daily. Upon positive response after 4 weeks, continuation of treatment up to a total of 8 weeks, with 2 weeks of 20 and 10 mg per day, respectively, was performed. Negativation of pull-test and hair re-growth were defined as response criteria. After 4 weeks, the pull-test turned negative in 48% and after 8 weeks in 50% of treated patients. Hair re-growth was observed in 60% after 4 weeks and in 81% after 8 weeks. About 60% of the patients showed no side effects, while 34% showed gentle to slight side effects like sweating, slight body weight or blood pressure increase, stomach acidity, itching, or acne. Moderate side effects (weakness, hot flush, sleep disturbance) occurred in 3.8%. One patient (1.8%) showed severe side effects (vomiting and loss of power). However, all side effects resolved after therapy end. In conclusion, the presented protocol of oral medium-dose prednisolone pulse therapy of up to 8 weeks is a successful therapy with high responding rates and relatively small side effects and therefore represents an effective, safe, and time-saving treatment option in AA patients.

P010

Optimization of severe forms' treatment of alopecia areata using corticosteroids

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Aim: To optimize the treatment of severe forms of AA.

Study material: 49 patients with AA, phenotypes S2–S5, male 9, female 40, age 18–56 years.

Compared methods of treatment: 1st group: 27 patients treated with prednisolone given as tablets. The starting dose depended on the weight of the patient: up to 60 kg 35 mg per day; >60 kg 40 mg per day.

2nd group: 22 patients treated with combined therapy of prednisolone 15 mg per day and cyclosporine A 3.5 mg per kg of weight per day. The treatment lasted 4–6 months depending on effect.

Results: Hair growth was observed after 2–3 weeks of treatment in group 1, and after 3–6 weeks of treatment in group 2. The efficacy: no response: 1st group 2/27 (7.4%), 2nd group 2/22 (9%); partial response: 1st group 15/27 (55.6%), 2nd group 9/22 (41%); complete response: 1st group 10/27 (37%), 2nd group 11/22 (50%). The effect was worse in patients of both the groups who had an early disease onset (in the childhood), alopecia persisting for more than 5 years, and more than four episodes of AA in the course of life. Side effects were most frequently reported in the patients of group 1 compared with group 2. A weight gain of 2–8 kg (15/27), steroid-induced acne (9/27), facial cushingoid with lipopexia in the area of the upper shoulder girdle (4/27), and increased appetite (13/27) were observed only in group 1. Arterial hypertension (1/22) and dysesthesia (12/22) were observed only in patients of group 2. Hypertrichosis of the face and extremities were observed in 11.1% of patients in group 1 and 36.4% in group 2.

Conclusion: The combined therapy stimulates hair regrowth more effectively, because this is a result of combined effects of the two drugs with pathogenetic activity against AA. The use of suboptimal doses of prednisolone and cyclosporine A allows to minimize the side effects of either drug and simultaneously extends the scope of pathogenetic treatment, which results in extended remission.

P011

Statistical analysis of prognostic factors in patients with rapidly progressive alopecia areata

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Alopecia areata (AA) is a common acquired alopecia showing variable hair loss. Although various prognostic factors have been reported, no evident factors for determining prognosis and appropriate treatment are known. To identify prognostic factors in AA patients, especially those with rapidly progressive AA (RPAA), defined as those with positive hair-pull test results across the entire scalp on the first visit or with a history of onset or rapid worsening of hair loss across the entire scalp within 6 months prior to first visit, 1,030 patients diagnosed with AA at Tokyo Medical University Hospital were retrospectively examined for 3 years, and their prognosis was assessed on the basis of various indices using multivariate analysis. Patients with regenerated vellus hairs initially observed on hair loss areas showed a significantly higher improvement or cure rate regardless of the type or severity of AA, including RPAA, assessed on the first visit. Early onset was associated with a significantly lower cure rate, and long disease duration was associated with a significantly higher risk of relapse among all AA patients.

RPAA patients tended to show a good prognosis regardless of treatment modality or the severity on the first visit, and previous history of AA was significantly associated with a poor prognosis in RPAA patients. Our study suggests that these variables can aid in predicting prognosis among RPAA patients.

P012

Home treatment using diphencyclopropenone for alopecia areata: focused on efficacy, safety, convenience, and economic feasibility

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Background: Diphencyclopropenone (DPCP) immunotherapy is effective for alopecia areata, although there are some troubling side effects as DPCP is a potent contact sensitizer. Hence, most of the DPCP immunotherapy is directly conducted by dermatologists at a hospital weekly. However, frequent visiting to a hospital could result in considerable loss of time and money for patients that lead to decline in quality of life.

Objective: The aim of this study was to evaluate the efficacy, safety, convenience, and economic feasibility of DPCP home treatment.

Methods: A retrospective review was done for 87 patients with alopecia areata who received home treatment at Chonbuk National University Hospital. The procedure for sensitization and determination of optimal concentration was identical to that of conventional methods, which are based on weekly visits to hospital. Afterward, having received education regarding application methods and side effects, DPCP solutions were given to all the patients at each visit in order to let them apply themselves at home. Follow-up at hospital was done monthly. We evaluated regrowth of hair using SALT score, side effects, and patient's quality-of-life questionnaire. The results were compared with those of previously published papers in which DPCP immunotherapy was done at hospitals.

Results: Seventy-one patients (81.6%) presented clinical response during treatment period, with 33 patients showing complete response. Overall regrowth rate using SALT score was 54%. Forty-two patients (48.3%) experienced at least one complication, although there were no serious ones. Average score of questionnaire obtained at the end of home treatment was lower than that obtained during weekly visit to hospital; it signifies that home treatment is economic, convenient, and improves quality of life.

Conclusions: Our clinical outcomes were similar to those of previous conventional methods. Improvement in quality of life was shown through the questionnaire. Hence, home treatment of DPCP immunotherapy could be recommendable for patients with alopecia areata.

P013

Alopecia areata: a new treatment plan

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Background: Many therapeutic modalities have been used to treat alopecia areata (AA), with variable efficacy and safety profiles. If AA extent is more than 50%, topical immunotherapy with diphenylcyclopropenone (DPCP) is the first therapeutic option. For poor responders to DPCP or those who cannot use it, several review articles for AA therapy suggest topical minoxidil and topical corticosteroids, but the yield of these topical agents in treatment of extensive AA is limited. So what are the other therapeutic agents that can be used? Also, other treatment protocols suggest the use of systemic corticosteroids, but we know that corticosteroids have a high relapse rate once stopped.

Objective: There is a need to propose new therapeutic alternatives and a new treatment plan based on the safety and efficacy profiles of the available therapies.

Method: We conducted a systematic review of clinical trials evaluating the available therapeutic options for AA treatment.

Results: In this paper, the therapeutic agents are organized according to their efficacy and safety profiles into first-line, second-line, and third-line options. If alopecia areata involves more than 50% of the scalp, topical immunotherapy with DPCP is the first therapeutic option recommended by many experts in hair diseases. For patients who respond poorly to DPCP and those who cannot use it, second-line therapies can be used. Second-line therapies include use sulfasalazine with or without systemic corticosteroids and PUVA-turban. If these therapies fail or are not tolerated, third-line therapeutic options can be discussed with patients. These agents include methotrexate with or without a systemic corticosteroid, azathioprine, cyclosporine, and pulse therapy of corticosteroids.

Conclusion: In this review, the therapeutic agents are organized according to their efficacy and safety profiles into first-line, second-line, and third-line options. A suggested new AA treatment plan is presented in a practical algorithmic approach.

P014

Clinical and epidemiological study of childhood alopecia in two pediatric hospitals in Santiago, Chile

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Background: Scalp hair loss in children is a relatively rare event with a negative impact on the child's psychosocial wellbeing. International studies of alopecia in the pediatric population have reported multiple causes, usually acquired and non-scarring forms. Also, there may be etiologic differences according to age groups. No clinical or epidemiological surveys have been done in the pediatric population of Chile.

Objective: To describe the clinical and epidemiologic profile of alopecia in children from two Chilean pediatric hospitals.

Methods: Retrospective and prospective analysis of clinical records from Roberto del Rio and Luis Calvo Mackenna Children's Hospitals between January 2007 and June 2010. Patients under 15 years of age with diagnosis of scarring and non-scarring alopecia were included. Clinical and epidemiologic characteristics were recorded. Statistical analysis was performed using the SPSS 11.5 program.

Results: 345 clinical records were analyzed, 179 male (51.9%) and 166 female (48.1%). The median age of patients was 72 months. Overall, most of the cases were acquired forms of alopecia, 97.4% were non-scarring. The most common diagnoses were: alopecia areata (36.8%), tinea capitis (21%), nevus sebaceous (13.2%), telogen effluvium (8.7%), and trichotillomania (5.2%). Only telogen effluvium and loose anagen syndrome were statistically higher in girls. According to age groups the principal causes were aplasia cutis and nevus sebaceous in newborns; nevus sebaceous, alopecia areata, tinea capitis, and telogen effluvium in toddlers and preschoolers; alopecia areata, tinea capitis, tellogen effluvium, and trichotillomania in school years; and nevus sebaceous, alopecia areata, tellogen effluvium, and trichotillomania in adolescents.

Conclusions: This is the first study of alopecia in the Chilean pediatric population. As in international reports, most of the cases were acquired and non-scarring forms of hair loss. The most prevalent causes were alopecia areata, tinea capitis, nevus sebaceous, telogen effluvium, and trichotillomania. However, this may vary according to the age group analyzed.

P015

Alopecia areata and Down's syndrome: a coincidence?

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Alopecia areata (AA) is considered to be more frequent in patients with Down's syndrome (DS) in comparison to the general population. The increased prevalence rate implicated that a causative gene for this condition is located on chromosome 21. Nevertheless, only a small number of studies evaluated the link between these two conditions, and they differ in methodology and in their outcomes. Most importantly, none of these surveys has checked the familial incidence of AA in these cases. Since an increased familial incidence of AA in DS patients with AA would suggest that the association with chromosome 21 is coincidental, we evaluated patients with DS from our AA clinic. We found 14 DS patients with AA (age range 3–35 at examination), with a mean age at onset of 6.8 ± 5.1 (range, 1.5–16). Two patients suffered from alopecia universalis, one had alopecia totalis, and the others ($n = 11$) had patch-type AA. One patient had celiac disease, one had Graves' disease, two had a history of hypothyroidism, and three had elevated TSH. Of note, AA was reported in first- or second-degree relatives of eight patients (57%). In our series of patients, the age of onset was lower than the reported age in the literature, but clinical phenotype was not more severe than the normal population. DS patients are often under strict medical surveillance, and under these conditions skin diseases, such as AA, are more often diagnosed and reported. This may have resulted in a selection bias in previous reports linking DS to AA. The fact that many patients had a positive family history of AA suggests that the association to chromosome 21 might have been coincidental. Further studies are needed to clarify if indeed an association between AA and DS exists.

P016

Quality of life in alopecia areata

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Quality of life (QL) is defined as individuals' perceptions of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards, and concerns. This definition reflects the view that most dermatologic conditions do not imply a direct threat to life, but their chronic and incurable character has a powerful negative impact on the QL of afflicted patients. They affect patients in a multidimensional manner, ranging from emotional to social interactions, symptoms, and functional impairment. Many skin-specific QL measures have been developed, one of the first being the Dermatology Life Quality Index (DLQI), developed in the UK in 1994 by Finlay *et al*. Measuring the impact of dermatologic disease on QL allows patients to express their feelings and appreciate their physicians' concerns and improves doctor–patient communication. It also helps in disease management, including the risk/benefit assessment of alternative therapeutic interventions.

Objective: To assess baseline QL in alopecia areata patients referred to a dermatology department in Re-Medika hospital.

Methods: In order to measure the QL, DLQI survey (using the 10-item DLQI) was completed by 100 patients with alopecia areata (AA) during the period of January–November 2012. The DLQI score in patients with AA was compared with that of other dermatology illnesses.

Results: This is the first study in Macedonia to evaluate the QL in patients with AA. Women with AA had significantly higher mean DLQI scores than men. DLQI scores for AA were significantly higher than in patients with androgenetic alopecia.

Conclusion: The first step of improvement of QL is establishing a good doctor–patient relationship for the motivation of the therapy, especially in periods of insufficient success.

P017

Treatment of local forms of alopecia areata

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Aim: To optimize treatment of patients with local forms of alopecia areata (AA) using corticosteroids.

Study material: 96 patients with AA, phenotypes S1–S4; male 33, female 63, age 18–60.

Compared methods of treatment:

Group 1: $N = 38$. Intralesional injections of betamethasone dipropionate/betamethasone sodium phosphate not more than 2 ml once in 4–5 weeks.

Group 2: $N = 21$. Clobetasol propionate 0.05% externally in the form of ointment to alopecia foci with occlusion overnight during 6 days, with a break on the seventh day.

Group 3: $N = 35$. Clobetasol propionate ointment 0.05% × 2 times daily, combined with 5% minoxidil application after 30 min.

Duration of treatment: 2–4 months. Observation after treatment: 3 months.

Results: Efficacy of treatment. No response: group 1—6/38 (16%), group 2—3/21 (14%), group 3—1/35 (3%); partial response: group 1—11/38 (29%), group 2—5/21 (24%), group 3—12/35 (34%); complete response: group 1—21/38 (55%), group 2—13/21 (62%), group 3—22/35 (63%). The effect in the first group depended on the alopecia area and was maximal for a single lesion with area of 3–5 cm^2. In the second and third groups, the sizes of lesion area did not affect the treatment efficacy.

Efficacy of treatment is comparable to patients of the second and the third groups. But, the frequency of inadequate treatment responses was lower in patients of the third group, and in this group hair grew denser in foci, were thicker, and more pigmented as compared to the first and the second subgroups.

Conclusion: Clobetasol propionate in ointment can also be successfully used for treatment of subtotal or total forms of AA. Combined therapy using 5% minoxidil contributes to a more rapid restoration of pigmented hair (in case of extensive areas of alopecia) and optimizes treatment of AA.

P018

Association of alopecia areata with autoimmune diseases in Georgia

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Aim: To identify the association of alopecia areata (AA) with other autoimmune diseases.

Methods: Nine hundred patients (100 SLE; 100 systemic scleroderma; 100 myasthenia; 200 psoriasis; 100 autoimmune thrombocytopenia; 300 chronic autoimmune thyroiditis (CAT)) were screened to determine AA. Three hundred individuals were selected in the control group by simple randomization method.

Results: In all groups prevalence of AA was higher than in controls, but significant difference was observed only in the CAT group, with χ^2 value totalling 7.86; $P = 0.005$; odds ratio 12.46 (95% CI 1.68–258.18), where only extensive form of AA is much more frequent, than in control ($P = 0.025$).

Conclusions: There is strong association between AA and most other autoimmune diseases, but there was not enough evidence to prove this; we hence need more larger and stronger research in future. It seems there is also association of the autoimmune process with hormonal dysfunction and hypothyroidism in our case.

P019

Off the top of your head: what makes a wig work for alopecians?

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Alopecia UK quote that 1.7% of the British population may experience alopecia areata during their lifetime. A large percentage of this population chose to wear wigs to conceal their hair loss. Since 2008 NHS Scotland has spent over £1 million per annum on the provision of wigs to medical hair loss patients, including alopecia. However, little is known about the wig users' experience or how it affects their body image and quality of life. This study has conducted pilot focus groups, semi-structured interviews, online questionnaires, and physical testing of wig materials to gain a holistic understanding of wig users' experiences. The findings look at identifying any relationship with fiber technology and personal experiences, and how wig design could improve their quality of life.

P020

Low-dose diphenylcyclopropenone treatment in alopecia areata

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Objective: Diphenylcyclopropenone (DPCP) is the most commonly used contact sensitizer in the immunotherapy of alopecia areata (AA). Despite its relatively good treatment response, the common side effect of severe contact dermatitis with a current high sensitization dose of 1 or 2% DPCP decreases the compliance of the patients, which eventually causes failure in consistency of the treatment. We aim to investigate the effect of immunotherapy with low-dose DPCP for AA.

Methods: We reviewed the medical records of the patients who visited our hair clinic in the past 10 years. Among them, we enrolled 127 patients with AA who received immunotherapy with low-dose DPCP for at least 3 months, which starts with 0.1% sensitization. From a week after initial treatment, DPCP challenge starts with a dose of 0.01%, at increasing doses of 0.025, 0.05, and 0.1%. Patients who received other treatments were excluded.

Results: Of all 127 patients, 19 (15%) patients showed mild improvement, 55 (43.3%) patients showed moderate improvement, and 27 (21.3%) patients had great improvement. The average duration of initial treatment response of each group was 7.71, 7.68, and 7.52 months, respectively. There was no statistical significance with alopecia subtypes, past history, and laboratory results in terms of treatment response. Ninety-eight (77.2%) patients were reported with no side effects; 17 (13.4%) patients complained mild itching and 4 (3%) patients had folliculitis; 2 (1.6%) patients were reported with scale and erythema. Other side effects of moderate and severe itching, increased hair fall, rash, vesicles, and lymph node enlargement were reported with one case each.

Conclusion: Low-dose DPCP can be used safely and effectively as the first-line immunotherapy for AA.

P021

Analysis of personality traits in patients with alopecia areata

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Although the pathophysiology of alopecia areata (AA) is not fully understood, biochemical interaction between hair follicle and brain, when coping with psychological stress, has been proposed as a possible cause of AA. Furthermore, hair loss could cause profound psychosocial impact. Therefore, patients with AA have a higher prevalence of psychiatric morbidity and certain personality traits. However, there are few studies on personality traits in patients with AA. Thus, this study was aimed to clarify the personality difference in 100 patients with AA and a control population using a structured questionnaire based on the NEO-Five Factor Inventory (FFI). This study could provide more detailed information on the five basic personality factors: neurotism, extraversion, openness, agreeableness, and conscientiousness. This study could also be helpful in understanding the role of personality in pathophysiology and treatment of AA, as well as in giving a new insight into the personality traits of patients with AA.

P022

Cases of alopecia areata where invasion of mast cell is remarkable around hair follicles: including examination of our department's experience cases

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In recent years it has been suggested that mast cell contributes to the clinical condition of alopecia areata. Here we report one case of alopecia areata with remarkable mast cell invasion around the hair follicles in histopathology. The case is a 48-year-old Japanese woman. She noticed alopecia of the hair in the beginning of September 2012. She visited the Asaya Dermatological Clinic, because the alopecia area was spread, and was then referred to our department. She visited our department on November 7. She developed bronchial asthma and urticaria on October 22. A hen-egg-size, irregular-shaped, and imperfect alopecia lesion was visualized in her occipital area, and a thumb-caput-size, circular, perfect alopecia spot was shown in the macula. Fortunetelling depilation characteristics were remarkable in and around the alopecia spot and she experienced itching. On histopathology, telogen hairs with cellular infiltration around the hair bulbs were visualized. We diagnosed it as alopecia areata by its clinical features and histopathological image. Mast cells appeared in the infiltrate cells around hair bulbs. In the upper part of the dermis, a lot of mast cells were shown in the perivascular area. On blood examination, there was no eosinophilia, and the IgE showed a slight increase. We performed treatment by topical application of steroid and oral administration of an antihistamine and are currently observing an episode of care. We investigated factors such as presence of a merger of allergic diseases, results of blood examination, severity, and consequence of remarkable mast cell invasion observed on histopathology in this alopecia areata patient.

P023

Reduced level of vitamin D in chronic/relapsing alopecia areata

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Current observations apparently link vitamin D deficiency to many autoimmune diseases. Data are scarce on the vitamin D levels in alopecia areata, an autoimmune disease that, in our published experience, shows seasonality in most of its remitting-relapsing forms, with a pick of relapse in autumn–winter. Our results in this period demonstrate the presence of insufficiency of 25OH-D in many patients, independent of their clinical forms and correlated to the expected increase of the PTH values. This could suggest the possible clinical utility of vitamin D in the management of this highly frustrating disease.

P024

Alopecia areata in a patient with Turner's syndrome

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Turner's syndrome is a sex chromosomal abnormality characterized by gonadal failure, short stature, skeletal, and medical anomalies. Turner's syndrome is infrequently associated with alopecia areata (present in 3% of cases, compared with 0.3% of controls) and more recently with psoriasis (present in 17% of patients compared with 1.6% of controls). We report a first case of alopecia areata in a patient with Turner's syndrome in Korea.

An 11-year-old girl with Turner's syndrome was referred to our department by the Department of Pediatrics with four round-shaped, hair loss patches on the scalp that existed for 6 months. She had taken human growth hormone and estradiol for short stature and sexual immaturation, which did not provoke the onset of cutaneous disease. Her family history was non-contributory. Histopathologic feature was mild lymphocyte infiltration around the lower portion of the follicles. Dermoscopic findings were black dots, broken hairs, short vellus hair, and tapering hair. Histologic and dermoscopic findings were consistent with alopecia areata. Local treatments with low-dose intralesional triamcinolone acetonide had been carried out once weekly, but therapeutic response was slow, like in other cases of alopecia areata in Turner's syndrome patients.

Alopecia areata occurring in Turner's syndrome may indicate some genetic relationship and could support the concept that these patients have the tendency to develop autoimmune or immunological diseases.

P025

The effect of AIRE −207 polymorphism on AIRE transcriptional activity highlights the potential role of AIRE in the pathogenesis of alopecia areata

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Alopecia areata (AA) is an inflammatory hair-loss disorder characterized by the follicular infiltration of CD4+ and CD8+ T cells and is considered a tissue-specific autoimmune disease. AA is highly associated with autoimmune polyendocrine syndrome type 1 (APS-1), a disease caused by the malfunction of the *AIRE* gene. AIRE is expressed in the thymus, particularly in thymic medullary epithelial cells (mTECs), and is required for the ectopic expression of a diverse range of peripheral tissue antigens (PTAs) by mTECs, conferring on them the ability to perform the negative selection of T cells. The expression profile of PTAs is affected not only by AIRE deficiency but also by variation of AIRE activity in the thymus. To elucidate the mechanism underlying this, we screened 591 bp upstream of the *AIRE* transcription start site including the AIRE minimal promoter region for SNPSs and identified two SNPs at positions −655 and −230. To study the effect of these variants on AIRE promoter activity we generated a TEC 1A3 cell line stably transfected with a single copy of the reporter vector. Relative promoter activity was estimated by comparing the luciferase-specific activity for lysates of the different reporter cell lines, including *AIRE*-655G *AIRE*-230C, *AIRE*-655G *AIRE*-230T, and *AIRE*-655A *AIRE*-230C. The analysis showed that the commonest haplotype, *AIRE*-655G *AIRE*-230C, has the highest luciferase activity ($P<0.001$), whereas *AIRE*-655G *AIRE*-230T has an activity-specific activity value that approaches nil. This haplotype was not present in the homozygous state in 110 healthy individuals, whereas 4 patients were homozygous for *AIRE*-655G *AIRE*-230T out of 172 patients with sporadic AA genotyped for these variants. The *AIRE*-655G *AIRE*-230T genotype could dramatically alter AIRE transcription, leading to the defective negative selection, and allowing self-reactive immature T lymphocytes to escape clonal deletion, leading to the development of an autoimmune response as observed in AA.

P026

Investigation of selected cytokine genes suggests that *IL-2RA* and the *TNF/LTA* locus are risk factors for severe alopecia areata

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The hypothesis that AA is autoimmune in nature is supported by previous studies and a genome-wide association study. These report association with specific HLA alleles, as well as genetic variants of other genes implicated in autoimmunity, such as various cytokine genes. However, most of the cytokine genes cannot yet be considered proven susceptibility loci, since many of these association findings were derived from small patient samples. Accumulating knowledge of shared pathways in autoimmune diseases and findings of association between AA and other autoimmune disorders highlight the importance of investigating the role of other common autoimmune susceptibility alleles in AA. We therefore genotyped variants of cytokine genes using a sample of 768 AA patients and 658 controls of Central European origin. We genotyped 11 single-nucleotide polymorphisms (SNPs) from cytokine genes implicated in previous AA studies using the MassARRAY system and a Sequenom Compact MALDI-TOF device. These genes were *IL-1B*, *IL-1A*, *IL-1RN*, *MIF*, *IFNG*, and the *TNF/LTA* gene region. We also genotyped 15 SNPs selected from cytokine genes that have shown significant association with other autoimmune diseases. These genes were *IL-10*, *IL-1F5*, *IL-12B*, *IL-6*, *IL-2*, *IL-23*, *IL-2RA*, and *IL-4R*. Significant association was found for two variants within both *IL-2RA* and *TNF/LTA*. In the overall sample, the most significant results were obtained for the *IL-2RA* variant rs706778 ($P=0.00038$) and the *TNF/LTA* locus variant rs1800629 ($P=0.0017$). In subgroup analyses, according to severity, age of onset, and family history these effects were stronger in the severely affected patients, with the lowest *P*-values being obtained for rs706778 ($P=3.8\times10^{-6}$). Our results therefore point to the involvement of *IL-2RA* and the *TNF/LTA* region in the aetiology of AA—in particular severe AA—and provide further support for the hypothesis that AA is autoimmune in nature.

P027

Functional genomics and targeted next-generation sequencing point to ULBP6 as a critical node in the NKG2D axis in alopecia areata

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Our GWAS study in alopecia areata (AA) first revealed a role for NKG2D ligands encoded by the ULBP3/6 genes in disease pathogenesis, which we biologically validated by showing a marked upregulation of ULBP3/6 and the presence of CD8+NKG2D+ T cells surrounding lesional hair follicles. These findings, together with the previous demonstration of MICA overexpression in AA hair follicles, placed the NKG2D axis squarely at the center of AA pathogenesis, and invited a functional genomics approach to uncover causal variants predisposing to disease. In order to interrogate the distribution of genetic variants that reside within the NKG2D axis in AA, we first identified a comprehensive list of NKG2D axis loci by constructing a genetic network of 65 genes from a database of known and predicted protein interactions. Next, we performed whole-exome sequencing for 11 probands with severe AA from multiplex families from our linkage study and identified 280 variants within 57 of 65 NKG2D axis genes. Of these, 50 variants result in a protein sequence change, including 9 rare or novel mutations, which cluster in 7 genes, two of which reside within genomic regions with evidence for linkage in AA. Unexpectedly, one of these genes is ULBP6, which was previously identified in our GWAS ($P=5\times10^{-19}$). Given the convergence of linkage and GWAS evidence at this locus, we next performed targeted sequencing of the ULBP3/6 region in 80 AA patients using the Raindance platform and identified additional protein-coding variants that were reported to have very low population frequencies (<0.007), but, strikingly, were strongly overrepresented at a combined frequency of 10% among AA patients. The preponderance of rare, nonsynonomous variants affecting critical protein domains suggests that ULBP6 is an essential node in the genetic architecture of AA, and points to a role for variability in NKG2D-mediated cytotoxicity in disease pathogenesis.

P028

Next-generation T cell receptor sequencing for the identification and monitoring of pathogenic T cells in alopecia areata

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Alopecia areata (AA) is one of the most common autoimmune diseases, characterized by non-scarring hair loss. T cells are considered the critical pathogenic cells, since they abundantly infiltrate the hair follicles in AA, and are both necessary and sufficient to transfer disease in the C3H/HeJ mouse model of AA. Next-generation T-cell receptor (TCR) sequencing provides a platform to identify and track the frequency of pathogenic TCR clonotypes, representing a functional biomarker of disease activity. Using this technique for TCR β-chain sequencing, we have identified strikingly expanded T-cell clones in the lesional skin from five AA patients, which represented up to 9.5% of the total TCR sequences, supporting an antigen-driven process in AA, and providing evidence of dominant pathogenic T-cell clones. Comparative data from the C3H/HeJ mouse model of AA also strongly support this notion, as identical TCR sequences were dramatically expanded in new AA lesions of recipient mice grafted with lesional skin from the same donor. In human AA patients, we found that some T-cell clones that were expanded in affected skin also circulate at significant frequencies (>0.1% of total blood sequences) in the peripheral blood of the patient. By correlating the disease severity with the frequencies of circulating pathogenic clones, we are now testing the hypothesis that circulating pathogenic TCR frequency distinguishes patchy alopecia (AAP) and generalized alopecia (AU), and/or correlates with baseline disease severity or disease trajectory. In a longitudinal study, we will determine if increased circulating pathogenic TCR frequency precedes or occurs concomitantly with disease progression. The unique accessibility of clonally expanded pathogenic T cells within the hair follicle end organ represents an ideal clinical setting to examine the broad applicability of next-generation sequencing to identify and track pathogenic TCR clonotypes in human autoimmunity.

P029

Reversal of longstanding alopecia areata in C3H/HeJ mice using topical JAK inhibitors

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Alopecia areata (AA) is one of the most prevalent autoimmune diseases for which no targeted therapy exists. T cells have been previously implicated in the disease process, but the immune activation pathways and specific T-cell types involved have not been identified. Guided by our GWAS studies implicating NKG2D ligands (NKG2DL) in human AA, here we identify NKG2D-expressing CD8+ cytolytic T cells (CTLs) as the dominant immune effectors in the C3H/HeJ mouse model, which were both necessary and sufficient for disease induction. Moreover, we show that the NKG2DLs Rae1 and H60 are both upregulated in the AA mouse hair follicle (HF). We interrogated the cytokine pathways involved in the AA inflammatory response, identifying a dominant IFNγ signature as well as IL15. These two cytokines conspire to induce the pathogenic AA Type 1 cytotoxic T cell inflammatory response. IL15 produced by the HF promotes the activation of ''NKtype'' IFNγ-producing CTLs. In turn, IFNγ produced by alopecic CTLs activates the HF, closing a pathogenic circle by upregulating HF IL15, MHC molecules, and NKG2DLs, all of which target the HF for CTL attack. This type 1 cytotoxic pathway can be interrupted with either anticytokine biologics or small-molecule inhibitors of their downstream effector JAK kinases. We demonstrate that both IL15 blocking antibodies and systemic JAK inhibition prevent disease induction in grafted AA mice. Importantly, two recently FDA-approved JAK inhibitors, tofacitinib (JAK3i) and ruxolitinib (JAK1/2i), that inhibit signaling through the IL15 and IFNγ receptors, respectively, have been topically formulated and have demonstrated safety and efficacy in human psoriasis. We show that both topical JAK inhibitors eliminate local CTLs and induce hair regrowth, durably reversing AA in mice with established disease, thereby providing a strong rationale for their clinical evaluation in human AA.

P030

Endogenous retinoids in the pathogenesis of alopecia areata

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Alopecia areata (AA) is an autoimmune disease that attacks anagen hair follicles. Gene array in graft-induced C3H/HeJ mice revealed that genes involved in retinoic acid (RA) synthesis were increased, while RA degradation genes were decreased in AA compared to sham controls. This was confirmed by immunohistochemistry in biopsies from patients with AA and both mouse and rat AA models. RA levels were also increased in C3H/HeJ mice with AA. C3H/HeJ mice were fed a purified diet containing one of four levels of dietary vitamin A, or an unpurified diet 2 weeks before grafting and disease progression followed. High vitamin A accelerated AA, while mice fed no vitamin A had more severe disease by the end of the study. More hair follicles were in anagen in mice fed high vitamin A. Both the number and localization of granzyme B-positive cells were altered by vitamin A. Protein levels of IFNG were also the lowest and IL13 highest in mice fed high vitamin A. Protein levels of IL10, IFNG, IL17, IL21, IL22, and PDL1 were reduced and CXCL9 and CCL5 increased as the disease progressed, but no additional effects of vitamin A were seen. Combined, these results suggest that vitamin A regulates both the hair cycle and immune response to alter the progression of AA.

P031

Type I cytokines and chemokines are targetable immune pathways in alopecia areata

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Alopecia areata (AA) is pathologically characterized as a ''swarm of bees'', with recruitment of T cells around and invading the hair follicle. We recently defined the phenotype of these cells in the C3H/HeJ alopecic mouse. These IFN-γ-producing ''NK-type'' CD8 killer T cells infiltrate the hair follicle, are massively expanded in the cutaneous lymph node, and are necessary and sufficient for disease transfer in adoptive recipients. In alopecic mice, the inflammatory signature is dominated by the Type I response, and the CXCL9-11 chemokines are among the most highly upregulated genes in lesional skin. The FDA-approved small-molecule JAK1 inhibitor ruxolitinib, which blocks IFN signaling, eliminated the Type I cytokine signature and durably reversed AA development in mice with established disease when given topically. Interestingly, the CXCR3 receptor, known to bind these IFN-inducible chemokines, is upregulated on the key pathogenic alopecic CTL effectors. To demonstrate whether CXCL9-11 chemokines were pathogenically required, we treated mice with blocking antibodies to CXCR3. Importantly, CXCR3 blockade prevented AA development in the AA graft model and notably abrogated the expansion of CXCR3 + alopecic killer T cells in the skin and cutaneous lymph node. We show here using transcriptional profiling of human and mouse alopecic skin that the IFN pathway is the dominant signaling pathway involved in AA. Transcriptional profiles of 12 AA patients and 9 normal controls show that IFN-inducible chemokines (CXCL9-11) are massively transcriptionally upregulated in the skin of human AA lesions. Taken together, these data demonstrate proof-of-principle of interfering with the Type I response in human AA, via blockade of IFN-inducible chemokines. CXCR3 blockade could be approached clinically with either antibody or small-molecule blockade, the latter being particularly intriguing as a topical therapeutic.

P032

Identification of autoantigen epitopes in alopecia areata

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Alopecia areata (AA) is believed to be an autoimmune disease that results in non-scarring, inflammatory hair loss. Both CD4 and cytotoxic CD8 T cells (CTLs) have been found to be important for the onset and progression of AA both in humans and in rodent AA models. Hair follicular (HF) keratinocyte and/or melanocyte antigen epitopes are suspected as potential targets of auto-reactive CTLs, but the specific epitopes have not yet been identified. We investigated the potential for a panel of known epitopes, expressed by human HF keratinocytes and melanocytes, to induce activation of CTLs. Peripheral blood mononuclear cell (PBMC) populations were isolated from AA-affected and healthy subjects with HLA-A2 serotypes. PBMCs were cultured with synthesized HLA-A2 restrictive peptide with specific sequences for trichohyalin, melanin, MART-1, tyrosinase, tyrosinase-related protein-2 (TRP2), and GP-100. The frequency of CTL activation in PBMC was measured by using enzyme-linked immunosorbent spot (ELISpot) assays, where activated IFNgamma-secreting cells are visible as spots. Specific epitope cocktails derived from trichohyalin, MART-1, and TRP2 induced significantly higher responses in human AA CTLs compared to healthy controls. Investigation into CTL activation via single trichohyalin epitopes showed highly variable results, suggesting patients with different stages of AA may have different primary targets. AA-affected C3H/HeJ mouse lymph node cells (LNCs) showed significantly higher responses to mouse antigen epitopes like keratin-16 (K16) and MART-1. However, in contrast to human PBMCs, trichohyalin epitopes did not induce a significantly higher amount of CTL activation in AA mouse LNCs, though the average response was greater than control mouse LNCs. The data indicate that AA-affected subjects present with an increased frequency of CTLs responsive to antigen epitopes originating from keratinocytes and melanocytes. Potentially, trichohyalin, MART1, and K16 could be specific targets for CTLs that cause AA.

P033

Remission of longstanding alopecia universalis after HIV infection

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Alopecia areata (AA) is a common disorder leading to non-scarring hair loss. Infrequently, the disease may progress to alopecia universalis (AU), the most severe form of the disease, affecting all scalp and body hair. AU is highly resistant to treatment, and only rarely spontaneous remission occurs. Its exact pathogenesis is still obscure, although it was suggested to be an autoimmune-mediated disease. Infection with human immunodeficiency virus (HIV) has been associated with autoimmune diseases, and several reports described the induction of AA by HIV infection. We report here a 46-year-old male patient with AU for 20 years, who experienced dramatic regrowth of the scalp and body hair following infection with HIV. Hair growth increased with lowering of CD4 counts, reaching a maximum of 90% regrowth of hair with CD4 counts of 400 cells mm^{-3}. The patient did not have recurrence of hair loss after starting treatment for associated syphilis and with increase in CD4 counts. To our knowledge, this is the first report of induction of hair growth in an AA patient after HIV infection. This finding is especially noteworthy, considering that remission occurred in longstanding AU, the most severe and recalcitrant form of AA. This report raises interesting questions regarding AA pathogenesis, and emphasizes the importance of systemic immune dysfunction for the induction of the disease. Further insight into the pathogenesis of AA remission in HIV patients may help us elucidate the underlying mechanism of AA in the general population.

P034

Correlation between serum thymus and activation-regulated chemokine (TARC), and severity and activity in alopecia areata without active atopic symptom

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Serum thymus and activation-regulated chemokine (TARC), a chemokine involved in Th2 cell migration, is a good indicator of atopic dermatitis (AD) severity. Here, we report serum TARC in our 130 alopecia areata (AA) patients without active atopic symptoms such as AD, allergic rhinitis, conjunctivitis, and asthma. Patients with past history of AD who had only mild xerosis were included, while those with eczematous lesions excluded. Using the SALT score the patients were classified as follows: S0: no ($n=10$); S1: 1–25% ($n=29$); S2: 26–50% ($n=13$); S3: 51–75% ($n=10$); S4: 76–99% ($n=8$); S5: 100% scalp hair loss ($n=39$), rapidly progressing diffuse type ($n=6$), and ophiasis type ($n=15$). As a result, serum TARC was 302.3 ± 90.7 pg ml^{-1} in S0, 272.5 ± 116.3 pg ml^{-1} in S1, 310.8 ± 119.0 pg ml^{-1} in S2, 316.8 ± 204.5 pg ml^{-1} in S3, 431.9 ± 407.4 pg ml^{-1} in S4, 454.7 ± 250.7 pg ml^{-1} in S5, 663.3 ± 490.7 pg ml^{-1} in the diffuse type, and 334.6 ± 165.3 pg ml^{-1} in the ophiasis type group. The serum TARC positively correlated with the SALT grade (S1–S5) severity ($P<0.001$, Spearman rank-order correlation coefficient by rank test). Moreover, when comparing serum TARC in the S2 and rapidly progressing diffuse type with 26–50% scalp hair loss (703 ± 537.8, $n=5$), that in the latter was significantly higher ($P<0.05$, Mann–Whitney's U-test). On the other hand, there was no significant difference between serum TARC in the S1 and 2 (284.4 ± 117.1, $n=29$) groups and ophiasis type with 1–50% scalp hair loss (349.5 ± 172.7, $n=13$). Further, immunohistochemical study using anti-TARC and CD68 antibodies revealed that CD68-positive histiocytic cells produce TARC around the AA hair follicles. Together, although AA is generally considered a Th1 disease, Th2 cells can play some additional roles in severity and rapid progression of AA.

P035

An association study of alopecia areata using microsatellite markers within the major histocompatibility complex

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Background: Many autoimmune diseases are associated with alleles of human leukocyte antigen (HLA) genes within the MHC (major histocompatibility complex) region. Associations with alopecia areata (AA) in HLA genes were also observed in different ethnic groups. However, the alleles associated with AA and the loci were completely discordant, and AA susceptibility gene within the MHC region has not been identified.

Objective: The aim in this study is to identify an AA susceptibility locus within the MHC region using a Japanese population that has not been exploited for genetic association study of AA to date.

Methods: We comprehensively selected 22 microsatellites spanning 2.4 Mbp (from *HLA-E* to *PSMB8*) in the MHC region and genotyped 163 cases and 560 controls. To decide alleles of the HLA-C locus, we used the LABType SSO typing test produced by ONE LAMBDA.

Results: The most significant association with AA was obtained for an allele ($P=1.45E-05$, OR: 3.25) of the D6S2811 locus that is 19.2 kb centromeric of the *HLA-C* gene within the HLA class I region. This association was the most statistically significant one after Bonferroni's correction out of all the microsatellites that were analyzed. Moreover, our haplotype analysis demonstrated that a haplotype strongly associated with AA ($P=3.28E-05$, OR: 3.75) was located around the HLA-C locus.

Conclusion: Multiple studies showed that the AA susceptibility locus was located within the HLA class II gene region in mainly European ancestry; however, our study showed a novel finding different from these studies.

P036

Identification of gene expression biomarker signatures for use as an Alopecia Areata Disease Activity Index (ALADIN)

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Alopecia areata (AA) is a highly prevalent autoimmune disease in which the hair follicle is attacked by cytotoxic T lymphocytes. The accessibility of the target organ within skin biopsies has provided us the opportunity to develop a novel disease activity score based on quantitative composite gene expression signatures. Using both Affymetrix microarrays and RNA-Seq data analyzed using Ingenuity Systems' IPA, we identified two striking gene expression signatures in total skin from both human AA and the C3H-HeJ mouse, namely the IFN response, including IFN-gamma and IFN-inducible chemokines, and a cytotoxic T cell (CTL) signature including CD8 and granzymes, implicating these effectors as the dominant inflammatory cells in AA pathogenesis. To generate a functional biomarker from the AA transcriptome, we developed the Alopecia Areata Disease Activity Index (ALADIN), a two-dimensional quantitative composite score. ALADIN was derived from expression levels of representative genes from both the IFN and CTL pathways to generate a measure of the distance of AA transcriptional levels from a baseline obtained from the skin of healthy individuals. The ALADIN score reduces the complexity of the transcriptome into a tractable bivariate index that can be used to quantitate and monitor patients' disease status and progression. We deployed ALADIN in our studies of mouse AA, and demonstrated that ALADIN scores provide a quantitative measure of observed disease reversal, and ''molecular distance to skin homeostasis'' during the course of successful prevention and treatment of disease. ALADIN is currently being assessed in cross-sectional studies to validate its utility in human AA. We anticipate that ALADIN will be useful as a dynamic functional biomarker to stratify and longitudinally track patients enrolled in observational and interventional clinical studies.

P037

Alopecia areata in mice: is it a family affair?

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Inbred mouse strains have been categorized into seven groups based on pedigree derivation and haplotype analyses. Mice, as is also the case for other mammalian species including humans, develop alopecia areata (AA). The C3H/HeJ inbred strain, a member of pedigree group 1, was the first to be described with spontaneous AA. Subsequently, all C3H substrains as well as the closely related CBA/J (also in group 1) carrying a mutation (*Btk*^{xid}) causing a form of B-cell immunodeficiency, were found to develop AA. A/J mice (also group 1) were later found to develop a form of AA. In a massive aging phenotyping project, MRL/MpJ (group 1), SJL/J (group 2), and SWR/J (group 2) were found to develop histological lesions consistent with AA. All six strains are relatively closely related. Using genome-wide association mapping methods, candidate genes were identified in the genetic intervals designated *Alaa1*, *2*, and *4* but not in *Alaa3* of the C3B6F2 quantitative trait loci (QTLs). Transporter 2, ATP-binding cassette, sub-family B (*Tap2*) was confirmed in *Alaa1*, verifying earlier haplotype mapping studies. Additional candidate genes were identified on mouse chromosomes 1, 2, 3, 5, 11, 12, and 18 confirming the complex genetic basis of AA. Genetically closely related mouse strains have an AA phenotype and many of these genes have been found in human AA genetic studies. These mice strains with an AA phenotype may be useful to clarify the role of these genes in human AA in various ethnic groups.

ANDROGENETIC ALOPECIA

P038

Differential gene expression patterns in frontal and vertex scalp in men with androgenetic alopecia-before and after the use of minoxidil topical foam

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The efficacy of 5% minoxidil topical foam (MTF) for hair regrowth in androgenetic alopecia (AGA) has been well established; however, its mechanism of action is poorly understood. In this double-blinded placebo-controlled prospective study, 16 healthy men aged 18–49 years with Hamilton–Norwood type IV–V thinning were enrolled. Ten men used 5% MTF twice daily for 8 weeks and 6 used placebo. Scalp biopsies were taken from the leading edge of hair loss from the frontal and vertex scalp at visit 0 and again after 8 weeks of active drug/placebo use. Microarray analysis was performed on all available samples. A comparison of vertex scalp biopsy specimens before and after use of MTF revealed the following upregulated genes: keratin-associated proteins (KRTAP 13-2, KRTAP19-3, KRTAP19-5, KRTAP7-1, KRTAP8-1, KRTAP19-1) as well as non-coding RNAs (SNORD116-22, SNORD25, SNORA5, VTRNA1-1). The downregulated genes included: epidermal differentiation and keratinization genes including late cornified envelope genes (LCE3D, LCE3E, LCE1C, LCE2A, LCE2C, LCE2D), small proline-rich proteins (SPRR2B, SPRR2E, SPRR2G, SPRR2A), loricrin (LOR), fillagrin (FLG2), and cornefelin (CNFN). Inflammatory genes (CCL18, S100A7, IL1F7, CD177) were also downregulated. These changes were not seen in the placebo samples. Gene expression changes indicated that the frontal scalp was responsive to minoxidil treatment in a manner similar to vertex scalp. These data suggest that the hair growth properties of MTF may be mediated through increased production of hair keratin-associated proteins, which are essential for the formation of a rigid and resistant hair shaft through their extensive disulfide bond crosslinking with abundant cysteine residues of hair keratins. MTF also reduces keratinization of the epidermis and inflammatory signals, which are known to be altered in AGA. Further studies are needed to understand the potential role of non-coding RNAs in follicular physiology. (This work was supported by an Independent Investigator grant form Johnson & Johnson Consumer Companies, Inc.)

P039

What is the meaning of the ''visible pigmented non-vellus hair count'' in FDA-approved clinical trial reports?

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During the past 30 years, the Canfield method was employed in minoxidil-Regaine/finasteride-Propecia clinical trials. However, unlike EU-based methods, we are unaware of any published critical validation data for this technique. This probably led to the Cochrane Review questioning the efficacy of topical minoxidil therapy. Pooling the Canfield data as changes from baseline in non-vellus hair counts between 16–48 weeks of treatment showed an increase of, respectively, 25 and 27 hair cm^{-2} for 2 and 5% minoxidil. Taking +26 hair cm^{-2} change as 100% therapeutic response, it follows that the +14 hair cm^{-2} increase obtained after using placebo lotion explains about 50% of the measured effect. Therefore the calculated net therapeutic benefit (active minus placebo) was +12 hair cm^{-2}. However, Canfield did not use immersion liquid before scalp hair imaging, while studies that did so found a decrease in hair count with placebo lotion, pointing to a confounder in Canfield's method. Furthermore, in the absence of lotion, Canfield's hair counts in two international finasteride trials (pooled data 24–96 weeks) showed negative changes from baseline with oral placebo, i.e., 5.7 and −8.1 hair cm^{-2}, with a net therapeutic benefit of +24 and +16 hair cm^{-2}, respectively. This indicates that the application of lotions affects the visibility of hair when employing the Canfield method. In our independent controlled study evaluating Propecia, employing a calibrated contrast-enhanced phototrichogram (immersion fluid; hair counted if thickness $\geqslant 30\,\mu m$) involving 15 male subjects with randomized topical lotion (1 ml per day) of either placebo ($n=8$) or minoxidil 5% ($n=7$), for 12 weeks, there was no statistically significant change from baseline with Propecia + placebo (+8 hair cm^{-2}), while a significant increase of +40 hair cm^{-2} was found with Propecia + minoxidil 5%. We advise the use of accurate validated methodologies to generate appropriate objective data!

P040

Pharmacodynamic of P-3074 (finasteride 0.25% topical solution) in subjects with androgenetic alopecia

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A new proprietary topical formulation, P-3074, containing finasteride 0.25% as active ingredient and hydroxypropyl-chitosan (HPCH) as film-forming agent, was developed for androgenetic alopecia. The present study was aimed at investigating the pharmacodynamic profile of finasteride in terms of dihydrotestosterone (DHT) concentrations in the scalp and in serum after multiple topical application of P-3074 or oral finasteride intake in subjects with androgenetic alopecia. Eighteen healthy men were randomly allocated to P-3074 or oral treatment after providing written informed consent. Twelve volunteers applied P-3074 topical solution for 7 days: six subjects once daily (o.d.) in the morning and the others twice daily (b.i.d.) in the morning and in the evening. The third group of six volunteers was administered 1 mg oral finasteride once daily in the morning for 1 week. Scalp (vertex) biopsies were collected at baseline and 6 hours after last dose administration, while serum samples were collected at baseline, before last administration, and 6 and 12 hours after the last multiple dose. A marked decrease in scalp DHT levels was observed: by 47.22% with P-3074 b.i.d., from 1.91 (±0.54) to 1.01 ng ml^{-1} (±0.39), by 71.20% with P-3074 o.d., from 1.52 (±0.41) to 0.44 ng ml^{-1} (±0.08), and by 51.11% with the oral formulation, from 1.39 (±0.25) to 0.68 ng ml^{-1} (±0.34). Serum DHT was reduced by 69.3–74.0% with P-3074 b.i.d., 67.6–80.4% with P-3074 o.d., and 69.7–76.1% with the oral formulation. These results showed a similar inhibition of serum DHT after 1 week of finasteride administration with the three dose regimens and were consistent with the results obtained in a previous P-3074 PK study. These findings show that DHT concentration in the scalp, after 7-day treatment course of P-3074 o.d., was more reduced (about 40%) than after 1 mg oral finasteride administration for the same treatment period.

P041

Genetic variations associated with efficacy of dutasteride, the dual 5a-reductase inhibitor in the treatment of male pattern hair loss

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Male pattern hair loss (MPHL) is an androgen-induced progressive hair loss caused by shortening of anagen duration representing as miniaturization of involved hair follicles. A main treatment target for MPHL is inhibition of 5α-reductase (5AR) because dihydrotestosterone (DHT), which is converted from testosterone by 5AR in hair follicles and sebaceous glands, is the most responsible androgen for MPHL. However, the effect of 5AR inhibition differs for each individual and the genetic causes of the individual difference in the treatment efficacy have not been identified. In this study, we aimed to search for exonic variants associated with response to dutasteride, a dual inhibitor of type I and II 5AR. A total of 42 men with MPHL who had been treated with dutasteride for 6 months were recruited and classified into good- (above the 75 percentile in efficacy, $n=10$) and poor-efficacy groups (below the 25 percentile in efficacy, $n=10$) according to the change in hair count after treatment. Quantitative regression analysis on all subjects yielded 16 single-nucleotide variants (SNVs) to be associated ($P<0.05$), and quantitative regression analysis by classifying good- and poor-responding groups left 10 SNVs out of 16. On treating the poor/good responding groups as cases and controls, three SNVs were found significant to be associated. A novel SNV (chr7:91763660:T>C) was located on 7q21.2, the first exon of CYP51A1. The others were rs72623193 on 2q31 and rs1064796 on 19p13.12, and the associated genes were DHRS9 and CYP4F11, respectively. Our results suggested that CYP51A1, DHRS9, and CYP4F11 are potential candidate genes associated with the efficacy of dutasteride on MPHL.

P042

Analysis of androgenetic alopecia in Amerindian people (mapuche) from Southern Chile

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Background: Androgenetic alopecia (AGA) is a common cause of hair loss in men and women. Studies in Caucasian and Asian populations have established racial differences in prevalence and clinical types of AGA. Investigators have suggested that native American (Amerindian) populations would have a lower prevalence and less severe type of AGA.

Objective: To determine the prevalence and clinical patterns of AGA in adult males and females in an Amerindian (mapuche) population of Chile.

Methods: Sample size was calculated using an estimated population proportion. Individuals included adults with two or more surnames of mapuche origin who attended outpatient clinics for general morbidity. Participants were excluded if they had scarring or other causes of non-scarring alopecia or known triggering factors of effluvium. The Norwood-Hamilton and Ludwig classifications were used for clinical pattern evaluation.

Results: 231 patients (88 males and 143 females) were evaluated, men averaging 46.2 years and women 40.7 years. The prevalence of men with AGA was 32.9% and that of women 8.29%. The percentage of AGA increased with age: in the 20-29-year group, 6% affected males and none in females; in the 30–39-year group, 10.5% affected males and 5% in females; in the 40-49-year group, 20% affected males and 7% in females; in the 50–59-year group, 21.4% affected males and 6% in females. In the sixth decade, 46.6% of men and 27% of women were affected. In subjects older than 70 years, all of the men and 42.8% of the women had AGA. Regarding the clinical pattern of AGA in men, 27.6% (8/29) had a female pattern, 20.7% (6/29) had type IV, 17.2% (5/29) were types III vertex or VI, 10.3% (3/29) had type V, and 6.9% (2/29) had type VII. All of the women with AGA showed a female pattern classified as type I.

Conclusions: Chilean mapuche men and women showed a lower prevalence of AGA compared to Caucasians, but higher than reported in Asian populations. Likewise, the prevalence of AGA in our Amerindian population increased with age. Unlike Caucasian men, the female pattern was the most common clinical pattern in mapuche men. As in Asian reports, Ludwig type I was the most common pattern in mapuche women.

The similarities of AGA prevalence and clinical pattern of our study group with Asian studies may support the genetic and anthropologist evidence of a common genetic link.

P043

Oxidative stress and cell senescence in androgenetic alopecia (AGA)

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The hair follicle dermal papilla (DP) is essential for hair growth and is known to be a target tissue for androgens. However, the mechanism for decreased hair growth in AGA is unknown. We have shown that DP cells from balding scalp undergo premature senescence *in vitro* when compared to matched DP cells from non-balding scalp, and that this is associated with upregulation of $p16^{INK4a}$ and downregulation of BMI-1. Patient-matched DP cells from balding scalp have significantly higher levels of reactive oxygen species (ROS), undergo fewer population doublings, and have higher levels of cell senescence when cultured at 21% O_2 compared to 2% O_2, and these differences correlate with changes in $p16^{INK4a}$ and BMI-1 expression. We also show that growth factor secretion by the DP is affected by O_2 culture conditions differently in balding and non-balding DP cells. In addition, we show that the endogenous antioxidants catalase and total glutathione exhibit increased expression in balding DP cells, or cells grown under 21% O_2 conditions. Conversely, reduced glutathione was expressed at lower levels in balding DP cells compared to non-balding DP cells when cultured under 2% O_2. Oxygen conditions were also found to alter DP cell motility, with greater motility observed at 2% O_2 compared to 21% O_2. These data suggest that oxygen conditions significantly alter the function of DP cells and, by extension, oxidative stress may exacerbate the onset of androgenic alopecia by affecting senescence and DHT-induced TGF-β secretion, a known inducer of catagen and inhibitor of hair follicle growth.

P044

The therapeutic potential of naturally immune privileged hair follicle cells

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Naturally immune privileged hair follicle cells have great potential for hair restoration and cell transplantation. The objective of this study was to explore the potential of localized, non-toxic, cell-based immune modulation as an alternative to current immunosuppressive regimens in allogeneic tissue transplantation. Hair follicle–derived mesenchymal dermal sheath cells (DSCs) possess innate immunosuppressive properties that could potentially be utilized to create localized hyporesponsiveness in the context of allogeneic transplantation. Immune privilege-related gene expression was evaluated in cultured human DSCs by using quantitative real-time PCR (q-PCR) and western blot/ELISA, respectively. Immunomodulatory potential was characterized by using a peripheral blood mononuclear cell (PBMC) proliferation/activation assay. Both program death ligand 1 (PD-L1, 7.3 ± 3.2-fold increase at mRNA level, $n=4$) and transforming growth factor beta 2 (TGF-β2, 6.3 ± 3.0-fold increase at mRNA, $n=4$) were upregulated, whereas interleukin 1 receptor antagonist (IL-1RA) and HLA-A, -B were downregulated in DSC (12.5 ± 4.2, 2.8 ± 0.8, and 2 ± 0.5 decrease, respectively) compared with non-follicular fibroblasts (FB). Activation of T cells was measured by the production of IFN-γ in the co-culture of PBMCs (as responders) and allogenic human islets (as stimulators). The secretion of IFN-γ from PBMCs was significantly reduced in the presence of DSCs (23.0 vs. 5.7 pg ml^{-1}, $P<0.01$) or DSC-conditioned medium (18.5 vs. 2.15 pg ml^{-1}, $P<0.04$) compared to FB. The data suggest a limited capacity of T-cell activation in the presence of DSCs or their conditioned medium in response to alloantigen stimulation. This study demonstrates that immune-privileged DSCs may create a localized immunosuppressive microenvironment that may be exploited to reduce the need for chronic systemic immunosuppression with drugs in clinical transplantation.

P045

Follicular unit extraction using the robot system (ARTAS) in an Asian population

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Recently, follicular unit extraction (FUE) has become a popular harvesting method for hair restoration. FUE provides many advantages over the strip method, including absence of linear scar, much less pain, and short recovery time. However, FUE is a time-consuming, exhausting, and technically more challenging job to doctors.

The ARTAS system (Restoration Robotics, Mountain View, CA) is an interactive, computer-assisted, and physician-controlled robotic system for use with the FUE harvesting technique for surgical hair restoration. Each follicular unit is digitally tracked, and removed in a random fashion. The authors introduced the ARTAS system for the first time in Asia, and seek to share their experiences of using this system in the Korean population in this paper.

In the Seoul National University Bundang Hospital, a total of 13 patients underwent FUE hair restoration surgery using ARTAS system from September 2013 to December 2013. The mean age of patients was 46.77 ± 16.10 years. Twelve patients were male with androgenetic alopecia, and the other one patient was female with female-type baldness. The mean number of punching done by the ARTAS system for each patient was 1016.2 ± 111.2. Transection rate ranged from 0.57 to 12%, with a mean value of 4.37 ± 3.17%. No significant side effect or complication was detected during and after the surgery. The surgeons felt much less fatigue compared with manual FUE during the procedure and more precise extraction could be done all through the harvesting time. The ARTAS system is a good advancement in FUE graft harvesting in terms of reducing surgeon's fatigue and improving graft quality. Unfortunately, there are no clinical or experimental Asian data about this system yet. Further sharing of experiences and investigation for this system would be necessary in the future.

P046

Platelet-rich plasma promoting hair growth in human hair follicle dermal papilla cells associated with VEGF and VEGFR-2

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Aims: Autologous platelet-rich plasma (PRP) has been used to *accelerate* wound repair. It has been reported that PRP contains various growth factors such as platelet-derived growth factors (PDGF), transforming growth factors (TGF), fibroblast growth factors (FGF), and vascular endothelial growth factors (VEGF). Previously, we have proposed that PRP promotes hair growth in male-pattern baldness surgery. In this study, we investigated the effect of PRP in enhancing hair growth on human hair dermal papilla (DP) cells.

Method: We assessed cell proliferation in cultured human DPCs by MTT assay and measured the expression levels of VEGF, VEGFR-2, p63, Wnt5a, Wnt10b, and β-catenin by western blot analysis. This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2011-0008687).

Results: To elucidate this hair growth effect related to the proliferative effect of PRP on DP cells, we treated human dermal papilla cells with PRP in a time- and dose-dependent manner. We showed that PRP increased DP cell proliferation in a time- and dose-dependent manner. We further evaluated the expression of VEGF and VEGFR-2 on human hair follicle DP cells. The expression of VEGF and VEGFR-2 on DP cells was examined by western blot analysis. We found that PRP strongly enhanced VEGF and VEGFR-2. We also found that PRP increased expression of β-catenin, Wnt5a, and Wnt10b, a potent hair growth factor, and enhanced the level of p63, a stem cell factor.

Conclusions: These results suggest that the PRP hair growth effect in DP may result from upregulation of β-catenin, Wnt5a, and Wnt10b expression due to increased levels of VEGF and VEGF-R. Thus, our results provide support for possible therapeutic materials based on autologous PRP to promote hair growth.

P047

Aspirin inhibits minoxidil-induced proliferation of human keratinocytes

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Minoxidil is one of the most commonly used drugs for treating androgenetic alopecia, though its exact mechanism of function is not well understood. A number of *in vitro* studies suggest it involves the stimulation of cyclooxygenase1 (COX-1) activity, which increases prostaglandin E2 (PGE2) production, leading to a reversal of follicular miniaturization, and promotion of hair follicles into the normal anagen phase of the hair cycle. However, approximately one-third of the minoxidil users show no response to treatment and their hair loss progresses.

Aspirin, also known as acetylsalicylic acid (ASA), is a commonly used non-steroid anti-inflammatory drug. It suppresses the synthesis of prostaglandins through irreversible inactivation of cyclooxygenase enzymes, particularly COX-1. The regular use of a low-dose ASA is widely accepted as a preventative measure against myocardial infarction (MI) and stroke for certain subsets of the population in the United States and elsewhere. Estimates suggest that about one-fifth of US adults take ASA daily or every other day.

In this *in vitro* study, the objective was to investigate the effect of the COX-1-suppressing property of ASA on the COX-1-stimulating activity of minoxidil, mimicking the situation when both drugs are taken concurrently. To evaluate the potency of minoxidil in keratinocyte proliferation in the presence of ASA, human immortalized keratinocytes (HaCaT cells) were cultured in minoxidil sulfate with increasing concentrations of ASA (1, 3, and 5 mM) for 72 h. Compared to respective controls, the minoxidil sulfate growth-promoting effect on HaCat cells was significantly hindered by the presence of ASA in a dose-dependent manner. This preliminary result concurs with our speculation that ASA could diminish the minoxidil-induced hair growth response in some minoxidil users. Further studies are needed to determine the effectiveness of minoxidil in those who are concomitantly using ASA.

P048

Quantitative assessment of female-pattern hair loss by estimating the width of a reshaped baldness area

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Aims: The Ludwig and Savin scale categorize the severity of female-pattern hair loss (FPHL), but it is insufficient in describing patient progress. The appearance of baldness area should be clarified with a quantitative parameter so that even a minor difference among individuals can be decently detected and recorded for both follow-up assessment and treatment response.

Methods: Images of 44 FPHL patients with Savin scale stage I and II (from I-1 to II-2) were enrolled, and every image was normalized before the segmentation of baldness area. With level-set algorithm and principal component analysis, the baldness area was delineated and reshaped to an equivalent ellipse. The minor axis of the ellipse, indicating the width of the baldness area, was revealed. After estimating and declaring the appropriate interval of baldness width for each Savin scale, the severity of FPHL can be more precisely described.

Results: The image normalization process was verified to reduce the diagnostic discrepancy by 7.5% for physicians. With baldness width as the primary parameter, a stable diagnostic accuracy (82%) was achieved, while the diagnostic accuracy of the four physicians ranged from 40 to 64%.

Conclusions: The image normalization is approved to reduce diagnostic discrepancy and diagnosis of FPHL becomes a quantitative comparison of the baldness feature. By monitoring minor changes of the same individual, a customized diagnosis of FPHL for both follow-up assessment and treatment response can be facilitated in clinical practice.

P049

Racial differences of hair steroid profiling in male-pattern baldness between Korean and Caucasian populations

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The hair loss in male-pattern baldness (MPB) is the result of miniaturization of the hair follicle and shortening of the anagen phase of the hair growth cycle mediated by dihydrotestosterone (DHT), which is metabolized from testosterone (T) and catalyzed by 5α-reductase. However, the androgen activities in MPB within different racial groups are not completely understood. As biological fluids demonstrate no correlation between androgen levels and MPB, steroids extracted from the hair shaft were compared in balding and normal Korean and Caucasian subjects. Human hair fibers were obtained by cutting the proximal part hair from the vertex and occipital scalp, and the 12 steroid levels were evaluated in balding and normal Korean and Caucasian subjects. The balding groups in both populations had significantly higher DHT and T levels than the control groups. Balding Caucasians had twofold increased epitestosterone levels compared with normal Caucasians, but only slight increases compared with normal Koreans. 5β-Dihydroprogesterone levels were significantly increased in balding Koreans compared with normal Koreans, but were not significantly different from those in the Caucasian groups. Both Korean groups had higher vertex androgen levels than those of the Caucasian populations. However, levels of pregnenolone, a DHEA, and A-dione precursor were markedly higher in the Caucasian groups than in the Korean groups. For 5α-reductase, the metabolic ratio of DHT/T was increased slightly in both balding populations. The activities of 3β-hydroxysteroid dehydrogenase showed racial differences between normal and balding subjects. The quantitative results obtained from the occipital hair were not consistent with those of the vertex hair. Our findings confirm the existence of racial differences in hair steroid levels.

P050

Leukocyte telomere length is related to age but not to progression of androgenetic alopecia in Japanese males

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Background: Androgenetic alopecia (AGA) is strongly related to aging. Leukocyte telomere length shortens over time and it is known as a parameter of biological aging. We assessed the association of leukocyte telomere length with the progression of AGA.

Methods: Median terminal restriction fragment length as average telomere size was compared in the leukocyte DNA of 23 males, 20–35 years old (mean ± SE: 28.5 ± 4.3 years), with healthy hair (I in Hamilton–Norwood classification), and 23 age-matched males (28.0 ± 4.7 years) with AGA (IV and V in Hamilton–Norwood classification) by using the TeloTAGGG Telomere Length Assay kit (Roche, Mannheim, Germany) according to the manufacturer's instructions.

Results: Median telomere length between AGA males and healthy-hair males did not show significant difference (8.27 ± 0.58 vs. 8.23 ± 0.62, $P = 0.78$). Moreover, we compared the median telomere length between 30–35-year-old males ($n = 22$, 32.4 ± 1.6 years old) and 20–29-year-old males ($n = 24$, 24.6 ± 2.5 years old) to evaluate the association of telomere length with age. The length in 30–35-year-old males was significantly shorter than that in 20–29-year-old males (7.84 ± 0.35 vs. 8.63 ± 0.35, $P < 0.001$).

Conclusion: We demonstrated that the telomere length of leukocytes is associated with age, but not with progression of AGA in Japanese males. It is the first report on telomere length in leukocytes in AGA.

P051

Why care about linear hair growth rates (LHGR)?

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We found a positive correlation between hair thickness (μm) and linear hair growth rates (LHGR; μm per 24 hours) as well as *in vivo* on the scalp of humans and scalp grafts maintained of nude mice (*Ann NY Acad Sci* **642**: 480–482, 1991). Because uncertainties subsist in the technology (micrometry) and time window (30 days supposed continuous growth between clipping sessions for length measurement), especially with shorter hair cycling, we wished to control such information with up-to-date methods.

We used the contrast-enhanced phototrichogram method with exogen collection (CE-PTG-EC), an accurate technology (www.skinterface.be) for hair growth and thickness measurement. Sites on the top of the head were investigated in male subjects: 13 healthy controls and two groups of patients with male-pattern hair loss (MPHL; 39 (IHN 08 study) and 22 (P22 12 study) subjects, respectively, with Hamilton III–V patterns).

We confirm that there is a statistically significant (ANOVA;$P < 0.0001$) correlation between thickness and rate of growth in all tested subjects. In MPHL there were statistically significant and systematic slower growth rates in hair ⩽80 μm ranging from −18 to −7%. There was a non-significant difference between controls and MPHL with a range of −4 to +6% for thicker hair (∅ > 80 μm).

The reduced LHGR, together with reduced thickness, shorter duration of anagen, and increased ''lag'' between cycling phases, might contribute to the global impression of decreased cumulative growth and time-related difference in terms of reduction of hair volume on the top of the head as compared with the crown. This finding further explains why after 2 to 3 months patients become usually unhappy with their hairstyle and ask for ''equalizing'' the hair mass with a haircut!

More investigations on linear hair growth rate in individual patients might shed some light in terms of severity of hair loss and/or response to treatment.

P052

Observation of hair characteristics in the so-called 'permanent zone of scalp' containing part of the occipital and temporal areas according to BASP and Norwood–Hamilton classification in an Asian population with androgenetic alopecia

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Aims: Since previous studies reported the hair characteristics of a normal population, differences were noticed in practical use on treating alopecia patients. In addition, the hairs of occipital and temporal scalp are known to persist in androgenic alopecia patients, but there are no data about the follicular changes of the patients on such anatomical areas.

Methods: Our center divided 200 participants according to the BASP and Norwood–Hamilton classification, and analyzed the hairs of the occiput and 2 sites of the temporal regions with noninvasive digital phototrichogram. We examined hair density, numbers of follicular unit, ratio of compound/single hairs, hair diameters, and the ratio of terminal/vellus hairs.

Results: According to the BASP classification, statistical decrease of hair density was shown in the anterior aspect of the temporal region among U type and other groups of BASP ($P < 0.05$), although the hair densities of the occipital areas were statistically insignificant among the BASP subtypes. The diameters of hairs showed definite differences between U type and M, V, F types in all anatomical regions. The ratio of compound/single hairs and of terminal/vellus hairs was statistically insignificant.

Among the Norwood–Hamilton subtypes, only hair thickness in the temporal region showed statistically significant change as the disease reached the most severe form.

Finally, the age factor was divided into five groups (<30, 30–39, 40–49, 50–59, and ⩾60 years old). Statistically significant differences were observed in the hair density of the temporal region, measured above the ear helix, and in the hair thickness of the temporal regions ($P < 0.05$). Patients over 60 showed looser and thinner hairs than the younger population.

Conclusions: According to this observation, hair density and hair thickness of known permanent scalp regions showed significant differences depending on the severity, the BASP subtypes, and age in Asian people with male-pattern baldness.

P053

Epidemiologic and clinical profiles of Korean androgenetic alopecia patients

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Objectives: Androgenetic alopecia (AGA) is the most common form of hair loss. Previously, several studies had investigated the epidemiologic characteristics of AGA. The objective of this study was to evaluate the clinical features, medical history, and family history of AGA patients in Korea.

Methods: A total of 1,320 patients were enrolled. The demographics, past medical history, and past history of alopecia were investigated with medical records, laboratory results, and interviews.

Results: There were 862 male patients and 490 female patients (M:F = 1.7:1). The most prevalent age groups were the patients in their thirties (24.8%) and forties (20.8%). About 14% of the patients had a family history of AGA. Three hundred ninety patients were assessed by Basic and Specific (BASP) classification. The M type (55.9%) was the most common basic type and V type was the most common specific type. In all, 18.5% of the patients already had past history of medical problems. The most commonly associated diseases were hypertension (HTN) (13.8%), followed by diabetes mellitus (DM) (8.0%) and hypercholesterolemia (3.4%). Serum cholesterol, triglyceride, and glucose levels were increased in 24.1%, 12.9%, and 24.7% of the patients, respectively.

Conclusion: The prevalence of HTN, DM, and hypercholesterolemia did not show significant difference compared to the prevalences in the general population. These findings are different from previous studies conducted in individuals of Caucasian ethnicity, which showed a higher prevalence of associated diseases (HTN, DM, hypercholesterolemia) in AGA patients. We assume that these findings may be related to racial characteristics.

P054

An epidemiologic study of androgenetic alopecia using the basic and specific (BASP) classification in 3,114 Korean patients

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Background: Androgenetic alopecia (AGA) is the most common type of hair loss characterized by the transformation of terminal scalp hair into vellus hair. The epidemiology of AGA is not fully understood. A strong genetic basis has long been identified, while little is known of its non-genetic causes.

Objective: We investigated the clinical status using the Basic and Specific (BASP) classification and some environmental factors.

Methods: We examined 3,114 Korean AGA patients who visited 1 of 17 dermatologic clinics in South Korea from March 2011 to February 2012. Epidemiologic data were collected using a standard questionnaire.

Results: According to the basic type of the BASP classification, type M was the most common in both male (82.2%) and female (52.7%) patients. Type F was observed in 24.2% of Korean males with AGA. No associations were seen between eating or sleeping habits and severity of hair loss. However, drinking and smoking were associated with the severity of AGA in male patients. We observed that patients with a family history had more advanced types of hair loss in both genders. Moreover, the age of onset of AGA in male patients with a family history was earlier than in male patients without a family history.

Conclusions: Although there is very little evidence for environmental influences on AGA, the present study showed an association between hair loss severity and environmental factors, such as smoking and drinking. Also, the associations of family history with a higher degree of severity in hair loss and with the age of onset were observed.

P055

Investigator-initiated double-blind, two-armed, placebo-controlled, randomized clinical trial with an open-label extension phase, to investigate the efficacy of 5% Minoxidil topical foam twice daily in men with androgenetic alopecia in the fronto-temporal and vertex regions regarding hair volume over 24/52 weeks

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Background: 5% minoxidil topical foam (5%MTF) twice daily has shown effectiveness in male androgenetic alopecia (AGA) in the vertex area over 16 weeks of application. No long-term data on the efficacy of 5% MTF exist. Furthermore, there are little data on the efficacy of minoxidil in the fronto-temporal region in men with AGA.

Objectives: To assess the efficacy of twice daily application of 5% MTF compared with placebo in the fronto-temporal region of male patients with AGA after 16 and 24 weeks, as well as to gain long-term data on the efficacy and safety of 5% MTF BID in the fronto-temporal compared with the vertex region, over a period of 52 weeks.

Methods: Seventy male patients with moderate AGA (Hamilton–Norwood III_{vertex}–VI) were included. 5% MTF or placebo foam was applied twice daily for 24 weeks in the vertex and fronto-temporal regions, with changeover to a 28-week open-label phase with application of 5% MTF for all subjects. Measurements of non-vellus target area hair count (TAHC), non-vellus target area hair width (TAHW), global expert panel rating, and subject's rating were performed.

Results: Results were analyzed after 16 and 52 weeks. TAHC in the fronto-temporal area increased significantly at both time points compared to baseline (16 weeks: $P<0.0005$; 52 weeks: $P<0.02$). TAHW in the fronto-temporal and vertex regions showed a significant increase in both areas after 16 ($P<0.0005$) and 52 weeks ($P=0.04$). The occurrence of local intolerances and adverse events was similar in both groups. No major adverse events were recorded.

Conclusions: This is the first study proving the efficacy of 5% MTF twice daily in the fronto-temporal region. 5% MTF is a safe and effective treatment in men affected by moderate stages of AGA in the fronto-temporal and vertex areas over 52 weeks. (This work was supported by an Independent Investigator grant form Johnson & Johnson Consumer Companies, Inc.)

P056

Systemic growth factor treatment on the patients with androgenetic alopecia

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Background: Among the various stimulants of treatment in androgenetic alopecia (AGA), growth factor is known as an effective agent in hair regeneration. Systemic growth factor (SGF) is mainly composed of β-fibroblast growth factor (β-FGF), vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), and others.

Purpose: The aim of this study is to evaluate the efficacy of systemic growth factor treatment in patients with AGA.

Methods: SGFs were topically applied using medical devices containing a microneedle and by electroporation in a 2–4-week interval. The efficacy was evaluated by phototrichogram and digital photograph analysis after 10 times of treatment within 6 months. In total 116 patients, aged between 19 and 60 years, were enrolled (MPHL II–V: 54 patients, FPHL I and II: 62 patients) through 12 months, from October 2011 to September 2012.

Results: Phototrichogram showed 9.85% increase in hair density and 9.11% increase in hair thickness. In hair density, 30.1% showed 5–10% increase, and 25% of the patients showed more than 15% increase. In hair thickness, 35.3% presented 0–5% increase, and 21.5% patients presented more than 15% increase. MPHL cases were more effective than FPHL. Adverse effect was not observed except for a mild tingling sensation.

Conclusion: Systemic growth factor therapy is effective and safe for the treatment of AGA and this will be one of the treatment options for AGA.

P057

Male and female androgenetic alopecia indications for minoxidil, PRP, and follicles unit transplantation according to the phototrichogram and the multifactorial classification

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Introduction: It is known that hair growth is influenced by several parameters. Therefore, any alopecia consultation justifies a good clinical examination and a full evaluation analysis to establish a reliable diagnosis that is a prerequisite for any treatment.

Objective: The *Dynamic Multifactorial Classification* with various parameters that can be quantitated and computerized allows a precise evaluation of parameters such as fixed distances of the face, measurements of the balding area and the hairy area, scalp laxity and thickness, and covering power of hair. This includes density, caliber, shape, length, growth rate, and hair color. The *digitalized phototrichogram* allows a precise hair count for density and anagen–telogen ratio.

Results: All these measures proved to be efficient during the fluctuations of various parameters in hormonal, finasteride, minoxidil, and platelet-rich plasma (PRP) treatments. It also helped to determine precisely the surgical indications of hair transplant and allowed the best choice between the follicular unit extraction (FUE) or the follicular unit long hair (FUL) and the stage of maximal baldness according to a better assessment of the baldness evolution during the patient's life.

Conclusion: This approach will lead to a better evaluation of the evolution of androgenetic alopecia in both sexes, either spontaneously or under medical and surgical treatments.

P058

A randomized, double-blind, and placebo-controlled 12-month efficacy study of CG 210 on hair diameter in male alopecia subjects already using Finasteride 1 mg

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Study rationale: Finesteride is often regarded as first-line treatment for male patients with androgenic alopecia (AGA). However, after several years of treatment, patients often reach a plateau.

CG210 is a GMP-grade topical botanical blend that has clinically demonstrated its efficacy to reestablish regular A/T ratio (and hence normal hair cycle) in AGA sufferers within 44 days. Its unique clinically validated mechanisms of action are: preventing premature apoptosis of cells in hair follicles; reducing micro-inflammation in the scalp, and increasing collagen content. We would expect to get a beneficial effect when combining oral Finasteride 1 mg (acting on androgens) with topical CG210 (acting on apoptosis and micro-inflammation, two key factors causing hair loss).

Hair diameter is an accurate clinical parameter for hair miniaturization (larger diameter = longer anagen phase = improved proportion of anagen vs telogen hair (A/T ratio)). An increase in hair diameter should also affect the cosmetic appearance and hair volume.

Objective: To evaluate the clinical effect of the topical anti-hair loss CG210 vs placebo on hair diameter in Japanese AGA patients already using Finasteride 1 mg for > 3 years.

Study design: Randomized, double-blind, placebo-controlled, single-center, prospective, with two parallel groups.

Group A: Topical placebo provided to nine male alopecia subjects already taking Finasteride 1 mg treatment for > 3 years.

Group B: Topical CG210 provided to seven male alopecia subjects already taking Finasteride 1 mg treatment for > 3 years.

Results: Mean diameter of hair in the Finesteride + topical CG210 group versus Finesteride + placebo group increased by 36.9%. No adverse events were observed.

Conclusion: Application of topical CG210 in combination with Finasteride 1 mg demonstrated a statistically and clinically significant improvement in hair diameter and overall cosmetic appearance. Therefore, CG210 offers AGA sufferers an effective option to be used either alone to prevent and stop excessive hair loss or in combination with Finasteride to enhance patient outcome.

P059

Lifestyle evaluation of Korean androgenetic alopecia patients

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Background: Androgenetic alopecia (AGA) is the most common type of hair loss, and androgens and genetic predisposition are believed to be the major factors that influence the development of AGA. Although there have been previous reports about the relationship of family history, smoking, and insulin resistance with AGA, no previous study has investigated the lifestyle, such as eating habits, in AGA patients.

Objectives: The aim of this study is to investigate the family history, lifestyle, including smoking and eating habits, in AGA patients and to compare with the data from Korea Health Statistics 2009: Korea National Health and Nutrition Examination Survey (KNHANESIV-3).

Methods: We retrospectively evaluated a total of 347 male patients with AGA who visited the Department of Dermatology, Inha University School of Medicine, from September 2010 to August 2012.

Results: AGA with paternal family history was the most common (49.9%) and both (paternal and maternal) family history was the least (10.1%). BMI and smoking did not show significant differences, but eating habits showed statistically significant difference according to duration of AGA. Patients with longer disease duration (> 5years) showed more intake of meat but less intake of fish than patients with shorter disease duration. Also, AGA patients showed less intake of beef, bean, and squid in comparison to the average Korean population.

Conclusions: This is the first study to investigate lifestyle, such as eating habits, in AGA patients and to compare the result with data from Korea Health Statistics. However, further study is needed to define the casual relationship between eating habits and the development of AGA.

P060

Patients' satisfaction with the phototrichogram in androgenetic alopecia patients

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Objectives: Phototrichogram has been employed widely as a standard method for the determination of hair count, length, and thickness. The objective of this study was to evaluate patients' satisfaction with phototrichogram.

Methods: Patients with androgenetic alopecia (AGA) who were measured with a phototrichogram (Folliscope (LeedM) 2.8, Republic of Korea) participated in this study. The patients answered to questionnaires composed of three domains: patients' satisfaction, the convenience, and comparison to other treatments not using a phototrichogram. A 5-point scale was used.

Results: One hundred nineteen patients with AGA (male 77, female 42) were enrolled. In the patients' satisfaction domain, about 55% of the patients answered that they could realize accuracy in diagnosis and receive objective information about their conditions. Overall 63% of patients were satisfied with the phototrichogram. In the domain of convenience, most patients had no complaints about the process. 4.2% of patients replied that the process was time consuming and 13.4% of them felt discomfort in the method of measurement. 55.6% of the patients experienced an increase in satisfaction compared to the treatments not using phototrichogram.

Conclusion: Clinicians have considered phototrichogram as a standard tool for evaluation of AGA. But overall, only 63% of the patients were satisfied with the phototrichogram in this study. This percentage was certainly as per our expectation. These results may be related to the fact that the patients were not fully informed about its uses and importance in the process of diagnosis and treatment of AGA. We expect an increase in patient satisfaction on providing sufficient information about the phototrichogram.

P061

The frequency of antinuclear antibody in patients with patterned hair loss and alopecia areata

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Alopecia areata (AA) is an inflammatory disorder of the scalp that shows acute and sometimes chronic hair loss. Although the cause of AA is unknown, it is suspected to be an autoimmune disease. Although the significance of antithyroid antibodies in AA patients has been reported, few studies have examined the significance of antinuclear antibodies (ANA) in AA patients. Most studies reported no significant difference in terms of ANA titers between AA patients and normal controls. Meanwhile, pattern hair loss, also called androgenic alopecia, appears to result from androgen hyperactivity and genetic predisposition. However, the whole mechanism of pattern hair loss has not been proved. Even in pattern hair loss, there has not been any report about its relation with ANA. We retrospectively reviewed our patients who visited our hospital and checked the ANA in serum during 2009–2011. A total of 107 patients (51 females and 56 males) with patterned hair loss and 17 patients with AA (12 females and 5 males) were included. Their median ages were 38.3 and 38.9 years, respectively. Hematologic tests, thyroid function tests, and autoantibody tests were performed. In AA patients, 35.3% of 17 AA patients showed a positive ANA test. Among them, 23.5% were female and 11.8% male. In pattern hair loss patients, 30.4% of 105 patients (female: 19.1%, male: 11.3%) showed positive ANA. Our study suggests that ANA might be related to alopecia areata and pattern hair loss, which means that the anti-nuclear antibody's immunologic effect may act through the hair loss mechanism. Futhermore, we analyzed the relation between the severity of patterned hair loss and ANA using data from the patients' hair phototrichogram.

P062

Towards a cell-based treatment for androgenetic alopecia in men and women: 12-month interim safety results of a phase 1/2a clinical trial using autologous dermal sheath cup cell injections

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Cultured dermal sheath cup cells (DSC) from mouse vibrissae induced hair follicles *de novo* equal or superior to dermal papilla cells and also integrated with resident hair follicles in mouse ears producing larger follicles with longer, thicker hair. These observations have been developed into a GMP-manufacturing protocol for the treatment of androgenetic alopecia in humans (clinicaltrials.gov identifier: NCT01286649). Ten men and nine women with mild to moderate androgenetic alopecia were recruited to a phase 1/2a clinical safety trial with informed consent. For each subject, a biopsy from the scalp occiput was processed to isolate DSC cells from terminal hair follicles. Using a randomized, double-blind, placebo-controlled design, subjects subsequently received, via a validated semi-automatic injector, autologous, cultured DSC or vehicle alone in two separate predefined, tattoo-marked, contralateral scalp areas with thinning hair. Subjects are to return to the clinic for safety/tolerability and histopathological assessments, as well as global, macro, and TrichoScan images of the scalp and injection sites at regular intervals over 24 months. Injection site macro images are to be assessed by independent reviewers for local reactions and tolerability. Efficacy is to be assessed by determining cumulative hair thickness per area (mm cm^{-2}), mean hair thickness (μm), and terminal, vellus, and total hair density (n cm^{-2}) in the injected regions. Here we report the clinical development of this program and present the safety data at 12 months post injection.

CICATRICAL ALOPECIA

P063

Direct immunofluorescence of plucked hair in cicatricial alopecia: a preliminary case report

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Background: Direct immunofluorescence (DIF) is conventionally an important diagnostic tool in certain types of cicatricial alopecia. Recently, it has been shown that plucked hair can be used as substrate for DIF in the diagnosis of pemphigus. It is also crucial to perform this research in primary scarring alopecia, an immunological disease that target hair follicles.

Objective: Our aim was to assess the DIF of plucked hairs in a patient with scalp discoid lupus erythematosus (DLE), which is one of the common causes of scarring alopecia.

Materials and methods: We performed DIF on plucked hair in a case of DLE with typical clinical and histological findings and positive lupus band test of skin biopsy. Fifteen hairs were obtained in the same way as for the trichogram. Selected intact anagen plucked hair were processed for DIF. Five microns of cryosections obtained from plucked hairs were fixed in methanol-acetone and were then processed for DIF. The sections were incubated for 1 hour with the following fluorescein isothiocyanate (FITC)-conjugated antibodies: IgG, C3, IgM, IgA, and fibrinogen. Propidium iodide (PI) was used as a nuclear stain.

Results: IgG and C3 were immunopositive in diffuse granular pattern in the basal membrane of the epidermis, and IgG expression was also present in the outer sheath cells of the hair follicle. On the other hand, IgA, IgM, and fibrinogen expressions were not present in the hair follicle.

Conclusion: It seems that in DLE, a prototype of scarring alopecia, DIF of plucked hair has diagnostic value by utilizing horizontal cryosectioning. This is the first report about DIF of plucked hair in a DLE patient that demonstrates the possible usage of hair plucking as an alternative and less invasive technique for obtaining specimens for DIF, as well as contributing to its the rapid diagnosis.

P064

Frontal fibrosing alopecia: a genetic disease?

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Frontal fibrosing alopecia (FFA) is a primary lymphocytic cicatricial alopecia characterized by progressive hair loss from the frontal hairline, often involving the eyebrows. The disease is reported almost exclusively in post-menopausal women. The incidence of FFA appears to be increasing, but the etiology is uncertain. Seven reports of familial FFA have been reported in the literature. We report a further family in which three females in two generations are affected by FFA. The results of whole-exome analysis of affected and unaffected members will be presented. A 43-year-old woman presented with a 1-year history of recession of the frontal hairline, associated with eyebrow thinning. On examination, there was scarring alopecia and hair recession at the frontal and left temporal areas with perifollicular erythema at the affected hairline. Biopsy of the affected scalp showed a perifollicular lymphohistiocytic infiltrate with follicle destruction and scarring. The clinical and histological appearances were in keeping with FFA. On closer questioning, a similar pattern of hair loss was reported in both her mother and maternal aunt. Subsequent examination of these individuals also revealed frontal fibrosing alopecia. Male family members were unaffected. Blood samples were drawn from all three female affected members, as well as from the patient's unaffected brother and father, and sent for whole-exome gene sequencing. Exome sequencing is an efficient technique to selectively sequence the coding regions of the genome. It is a cheaper but effective alternative to whole-genome sequencing in identifying the causal variants associated with a particular disease.

The results of this testing are currently pending and will be available at the time of presentation. To our knowledge, this is the first report of genetic testing in a familial case of FFA and adds to the small number of familial FFA cases.

P065

Is hair transplantation indicated in frontal fibrosing alopecia?

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Frontal fibrosing alopecia (FFA) is an acquired scarring alopecia of unknown etiology. Medical treatment is usually non-effective and many patients with FFA become highly distressed by the typical band-like alopecia involving the frontal and temporal hairlines, and as the disease progresses they find it difficult to camouflage. This is the reason why more and more patients look into the possibility of a hair transplant as a permanent solution. However, there is little experience about the outcome of hair transplantation in patients with FFA.

In this study we describe the long-term follow-up of three patients with biopsy-proven FFA in whom test hair grafting was performed. The results of the hair transplant tests showed that the transplanted hair follicles initially grow well, but after 3–5 years most of them had been slowly but progressively destroyed.

A punch biopsy of one of the remaining transplanted FUs was carried out, showing the typical histopathological findings of FFA. The histological confirmation of FFA in the transplanted follicles suggests that FFA displays ''recipient dominance'', since the recipient area appears to have an effect on the survival rates of the transplanted hairs. It is conceivable that a local factor present in the frontotemporal skin region might be a determinant factor of the follicular destruction.

In conclusion, our experience shows that the long-term outcome of hair transplantation in FFA patients can be disappointing, and all potential patients should be advised of the possibility of hair graft loss over time. It is important to note that the initial growth of the hair grafts should not be interpreted as a successful outcome.

P066

Scarring alopecia of the sideburns: a unique presentation of frontal fibrosing alopecia in men

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Objective: To describe the clinical and histopathological features of four men with scarring alopecia of the sideburns.

Case 1: A 51-year-old black man presented with symmetric thinning of the bilateral sideburns. Examination revealed alopecia with loss of follicular ostia, but no perifollicular erythema or scale. A punch biopsy of the right sideburn revealed features consistent with lichen planopilaris (LPP).

Case 2: A 58-year-old white man presented with thinning hair on the frontal scalp and loss of sideburns and body hair. Examination revealed loss of lateral eyebrow hair and sideburns, alopecia with loss of follicular ostia on the crown, and patchy non-inflammatory alopecia of the face, trunk, and extremities. A punch biopsy of the left sideburn revealed LPP.

Case 3: A 40-year-old black man presented with symmetric thinning of the lateral eyebrow hair and sideburns bilaterally. Examination revealed no perifollicular erythema or scale. A punch biopsy of the left sideburn revealed LPP.

Case 4: A 53-year-old white man presented with scalp pruritus and hair loss from the sideburns and temporal scalp. Clinically, patchy alopecia and mild perifollicular erythema was present at the sideburns, and perifollicular scale on the scalp. A punch biopsy of the sideburn skin revealed late-stage LPP.

Discussion: Frontal fibrosing alopecia (FFA) is a variant of LPP that predominantly affects postmenopausal women. It causes scarring alopecia of the frontal and preauricular hairline, and often causes loss of eyebrows as well as body hair. Hair loss in non-scalp sites tends to have less perifollicular erythema and scale. We report four men with scarring alopecia of the sideburns, whose biopsies revealed LPP. This brings the total of histologically confirmed men with FFA to 7. Only one of the seven patients had perifollicular erythema. LPP should be considered in the differential diagnosis of sideburn alopecia, even in the absence of inflammation.

P067

Cicatricial alopecia—a retrospective review of 52 cases

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Aims: To review the clinicopathological correlation of clinical and histological findings, and therapeutic options in cicatricial alopecia (CA).

Methods: We retrospectively analyzed 52 patients with CA (M:F = 10:42, mean age 58.8 years, range 28–92 years) from three district general hospitals.

Results: The clinical diagnoses included lichen planopilaris (LPP) ($n = 21$, 40.4%), chronic cutaneous lupus erythematosus (CCLE) ($n = 11$, 21.2%), frontal fibrosing alopecia (FFA) ($n = 6$, 11.5%), folliculitis declavans (FD) ($n = 10$, 19.2%), and pseudopelade of Brocq (PB) ($n = 3$, 5.8%). One case remained undiagnosed. Thirty-seven patients (71.2%) underwent scalp biopsies. Correlation between clinical and histological findings was present in 70.3% (26/37) of cases (LPP, 14/18 (77.8%); CCLE, 5/6 (83.3%); FD, 6/7 (85.7%); FFA, 0/2 (0%); PB, 1/3 (33.3%); undiagnosed, 0/1 (0%)). In LPP, topical corticosteroids (TC) were used in 81.0% (17/21) of patients, with control of disease in 35.3% (6/17) and regrowth in 23.5% (4/17). Topical calcineurin inhibitors were used in 23.8% (5/21), and stabilizing disease in 60% (3/5). Oral/intralesional corticosteroids were used in 19.0% (4/21), and stopping progression in one case. Antimalarials were prescribed in 9.5% (2/21), with regrowth in one. All patients with CCLE were treated with TC, halting disease progression in 45.5% (5/11). Among those treated with antimalarials, 62.5% (5/8) were stabilized. Ciclosporin stopped progression in one case. FD was managed with systemic antibiotics (tetracyclines, macrolides, and rifampicin + clindamycin), with success in 70% (7/10). Treatment for FFA included TC in 83.3% (5/6), which halted disease progression in three patients. PB was effectively treated with antimalarials in one case.

Conclusions: In our limited review, we have noted that TC is beneficial in LPP, FFA, and CCLE, with an overall response rate of 54.5% (18/33). The addition of antimalarials is helpful in CCLE. Systemic/topical antibiotics are the mainstay of treatment for FD. Multi-centre, prospective studies are required to elucidate the gold standard treatments for each type of CA.

P068

Afro-American alopecia: specific treatments and transplantation

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Introduction: Afro-American hair and scalp present typical aspects in ethnic hairdressing and grooming, morphology, and physiology (coiled and helical hair shaft, elliptic or flattened cross section, curved hair root, low hair density, and slow hair growth), leading to specific pathological problems and to adapted treatments.

Materials and methods: Specific Afro-American hair pathologies include traction alopecia, hot-comb alopecia, folliculitis and pseudofolliculitis, acne keloidalis, dissecting cellulitis, and cicatricial alopecia. Medical and cosmetological treatments should be adapted to dry and brittle hair shafts and to the specific inflammatory sensitivity of black patients' scalp.

Discussion: Surgical treatments, procedure, instruments, and techniques should be adapted according to alopecia area and the scalp and hair ethnic specificity. Before doing any treatment, all the traction of the hair roots and hair shafts is completely stopped.

For Afro-American patients it is easier to achieve a good and dense esthetic result with the FUL (follicular unit long) hair rather than the FUE (follicular unit extraction) procedure. This is almost due to the curved hair roots and the lowest donor hair density.

Afro-American patients are notably susceptible to postinflammatory hyperpigmentation or hypopigmentation, and hypertrophic or even keloidal scarring process.

Conclusion: For quiescent alopecia and non-aggressive hair styling in Afro-Americans, it is possible to get a definitive and aesthetic reconstruction with follicular unit transplant.

P069

Frontal fibrosing alopecia: a case-note review over a 12-year period in a tertiary center

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Frontal fibrosing alopecia (FFA) is a distinctive cicatricial alopecia with a rising incidence. It mainly affects postmenopausal women, with a typical clinical picture of frontotemporal scarring hair loss, follicular hyperkeratosis, and variable peri-follicular erythema. Eyebrow involvement is common with occasional involvement of body hair loss (particularly the forearms) and, rarely, eyelash loss. The condition is extremely challenging to manage with an often limited treatment response. A wide variety of topical and systemic pharmacological modalities have been reported in the literature.

We sought to explore the approach to management of this condition in our tertiary center for a 12-year period between 2000 and 2012. Patients coded with a diagnosis of ''frontal fibrosing alopecia' on the electronic medical record system were identified. Fifteen patients had clinical and histological confirmation of FFA, and a case-note review was undertaken to determine the treatments used and their documented efficacy.

The commonest modality employed was super-potent topical steroids in 9/15 (60%) of patients. This resulted in stabilization or improvement in 4/9 (44%) of cases used. Lymecycline 408 mg once daily was used in 4/15 (26%) of patients and led to improvement in two cases. Dutasteride was used in three patients, with one patient not tolerating the medication, one deriving some benefit, and one experiencing progression on treatment. 2/15 patients were subsequently escalated to immunosuppressive treatment with either azathioprine or ciclosporin and an improvement was seen in both cases.

FFA is a condition with significant morbidity and impact on quality of life. It has an as yet uncharacterized aetiology and is likely to be underdiagnosed in the general population. Treatment remains a challenge, but in our small, retrospective case series super-potent topical steroids were most helpful.

P070

First evidence of bacterial biofilms in the anaerobe part of scalp hair follicles: a pilot comparative study in folliculitis decalvans

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Background: The cause of folliculitis decalvans (FD) remains unknown. We hypothesized that a bacterial biofilm could be involved in its pathogenesis.

Objective: To assess the presence or not of a bacterial biofilm in the hair roots of the scalp in FD.

Patients and methods: Hairs plucked from four patients and three controls were examined by field emission scanning electron microscopy (FESEM) and confocal laser scanning microscopy (CLSM).

Results: Bacterial communities organized as biofilms were observed both by FESEM and by CLSM in the infra-infundibular part of hair follicles in all patients and in two of the three controls. In patients and controls, these biofilms were formed exclusively of bacilli of comparable shapes compatible with *P. acnes*.

Discussion: As the growing knowledge about biofilms shows, it seems possible that the normal and pathological scalp hair follicle microbiota are organized in biofilms as suggested by our observations and recent literature data. In line with our hypothesis, this study has shown the presence of biofilms in the hair follicles of patients with FD and raises the question of its causal role in FD pathogenesis. The hypothesis of a follicular biofilm in FD could explain the recurrences despite appropriate antibiotic therapy for *S. aureus* obtained from the lesions (which are also active against *P. acnes*), histology (influx of polymorphonuclear neutrophils that secrete cytokines that destroy the hair follicles without destroying the biofilm), and the usual normal immune and genetic background of patients. Thus, *S. aureus* observed frequently on the skin in these patients would only be opportunistic.

Conclusion: This pilot study provides the first evidence of the presence of bacterial biofilms in the infra-infundibular part of human scalp hair follicles. These biofilms were detected both in FD patients and controls, suggesting their ubiquity as a commensal biofilm with a possible pathogenic shift in FD.

P071

Vulval and oral lichen planus in frontal fibrosing alopecia

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Lichen planus (LP) is an inflammatory dermatosis affecting many cutaneous and mucosal sites. This study describes the patterns of alopecia observed in patients with vulval lichen planus.

In the 4-year period from January 2009 to December 2012, 83 new patients referred to the specialist Vulval Clinic were diagnosed with vulval lichen planus. All patients were routinely asked and examined for any hair loss. There were 16 patients (19.2%) with scarring alopecia of the scalp, and these were grouped into those with diffuse alopecia, one or more localized patches of alopecia, or with the patterned variant of frontal fibrosing alopecia (FFA).

The commonest pattern encountered was that of FFA, which was present in 8 women (50%), with 6 of them also exhibiting loss of eyebrow hair. All of these patients also had oral LP, and in 7 of the 8 cases the patients had the erosive scarring variant of vulval LP. Four patients had a single patch of alopecia localized at the crown, two patients had multiple patches, and two patients had diffuse scarring alopecia. In all those patients who had a scalp biopsy, the characteristic histopathological features of lichen planopilaris were identified.

Our study documents that a significant percentage of patients with vulval LP have associated alopecia, with FFA being the commonest clinical pattern. This suggests a further expansion of the FFA clinical spectrum, identifying a specific subgroup with concomitant vulval involvement.

P072

Reassessing the histopathology of primary cicatricial alopecias: an evaluation of 256 biopsies

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Despite years of study, many dermatopathologists find it difficult to render specific diagnoses based solely on histopathological features. A small study demonstrated that histopathological findings in biopsies from 20 cicatricial alopecia patients were separable into lymphocyte-mediated and neutrophil-mediated cicatricial alopecia.

With a significantly larger sample, this study aimed to test whether certain histopathological features are characteristic of specific clinical variants. It also aimed to compare the histopathology of cicatricial and non-cicatricial alopecias.

Cases were retrieved and reviewed from records of the UCSF Dermatopathology Service and the UCSF Hair Clinic. Clinical and histopathologic diagnoses were tabulated and matched to the NAHRS working classification. Consecutive cases were included up to a maximum of 30 per entity. Histopathologic assessment was blinded. Cases of chronic cutaneous lupus erythematosus (CCLE) were included. Non-cicatricial alopecia cases served as controls. Over 25 histopathologic attributes were scored, tabulated, and compared.

A total of 256 biopsies were scored, including 198 from 109 cases of cicatricial alopecia, 27 from CCLE, and 31 from non-cicatricial alopecia cases. Irrespective of the clinical diagnosis, cicatricial alopecia was typified by concentric perifollicular fibrosis, loss of sebaceous glands, inflammation involving the upper segment, compound follicle formation, and scarring of fibrous tracts. A plasmacyte-rich infiltrate (49% vs. 1%), extra-adventitial inflammation (80% vs. 22%), and fibrosis (78% vs. 5%), and extensive compound follicle formation (60% vs 10%) favored a diagnosis of neutrophil-mediated rather than lymphocyte-mediated cicatricial alopecia. Features that have been suggested to be specific to certain clinical variants, like squamotization and premature desquamation of the inner sheath, were not limited to lichen planopilaris or central centrifugal alopecia and were identified in other cicatricial alopecias.

We contend that many attributes held to be characteristic of certain clinical variants of cicatricial alopecia are not specific. However, a distinction between lymphocyte-mediated and neutrophil-mediated cicatricial alopecia is generally possible.

P073

Impaired ubiquitin–proteasome system in cicatricial alopecia: implications for inflammatory response

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Primary cicatricial alopecia (PCA) is a group of inflammatory disorders that cause scarring and permanent hair loss. Mechanisms that trigger the inflammatory response in PCA are poorly characterized. Here, the goal was to determine whether the ubiquitin–proteasome system (UPS), a major ATP-dependent protein degradation pathway, is defective in PCA. The UPS consists of concerted actions of enzymes that tag ubiquitin onto proteins to mark them for degradation. This leads to their recognition by the 26S proteasome complex that degrades ubiquitinated proteins. TEM studies of PCA subtypes showed the accumulation of protein aggregates in hair follicle cells. Studies aimed to identify protein aggregates tagged or untagged with ubiquitin from diseased cells are currently in progress. Evidence of protein aggregation suggests for the first time that the proteasome system may be impaired in PCA. We used an integrated proteomics and transcriptomics approach to characterize the UPS in centrifugal cicatricial alopecia (CCCA), frontal fibrosing alopecia (FFA), and lichen planopilaris (LPP). Our analysis revealed that the gene and protein expression associated with the proteasome complex is deregulated in unaffected scalp tissue in all subtypes. In addition, enzymes required for protein ubiquitination are de-regulated, suggesting the impairment of the UPS in PCA. To determine if oxidative stress causes UPS inactivation, we carried out western blotting with four hydroxynonenal antibodies. Mass spectrometric analysis of the bands obtained showed that enzymes of the ubiquitin–proteasome complex are subject to oxidative modification, suggesting that this post-translational modification causes impairment of UPS. Intriguingly, proteome analysis identified the major histocompatibility complex (MHC) and inflammasome proteins to be de-regulated in PCA. Our data suggest that accumulation of damaged proteins due to UPS impairment may serve as the primary trigger for inflammatory responses in PCA. Thus, UPS activation may be a promising new treatment strategy for PCA.

P074

Small fiber neuropathy in symptomatic lichen planopilaris (LPP) and frontal fibrosing alopecia (FFA)

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LPP is a cicatricial alopecia that is commonly associated with scalp symptoms such as pain, burning, paresthesias, and pruritus. FFA is a clinically distinct cicatricial alopecia but is histologically very similar to LPP. There has been little research on the role of neurogenic inflammation in these diseases. While peroxisome proliferator-activated receptor gamma (PPARγ) expression has been found to be decreased in lichen planopilaris (LPP), neuropeptide-induced inflammation may also be a major contributor to disease pathogenesis and symptomatology. Calcitonin gene-related peptide (CGRP) is elevated in chronic inflammatory conditions, while PPARg is usually decreased. Substance P (SP) induces production and release of cytokines such as IL-1, IL-6, and TNF-α in addition to PPARγ through the neurokinin 1 (NK1) receptor. To evaluate the role of neurogenic inflammation in LPP and FFA, 4-mm skin biopsy specimens were obtained from the affected scalp skin of four LPP patients, three FFA patients, and six control subjects. The biopsy samples were stained for Protein Gene Product (PGP) 9.5, CGRP, and SP using an immunofluorescence technique. The distribution and quantification of CGRP and SP expression in the subepidermal nerve plexus and PGP 9.5 in epidermal nerve fibers were evaluated using confocal microscopy. We found a statistically significantly lower epidermal nerve fiber density ($P<0.05$), a trend toward lower CGRP expression, and similar SP expression in patients with both LPP and FFA as compared to normal control samples. These findings suggest the pain-affected patients' experience could be related to the loss of epidermal nerve fibers, characteristic of a small fiber neuropathy. Treatment with medications such as topical gabapentin or clonidine could potentially be beneficial in symptomatic patients.

P075

Epidemiology of patients with central centrifugal cicatricial alopecia

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Central centrifugal cicatricial alopecia (CCCA) is a form of scarring hair loss primarily seen among African American women. Symptoms and the severe disfigurement caused by this form of hair loss lead to large numbers of patients seeking treatment. This study examines common hair care practices, family history, health history, and stage of disease in CCCA patients.

A survey was administered including: severity of CCCA, age of onset, familial prevalence, concurrent medical conditions, efficacy of treatments used, and history of all hair care practices employed. Stage of hair loss was recorded and photographs of each subject's scalp were taken. Thirty-eight subjects were recruited. Subjects presenting with stage 5 hair loss according to the Central Scalp Scale report an average duration of hair loss that is longer than those with stage 1. Subjects with more severe CCCA tend to shampoo more frequently. Steroids, topically applied or given as injections to the scalp, are both the most commonly used treatment for CCCA and are reported to have helped more than topical minoxidil ($P<0.001$). Of the many listed concurrent medical conditions, over 50% of subjects reported experiencing facial hair growth, vaginal yeast infections, and seborrheic dermatitis. Of the 38 subjects, 37 reported a history of using a chemical relaxer on their hair and 21 subjects continue to use a relaxer. Using a hot comb, and other styling techniques, also has a high prevalence in the subjects' history but is not used in their current routine.

The study was conducted with subjects seen in one medical center, but these data will be combined with that from other sites, totalling 250 subjects as part of the NAHRS Cicatricial Alopecia Work Group. A larger sample size from regions across the United States will further provide valuable information and add strength to the trends we have found.

P076

Facial papules in women with frontal fibrosing alopecia

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Introduction: A connection between facial papules and frontal fibrosing alopecia was recently introduced to the literature. Papule histology resembles that of lichen planopilaris.

Objective: To review the frequency of facial papules in patients seen in the University of Toronto Hair Clinic over the years 2010–2012 and to identify risk factors and response to treatment.

Materials and methods: A retrospective review of clinical and photographic records of patients with FFA was performed.

Results: Thirty-four patients with frontal fibrosing alopecia were identified. Thirty patients were post-menopausal and four were pre-menopausal. Facial papules were present in 10 (32%). In eight women the facial papules developed slowly and in two women they developed rapidly associated with intense erythema. Compared to women without the facial papules, women with facial papules were more likely to have indicated loss of body hair (80% vs. 17%, $P<0.005$). Facial papules were also more likely in women with more than 1.5 cm of frontal loss (62% vs. 10%, $P=0.002$). Topical treatment of the facial papules was uniformly poor, including those involving topical steroids, retinoids, metronidazole, and tacrolimus. One woman developed facial papules while concurrently receiving oral hydroxychloroquine and experienced resolution without further treatment.

Conclusion: Facial papules are associated with frontal fibrosing alopecia in a proportion of women, and, like the scalp condition itself, present particular treatment challenges.

CLINICAL CASES

P077

Review of the use of diphencyprone in the treatment of alopecia areata: the last 20 years in Glasgow

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Immunotherapy with diphencyprone (DCP) can be used to treat alopecia areata (AA); however, its efficacy is variable within the literature and response to treatment cannot currently be predicted. We reviewed the patient data from our DCP service from 1991 to 2010, including outcome and patient variables that may affect treatment response.

During this time we carried out 205 treatments with available data. A total of 162 courses of treatment (on 133 patients) were completed and overall 19.1% (31/162) had a response of 90–100% cosmetic satisfaction or complete re-growth (>90% cosmetic satisfaction).

Overall 62.4% (101/162) of patients had some response to DCP treatment (including vellus hair) detectable at the end of the treatment course, but 23.5% (38/162) did not respond. An additional 13% (21/162) responded to DCP but relapsed during treatment. Our data suggested that patients with mild AA, a shorter history of AA, and no history of atopy are more likely to achieve >90% cosmetic satisfaction. Additionally, on average, patients achieving >90% cosmetic satisfaction had been exposed to a higher median concentration of DCP compared to non-responders.

In all, 5.9% (12/205) of patients commencing DCP treatment terminated treatment early due to side effects. Notable side effects included a type-I urticarial reaction to DCP, severe facial swelling, and an acneiform eruption.

Overall our data, which appear to be one of the largest consecutive series of patients treated with DCP, suggest that in our unit approximately 1 in 5 patients achieve >90% cosmetic satisfaction with DCP treatment; however, 1 in 5 patients does not respond. In addition, it may support previous reports of the associations of atopy, longer history of AA, and more extensive AA with a less favorable outcome to DCP and appeared to be well tolerated by most.

P078

Analysis of quantitative changes in hair growth during treatment with chemotherapy or tamoxifen in breast cancer patients: a cohort study

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Aims: Chemotherapy-induced alopecia (CIA) is widely dreaded by breast cancer patients. The onset, pattern, and amount of hair loss differ depending on the treatment regime and have not yet been quantified using standardized techniques. This study aimed at quantifying and comparing the effect on hair loss in women receiving either chemotherapy or tamoxifen, by evaluating hair growth parameters.

Methods: In a monocentric, prospective observational cohort study, 34 breast cancer patients, after surgery treated with either chemotherapy or tamoxifen, were included. Patients were evaluated once before, twice during, and twice after therapy. At each visit, telogen–anagen hair ratio and hair density per cm^2 were quantified by phototrichogram in two defined areas of the scalp, one frontal and one occipital. The course of hair loss and regrowth was visualized with global photography.

Results: Telogen hair ratio increased on both investigation sites after 3 and 6 weeks of chemotherapy. Two weeks after cessation of chemotherapy, telogen hair ratio decreased and hair density was lower compared to baseline. Twelve weeks after cessation of chemotherapy baseline levels of hair density were reached. Patients treated with tamoxifen showed no significant differences in telogen–anagen hair ratio. Hair density decreased in the frontal areas 18 weeks after therapy onset, but not in the occipital area.

Conclusions: This study reveals that the type, course, and quantity of hair loss differ comparing chemotherapy and tamoxifen. Evaluation of CIA using objective methods is mandatory to evaluate preventive or therapeutic outcomes in the future.

P079

Adalimumab to treat recalcitrant dissecting cellulitis of the scalp

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Dissecting cellulitis of the scalp (DCS) is an uncommon condition, mostly seen in young men. Treatment is often disappointing and includes antibiotics, oral isotretinoin, topical, intralesional and systemic steroids, radical surgical excision with skin grafting, carbon dioxide laser, and, more recently, tumor necrosis factor (TNF) antagonists. To date there have been six reports of DCS treated with TNF antagonists (adalimumab and infliximab). We report our recent experience using adalimumab to treat recalcitrant DCS, and review the literature in this area.

A 48-year-old Afro-Caribbean man presented with a 20-year history of tender plaques, sinuses, and pustules with foul-smelling discharge on the scalp leading to scarring alopecia. He was under a hepatologist for fatty liver disease, with GGT levels up to 37 times the upper limit of normal, and ALT twice the upper limit. Skin biopsy was in keeping with DSC.

Various treatment modalities had been unsuccessful, including topical and intralesional steroids, long-term prednisolone up to 20 mg daily, systemic antibiotics (doxycycline, flucloxacillin, ciprofloxacin, amoxicillin, erythromycin, and clarithromycin), dapsone, isotretinoin, and surgical drainage. The involved area was too extensive for surgical resection alone and therefore anti-TNF-α agents were considered with or without surgery for any residual disease. Most cases of liver toxicity associated with TNF antagonists have been linked to infliximab and to a lesser extent to etanercept; therefore we chose adalimumab. This was initiated 7 weeks ago with a partial response so far and we plan to update you on his further progress. Reassuringly, his liver function has improved since initiation. There have been four previous reports of treatment of DCS with adalimumab, however, none in a patient with liver disease.

The treatment of DCS remains challenging, but anti-TNF-α agents promise at least palliative treatment of this disfiguring disease, thus potentially reducing the extent of scarring alopecia and reducing the need for extensive surgical excision.

P080

Clinical characteristics and prevalence of chemotherapy-induced alopecia in childhood

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Chemotherapy-induced alopecia (CIA) is a frequently observed complication with high psychological morbidity in patients who underwent chemotherapy. In addition, there is an increasing number of reports of permanent CIA, which usually occurs under high-dose conditioning chemotherapy and subsequent hematopoietic stem cell transplantation (HSCT). We investigated the clinical characteristics of CIA, including permanent CIA, in pediatric patients and analyzed factors that might be associated with development of CIA. We conducted a questionnaire survey in pediatric patients who had been treated with high-dose conditioning chemotherapy followed by HSCT due to various underlying diseases. In addition, we obtained clinical details from reviews of medical records and evaluated the status of hair regrowth by global photography and phototrichogram.

A total of 159 patients were included. The mean age of participants was 12.1 ± 5.3 years (mean ± SD) and age at diagnosis and HSCT were 6.2 ± 4.7 and 7.4 ± 4.9 years, respectively. Mean hair density was 198.3 ± 47.4 cm^{-2}. CIA began at 1.5 ± 1.4 months and sustained till 2.2 ± 1.6 months after the initiation of chemotherapy.

Hair regrowth started 2.6 ± 1.6 months after chemotherapy ceased and lasted for 7.3 ± 4.9 months. When hair regrew following chemotherapy, 67.1%, 58.3%, and 78.8% of patients experienced density, color, and texture change compared with hair status before chemotherapy, respectively. Regrown hair was thinner (80.4%) and lighter (79.8%) than the original in texture and color. Out of 159 patients, 19 (12%) had extensive hair loss (Olsen criteria 4 or more) at 6 months post chemotherapy, namely permanent CIA. Increasing age at HSCT is a significant risk factor for permanent CIA by multivariable logistic regression analysis ($P<0.05$).

Although CIA in the pediatric patients has been not investigated compared to adult patients, it surely is a very distressing side effect in children at an impressionable age. Therefore we must provide appropriate information before and after chemotherapy and support the patients psychologically.

P081

Loose anagen hair syndrome: a survey of 171 patients

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Background: The loose anagen hair (LAH), which is also known as loose anagen syndrome (LAS), is a disorder of abnormal anagen hair anchorage. The LAH is a sporadic or autosomal dominant disorder with variable expressivity that primarily affects children. The typical complaint of the parent is that the child's hair is lusterless and does not grow. Gentle traction results in hair that is painlessly removed. Diagnosis of LAH syndrome relies on the number and percentage of LAH at the hair-pull test and on trichogram. Olsen *et al* and Tosti *et al* proposed diagnostic criteria for LAS on the basis of the hair pull test and trichogram. Pull-test results with a painless extraction of at least 10 LAHs and the presence of more than 80% LAH on trichogram were regarded as positive.

Patients and methods: Between 2000 and 2012, 450 consecutive children who applied to a specialist hair clinic were assessed for loose anagen hair syndrome (LAS). The diagnosis included a complete medical case history, clinical examination, hair pull test, and trichogram administered to each patient. Scanning electron microscopy (SEM) was performed on selected cases. A total of 171 children with LAS having the minimum percentage of 80% LAH were chosen from 450 children who had a percentage of LAH >50%.

Results: Children with LAH included 131 girls (76.6%) and 40 boys (23.4%). More statistical analysis will be presented in the poster. We will show association with hereditary or developmental disorders that we found.

Conclusion: LAH syndrome occurs in children and could be on the one hand underdiagnosed; on the other hand, unaffected individuals may present with the same mutation as observed in the affected. The condition is not rare. LAS should be diagnosed only when the pull test shows 85% or more LAH and trichogram shows 80% or more LAH.

P082

A pilot clinical study of hair grafting in chronic leg ulcers

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Epidermal sheets spread centrifugally post injury from the hair follicle infundibulum to reepithelialize the wound bed. Healing progresses faster in skin areas rich in terminal hair follicles. These observations are consistent with the role of the hair follicle as a major reservoir for progenitor cells. To evaluate the feasibility and potential healing capacity of autologous scalp follicular grafts transplanted into the wound bed of chronic leg ulcers, 10 patients with ulcers of an average size of 36.8 cm^2 and a 10.5-year duration were included in this pilot study. Within each ulcer we randomly assigned a 2 cm × 2 cm "experimental" square to receive 20 hair grafts and a nongrafted "control" square of equal size. The procedure seemed to be safe. At the 18th-week end point, we observed a 27.1% ulcer area reduction in the experimental square as compared with 6.5% in the control square ($P=0.007$). Histological analyses showed enhanced epithelialization, neovascularization, and dermal reorganization. We conclude that terminal hair follicle grafting into wound beds is feasible in an outpatient setting and represents a promising therapeutic alternative for nonhealing chronic leg ulcers. We conclude that terminal hair follicle grafting into wound beds is feasible in an outpatient setting and represents a promising therapeutic alternative for nonhealing chronic leg ulcers.

P084

Frontal fibrosing alopecia and lupus overlap in a man

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A 46-year-old Caucasian man, with an otherwise unremarkable past medical history, presented with a 3-months history of eyebrow thinning. On examination he was found to have erythematous and pruritic eyebrows, associated with preauricular and supraauricular hair loss. One year later he reported the development of photosensitivity and photo-aggravation at the sites of hair loss. An initial biopsy of the left temple showed decreased density of terminal hair follicles and focal perifollicular fibrosis, consistent with scarring alopecia. Direct immunofluorescence study was negative. Two years later, he represented with worsening hair loss. On examination, almost complete loss of the eyebrows, beard, and hair thinning bilaterally at the temples, as well as hair loss of the forearms were noted. Biopsies from the scalp, eyebrow, and arm all showed the features of lichen planopilaris. This pathology, combined with the clinical picture, was consistent with frontal fibrosing alopecia (FFA). However, basement membrane zone thickening was highlighted by PAS on both the scalp and eyebrow biopsies. Direct immunofluorescence study demonstrated a bright granular basement band staining with IgG and, to a lesser extent, IgM, IgA, and fibrin, consistent with lupus. The patient's serology subsequently revealed positivity for antinuclear antibodies, anticardiolipin antibodies, and lupus anticoagulant. There were no systemic symptoms of lupus. This case is noteworthy, as it illustrates the rare occurrence of frontal fibrosing alopecia in a man. Also, this is only the seventh case of biopsy-proven FFA in a male reported in the literature and, moreover, the first male case with coexisting lupus. Our case highlights the importance of multiple biopsies and follow-up in the diagnosis of FFA-lupus overlap syndrome.

P085

Sebaceous gland atrophy: a potential pitfall in the histopathologic evaluation of non-scarring alopecia associated with psoriasiform dermatitis of the scalp

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Sebaceous gland atrophy of the scalp was originally reported by Headington *et al.* in 1989 in association with psoriasis. This finding is seldom taken into consideration when evaluating scalp biopsies in the context of nonscarring alopecia. Sebaceous glands undergoing atrophy show loss of mature sebocytes, leaving basaloid epithelial aggregates that can morphologically resemble telogen hair follicles. Furthermore, advanced sebaceous gland atrophy resulting in complete loss may mimic scarring alopecia. We searched our alopecia histopathological database between 2006–2012, looking for scalp biopsy reports containing the terms ''psoriasiform epidermis'', ''psoriasis'', and ''seborrhoeic dermatitis''. Ten cases were identified, eight of which were retrieved and studied. All biopsies had been sectioned horizontally and vertically. Clinically, all patients had a history of hair loss but only one case provided a history of psoriasis and one of severe eczematous dermatitis involving the scalp. Histologically, all cases showed nonscarring alopecia with overlying psoriasiform epidermis and variable degrees of sebaceous gland atrophy simulating telogen hair follicles and scarring alopecia. Our study highlights the importance of being aware of sebaceous gland atrophy when evaluating scalp biopsies for nonscarring alopecia. We also draw attention to potential diagnostic pitfalls that may occur in the absence of adequate clinical–pathological correlation.

P086

Trichorrhexis nodosa after hair transplantation: dermoscopic, pathologic, and electron microscopy analyses

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Trichorrhexis nodosa may have a genetic etiology but appears to result from repeated physical and chemical trauma, such as hair permanent liquid and blow drying. Although repeated hair shaft trauma is the major cause of acquired trichorrhexis nodosa, the other factors that cause acquired trichorrhexis nodosa remain inconclusive. Hair transplantation is a relative safe procedure compared to other surgical corrections for alopecia, but mild or severe complications can occasionally result from malpractice or poor quality control. Herein, we report a previously unpublished case of trichorrhexis nodosa induced by hair transplantation and describe the evaluation of this condition with blood test, dermatoscopy, scanning electron microscopy, skin biopsy, and hair mineral analyses. A 38-year-old man visited our dermatology clinic with breakage and lack of growth in hair that had been transplanted 3 years previously. The transplanted hairs failed to grow and broke off easily, although 1 year had passed since the hair transplantation. The overall survival rate of the transplanted hairs was very low. Transplanted hairs were distributed sparsely, and were short and lusterless on the recipient frontal area of the scalp. On dermoscopic examination, intermittent whitish discolored nodes of hair shaft were apparent, and the ends of the hair appeared weathered on close observation. There were many transplanted hairs with pits on the recipient area. The tips of hairs were severely split and the nodular swelling of the hair shaft exhibited the so-called 'crushed paint brush' appearance upon scanning electron microscopy. Histopathologic examination of the scalp biopsy showed a fibrous tract around the previous transplanted follicles. We also assessed if the transplanted hairs were located more deeply than the surrounding existing normal hairs. The blood and hair mineral analyses, which included a thyroid function test and copper and sulfur determinations, were normal.

P087

Invisible bleeding from clean-shave haircuts

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Blood-borne viruses such as the human immunodeficiency virus (HIV) are a major public health concern affecting millions of people worldwide. The risk of virus transmission through non-sexual routes such as contaminated barbering equipment could easily be eliminated with adequate sterilization. A history of at least one episode of visible bleeding after a clean-shave haircut has been reported in 32% and 24% of men from a population and an HIV study respectively. Although hairdressers have recommended practice, there is little to no evidence of equipment sterilization. Specific RNA markers are used in forensic medicine to detect invisible blood; this study aimed to investigate whether these markers could be used to detect microscopic bleeding resulting from clean-shave haircuts. An initial pilot study including five men of unknown HIV status was conducted in 2011. Two swab samples were collected from the scalp of each participant immediately after a clean-shave haircut performed by a professional barber with no visible surface exudate; one sample was tested for RNA markers hemoglobin beta (HBB), spectrin beta (SPTB), and porphobilinogen deaminase (PBGD), and the second for albumin. A follow-up study included eight HIV-positive males; two scalp swab samples were taken from each person and tested for HBB and HIV PCR using a commercial test. In the first pilot study 4/5 samples were positive for both HBB and albumin. Thus, HBB was considered the more reliable marker and used for the second study, in which 1/8 participants tested positive for HBB. HIV was not detected in any of the eight HIV-positive participants. No visible bleeding was observed by the volunteers, barbers, and doctor collecting the samples. However, evidence of bleeding was found in 46% of subjects, a concerning finding that requires further scrutiny in our country with its high HIV prevalence.

P088

Appearance of mycosis fungoides (MF) localized on the scalp with clinical signs of alopecia areata

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A 67-year-old woman presented in our weekly hair disease clinic with massive, patchy hair loss and strongly positive pull test. She had a history of 30 years of parapsoriasis on the trunk and extremities. On the scalp, several slightly reddish, up to 8-cm-large, scaly patches of nearly complete, diffuse hair loss without signs of scarring were visible. One of the differential diagnoses was an initial alopecia areata (AA), but the slight erythema and the whitish dandruff did not support this diagnosis. On the extremities and the trunk there were multiple oval, livid-red macules. A biopsy from the scalp showed histologically a folliculotropic mycosis fungoides (F-MF) with a peri- and intrafollicular infiltrate of mostly CD4-positive, atypical T-lymphocytes. Prior biopsies of the trunk showed the histological picture of a parapsoriasis. Not only histologically there are parallels between F-MF and AA, but also clinically, an AA-like appearance of hair loss in patients with MF has been described. In patients with AA we could recently demonstrate that an oral prednisolone pulse therapy descending from 50 to 10 mg per day over 8 weeks showed good results with hair regrowth in 81% of the cases. In the case presented here, we therefore applied this treatment scheme and observed a stop of hair loss with negativation of pull test after 8 weeks of therapy. Dense hair regrowth occurred another 8 weeks later. In conclusion, F-MF should be considered and a biopsy should be taken if the diagnosis of AA is clinically doubtful. Therapeutic options are glucocorticoids or bexarotene, both topical and oral.

P089

Dermatomyositis and scalp pruritus is associated with small fiber neuropathy

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Scalp involvement in dermatomyositis (DM) is common and manifests as an erythematous to violaceous psoriasiform dermatitis. Pruritus is common. We report a 40-year-old Caucasian woman with a diagnosis of DM who was referred for evaluation and treatment of severe scalp pruritus of 4 years duration. Examination of the scalp revealed diffuse erythema and fine scale; examination of the rest of the integument revealed the presence of Gottron's papules and a diffuse poikiloderma of her neck, face, and upper back. Despite multiple topical and systemic treatment modalities including topical and systemic steroids, mycophenolate mofetil, dapsone, methotrexate, IVIG, and hydroxychloroquine the patient's scalp pruritus persisted. At the time of presentation, she was taking hydroxychloroquine 200 mg twice per day. Examination of herscalp biopsy revealed hyperkeratosis, epidermal effacement, and a thickened basement membrane. No significant dermal inflammatory component was seen. A 4-mm scalp biopsy was also obtained from affected scalp skin and from a normal healthy control for nerve studies. The samples were stained for Protein Gene Product 9.5 (PGP 9.5) using an immunofluorescence technique. The distribution and structure of PGP-immunoreactive nerve fibers were evaluated in the upper dermis and epidermis using confocal laser microscopy. Visual microscopic examination and quantitative analysis of the microscopy images suggested morphological abnormalities characterized as ''sprouting'' or ''tufting'' abnormalities of epidermal nerve fibers along with a significant decrease of epidermal nerve fiber density similar to that observed in some cases of small fiber peripheral neuropathies. Further studies are needed to confirm these changes in a larger number of patients with DM and scalp pruritus. These results provide an opportunity to consider novel treatment approaches with agents such as topical gabapentin or oral medications that target neuropathic changes such as pregabalin, the selective serotonin reuptake inhibitors (SSRIs), or mixed serotonin noradrenaline reuptake inhibitors.

P090

Inverse Koebner reaction observed in alopecia areata: the Renboek phenomenon

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Psoriasis and alopecia areata (AA) may be present in one patient. In some patients the development of psoriasis in AA lesions may be followed by the Renboek phenomenon (hair regrowth starting from the psoriatic plaques within the AA area). In Koebner phenomenon psoriasis appears in traumatic or inflammatory regions. In Renboek phenomenon the inflammatory process is inhibited by psoriasis. We describe a 23-year-old woman who developed psoriasis of the trunk and elbows at age 13, and scalp AA at age 15, both of which resolved following topical treatment with steroid creams. In April 2012 psoriasis recurred, involving the trunk, extremities, and the scalp. In June 2012 the psoriasis on the central scalp completely resolved following the appearance of a focus of AA in this region. Later, in August 2012, the relapse of psoriasis in the scalp was followed by partial hair regrowth in the AA region. The transition from psoriasis to other immune-mediated inflammatory disease may be induced by cytokine balance changes. Consequently, in some cases psoriasis may be the cure for AA (Renboek phenomenon). Nevertheless, other potential mechanisms should be further investigated, as it might be the clue to new specific pathogenic treatment of AA.

P091

Drug-induced hair loss—rational diagnostics and therapeutic options

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Hair loss induced by drugs is often discussed controversially despite it being caused by antineoplastic agents. But, besides the neoplastic agents, there are many other different drugs that can cause diffuse hair loss and consequential alopecias. Usually, only scalp hair is involved, and patients note hair loss 3-4 months after beginning a medication. The hair loss is of the diffuse type and is dominated by a telogen effluvium if the drug has only a moderately inhibiting potency. In cases of strong inhibition, such as with antineoplastic agents, severe alopecias can occur within the first 3 weeks of medication, characterized by telogen/dystrophic hair root patterns. In most cases hair regrowth starts after discontinuation of the medication. But in certain cases alopecia can remain. To confirm drug-induced hair loss, all medicines taken in the past 4 months must be analyzed as possible agents of disturbing hair growth. When there is a temporal association between the onset of hair loss and the commencement of a medication, the medication can be taken into account as a possible cause of the hair loss, especially when other triggers of telogen effluvium or diffuse hair loss are excluded. Possible causes and diseases that induce and imitate diffuse hair loss can be very different, e.g., dermatological, internal, and endocrinological diseases, childbirth, fever, iron, zinc, or biotin deficiency, or hair damage through cosmetic procedures. A thorough exclusion of these potential confounders is necessary before hair loss can be attributed to the medication. What evidence-based diagnostic and relevant therapies should be taken into consideration to solve this problem? This presentation summarizes 20 years of experience in the management of drug-induced hair loss, and discusses the relevance of specific diagnostic procedures, as well as possible therapeutic options and their results.

P092

Weft hair extensions causing a distinctive horseshoe pattern of traction alopecia

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Traction alopecia (TA) represents a pattern of traumatic hair loss caused by a pulling force applied to the hair shaft over time. This excessive tensile force results from hair styling practices such as tight ponytails, braids, cornrows, chingons, or religious head coverings. Hair loss of the marginal hair line with a ''fringe sign'' is the most commonly recognized pattern of TA. Weft hair extensions, which have gained in popularity because of their ease and rapidity of application, may also cause TA, but in a distinct ''U'' or horseshoe pattern. Wefted hair extensions consist of multiple strands of hair held together by a band of fine threads. These extended-wear hairpieces are attached directly to the hairline by being sewn, bonded, glued, or clipped. Hair loss in a horseshoe pattern may mimic scarring alopecia; however, a detailed history will often reveal the cause of hair loss.

P093

Scalp eccrine porocarcinoma arising in nevus sebaceous of Jadassohn

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Nevus sebaceous of Jadassohn (NSJ) is a cutaneous hamartoma usually located in the scalp and may contain epidermal, follicular, sebaceous, apocrine, and eccrine elements. Up to 15 percent of them may develop benign or malignant tumors, most commonly syringocystoadenoma papilliferum, trichoblastoma, and basal cell carcinoma. Although surgical removal is indicated, the timing of excision is controversial, since malignant degeneration is rare before puberty. Localized enlargement and ulceration suggest malignant transformation in a preexisting NSJ. In this case report, an unusual porocarcinoma (PC) developed within a longstanding scalp NSJ. A 35-year-old healthy man consulted for a rapidly growing tumor in his scalp. He had this lesion since childhood, but in the last 4 months a nodule had grown in one of its margins. Physical examination showed a $1.5 \times 1.1\,cm^2$ yellowish-orange irregular alopecic plaque in the left parietal area. Complete surgical excision was performed and histopathology informed marked epithelial hyperplasia, and prominent sebaceous glands together with small and rudimentary hair follicles, suggestive of the NSJ. In the same sample, multiple intraepidermal nests of poroid cells were observed, with atypia, pleomorphism, and necrosis that also extended into the dermal stroma. These findings indicated a PC. Eccrine PC is uncommon and develops from intraepithelial or upper dermal acrosyringium of eccrine sweat glands. It may develop as a primary tumor or from malignant transformation of eccrine poroma. Less than 5% of them are reported in the scalp and it has the potential to disseminate locally and systemically. The treatment of choice is surgical excision with broad margins. This case emphasizes the importance of prophylactic removal or close clinical follow-up of NSJ in an adult.

P094

Hair trichotomy in a female patient with depression

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A 21-year-old female patient comes for consultation with what was described as a hair loss on the scalp top that had started about a year ago. On her first appointment, she reported her scalp hair was spontaneously falling out and that she was extremely upset about the problem. Concerning recent major events, she just mentioned the birth of her daughter about 2 years ago and that her husband abandoned the house approximately 18 months ago. She neither mentioned nor presented any kind of scalp alteration, such as pain, excessive oilness, itching, ulceration, or scaling. The patient showed good hair density on the area of complaint. There were no signs of hair miniaturization under the trichoscope. The most important clinical sign was the presence of scalp hair on the area of complaint that seemed to have been cut with some sort of instrument, such as scissors, trichotomy machine, or even a blade. On her first consultation, the patient reinforced the claim that her scalp hair loss was spontaneous and that she did not manipulate her hair.

During the second consultation, the patient's mental state was more compromised and her scalp hair seemed to have been cut even shorter. The patient confirmed that she trichotomized her hair every 3 to 4 days, and she also confirmed a still untreated depressive state, previously diagnosed by a psychiatrist. According to the new scenario we suggested the patient to visit the psychiatrist again to initiate the treatment for depression and evaluate the necessity of a psychotherapy.

P095

Tinea capitis—a case series

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Introduction: Tinea capitis is the commonest disease of the hair in childhood. It spreads by close contact in children and by sharing of combs. This condition can be properly managed only if it is diagnosed correctly. I shall be presenting 20 such cases seen in my private practice in Mumbai, India. This clinical case series classifies the patients on their clinical presentation.

Methods: Twenty cases (12 males and 8 females) were included. Only those cases where baseline clinical photographs were available were considered for the analysis. The clinical diagnosis was confirmed by microscopy and woods lamp examination, although all cases were given treatment based on clinical diagnosis alone. Fungal culture was not done due to cost factor. All were treated with oral terbinafine.

Observations: The inflammatory variant of tinea capitis was the commonest seen in 10 cases (50% of the total). This was followed by a gray patch seen in 8 cases. Black dot tinea capitis was seen in 2 cases. Scarring was seen in 5/10 cases (50%). Woods lamp examination showed fluorescence in 5 cases. The KOH mount was positive for fungal hyphae in 10 cases. The response to treatment was excellent in all the cases. Post-treatment photographs were available in 6 cases.

Conclusions: This study highlights that prevalence of inflammatory tinea capitis is common. Fifty percent of inflammatory tinea capitis had scarring alopecia. The response to treatment was excellent, implying that there was no terbinafine resistance in the cases studied.

P096

Low-level laser therapy for alopecia areata—case relate

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Alopecias are diseases that cause more hair loss than what is considered to be normal. In regular bases alopecias do not induce systemic disorders. But sometimes the intensity and the clinical appearance have a social and psychological negative impact on patients' lives.

Alopecia areata is a non-cicatricial form of alopecia that promotes round hair-loss areas in any region of the hairy body, with higher incidence in the scalp and beard. So far the etiology of this problem has not been fully elucidated, but studies point toward autoimmune issues. Given these gaps in the explanation of its etiology, treatments also have variable effectiveness, and so far corticosteroids have been the leading choice in clinical therapy as a way to decrease the activity of the immune system.

The low-level laser therapy (LLLT), one proposal in the field of non-invasive and non-drug form of treatment, has shown a promising option in the regrowth of the affected areas.

This study case shows very positive results in the replacement of the hair in areas of alopecia in a male patient with alopecia areata after ten sessions of LLLT with red light.

FACTORS INFLUENCING HAIR GROWTH

P097

Primary cilia-mediated cellular signaling in dermal papilla cells

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Dermal papilla cells (DPC) produce various intercellular signaling molecules and thereby play essential roles in hair growth and development. Great amount of attention has been paid to an antenna-like organelle (primary cilium) as a signaling center in mammalian cells. However, little is known about the roles of primary cilia in DPC. Here we show that the DPC cilium is involved in producing cell growth signals. Immunochemistry, together with electron microscopic observations, showed that primary cilia protruded from cultured human follicle DPC. The ciliary length was distributed around 2 μm and the diameter was approximately 0.2 μm, which is in good agreement with previous observations in other mammalian cells. In order to investigate a role of the organelle in intercellular signaling, mesenchymal stromal cells were grown in DPC-pretreated media under different conditions and the cellular viability was quantified. In the presence of lithium chloride, the cilia length of DPC became longer and the mesenchymal stromal cells became more viable. In contrast, shortening of DPC cilia by KIF3A knockdown reduced the viability of the mesenchymal cells. These results suggest that cilium-based signaling in DPC plays a key role in maintaining other cells. We are currently trying to identify candidates that regulate cilia structure and its signaling function. Understanding the primary cilia of DPC could provide fundamental insight into hair regulation and a clue towards the future clinical application.

P098

Prospective case series to evaluate hair shaft abnormalities after chemotherapy and during tamoxifen therapy in breast cancer patients evaluated by optical coherence tomography

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Background: Antineoplastic cancer treatment is frequently associated with alopecia. In literature and private practice, changes in texture and shape of regrowing hair after chemotherapy have been reported, but an evaluation on a scientific base is lacking. Optical coherence tomography (OCT) provides highly reproducible measurements of hair shaft parameters, similar to those produced in horizontal sectioning of embedded hair shafts.

Objectives: The aim of this study was to evaluate hair shaft alterations and differences in diameter before breast cancer chemotherapy and after regrowth, as well as before and during anti-hormonal tamoxifen treatment using OCT.

Methods: 34 women, aged 29-68 years, were included via consultations in an interdisciplinary breast center; they were receiving either tamoxifen or chemotherapy. Before treatment and a minimum of 12 weeks after end of chemotherapy or 28 weeks after start of tamoxifen intake, 20 hairs were cut from two different sites of the scalp (frontal, occipital) and examined by OCT technique. The cross section (CS) and the form factor (FF) were evaluated as hair shaft parameters. The ratio of the maximal and minimal hair shaft diameters was used to determine the FF.

Results: 12 weeks after the end of chemotherapy, the cross section of hairs was significantly lower compared to hairs taken at baseline ($P<0.001$). The FF varies between these two time points for the occipital area but not for the frontal. For tamoxifen-treated patients, neither change in CS nor change in FF was observed. Comparing both groups, there were significant differences in CS and in FF after 28 weeks of tamoxifen intake, but not for baseline.

Conclusions: The reported changes in hair structure after chemotherapy may be due to reduction of hair shaft caliber and increase of FF in regrowing hairs. The OCT technique is a promising method to gain more insight into chemotherapy-induced changes of hair morphology.

P099

Apoptosis causes inactivation of vitamin D receptor in human hair derma papilla cells and human keratinocyte through VDR ablation

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Aims: Vitamin D receptor (VDR) is a ligand-dependent transcription factor that mediates regulation of epidermal homeostasis and hair growth. In contrast, VDR ablation hinders the maintenance of normal hair follicle. In recent studies, we found that VDR was decreased in alopecia areata (AA) and alopecia universalis (AU) lesions compared with the non-lesion areas. In this study we investigated the mechanism of VDR in cultured human hair dermal papilla cells (DPCs) and human keratinocytes.

Methods: Cell viability was assessed by MTT assay. We analyzed the expression levels of VDR, β-catenin, Wnt3a, Wnt5a, and Wnt10b by western blot analysis. Also, VDR silencing was conducted using small interfering RNA (siRNA) for VDR in human hair (DPCs) and human keratinocytes. Also, VDR and cleaved caspase-3 expressions were determined using immune-fluorescence assay. (This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0021960).)

Results: We showed that VDR was expressed in human hair dermal papilla cells (DPCs) and human keratinocytes. Lithocholic acid (LCA) is a ligand of the vitamin D receptor (VDR); LCA treatment induced upregulated expression of VDR and enhanced cell proliferation in a dose-dependent manner. We also showed that VDR silencing downregulated protein VDR and β-catenin and attenuated expression levels of the VDR in both cells. Moreover, we found that VDR silencing dramatically decreased the viability, whereas co-administration with LCA significantly restored the viability, which is associated with VDR repression by LCA. Correspondingly, immune-fluorescence analysis showed that VDR silencing activated cleaved caspase-3 in human keratinocytes.

Conclusion: These results indicate that VDR plays a critical role in human hair DPCs and human keratinocyte cell survival.

P0100

Localization of mucins in human skin and hair follicles

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Background and aim: Mucins are high-molecular-weight glycoproteins. There are at least 18 human mucins in skin and hair follicles, but their localization and functions are unclear. Secreted mucins colocalize in the immune system and several recent studies have indicated that membrane-bound mucins have evolved from the secreted type and have important roles in cellular signal transduction. Secretion of mucins is controlled by cytokines and cell adhesion factors related to the hair cycle. The purpose of this study was to investigate the localization of mucins in human skin and hair follicles.

Subjects and methods: Scalp skin specimens were obtained from six subjects. The epidermis, dermis, bulb, and proximal hair follicles were isolated and RT-PCR for mucin (MUC) genes was performed. Alcian blue and immunohistochemical staining were also performed.

Results: In the anagen VI stage, mucins were strongly deposited in the dermal papilla and connective tissue sheath in proximal hair follicles. Mucins were present in the thickened vitreous membrane in the catagen stage, but were not observed in the telogen phase. Various MUC mRNAs were detected in the skin and hair follicles, and the expression patterns of MUC1, 3, 4, 8, 13, 15, and 17 were analyzed immunohistochemically. Several kinds of MUC were observed in the epidermis. MUC was present in the dermal papilla in the anagen III stage, and MUC1, 3, 4, 8, 13, and 15 were observed in the dermal papilla and hair bulb in the anagen VI stage. The outer sheath cells of proximal hair follicles strongly expressed MUC1, 8, and 15, while MUC3, 4, 13, and 17 were detected in the cell membrane of a few of the outermost strata. In the catagen phase, most MUC had disappeared, but MUC3 was deposited in the thickened vitreous membrane.

P101

Placental growth factor-induced hair growth promotion

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The dermal papilla (DP) comprises the specialized mesenchymal cells at the bottom of the hair follicle and plays a pivotal role in hair formation, hair induction, and cell cycle. Abnormality of the DP function is the main cause of the imbalance of hair growth cycle and hair loss. Dermal papilla cells gradually lose their distinct characteristics following subculture of the cells. In this study, dermal papillae were isolated from human hair follicles and were serially subcultured. In each subculture passage of #1, #3 and #5 ($n=4$), we compared the gene expression profiles using transcriptome analysis. We could obtain a list of 2,249 downregulated genes following the subsequent passages. Among the downregulated genes, placental growth factor (PlGF) was selected. Our purpose was to elucidate the function of PlGF in hair follicle cells and to evaluate the biological role of PlGF. First, we could confirm that mRNA and protein expression levels of PlGF were significantly decreased following subsequent passages. PlGF induced proliferation of hDPCs in BrdU-ELISA assay and in western blot analysis; it was revealed that PlGF would prevent cell death by increased phosphorylated Erk and cyclinD1 and promote the survival by upregulation of phosphorylated Akt and bcl2. SiRNA of PlGF downregulated the mRNA expression of some representative genes related to the stimulation of hair growth, such as IGF1, HGF, and FGF2. We also found that PlGF could enhance hair shaft elongation in *ex vivo* hair organ culture. In summary, these *in vitro* results indicate that PlGF would have a certain role in hair growth promotion and therefore may serve as an additional therapeutic mechanism for the treatment of alopecia.

P102

Influence of ceramide on the human hair follicle and its role in hair cycle

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Objective: Ceramide, a major class of hair lipid, may help determine the physicochemical properties of human hairs. It also triggers apoptogenic signals in many cell lines. Transformation of anagen to catagen is considered to be caused by apoptosis and terminal differentiation of hair follicles (HFs). However, the influence and role of ceramide in HFs and hair cycle has not yet been studied.

Methods: The purpose of the current study was to investigate the role of ceramide in human HFs, especially its association with the hair cycle. We observed that HFs express ceramidase, an enzyme that catalyzes the hydrolysis of ceramide, at mRNA and protein levels. Furthermore, we examined whether ceramide accumulation induced by *N*-oleoylethanolamine (NOE), a competitive inhibitor of ceramidase, influences human hair growth, a change in hair cycle, and proliferation/apoptosis-related molecular expression.

Results: During the transformation from anagen to catagen, ceramidase expression appeared to be downregulated. NOE inhibited the human hair growth and promoted transformation from anagen to catagen in organ-cultured human HFs.

Conclusion: Altogether, these results indicate ceramide might be an important regulatory factor for the human hair cycle. The effect of apoptosis induced by ceramide accumulation in the catagen stage is affected by modulating the ceramide hydrolytic pathway.

P103

Functional presence of the endocannabinoid system in human hair follicle-derived outer root sheath keratinocytes

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Aims: We have previously shown that the endocannabinoid anandamide (AEA) inhibits hair shaft elongation and induces apoptosis-driven catagen regression of human hair follicles (HFs) via the activation of cannabinoid receptor-1 expressed by epithelial cells of the HF. Since HFs were also shown to produce endocannabinoids, in the current study, we aimed at investigating the functional presence of the endocannabinoid system (ECS) in cultured human HF-derived outer root sheath keratinocytes (ORSKs).

Methods: To investigate the proliferation, viability, and cell death, a series of colorimetric (MTT-assay) and fluorescent assays (CyQUANT, DiIC$_1$(5)-SYTOX Green staining) were employed. Furthermore, expression of elements of the ECS was identified by immunocytochemistry and quantitative ''real-time'' PCR (RT-qPCR), whereas alterations in the gene expression levels following various treatments were assessed by RT-qPCR.

Results: Expressions of enzymes involved in the synthesis (*N*-acyl-phosphatidylethanolamine-specific phospholipase D, diacylglycerol lipase-α and -β) and degradation (fatty acid amide hydrolase and monoacylglycerol lipase) of the endocannabinoids were identified in the ORSKs. Moreover, AEA was shown to inhibit cellular proliferation and induce apoptosis-dominated cell death in a concentration-dependent manner. Of further importance, AEA exerted a remarkable anti-inflammatory effect (significant suppression of the basal and/or poly-(I:C)-induced expression of HLA-DRA and various pro-inflammatory cytokines, e.g. interleukin (IL)-1β, IL6, IL8, and tumor necrosis factor-α) and induced differentiation. Finally, according to our preliminary results, elevation of the endogenous AEA ''tone'' of the ORSKs by selective inhibitors of the cellular uptake (AM404) or degradation (URB597) of AEA appeared to mimic the above effects of the exogenously applied AEA.

Conclusions: Collectively, our data strongly suggest that a functional ECS is expressed in the ORSKs and that AEA may be a key endogenous regulator of their biological processes. Therefore, pharmacological modulation of the endocannabinoid ''tone'' could be a promising, novel tool for the management of relevant hair growth disorders and HF-related inflammatory conditions.

P104

Deletion of the *Sox21* gene drastically affects hair lipids

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Objective: The effects of *Sox21* gene deletion on the hair lipid, 18-methyleicosanoic acid (18-MEA), which is bound to the surface of each cuticle cell, and cholesterol (CH), cholesterol sulfate (CS), and ceramides (CERs), which play crucial roles in the chemical diffusion barrier, water holding, and cell cohesion, have been studied. *Sox* genes encode a family of transcription factors and are defined as containing the high-mobility group box of a gene involved in sex determination called SRY, which resides on the Y-chromosome. In *Sox21*$^{-/-}$ mouse hair, generated by knockout of the *Sox21* gene, some structural proteins like keratins and keratin-associated proteins are downregulated, but seemingly only in the hair shaft cuticle. This leads to improper cuticle formation and a loss of interdigitation with the inner root sheath, with the result that cyclic alopecia occurs.

Methods: Lipids extracted from *Sox21*$^{+/+}$ and *Sox21*$^{-/-}$ hairs of mice were measured by liquid chromatography-mass spectrometry. The distribution of 18-MEA was observed by time of flight secondary ion mass spectrometry.

Results: For the cuticle-specific bound lipid 18-MEA, which was found to predominantly exist as the free form in *Sox21*$^{-/-}$ hair, total levels and distribution were unexpectedly unchanged. This indicates that while the biosynthesis of 18-MEA is unaffected its covalent attachment to the cuticle surface is disrupted by loss of keratin-associated protein binding partners. Although the class compositions differed, the total CER levels were found to be comparable between *Sox21*$^{+/+}$ and *Sox21*$^{-/-}$ hairs. Deletion of the gene was also found to increase cholesterol sulfate (CS) levels.

Conclusions: The biosynthesis process might be associated with cuticle keratinocyte maturation, since both CS and CERs are known bioactives in keratinocyte differentiation.

P105

Nrf2 in the hair follicle: activation and protection against oxidative insult

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Nrf2 (nuclear factor (erythroid-derived 2)-like 2) is a transcription factor that has received widespread attention in recent years. This ''master regulator'' is responsible for controlling the expression of hundreds of downstream genes, yet its predominant role is in defense against redox imbalance. Activators of Nrf2 are currently under investigation as potential therapeutic agents in a number of inflammatory conditions and pathologies in which redox imbalance is implicated. The role of Nrf2 in hair follicle (HF) biology is currently unknown, yet the scalp and hair are frequently exposed to oxidative stress in the form of UV radiation. We therefore sought to examine the impact of Nrf2 activators in the regulation of downstream target genes in the human HF, with a view to understanding its role in HF biology. Full-length human HFs were obtained following hair transplant surgery. HFs were cultured for 24 hours in the presence of known Nrf2 activators, Sulforaphane and tBHQ (tert-butylhydroquinone). The expression of downstream targets (heme-oxygenase 1, HMOX-1; NADPH quinone reductase 1, NQO1; and catalase) were investigated on the gene (qPCR) and protein (IHC) levels. The impact of sulforaphane and tBHQ treatment on proliferation and apoptosis was assessed by Ki67-TUNEL staining. Activation of Nrf2 resulted in significant increases in HMOX-1 and NQO1 gene and protein expression, with no change in catalase levels. In the absence of oxidative insult, activation of Nrf2 did not alter levels of proliferation or apoptosis in the hair follicle matrix keratinocytes. These results indicate that the Nrf2 pathway is active in the HF and may play a role in protecting the HF against redox insult. As such, targeting Nrf2 may be a promising mechanism for protecting HFs against oxidative damage. Future work aims to further elucidate the biological impact of Nrf2 activation on HF growth during oxidative stress.

P106

Hepcidin, the iron-regulating peptide—what you need to know about its role in hair loss and, in particular, chronic telogen effluvium (CTE)

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Relatively low serum ferritin concentrations are associated with increased hair shedding in some (34%) but not all healthy individuals. Why do some iron-deficient/anemic females experience increased hair shedding while others do not? Could the answer lay with hair follicle iron metabolism and the iron-regulating peptide hormone, hepcidin? The homozygous '*Mask*' mouse (a hepcidin knockout mutant that inhibits iron absorption) fed on a normal mouse diet develops a gradual loss of body but not facial hair, leading to almost complete trunk nudity within 4 weeks. In addition, they become iron deficient, anemic, and the female is infertile. However, following iron supplementation all symptoms completely resolve. In humans hepcidin regulates iron absorption and in hereditary hemochromatosis hepcidin expression is suppressed, resulting in unregulated iron uptake. Furthermore, hemochromatosis affects only certain populations (northern Europeans) with variable penetrance. Serum ferritin concentrations in menstruating females expressing hemochromatosis are significantly lower than age-matched affected males, but are significantly higher than unaffected females. This highlights the role of menstrual blood loss in measured serum ferritin concentrations when determining the so-called 'normal' reference ranges for iron-related variables in women of reproductive age currently employed by hospitals and reference laboratories. In 35 years of studying unexplained CTE in premenopausal women, we have never found a fasting serum ferritin concentration above 130 μg l^{-1} (with an erythrocyte sedimentary rate (ESR) < 10 mm per hour), indicating women with hemochromatosis do not experience iron-related CTE. In iron-deficient individuals, an increase in follicular hepcidin expression would decrease iron uptake, releasing iron for the essential tissues. Conversely, no change in hepcidin expression leaves follicular iron uptake unaffected, even in individuals with a low serum ferritin concentration. It is hypothesized that hepcidin regulation of hair follicle iron metabolism might operate in CTE, with variable penetrance, explaining why only some iron-deficient individuals are affected.

P107

Dickkopf-1, an inhibitor of Wnt, regulates hair follicle growth degeneration in human hair dermal papilla cells and is associated with downregulation of VDR through DKK1-released inflammatory cytokines

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Aims: Dickkopf-1 (DKK-1) inhibits canonical Wnt/β-catenin signaling and maintains follicle anagen. In previous study, we found that DKK-1 suppressed the expressions of VDR and β-catenin in dermal papilla cells. This study examined the role of DKK1 in hair growth degeneration in human dermal papilla cells (DPCs).

Methods: Cell proliferation in human DPCs was assessed by MTT assay. We determined the levels of VDR, GSK-3β, and β-catenin by western blot analysis. Also, VDR expression was determined using immune-fluorescence assay in DKK1-treated cells. The role of DKK1 in human DPCs was examined by determining the levels of IL-1β, IL-6, and TNF-α using enzyme-linked immunosorbent assays (ELISA) and the expression levels of VDR, GSK-3β, and β-catenin using reconstruction of living skin equivalents (LSEs). (This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0021960).)

Results: We found that DKK1 did not alter DP cell proliferation in a dose-dependent manner. However, we found that DKK1 treatment reduced the expression of VDR and β-catenin in human DPCs in a time- and dose-dependent manner. As shown by immune-fluorescence analysis, DKK1 attenuated levels of VDR expression at a concentration of 50 ng ml^{-1}. In addition, the LSE system containing human keratinocytes and DPCs showed that DKK1 downregulates VDR and β-catenin, whereas it upregulates GSK-3β. We observed that DKK1-treated cells showed upregulation of TNFR2 expression and enhanced cytokine levels of IL-1β, IL-6, and TNF-α compared with non-treated cells.

Conclusion: In summary, the results of the present study demonstrate that DKK1 reduced expression levels of VDR and β-catenin in DPCs through the cooperative effect of inflammatory cytokines. Therefore, we suggest that DKK1 promotes hair loss through the inflammation factor in human hair DPCs.

P108

Tissue damage after ionizing radiation in skin and hair follicles: short- and long-term effects after a single high dose and multiple low doses in mice

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Introduction: It remains unclear how ionizing radiation damages healthy skin and hair follicles in the short and long term.

Methods: C57BL/J6 mice (8 weeks old) were divided into three groups (control: CTR, 10 Gy once irradiation: 10Gy × 1, 5 Gy twice irradiations: 5Gy × 2). After hair epilation, the groin area was radiated. At 0, 1, 2, and 4 weeks, 3 and 6 months after irradiation, the skin samples were evaluated histologically and macroscopically.

Results: At 1, 2, and 4 weeks, the epidermis was thicker in 10 Gy × 1 than in CTR and 5 Gy × 2, without increasing the layer number of epidermal keratinocytes. The number of capillaries in the dermis was fewer in 5Gy × 2 and 10Gy × 1 than in CTR at 1 and 2 weeks. At 4 weeks, the dermis was thicker in CTR than in 5Gy × 2 and 10Gy × 1. At 6 months, the subcutaneous fat tissue was thinner in 5Gy × 2 than in 10 Gy and CTR. At 3 and 6 months, capillaries in the groin fat of 5Gy × 2 and 10Gy × 1 appeared to be fewer than those of CTR and the size of adipocytes became smaller in 5Gy × 2. Many of the hair shafts disappeared at 1 week, followed by gradual regrowth of hairs by 3 months. The number and size of hair remained decreased in 5Gy × 2 and 10Gy × 1 than in CTR at 6 months when hairs were restored and the gray hairs were macroscopically observed.

Discussions/conclusions: In the short term (1–4 weeks), the number of hair follicles and capillaries decreased and the dermis became thinner because of acute radiation injury. In the long term (3–6 months), tissue damage was generally more severe in 5Gy × 2 than 10Gy × 1. These data provide clinical insights for minimizing short- and long-term radiation injury and revitalizing the radiated ischemic and fibrous tissue.

P109

TRPV1 antagonist, PAC-14028, can accelerate hair growth *in vivo*

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Transient receptor potential vanilloid type 1 (TRPV1) is a cation channel activated by diverse noxious stimuli like capsaicin, low pH, or heat. TRPV1 is expressed not only in peripheral sensory nerve fibers (C and Aδ) but also in the skin epidermal cells, including hair follicle. Recently, it was revealed that TRPV1 might be associated with hair growth control. Here we aimed to investigate if the blockade of TRPV1 might affect hair growth, by employing a new TRPV1 antagonist, PAC-14028. The effect of PAC-14028 on hair growth was evaluated *in vivo* using an anagen induction and a catagen induction model in C57BL/6 mice. In macroscopic evaluation, PAC-14028 accelerated the onset of anagen hair cycle (14 days after treatment) compared to vehicle (27–28 days after treatment). Mice treated with 1% PAC-14028 showed significantly increased hair weight in a dose-dependent manner (101.25 ± 16.51 and 56.81 ± 10.58 mg for PAC-14028 1% and vehicle control, respectively). In the catagen induction model, the 1% PAC-14028-treated group showed darker skin as determined by a skin chromameter, which was comparable to 2% minoxidil. In addition, 1% PAC-14028 increased the skin thickness, confirming the delay of catagen induction. These results demonstrate that TRPV1 antagonist could be useful for hair growth based on anagen elongation and induction *in vivo*.

P110

Effect of recombinant growth factors mixture on hair growth promotion *in vitro* and *in vivo*

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Background: Although hair loss may not be a life-threatening disorder, it has a great impact on a person's self-respect, mental health, and quality of life. Thus, it is important to develop new therapeutic materials that prevent hair loss and enhance hair growth.

Objective: This study was aimed to investigate the effects of recombinant growth factors mixture (RGFM) on hair growth promotion *in vitro* and *in vivo*.

Methods: We evaluated cell proliferation in cultured human DPCs by MTT assay and determined cell migration in DPCs using Phage contrast microscopy and measured the expression levels of extracellular signal-regulated kinase (ERK), Akt, GSK-3β, and β-catenin by western blot analysis. We also performed topical application onto the back skins of 7-week-old C57BL/6 mice after depilation. (This research was financially supported by the Ministry of Knowledge Economy (MKE), Korea Institute for Advancement of Technology (KIAT) through the Inter-ER Cooperation Projects.)

Results: RGFM treatment increased the proliferation of cultured human DPCs in a dose-dependent manner. Also, RGFM treatment enhanced the migration of cultured DPCs at a concentration of 1,000 ng ml^{-1}. The expression levels of phosphorylated ERK and phosphorylated Akt significantly increased at 1,000 ng ml^{-1} of RGFM. RGFM also decreased GSK-3β expression and increased β-catenin expression. In addition, RGFM treatment on the back skins of C57BL/6 mice significantly promoted hair growth compared with control treatment.

Conclusion: RGFM promotes the proliferation, migration, and survival of human DPCs by activating both ERK and Akt. Moreover, RGFM induced the proliferation of human DPCs by the upregulation of β-catenin accompanied by inhibition of GSK-3β. Thus, we suggest that RGFM promotes human hair growth through these proliferative effects on human DPCs.

P111

Enhancement effect on the transfollicular delivery of sodium fluorescein using iontophoresis

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In order to enhance the effect of hair growth drugs, the transfollicular delivery of drugs has been intensively investigated with various chemical and physical enhancing methods. To find a better way for transfollicular delivery, we studied the penetration route of sodium fluorescein (SF) using iontophoresis on hairy C57BL/6 and hairless SKH1 murine skin. SF is highly hydrophilic substance that has two oxyanions within one molecule. SF did not penetrate much by passive diffusion at 8 hours after 20-minute topical application. However, when applying the cathodic conduct for SF iontophoresis at −0.4 mV cm^{-2} for 20 minutes, strong green fluorescence of SF was observed along the transfollicular pathway in hairy skin regardless of hair cycle stage (anagen vs. telogen). Also, significant amounts of SF were found under the hairless skin by iontophoresis, but less and lower delivery than shown in hairy skin. In hairy skin, electric intensity (−0.1 to −0.4 mV cm^{-2}) and application time (5–30 minutes) correlated well with the amounts and the depth of SF in transfollicular delivery. In the combination of various types of liposome (neutral, cationic, anionic type) and iontophoresis, only cathodic conduct significantly enhanced the transfollicular delivery of SF regardless of liposome types, while anodic conduct did not increase it even in cationic liposome formulation. The ionic polarity of the liposome in this study did not influence the amount or direction of SF delivery in the transfollicular pathway by iontophoresis. This result might be due to lack of sufficient electric charge of liposomes that could entrap the whole charge of SF molecule, or the low entrapment efficiency of SF within the liposomes due to the high hydrophilicity of SF. Finally, in the combination of hydrogel and iontophoresis, high viscosity rather reduced the iontophoretic transfollicular delivery of SF without hydrogel.

P112

Genomewide expression analysis for the impact of a hair-growth-promoting formulation in an *in vitro* model for situations of diminished hair growth

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Diminished hair growth is believed to be associated with reduced activity of follicular keratinocytes. We previously described an *in vitro* model using normal, human, epidermal keratinocytes (NHEKs) mimicking the situation of diminished hair growth for screening the effect of hair-growth-promoting agents and formulations on cellular proliferation and metabolic activity. An *in vitro* correlate of the hair-growth-promoting formulation Panto(vi)gar was shown to have a positive impact on the metabolic activity and cell proliferation of NHEKs in this test system. In a systematic approach to identifying the underlying molecular mechanisms of Panto(vi)gar-mediated improvement of keratinocyte physiology, we performed a genomewide DNA-chip-based expression profiling (covering 27.958 Entrez gene RNAs) for the identification of potential Panto(vi)gar-regulated genes.Differentially expressed genes were identified by comparing the expression profiles of NHEKs under minimal growth conditions and after cultivation with a Pantogar *in vitro*-correlate (P-IC). The treatment with P-IC resulted in a significant modulation of gene expression leading to expression differences ranging from 2- to 207-fold. Inter-experimental correlation analysis revealed good reproducibility of the *in vitro* test system. Stringent parameters for the identification of differentially expressed genes reduce significantly the identification of false-positive genes. In summary, we identified 866 genes which were upregulated, while 411 genes were downregulated by P-IC treatment and showed ⩾4-fold expression difference. A functional group analysis identified a statistically significant induction of genes associated with cell cycle and cell proliferation. Thus, the identified P-IC differentially regulated genes fit well to the observed phenotype of the cells. In addition, P-IC appears to regulate also genes associated with cell death, extracellular matrix, response to oxidative stress/DNA damage, and genes associated in general with regulation of hair growth. The established pool of Panto(vi)gar-regulated genes provides the foundation for further analysis of the molecular basis of the beneficial effect of this hair-growth-promoting formula on hair growth.

P113

IL-6 inhibition *in vitro* using protease inhibitors of exogen

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The hair growth cycle consists of a number of phases, including anagen (the active growing phase), catagen (regression phase), and telogen (the resting phase). Exogen is the term used to describe the process of hair fiber shedding, and has been proposed to be an independent, yet active event of the hair cycle. There exists limited understanding of the biochemical events involved in exogen. It has been proposed to start after the hair follicle has been in telogen, and to continue until the ultimate release of the club fiber from the follicle. It has also been suggested that exogen involves proteolytic processes. We have previously reported localization of proteolytic activity around human club root tips of exogen fibers and *ex vivo* inhibition of this activity using Trichogen and climbazole. Here we present data showing the anti-inflammatory potential of the above materials *in vitro*. Primary human keratinocytes were treated with Trichogen or climbazole for 24 hours in the presence of TNFα. Following treatment, conditioned medium was collected and analyzed for IL-6. Our investigations showed that both Trichogen and climbazole reduced TNFα-induced IL-6 secretion in the above *in vitro* model. IL-6 has recently been observed to be upregulated in balding dermal papilla (DP) cells compared to non-balding, and IL-6 inhibited hair shaft elongation *in vitro*. These data suggest that Trichogen and climbazole may impart the additional benefit of attenuating the inflammatory cytokines apart from their potential to inhibit proteolytic activity. Further work is required to determine whether these benefits can be observed *in vivo* in subjects with scalp or hair follicles under inflammatory states.

P114

Mycophenolic acid has concentration-controlled effect on the hair growth-related genes

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Mycophenolic acid (MPA) has several biological effects as an immunosuppressant, but little is known about its biological activity as a hair growth effector. Recently, it was reported that MPA has been shown to be effective in the treatment of inflammatory disorders such as lichen planopilaris (LPP) and psoriasis by selectively inhibiting T lymphocyte activity. The Microneedle Therapy System (MTS) needle roller is a procedure that stimulates the skin to produce new collagen formation and to help cell revitalization. In a previous study, we found the effects of MTS roller on hair growth.

We hypothesized that MPA has either direct or indirect effects on the proliferation and apoptosis of dermal papilla cells (DPCs) of the human hair follicle. To determine the effects of topical MPA in stimulating hair growth, we designed an experiment applying topical MPA plus MTS roller in mice, and then the measurement of hair growth was compared with 5% Minoxidil, used as positive control. We also performed the cell proliferation in cultured DPCs by diphenyl tetrazolium bromide (MTT) assay and measured the elongation of hair follicles in organ culture. Hair growth after microneedle stimulation was evaluated with a photograph and hand-held digital microscope.

MPA significantly increased the proliferation of DPCs and elongation of hair follicle in organ culture. Hair elongation was significantly increased (by 115%) at 0.75 μM of MPA. However, high concentrations of MPA significantly decreased the hair growth effects. MPA plus MTS roller-applied groups showed strongly increased gene expression compared with the positive control in RT–PCR. We suggest that the MPA plus MTS roller stimulates hair growth *in vivo* and high concentration of MPA affects the toxicity on DPCs. If we adjust and select the proper concentrations of MPA, it might be one of the therapeutic options for the treatment of hair loss.

P115

Hair growth inhibition by topical application of a type II ribosome inactivating protein (RIP)

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Introduction: The very few topical treatment options available for Hirsutism patients led us to look for an effective solution to this unmet need. Cytotoxic lectins like Ricin are widely considered to be dermally inactive, as the amount being absorbed is minimal. However, our studies are the first report showing the role of the topically applied type II ribosome inactivating protein Ricin in inhibiting the growth of mammalian hair. This study is expected to lead to a new tool in dermatology.

Aims: To assess the role of topically applied Ricin on growth of hair.

Methods: Ricin was purified using crosslinked guar gum matrix and galactose gradient. Purity of ricin was confirmed using SDS–PAGE. Ricin of varying concentrations was applied on skin patches prepared by removing hair by waxing of albino mice. Skin biopsies and histopathology (hematoxylin-eosin staining) were done after 10 days of treatment for the first group and after 30 days for the second group.

Results: Hair growth on all the test patches was delayed and sparse when compared to the control patches. No adverse reaction to the skin was observed. Histopathology sections studied from all the biopsies showed no adverse reaction to other skin structures. All the sections marked as test samples showed reduced number of hair follicles as compared to the control. Some of the hair follicles from the test samples appeared empty, whereas some of them showed hair follicle dystrophy wherein the normal ladder-like structure of the hair follicle was disturbed.

Conclusions: Ricin, which represents Type II RIPS, is a promising lead candidate for hair growth inhibition. Our lab is pursuing further qualitative and quantitative studies for inhibition hair growth by ricin and related proteins.

P116

Mechanisms of caffeine antiandrogenic on human dermal papilla cells *in vitro*

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Objective: To investigate the possible antiandrogenic effects of caffeine on human dermal papilla cells *in vitro*.

Methods: Human dermal papilla cells, taken from the vertex, occiput, and pubis, were cultivated for 48 hours *in vitro* with 0.0005% caffeine and 10 nM testosterone separately or in combination. We measured the relative growth and apoptotic rate by MTT assay and flow cytometry. Real-time PCR was applied to analysis of the mRNA of 10 candidate genes connected to the possible signaling pathway of AGA.

Results: 0.0005% caffeine stimulated the proliferation and inhibited the apoptosis of human DPCs *in vitro*. AR, SRD5A2, P53, FasR, GSK-3β, and TGF-β2 showed significant regulation in vertex DPC culture treated with concentrations of 10 nM testosterone; significant gene expressions were analyzed for Bcl-2 and β-catenin in the combined treatment group. In pubic cell culture P53 and FasR were downregulated with testosterone; further apoptosis suppression can be achieved by caffeine treatment.

Conclusion: Caffeine probably plays a role in the antiandrogenic effect on human dermal papilla cells *in vitro* by acting on different signaling pathways.

P117

From bacterial quorum sensing a possible treatment for hair

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Quorum sensing is one of the most important mechanisms for bacterial cell-to-cell communication, which is perhaps the most important tool in the battle for survival. Plantaricin A (PlnA) is a quorum sensing signal molecule produced by *Lactobacillus plantarum* with antimicrobial and pheromone activities. In our previous study we demonstrated the acceleration of wound healing by PlnA used as pure peptide or biologically synthesized during co-cultivation of two strains of *Lactobacillus*. Considering the deleterious role of oxidative stress and the importance of the vascular network in hair loss and growth, we studied the possible modulations induced by chemically or biologically (by *L. plantarum* in association with *L. rossiae*) synthesized PlnA in these aspects. The effect on transcriptional regulation of vascular endothelial growth factor (*VEGF*) gene was assayed through RT-PCR and ELISA, while the cellular antioxidant protection was tested by MTT and dichlorofluorescein assays after H_2O_2 oxidative stress induction.The results obtained showed that VEGF expression is remarkably induced both by synthetic PlnA treatment and by treatment with a co-culture supernatant containing PlnA.For all treatment times treatment with co-culture PlnA at 10 μg/ml resulted in the highest VEGF increment. Particularly, at 10 μg/ml the induction by co-culture supernatant PlnA was higher than that by synthetic PlnA at the same concentration. As regards oxidative stress protection, compared with H_2O_2-stressed control cells, cells treated for 2, 4, 16, and 24 hours with PlnA preparations showed increased survival. These results were confirmed by determining the concentration of intracellular reactive oxygen species. Compared with H_2O_2-stressed control cells, cells treated with chemically or biologically synthesized PlnA showed decrease in ROS concentrations. These findings suggested that antimicrobial peptide pheromone PlnA was positively sensed by human cells promoting certain important activities useful in the treatment of hair loss.

P118

Effects of ectoine on follicular aging processes

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Oxidative stress followed by DNA damage and changes in mitochondrial functionality has been considered to be etiological for several aging processes in human beings. Additionally, it has been shown that also hair follicles, similar to other human tissues, exhibit age-related changes at the molecular level, such as an altered gene expression of structural proteins. As further studies suggested a link between cellular aging parameters in the hair follicle and oxidative stress, we evaluate the different bioactivities regarding their potential to counteract typical aging characteristics in follicular cells. Ectoine is a natural compound that is produced in high concentrations, e.g., by halophilic microorganisms and confers resistance towards salt, temperature, and UV stress by stabilizing cellular biopolymers such as proteins. Our studies showed that Ectoine furthermore reveals a high potential to protect cellular functions against oxidative stress, the resulting DNA damage, and impaired mitochondrial function. We treated follicular outer root sheath keratinocytes with sublethal doses of hydrogen peroxide and checked the expression of structural proteins and mitochondria-relevant genes with and without pre-treatment with Ectoine. Additionally, we measured the amount of produced reactive oxygen species and determined the degree of DNA damage using the COMET assay. In these assays Ectoine exhibited not only a significant restoration of essential cellular parameters under oxidative stress but also a reduction of DNA damage up to 80%. These data suggest that Ecotine shows a high potential to antagonize follicular aging processes.

P119

Klotho expression pattern in human scalp skin and hair follicles

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Background: Klotho is a newly identified anti-aging protein that plays a pivotal role in regulating aging. However, whether klotho is expressed in human hair follicles (HFs) and whether klotho expression correlates with hair growth have not yet been clearly shown.

Objectives and methods: In this study, we examined the expression of klotho in human scalp skin, human HFs, and its expression change in organ-cultured human HFs using reverse transcriptase-polymerase chain reaction and immunofluorescence.

Results: Klotho was expressed in human scalp skin and HF at both gene and protein levels. In human scalp skin, prominent klotho expression was observed in the epidermis. Klotho expression in the epidermis was increased with keratinization from the basal layer to stratrum corneum. In human anagen HFs, prominent klotho expression was observed in the epithelium. Klotho expression in the epithelium was increased with keratinization in henle layer and hair cuticle. In human catagen HFs, klotho expression was observed in the epithelial strand.

Conclusion: Altogether, these results indicate that klotho might be an important regulatory factor for human hair growth and hair cycle change.

P120

Microcirculation disorders of scalp skin in patients with diffuse alopecia

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Normal functioning of scalp skin vascular system provides full-blown nutrition and development of dermal papilla in the bulge zone of hair follicle, thereby playing an important role in hair cycle regulation.

Purpose: To estimate scalp skin microcirculation disorders in patients with diffuse alopecia.

Materials and methods: We examined the scalp skin in 120 patients with complaints on loss of hair by the ''Lazer Doppler flowmeter'' method. Active factors of microcirculation control were investigated (the factors directly influencing the microcirculation system)—endothelial, myogenic, and neurogenic mechanisms of vessels' lumen and tonus regulation. These factors of regulation control and modulate the blood flow on the part of the vascular wall and are realized through its muscular component. Active mechanisms influence the blood flow transverse oscillations as a result of vessel muscles' alternating contraction and relaxation (taking turns one after the other in episodes of vasoconstriction and vasodilation). Microhemodynamics testing of scalp skin in patients with diffuse alopecia has made it possible to reveal the different disorders of vascular tone regulation and to prescribe the differentiated treatment that has raised the efficiency and reduced the time of treatment in such patients. Topical preparations (Mynoxidil or Aminexil), which are peripheral vasodilators, were prescribed for disorders of myogenic and neurogenic oscillations, which are connected with the sympathetic adrenergic (mainly, thermoregulating) influences on the smooth muscle of arterioles and arteriole areas' arteriole–venule anastomoses. Topical irritants (the preparations, containing alcohols, tincture of red pepper, diethyl ether, etc.) and massage of scalp were used at signs of increase in vessels' endothelial tone. Regular physiotherapeutic procedures were recommended for these patients. This treatment promoted the release of vasoactive substances (the nitrogen oxide and histamine)influencing the microvascular endothelium.

P121

Evaluation of diffuse hair loss in premenopausal women in the Indian scenario

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Methodical evaluation of 50 women in the premenopausal age group from the Indian scenario suffering from diffuse hair loss was done considering the following hematological criteria: (1) hemoglobin concentration; (2) RBC indices; (3) transferrin saturation; (4) serum vitaminB12 level; and (5) serum vitaminD3 level. The diagnosis of alopecia was confirmed by clinical tests. In a few patients, where indicated, thyroid function tests and pelvic ultrasonography were done. The results were tabulated and analyzed. Most patients were in the age group 26–40. We found that there was a significant percentage of patients having deficiency of Vitamin D3 and Vitamin B12. Hemoglobin levels were low in a significant number of patients. The patients were treated for the underlying deficiencies and the patients were then followed up from 3 to 6 months. The treatments included hematinics, B12 injectables, or Vit D3 supplements, as per the need. We could follow up with 40 patients, and subjective and clinical evaluation of improvement in hair fall was noted. There were a significant number of patients showing moderate to dramatic improvement in the hair fall.

P122

Usage of platelet-rich plasma in alopecia treatment

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Human hair has an important role in visual appearance and social communication. An alopecia is a common disorder affecting both men and women. Minoxidil and finasteride are effective treatment methods, but those who have poor response to them have limited treatment capabilities. One kind of cell therapy that is in use nowadays is autologous platelet-rich plasma (also known as PRP). PRP is a component of whole blood that contains concentrated platelets that play a key role in a human life preservation-bleeding blockage. As a source of platelets, PRP contains several growth factors and other cytokines that stimulate healing of bone and soft tissue. There are a lot of technologies for obtaining PRP, which are in use in different countries around the world. We have been using PRP in combined treatment of more than 150 patients with good effect. The obtained results are inspiring, but there is a necessity of uniting our colleagues' efforts to investigate this issue deeper and in a more profound way. PRP might be a safe, effective, and well-tolerated treatment for alopecia.

P123

Increased rates of alopecia and hirsutism in obese compared with non-obese children/adolescents: a population-based study

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Study design: We conducted a retrospective population-based study by analyzing the electronic medical records of the Kaiser Permanente Northern California (KPNC) Managed Healthcare System. All members aged 5–17 years with at least one recorded body mass index (BMI) value in 2009 were eligible. Diagnoses of inflammatory alopecias were excluded. Patients were classified as normal BMI, overweight, or obese according to age- and sex-specific criteria established by the Centers for Disease Control (CDC). Obesity was defined as a BMI >95th percentile, overweight as 85th–95th percentile, and normal BMI as <85th percentile.

Results: Data from a total of 248,775 subjects were analyzed. The prevalence of alopecia and hirsutism in this cohort was low: alopecia (normal BMI 0.34%, overweight 0.42%, and obese 0.42%); hirsutism (normal BMI 0.06%, overweight 0.15%, and obese 0.17%). Alopecia was not associated with being overweight or obese. However, rates of hirsutism were significantly higher in those subjects who were overweight or obese as compared to those who had a normal BMI ($P<0.0001$). Among girls who had a diagnosis of hirsutism, the rates of PCOS were high (31.0%), whereas the frequency of PCOS in girls with a diagnosis of alopecia were lower (2.8%).

Conclusions: Obesity results in an increased mass of adipose tissue with a corresponding increase in secretion of peptide hormones, cytokines, and paracrine transmitters, among others. Thus, it is possible that in obese patients, androgen as well as non-androgen or inflammatory signals may induce facial hirsutism in children and adolescents. Further study of the effects of adipose tissue as an endocrine organ capable of influencing the pathophysiology of the hair is warranted.

P124

Effect of several anthraquinone derivatives on hair growth

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Hirsutism affects 5–15% of women. It is defined as the excessive growth of coarse hairs in women, which may appear in a male-like pattern, i.e. face, chest, stomach, and thighs. It is therefore primarily of cosmetic and psychological concern. Thus, it is important to develop new therapeutic materials to stop hair growth or to delay hair growth. Anthraquinone has gained lots of interest because of its various biological activities, including anti-tumor, anti-bacterial, and anti-inflammatory effects. In this study, we tested the effects of several anthraquinone derivatives, including emodin, aloe-emodin, and physcion, on hair growth using *in vivo* and *in vitro* test models. After topical application of emodin onto the back of C57BL/6 mice, earlier conversion of anagen to telogen was induced. To investigate the action mechanism, we treated cultured dermal papilla cells with anthraquinone derivatives. The growth of dermal papilla cells, however, was not affected by anthraquinone derivatives. Among them, RT-PCR analysis showed that emodin induced mRNA levels for transformation growth factor-β (TGF-β), suggesting that the effects of emodin on hair growth may be mediated through the regulation of growth factors in dermal papilla cells. We further investigate the effect of emodin on the TGF-β signaling pathway. Interestingly, emodin induced Smad 2 and 3 expresion in a dose-dependent manner. Taken together, our results showed that emodin delayed hair growth via the Smad-mediated TGF-β signaling pathway and can be used for helping in hair growth control.

P125

Corticotropin-releasing hormone downregulates hair growth-related cytokines in cultured human dermal papilla cells

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Corticotropin-releasing hormone (CRH) is involved in the stress response and there is increasing evidence that stress influences skin diseases such as hair loss. In cultured human hair follicles, CRH inhibits hair shaft elongation, induces premature regression, and promotes the apoptosis of hair matrix keratinocytes. We investigated whether CRH influences the dermal papilla cells that play pivotal roles in hair growth and cycling. Human dermal papilla cells were treated with CRH, ACTH, and cortisol, key stress hormones along the hypothalamic–pituitary–adrenal (HPA) axis, for 1–24 hours. Interestingly, CRH modulated expression of cytokines related to hair growth (KGF, Wnt5a, TGFb-2, Nexin) in cultured dermal papilla cells. CRH receptors are downregulated by negative feedback systems. Pretreatment of antalarmin and astressin2B, CRH receptor antagonists, prevented the CRH-induced modulation of cytokines. Furthermore, CRH increased cAMP production and the influence of CRH is also abolished by PKA inhibitor. Since the CRH induces POMC expression through the cAMP/PKA pathway, we analyzed POMC mRNA, ACTH, and cortisol levels. Interestingly, while CRH induced POMC expression in cultured human dermal papilla cells, alteration of protein levels of ACTH and cortisol was not observed. These results indicate that CRH operates within dermal papilla cells through CRH receptors along the classical CRH signaling pathway and provide a novel insight into the mechanism triggered by CRH stimulation in human dermal papilla cells.

P126

A novel PPARγ modulator (GMG-43AC) inhibits human hair growth and inflammatory response in keratinocytes, and upregulates expression of epithelial stem-cell-associated keratins, K15 and K19

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In the skin, signaling through peroxisome proliferator-activated receptors-gamma (PPARγ) exerts anti-inflammatory effects, and modulates keratinocyte and sebocyte differentiation. PPARγ signalling may also be needed for the maintenance of hair follicle (HF) epithelial stem cells. We have investigated whether a newly designed PPARγ modulator, GMG-43AC, alters human hair growth *in vitro*, and/or the expression of stem cell-associated keratins. In addition, we have assessed the capability of this compound to inhibit induction of IL-6 in primary culture of normal human keratinocytes (NHKs). Scalp HFs from 6 healthy females were cultured with 0.01–1 mM GMG-43AC or vehicle for 6 days, and NHKs were treated with 10 ng/ml TNF-α and 30 ng/ml IFN-γ with or without the addition of 0.5 mM GMG-43AC for 6 and 24 hours. The expression of IL-6 at mRNA and protein levels was evaluated by real-time PCR and ELISA, respectively. Despite substantial interindividual variations in the HF response to GMG-43AC, overall, 1 mM of GMG-43AC inhibited hair shaft elongation, enhanced catagen development, and increased hair matrix keratinocyte apoptosis. Yet, GMG-43AC also upregulated expression of keratins K15 and K19. Microarray analysis revealed several candidate GMG-43AC target genes that invite subsequent mechanistic studies. In the NHKs model, GMG-43AC inhibited inflammation. The observed hair growth-inhibitory effects of GMG-43AC associated with the anti-inflammatory activity on keratinocytes, together with its favorable toxicological profile, make this compound an interesting candidate for development as a future anti-hirsutism agent. Interestingly, GMG-43AC appears to rather stimulate the HF's vital epithelial stem cell pool and thus may help to preserve the HF's regeneration potential. Together with its anti-inflammatory effects, this effect may also be interesting for the management of lichen planopilaris, where PPARγ signaling is deficient.

P127

Influence of oxygen tension on hair growth and cycle and the therapeutic potential of systemic normobaric oxygenation

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Introduction: The influence of magnitude of oxygen tension on the hair follicle remains unclear, though ischemia clearly induces apoptosis and hair loss. We investigated the relationship between tissue oxygen tension and hair growth and cycle, and evaluated the potent value of oxygenation therapy.

Methods: Organ culture of human hair and rat whisker follicles was performed under three different oxygen concentrations (1%, 6%, and 20%). The back skin of 8-weeks-old C56BL/6 mice was epilated (anagen induction), and then they were divided into two groups and were housed under normobaric 20% or 60% oxygen. Hyperoxygenation was performed during early anagen, mid-anagen, and catagen phase, and the hair was evaluated macroscopically and histologically.

Results: Hair growth was significantly faster under higher oxygen concentration in organ culture of both rat and human hair follicles. This indicates the sensitivity of hair follicles to oxygen and the importance of skin vascularity and tissue oxygen tension. In animal experiment, ki67+ cells in hair follicles appeared to be more increased under 60% oxygen than under 20% oxygen between 2 to 4 days after epilation. At 1 week and 2 weeks after epilation, the length of hair was significantly extended under 60% oxygen. It was suggested that hyperoxygenation therapy accelerated anagen induction and promoted hair growth. On days 15 to 18 after epilation, the number of TUNEL+ cells in hair follicles appeared to be reduced under 60% oxygen, suggesting that oxygen therapy may delay catagen transition and extend anagen term.

Conclusion: These data indicated the strong influence of surrounding tissue oxygen tension on hair growth and cycle, which may partly explain scalp hair loss with aging and ischemia. Unlike hyperbaric oxygenation, normobaric oxygenation is safe and can be applied for a long period. The therapy may be beneficial to a variety of hair loss associated with scalp ischemia.

P128

Eplerenone stimulates human hair growth *ex vivo*

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Aldosterone and glucocorticoids bind to the mineralocorticoid receptor (MR). Although human skin also expresses MR and can generate glucocorticoids, the important role of the MR in regulating the water and ion homeostasis in the kidney is well described but remains unknown in human skin. However, overexpression of MR causes abnormal hair follicle (HF) development in mice, and the MR antagonist, spironolactone, can modulate human hair growth *in vivo*. We have explored the hypothesis that MR-mediated signaling impacts on this non-classical organ, the human HF, and modulates human hair growth *in vitro*. MR mRNA and protein expression and transforming growth factor beta 2 (TGFβ2) transcription of human scalp HFs were analyzed. Microdissected human scalp HFs were exposed in our serum-free human HF organ culture system to aldosterone (classical MR agonist), spironolactone (non-selective MR antagonist), and eplerenone (selective MR antagonist), and hair shaft elongation, HF cycling, proliferation, and apoptosis were assessed morphometrically. Human scalp HFs constitutively expressed MR mRNA and protein *in situ*. MR expression appeared to be downregulated during catagen development and by spironolactone. Eplerenone enhanced human hair shaft production and hair matrix keratinocyte proliferation, and retarded spontaneous HF regression (catagen). Since spironolactone reduced TGFβ2 transcription, downregulation of this potent hair growth inhibitor may underlie these effects, at least in part. These preliminary data provide the first indication that MR-mediated signaling functions as an important modulator of human scalp hair growth and that inhibitors of MR signaling need to undergo systematic exploration as novel candidates for anti-hair loss treatment.

P129

Age-related expression changes of wnt signaling-associated genes in human hair follicle

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The effect of age on various features of scalp and hair shaft, for example, hair loss, hair graying, and hair luster reduction, has been investigated previously. However, the mechanism and key regulators of the hair aging process were largely unknown. This study aimed to determine the major aging factors of hair aging in human hair bulb area using microarray analysis. We collected ten scalp hairs plucked from the vertex area of each of 26 young (22–29 years) and 29 old (61–69 years) Korean women. Total RNA isolated from the hair bulb region were pooled in two age groups as young (20 s) and old (60 s). The gene expression profile of the hair bulb area of each group was analyzed using microarray (Affymetrix GeneChip Scanner 3000 7G) and real-time PCR. From 54,675 human genome probes, 1,179 genes showed >2-fold differential expression with aging, including 649 upregulated and 530 downregulated genes. Among the changed genes, the expression of five genes associated with the wnt signaling pathway, *WIF1*, *DKK3*, *FRZB*, *SHH*, and *GSK3B*, was confirmed with real-time PCR. As a result, three genes known as wnt inhibitors, *WIF1*, *DKK3* and *FRZB*, showed consistently increasing expression pattern in the aged hair bulb area. In conclusion, the wnt pathway in normal hair might be inactivated with aging following aging-related hair loss or senescent alopecia. This study suggested that the wnt signaling pathway plays an important role in hair aging as well as hair development. Further studies are required to understand the specific role of wnt-related genes in hair follicle aging.

P130

Human scalp hair follicles possess the enzymes to synthesize prostaglandins and prostamides from phospholipids and contain $PGF_{2\alpha}$ *in vivo*

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Prostamides are recently discovered biological lipids closely related to the paracrine regulators, prostaglandins (PG). We demonstrated that a prostamide $F_{2\alpha}$ analogue, bimatoprost, developed as a glaucoma treatment, stimulates the growth of rodent back follicles *in vivo* and human scalp follicles in organ culture and that scalp follicles contain receptors for $PGF_{2\alpha}$ (FP) and prostamide $F_{2\alpha}$. Since this suggests that these molecules have biological roles in hair follicles, we investigated whether follicles contain the enzymes to synthesize prostaglandins and prostamides *de novo* from phospholipids. We also examined whether normal scalp follicles contain $PGF_{2\alpha}$. Scalp hair follicles were individually microdissected from non-balding scalp skin by elective cosmetic surgery with appropriate approvals and extracted for enzyme gene expression identification by RT-PCR, qPCR, and sequencing, or for $PGF_{2\alpha}$ measurement by electrospray tandem mass spectrometry coupled to liquid chromatography (LC/ESI-MS/MS). Enzyme protein expression was analyzed on frozen scalp skin sections using dual color immunofluorescence with FITC and DAPI nuclear fluorescence to confirm follicular structures. Isolated scalp hair follicles ($n=5$) expressed the genes for all enzymes to synthesize the key central PGH_2 and prostamide H_2 (from which other PGs and prostamides are made) *de novo* from phospholipids: PLA_2, NAPE-PLD, COX-1, COX-2, $FAAH_1$, and $FAAH_2$. Immunofluorescence confirmed their protein expression in the hair bulb. Follicles also contained measurable amounts of $PGF_{2\alpha}$ (13.39 ± 1.8 pg per mg of follicle protein; mean ± SEM, $n=3$). The enzyme that synthesizes prostamide $F_{2\alpha}$, prostamide/PGF synthase, was also present. Thus, scalp hair follicles possess the necessary enzymes for the local synthesis of both prostaglandin and prostamide mediators from phospholipids and contain $PGF_{2\alpha}$. This suggests that these paracrine mediators may have natural roles in hair follicles; further analysis of these should increase our understanding of hair follicle biology and may lead to further treatments for hair disorders.

P131

Closing K_{ATP} channels in human hair follicles inhibits hair growth, promotes catagen-like changes, and alters paracrine signalling; could this be a new therapeutic approach for excessive hair disorders?

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Excessive hair growth disorders, hirsutism and hypertrichosis, are currently poorly controlled. Since drugs that open K_{ATP} channels in plasma membranes often stimulate hair growth, e.g. minoxidil and diazoxid, we investigated the effect of closing such channels in human hair follicles. Human hair follicles contain two types of K_{ATP} channels, SUR1 and SUR2B, with differing sensitivity to K_{ATP} channel regulators. Therefore, we assessed the effect of tolbutamide, which closes both SUR1 and SUR2B channels, on hair growth in organ culture and paracrine signalling in human hair follicles and dermal papilla cells. Human hair follicles were microdissected, and observed, measured, and photographed daily for 9 days while being cultured with, or without, tolbutamide (10 nM–1 mM). After incubation with 1 mM tolbutamide, total RNA was extracted from hair follicles after 4 days (three pooled samples, each from five individuals) and dermal papilla cells after 48 hours. Differences in gene expression determined using DNA microarray analysis were confirmed using quantitative real-time PCR. Tolbutamide had no effect at 10 nM but at 100 nM–1 mM it significantly inhibited human follicle growth in organ culture and stimulated catagen-like changes, which were confirmed by histology. Tolbutamide at 1 mM altered follicular paracrine signalling, increasing gene expression of inhibitory TNF ($P<0.05$) and its receptor, TNFRSF1A ($P<0.01$), and decreasing stimulatory FGF-10 ($P<0.01$) and FGFR2 receptor ($P<0.05$). In cultured dermal papilla cells tolbutamide also reduced expression of FGF10 ($P<0.01$) and FGFR2 ($P<0.05$), while increasing TNF ($P<0.05$) and TNFRSF1A ($P<0.05$). Therefore, closing both SUR1 and SUR2B K_{ATP} channels decreased hair growth, induced catagen-like changes, and altered paracrine signalling in both follicles and dermal papilla cells. This strongly suggests that follicular K_{ATP} channels have an important biological role in hair growth. Further understanding should enable novel therapies for excessive hair disorders.

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Effect of the hair follicle environment on nanocarriers' stability and drug release properties

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Several types of particulate carrier systems are under investigation for transcutaneous drug delivery as well as hair follicle targeting. Previous work by our own group and others characterized the important role of hair follicles as reservoir, where particles can be stored for days as well as a shunt penetration pathway. Over the past years, it has become clear that particles can be used to target active compounds to different regions of the pilosebaceous unit. For a translation of such approaches into clinical and cosmetic applications, however, more detailed investigations on the way in which different particle types interact with the hair follicle environment are very important. Using human skin explants, we combined high-resolution imaging techniques and cell biology methods (magnetic cell separation, flow cytometry) to investigate particle distribution on the skin surface and possible translocation of different carrier classes ranging from rigid inorganic particles, like gold or silica particles, to fluorescent lipid and polymer particles and virus-like particles. We found a bright spectrum of penetration and release properties depending on the particle type. Rigid particles accumulated in skin furrows and hair follicle canals and were taken up by skin dendritic cells, when activated by mechanical skin barrier disruption. Biological particles, like virus-like particles, were detected by means of electron microscopy in the first epidermal layers in the proximity of hair follicles, pointing to very specific interactions of these particles with skin and the follicular epithelia. On the contrary, flexible lipid and polymer particles aggregated in the hair follicle canal, where they released their load to the different skin layers in a time-dependent manner. We conclude that a variety of drug release features and targeting functions can be achieved by choosing the appropriate type of particle in accordance to its physicochemical property and its interaction with the hair follicle environment.

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Thyrotropin-releasing hormone (TRH) modulates keratin expression in human skin

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Keratins are major structural components of the skin and hair, and their importance has been emphasized by recent evidence showing that keratins have a much wider spectrum of functions (e.g. wound healing, hair follicle (HF) cycling). Therefore, it is of utmost importance to understand their regulatory mechanisms. Although some evidence exists regarding steroid hormone regulation, little is known on neuroendocrine control of human keratin expression. Preliminary microarray-based evidence had suggested that thyrotropin-releasing hormone may modulate keratin expression in human HFs. Therefore, our aim was to elucidate whether TRH has modulating effects on keratin expression in human skin, under physiologically relevant conditions *in situ*. Serum-free organ cultures of male or female human scalp HFs and scalp skin were treated for 12 hours–6 days with 100 ng ml^{-1} TRH or vehicle, and the expression of selected keratins was assessed by quantitative immunohistomorphometry and RT-qPCR. Staining intensity of hair keratins K31 and K32 was increased, while that of K85 and K86 was reduced after 6 days in culture. Staining intensity of epithelial keratins K14 and K17 was also reduced. TRH also modulated expression of the epithelial stem cell-associated K15 and K19. In the interfollicular epidermis, TRH stimulated expression of K6, K14, and K17, both at the mRNA and protein levels. Stimulation of the same keratins was also evident in the eccrine sweat and sebaceous glands. These findings introduce the neuropeptide hormone TRH as a novel modulator of selected human hair keratins *in situ*. The keratin-modulating effect of TRH revealed here invites novel neuroendocrine strategy that utilizes neurohormones to therapeutically modulate keratin expression in human skin and its appendages.

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Systemic angiotensinergic stimulation modulates epithelial–mesenchymal transition and collagen deposition in skin and hair follicle

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The mechanisms of hair follicle scaring in inflammatory disorders such as primary cicatricial alopecia (PCA) are unclear. Expression profiling studies on scalp biopsies from PCA patients revealed that the upregulation of TGFβ and the fibrosis pathway is associated with either reduced PPARγ or increased AhR. Mechanistic studies in mice and in cultured hair follicle outer root sheath cells with specific agonists and antagonists of PPARγ and AhR indicated coordinated regulation of expression of TGFβ and COL1A1 genes as well as the vascular angiotensin receptor, AGTR1. Thus, scarring may involve AGTR1 signaling. In the present study we tested the hypothesis that system-wide angiotensinergic stimulation induces destruction of pilosebaceous units in the skin, which can be prevented by AGTR1 blockade. We infused C57BL6 mice ($n=10$, each group) with either the AGTR1 agonist ([Sar1]AngII, 1,400 ng kg^{-1} per minute) or the AGTR1 inverse agonist (Candesartan, 1,000 ng kg^{-1} per minute) or saline for 28 days by surgically implanting subcutaneous osmotic minipumps. Blood pressure was 160 ± 8 in the [Sar^1]AngII infusion mice, whereas it was 122 ± 10 in the saline infusion group and 118 ± 6 in the Candesartan infusion group. As expected, hypertrophy combined with increased collagen deposition and elastin lamina disruption was observed in the cardiac and vascular tissues in mice after [Sar1]AngII infusion, but these effects were not observed in the Candesartan-infused mice. Histopathology of the skin samples analyzed showed that the pilosebaceous units are significantly deformed and perhaps rendered dysfunctional, specifically in the [Sar^1]AngII infusion mice. The skin histopathology of the Candesartan-treated group was normal. Further molecular analysis for PPARγ, AhR, TGFβ, collagen synthesis, and scarring pathways in these samples is in progress. We will show evidence for the expression of epithelial to mesenchymal transition (EMT) markers, to demonstrate EMT in skin upon [Sar1]AngII infusion. Thus, systemic angiotensinergic stimulation may produce skin lesions involving EMT and collagen deposition in hair follicle that resembles scarring in PCA.

GENETIC HAIR DISORDERS AND HAIR FIBER SCIENCE

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Novel *KRT86* mutation in a Turkish family with monilethrix: first-time identification of a mutational mosaicism

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Monilethrix is a rare genetic disorder with a high intrafamiliar and interfamilial variability of hair loss. The clinical hallmarks consist of diffuse or localized occipital and temporal alopecia, hair fragility, and follicular keratosis of the occipital region. Patients show distinctive hair shaft anomalies with periodic constrictions, resulting in elliptical nodes and internodes, variation of hair shaft thickness, and a tendency to fracture at the constriction sites. The inheritance pattern is mostly autosomal dominant with pathogenic mutations in the keratin genes *KRT81*, *KRT83*, or *KRT86*, but an autosomal recessive form has also been described with mutations in *DSG4* (desmoglein 4). We investigated a consanguineous Turkish family with three affected children and clinically apparently unaffected parents. The siblings developed progressive hair loss within the first weeks of life. The mother had noticeably no hair loss but a self-reported history of mild hair fragility. The father showed no abnormalities in hair growth or structure. Sequencing of DSG4 revealed no pathogenic mutation. Therefore, we sequenced the coding exons of *KRT86* and revealed the novel mutation c.1231 G>T;p.Glu411X in exon 7 in the three affected children as well as in the mother. The mutational signal observed in the mother was much weaker than the signal observed in the children, pointing to a mutational mosaicism. SNaPshot analysis revealed a substantial mutation-level variation between the three children and the mother. To our knowledge, this is the first time that a mutational mosaicism was demonstrated for monilethrix or any other monogenic hair loss disorder. The mother who carried the *KRT86* mutation as a mosaic showed a less severe picture than her children, suggesting that a *KRT86* mosaicism leads to a milder form.

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An international repository for mouse models of human hair diseases

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The laboratory mouse is the premier mammalian biomedical model for most human diseases. The Jackson Laboratory is a private, nonprofit, research institution that developed a centralized, international repository for inbred and mutant mice in the 1940s and serves as the template for other repositories around the world. The Jackson Laboratory Rare and Orphan Disease Center was recently launched to focus on partnering with scientists, foundations, and other experts around the world to enable the development, standardization, optimization, and rapid distribution of preclinical mouse models for basic research and drug discovery. Being able to offer the resources and expertise to enable the design, construction, and management of preclinical mouse models of disease, in combination with a global delivery system, expertise in technical transfer issues, and genetic quality control, uniquely positions the Center to put new tools into the hands of scientists and thereby accelerate drug discovery. Complementing the Center's model development pipeline is one of the most comprehensive mouse repositories in the world, consisting of a growing collection of over 7000 spontaneous and engineered mouse lines, including several hundred dealing with skin and hair diseases. The wide array of easily accessible, well-characterized biomedical tools, and mutant allele strains serves as an unmatched companion resource for the building of novel disease models with applications in translational research. Contributing your disease model to the Rare and Orphan Disease Center enables researchers across the globe to have greater access to tools for drug efficacy testing and discovery. If you would like to donate your mouse strain to the Jackson Laboratory Mouse Repository, please see: www.jax.org/donate-a-mouse. To learn more about The Jackson Laboratory Rare and Orphan Disease Center, please visit our website at www.jax.org/rare.

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Mutation detection of type II hair cortex keratin gene hHB6 in a Chinese han family with congenital monilethrix

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Monilethrix (MIM 158000) is an autosomal dominant hair disorder characterized clinically by alopecia and follicular papules. In this study, we identified a Chinese family with monilethrix by light microscopic and scanning electron microscopic (SEM) examination. A heterozygous transversion mutation c.1204G>A(p.E402K) in the seventh exon of hHB6 was identified in both of two patients. Study of the relationship between the phenotype and the mutation of the Type II hair keratin gene hHB6 provided the molecular basis to further explore the pathogenesis of monilethrix.

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A novel deletion mutation in the TRPS1 gene underlies trichorhinophalangeal syndrome type I

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Tricho-rhino-phalangeal syndrome (TRPS) is a rare autosomal dominant disorder. Deletion or mutation of the *TRPS1* gene leads to the tricho-rhino-phalangeal syndromes type I or type III. In this article, we describe a Chinese patient affected with type I TRPS and showing prominent pilar, rhinal, and phalangeal abnormalities. Mutational screening and sequence analysis of *TRPS1* gene revealed a previously unidentified 4-base-pair deletion of nucleotides 1783–1786 (c.1783_1786delACTT). The mutation causes a frame shift after codon 593, introducing a premature stop codon after 637 residues in the gene sequence. This deletion is an unquestionable loss-of-function mutation, deleting all the functionally important parts of the protein. Our novel discovery indicates that sparse hair and metacarpal defects of tricho-rhino-phalangeal syndromes in this patient are due to this *TRPS1* mutation. In addition, these data further support the critical role of *TRPS1* gene in hair and partial skeleton morphogenesis.

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Combination of atrophoderma vermiculatum, KPA, hypotrichosis with palmar pits in a family—an interesting case report from South India

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Atrophoderma vermiculatum (AV) is a variant of kertosis pilaris atrophicans (KPA) otherwise called folliculitis ulerythmatosa reticulata or honeycomb atrophy. It occurs usually as a distinct entity, rarely with keratosis pilaris or as part of syndromes like Rombo. We report here a combination of AV with extensive keratosis pilaris (KP), KPA, hypotrichosis of the eyebrows, scalp, and beard region in a father and his two daughters, the latter also showing milia, palmar pits, and enamel defects. The mother was unaffected. Marriage was non-consanguineous. Two female children of 10 and 5 years, respectively, presented with asymptomatic skin lesions over the face since 5 months of age. Both had multiple follicular papules over the face admixed with milia, especially around the eyes and malar areas. Multiple atrophic scars were present over both the cheeks almost in a symmetric pattern. Loss of eyebrows was prominent in both the siblings. Also, the younger sibling had papules and scars over the frontal scalp with patchy loss of hair. Father (35 years old) had prominent atrophic scars characteristic of AV associated with total loss of eye brows, and sparse hairs on the mustache, beard, and chest with baldness in the frontal scalp. He also had prominent keratosis pilaris papules all over the body. Biopsy of the eyebrow and follicular lesion showed prominent follicular hyperkeratosis with dilated orifices associated with perifollicular chronic inflammation and fibrosis. Milia lesion showed the characteristic picture. AV lesion showed follicular atrophy as well as mid to deep dermal fragmentation of collagen (woolly appearance), which was more prominent underlying the follicular areas. Enamel defects and palmar pits were present in both siblings. AV with hypotrichosis and palmar pits have not been reported so far to the best of our knowledge. Hence, this could be a new combination and may point to an association with ectodermal dysplasia, possibly with autosomal dominant mode of inheritance, in these cases.

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Nonphotosensitive trichothiodystrophy: a report of two male sibs

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Trichothiodystrophy (TTD; MIM #601675) describes a group of autosomal recessive disorders characterized by brittle, sulfur-deficient hair. TTD can be associated with variable neuroecto-dermal manifestations including mental and developmental delay, ichthyotic skin, nail dystrophy, and cutaneous photosensitivity with a wide variety of phenotypes. The diagnosis of TTD is based on bright and dark areas known as ''tiger tail'' pattern observed by polarizing microscopy of the hair and low sulfur content. We report an 18-year and a 13-year-old male sibs with short and brittle hair who presented with mental retardation and ataxia. Clinical examination revealed widespread follicular keratosis mostly prominent on the trunk and extremities, short, dry, brittle hair, and sparse eyebrow and nail dystrophy. Apart from dermatological findings the patients had ataxia, dental caries, and malocclusion. Moderately decreased intelligence quotients (IQ) were noted in both of them. Growth development and osseous radiographies were normal. Polarizing light microscopic examination showed pathognomonic alternating light and dark bands in addition to the structural hair abnormalities including trichoschisis and trichorrhexis nodosa seen on light microscopy. Hair amino-acid analysis determined by using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) showed marked decrease in cystine content of hair compared with those of control hair. thus confirming the diagnosis of TTD. The name 'trichothiodystrophy' comprises several phenotypes with many cutaneous and neurological features on the basis of hair abnormalities with low sulfur content. These cases will contribute to the literature with additional features that have rarely been reported in trichothiodystrophy.

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Visual attention to and perception of undamaged and damaged versions of natural and colored female hair

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Female hair color is thought to influence physical attractiveness and, although there is some evidence for this assertion, research has yet not addressed the question how structural integrity of the hair shaft affects the optics and, thus, perception of female hair color. Here we investigate whether people are visually sensitive to subtle differences in hair images of natural and colored hair before and after a controlled cuticle damage process. We used eye-tracking technology to measure the visual attention of men and women to randomized pairs of standardized images of natural and colored hair tresses, each pair combining the same tress before and after controlled cuticle damage. The same hair images were then rated for perceived health, age, and attractiveness. Undamaged versions of natural and colored hair were perceived as significantly younger, healthier, and more attractive than the corresponding damaged versions. Visual attention to images of undamaged colored hair was significantly higher than to their damaged counterparts, while in natural hair the opposite pattern was found. We argue that the divergence in visual attention to undamaged colored female hair and damaged natural female hair and associated ratings is due to differences in social perception and discuss the source of apparent visual difference between undamaged and damaged hair.

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Follicular neogenesis in marie-unna hereditary hypotrichosis

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Marie-Unna Hereditary Hypotrichosis (MUHH) is an autosomal dominant genotrichosis due to a mutation in the 5-prime UTR (termed *U2HR*) of the *HR* gene. Affected individuals have coarse, wiry hair in childhood and then develop progressive hair loss in early adulthood. *U2HR* mutations are thought to cause increased translation of the main physiological HR ORF, but the consequent effect on hair follicle pathobiology has not been determined. We have studied biopsies from four patients with MUHH. The *U2HR* mutation was confirmed in two of these patients, whereas the other two patients were from families in which the *U2HR* mutation had been identified in other family members. All four biopsies showed similar features. The most striking finding was of numerous follicular buds arising from epidermis and from the outer root sheath of preserved terminal follicles. Most of these buds were at an early stage of anagen development, with few beyond Anagen 2. Dermal papilla morphology was often abnormal with a broad upper pole. Some preserved terminal follicles showed grossly excessive hyperpigmentation in hair bulb epithelium and in the dermal papilla. These histologic features of MUHH are highly distinctive and, to our knowledge, unique in human hair follicle pathology. They appear to show follicular neogenesis and resemble the changes seen in mice overexpressing beta-catenin. A study on a mouse model of MUHH indicated that the *HR* mutation causes the upregulation of the wnt signaling pathway (Kim *et al Hum Mol Genet* 2010;19;445–53) and our findings would support this concept in the human equivalent. The initial development of terminal follicles in MUHH and their subsequent loss remains to be explained.

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A position effect on *FGF13* underlies X-linked congenital-generalized hypertrichosis

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Hypertrichosis describes all forms of excessive hair growth for a given body location of an individual that does not depend on androgen stimulation. We and others have defined position effects involving the *TRPS1* and *SOX9* genes underlying autosomal forms of hypertrichosis; however, the genes that control increased hair follicle (HF) growth in X-linked hypertrichosis (XLH) remain unknown. Here we analyzed the DNA from a Mexican family with X-linked congenital-generalized hypertrichosis (MIM307150), as well as deafness and dental anomalies. Using whole-genome sequencing and SNP oligonucleotide microarray analysis, we identified a 389 kb intrachromosomal insertion at an extragenic palindrome site on chromosome Xq27.1 that completely cosegregates with the disease, and confirmed it by using FISH. Quantitative RT-PCR revealed that among the six genes surrounding the insertion, *FGF13* levels are significantly ($P<0.001$) decreased in the patients by fourfold, whereas mRNA levels of the neighboring genes remain unchanged. Importantly, RNA sequencing confirmed the selective decrease on *FGF13* expression in XLH. We localized FGF13 to the outer root sheath (ORS) of the human HF using *in situ* hybridization and immunofluorescence staining, and revealed a striking decrease in FGF13 localization throughout the ORS of patient HFs. As *FGF13* lies ~1 Mb away from the insertion in XLH, we postulate that a position effect occurs as a result of the insertion, and suggest that altered *FGF13* levels influence important downstream signaling pathways, which ultimately lead to the terminal hair overgrowth phenotype of XLH.

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Mutations in *SNRPE*, which encodes a core protein of the spliceosome, cause autosomal dominant hypotrichosis simplex

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Hypotrichosis simplex (HS) comprises a group of hereditary isolated alopecias that are characterized by a diffuse and progressive loss of hair starting in childhood with a wide phenotypic variability. We mapped an autosomal dominant form of HS to chromosome 1q31.3–1q41 in a Spanish family. By direct sequencing, we identified the heterozygous mutation c.1A>G (p.Met1?) in *SNRPE*, which results in the loss of the start codon of the transcript. We identified the same mutation in a simplex HS case from the United Kingdom, and an additional mutation (c.133G>A, p.Gly45Ser) in a simplex HS case originating from Tunisia. *SNRPE* encodes a core protein of U snRNPs, the key factors of the pre-mRNA-processing spliceosome. The missense mutation c.133G>A leads to a glycine to serine substitution and is predicted to disrupt the structure of SNRPE. Western blot analyses of HEK293T cells expressing *SNRPE* c.1A>G revealed an N-terminally truncated protein, and thus the mutation might result in the use of an alternative in frame downstream start codon. Subcellular localization of mutant SNRPE by immunofluorescence analyses, as well as incorporation of mutant SNRPE proteins into U snRNPs, was found to be normal, suggesting the function of U snRNPs in splicing, rather than their biogenesis that is affected. In this report, we link a core component of the spliceosome to hair loss, thus adding another specific factor in the complexity of hair growth. Furthermore, our findings extend the range of human phenotypes that are linked to the splicing machinery.

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Trichomegaly, or Hollywood "movie lashes", resulting from mutations in FGF5 and an elongated hair cycle anagen phase

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Trichomegaly, or Hollywood "movie lashes" (OMIM 190330), refers to an autosomal recessive disorder characterized by the growth of excessively long eyelashes; however, the genetic basis of this disease remains unknown. To identify molecular regulators of eyelash growth, we ascertained two consanguineous families from Pakistan who presented with familial trichomegaly. First, using whole-genome SNP genotyping and autozygosity mapping, we identified an extended region of high autozygosity on chromosome 4q21.21 within family 1. We then used whole-exome sequencing and identified distinct pathogenic mutations in both the families within the fibroblast growth factor 5 (FGF5) gene (c.158_159delTA and c.459+1delG), which lies directly within the region of autozygosity. Subsequent direct sequencing of FGF5 in several additional trichomegaly families identified a third mutation (c.T520C). To ascertain the effects of FGF5 mutations on human hair growth and anagen duration, we obtained and measured plucked hair fibers from patients' forearms and found them to be significantly longer than control hair fibers, indicative of a prolonged hair growth phase and a shifted anagen:telogen ratio. Moreover, we found that FGF5 protein was absent in plucked patient hair samples compared with controls using whole-mount immunofluorescence. Mutations in FGF5 underlie the *angora* phenotype in several mammalian species including mouse, dog, and rabbit among others, all of which exhibit an elongated anagen phase and excessive hair growth; however, until now, a human counterpart had not been described. We have identified FGF5 as a crucial regulator of hair growth in humans for the first time, and demonstrated a profound effect on the elongation of hair usually residing in telogen. Moreover, as this phenotype is strikingly visible in the eyelashes, this discovery raises the possibility of therapeutic targeting of FGF5 to selectively enhance the eyelash growth.

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Detection of chronobiological features on the surface of human hair through the multi-stage analysis of Wilhelmy-force profiles

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The natural surface of human hair is hydrophobic, consisting of a bilayer of heavily cross-linked proteins toward the cell inside, combined with a layer of fatty acids to the outside. The surface properties can be characterized through the wetting- or Wilhelmy-force in water. Wetting-force curves along segments of hair from female test individuals with lengths equivalent to about 1 month of growth (approx. 10 mm) are presented. In a multi-step analysis, applying curve smoothing, Fourier, and Principal Component Analysis for hair lengths comprising daily and weekly growth (2 mm), a compound circadian rhythm is observed, which through its typical bimodality can be linked to wake and sleep phases. The data set furthermore indicates systematic monthly changes with apparent relations to the menstrual cycle. This view is supported by observations of marked changes of this monthly pattern with the onset of pregnancy. Furthermore, consistent systematic changes on the same time scale are absent for male head hair. These results confirm that the wettability curves are nonstochastic in nature and that hair has a systematically nonhomogeneous, hydrophobic surface, which can be viewed in the context of the fluid mosaic model for cell membranes. This leads to the hypothesis that the hair surface preserves a rather detailed and long-term, individual chronobiological record. This record is "written" by the composition of the cell membrane of the cuticle cell before cell death. A practical application of this analytical approach is the determination of the pattern of distribution for cosmetically relevant material on the surface of human hair.

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Fractions of cortical cell types correlate with macroscopic bending modulus in human hair

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It has been shown that para-like and ortho-like cortical cells exist in human hair and the intermediate filaments (IFs) are arranged in parallel and helically in these cells, respectively. In this presentation, factors that correlate with macroscopic bending modulus of hair will be discussed from the viewpoints of cortical cells and macrofibrils. Bending stress and the diameters of the major and minor axes were evaluated for hair samples from 156 Japanese female donors. Although the bending elasticity was apparently well explained by the moment of inertia, which is a function of the major and minor axes, the calculated bending modulus was not constant and the difference between the maximum and the minimum values was more than double. Transverse sections of hair having various bending moduli were dual-stained with fluorescent dyes and observed under a fluorescence light microscope to evaluate the ratio of para- and ortho-like cortical cells. As the result, it was found that the hair of high bending modulus were rich in para-like cortical cells and those of low bending modulus were rich in ortho-like cells, and this trend was confirmed to be statistically significant. The moduli at the micro-scale, inside macrofibrils, in para- and ortho-like cortical cells were estimated by atomic force microscopy nanoindentation measurements. The difference in the modulus between the two types of cortical cells was only 6.4%, which was too small to explain the larger difference in the macroscopic bending modulus. This leads to a possibility that the difference in the morphology of macrofibrils is more plausible. It is suggested that the intermacrofibrillar material, of which the amounts are quite different in the para- and ortho-like cortical cells, is contributing to the difference in the modulus of cortical cell types, and this leads to the large difference in the macroscopic bending modulus of human hair.

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In vitro assembly of human hair keratin intermediate filaments: combination of keratin proteins and their properties

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Objectives: Keratins are intermediate filament proteins in epithelial cells and are classified into cytokeratins and hard keratins forming hair, nails and so on. There are 17 kinds of keratin proteins in human hair. The reasons for the existence of many species in hair and their molecular functions in the course of forming hair were not clear. To investigate these proteins' functions and properties, *in vitro* assembly of human hair keratin intermediate filaments was examined.
Methods: Five kinds of recombinant proteins of human hair keratin (hK35, hK36, hK38, hK81, and hK85) were prepared by using E. coli. Recombinant cytokeratins (hK14 and hK5) were also prepared as control. The recombinant proteins were purified by column chromatography and analyzed by two-dimensional electrophoresis. *In vitro* assembly was observed to form intermediate filaments under various conditions, such as when salt and urea concentrations varied.
Results: Under the optimized assembly treatment conditions, regular intermediate filaments with a 10-nm diameter and hundreds nano-meter length were formed, as confirmed by TEM. The optimum concentrations of salt and urea for the assembly were different between cytokeratins and hair keratins. Furthermore, as the complex of hK36 and hK81 was most stable among those tested, it was considered that the stabilities of the intermediate filaments formed may depend on the combination of particular hair keratins.
Conclusions: The assembly conditions of particular hair keratin intermediate filaments were optimized and the properties examined.

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ALOX12 inhibitors promote cuticle maturation that affects hair texture

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Aims: Hair cuticles are multiple scale-like layers that originate from a specific population of trichocytes in hair follicles. This specialized protective tissue has marked effects on the texture and resilience of hair fibers. During cuticle differentiation, specific citrullination of dimeric Ca^{2+}-binding S100A3 causes assembly of a S100A3 homotetramer. This study aimed to identify compounds that modulate the post-translational modifications of S100A3 associated with cuticle maturation in the SW480 cell line and in immature trichocytes of organ cultures.

Methods: Proteins were extracted from individual hair with characteristic types of cuticle damage, and from an SW480 cell line (EC87092801) and isolated human hair follicles, following culture in the presence of various compounds. The isoelectric variants of S100A3 that migrated on 2D-PAGE gels (pI range 3.9–5.1) were detected by western blot analysis.

Results: Acidic shifts of S100A3 (indicating post-translational modifications such as citrullination) in individual hair correlated with the mechanical strength of hair cuticles. S100A3 is expressed in the SW480 cell line, according to a public database. The 2D-PAGE pattern of S100A3 displayed an acidic form and an unmodified form. The level of post-translationally modified S100A3 in SW480 cells was significantly increased, following the supplementation of culture medium with arachidonate lipoxygenase (ALOX) inhibitors for 48 hours. The effects of ALOX12 inhibitors (Hinokitiol, Esculetin, and others) and an ALOX15 inhibitor (PD146176) on modification of S100A3 were compared in SW480 cells and cultured hair follicles. The effective concentration for S100A3 modification correlated with the previously reported IC_{50} value of the ALOX12 inhibitor *in vitro*.

Conclusions: Post-translational modification of S100A3 confers mechanical resistance to hair cuticles. Using an SW480 cell line and cultured hair follicles, several ALOX12 inhibitors that promote S100A3 modification were selected. Topical application of these ALOX12 inhibitors may improve the texture and resilience of hair.

HAIR FOLLICLE DEVELOPMENT, CONTROL OF THE HAIR CYCLE AND PIGMENTATION

P150

Mammalian target of rapamycin complex 1 (mTORC1) may modulate the timing of anagen entry in mouse hair follicles

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Mammalian target of rapamycin (mTOR) has a critical role in the regulation of cell growth and survival by integrating major signal inputs, such as nutrients, growth factors, energy, and stress. There is limited evidence that mTOR influences hair follicles (HFs), which undergo cycles of quiescence (telogen), growth (anagen), and regression (catagen). Activated mTOR was detected in telogen HFs and it is known that diverse molecules taking part in the mTOR signaling pathway, including Wnt, FGF-1 and Akt-related molecules, control HF proliferation. Thus, we sought to investigate whether mTOR, in particular mTOR complex 1 (mTORC1), regulates the hair growth cycle by using immunohistochemical, biochemical, and *in vivo* functional approaches. mTOR activity was quantitatively evaluated by measuring mTORC1-specific kinase activity throughout the hair cycle. The analysis of mTORC1 kinase activity showed phase-dependent changes: low in telogen, high in anagen, and late morphogenesis. The localization of activated mTOR in HFs was assessed by immunohistochemical staining using antibodies against phosphorylated mTOR at S2448 (p-mTOR) and phosphorylated p70S6K at T389 (p-p70S6K), a downstream target of mTOR. In telogen, only scattered p-mTOR/p-p70S6K-positive cells were detected in the bulge region; however, when anagen initiation approached, the number of stained cells was increased. These results were indicative for mTOR's role in hair growth initiation at the onset of anagen. *In vivo* pharmacological analysis using the specific mTORC1 inhibitor, rapamycin, showed a delay in the hair cycle initiation, reflecting mTOR's role in temporal regulation of anagen initiation. This study adds functional evidence that mTOR signaling is involved in the hair cycle regulation. We suggest that mTORC1 may modulate the timing of anagen initiation in HFs.

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Regulatory T cells increased in the area around and above the bulge of mouse vibrissa follicles in catagen

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Regulatory T cells (Tregs), a subpopulation of T cells expressing a transcription factor Foxp3, suppress immune responses of other immune cells and maintain tolerance to self-antigens. Inducible Tregs are generated in the periphery and exert their suppressor activity mainly by producing IL-10 and TGF-β. They also express LIF, which is known to have a pivotal role on maintaining stemness of ES cells. Therefore, we examined the changes of distribution and number of Tregs during hair cycle by immunohistochemistry for Foxp3 in the back skin of C57BL/6 mice. Tregs were mainly detected in shallow dermis less than 100 μm in depth from the skin surface. Their number was the maximum in early anagen, suddenly decreased until late anagen, and gradually increased again during catagen and telogen. Tregs also localized in upper hair follicles around and above the bulge. They markedly increased in number in catagen more than a 10-fold and diminished in telogen. To understand the function of Tregs on hair cycle progression, the back skin samples were collected at various time points of the hair cycle and mRNAs were isolated. Q-PCR revealed that the expressions of *tgf-β1* and *il-10* mRNAs were high in early anagen and low in late anagen. Their expression patterns coincided with the number of Tregs in the dermis. Interestingly, transcription of *lif* gene was markedly elevated in catagen. It was similar to the change of Treg number in the follicular epithelium around the bulge. Treg differentiation is thought to be dependent on TGF-β, which is known to have crucial roles both in inducing catagen and stimulating stem cell renewal. As Tregs secrete TGF-β and LIF, our data suggest that they could regulate the stem cell status in the vicinity of the bulge area by changing their number and localization during the hair cycle.

P152

A meeting of two chronobiological systems: *Period1* and *BMAL1* modulate the human hair cycle

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The hair follicle (HF) is a continuously remodeled mini organ that cyclically switches between growth (anagen), regression (catagen), and relative quiescence (telogen). As the anagen-catagen transformation of microdissected human scalp hair follicles (HFs) can be observed in organ culture, this permits one to study the as yet unknown oscillator system that drives this autonomous, rhythmic tissue remodeling activity at the intersection of developmental, chronobiological, and growth-regulatory mechanisms. Here we have tested the hypothesis that the clock system is involved in the anagen-to-catagen transition. We show that in the absence of central clock influences isolated, organ-cultured human HFs show circadian changes in the gene and protein expression of core clock genes (*CLOCK, BMAL1, Period1*) and clock-controlled genes (*c-Myc, NR1D1, CDKN1A*), and that *Period1* expression is also hair cycle-dependent. Knock-down of *BMAL1* or *Period1* in human anagen HFs significantly prolonged anagen and stimulated hair matrix keratinocyte proliferation. Moreover, individual silencing of these two core clock genes also stimulated HF melanogenesis in a hair cycle-independent manner. This provides the first evidence that peripheral core clock genes modulate human HF cycling and pigmentation under clinically relevant *in vitro* conditions, and are an integral component of the elusive ''hair cycle clock''. Specifically, our study identifies BMAL1 and Period1 as promising novel targets for the therapeutic modulation of human hair growth and pigmentation.

P153

Reprogramming regular skin fibroblasts into hair-inducing dermal papilla cells

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Cell-based regenerative therapies are still unavailable to restore hair follicles in hair loss patients and to generate new hair follicles in burn victims or patients with other debilitating skin disorders. Currently, there is a lack of know-how to expand fully functional DP cells in the culture dish for hair-inductive cell transplantations. To generate sufficient cell quantities for hair regenerative therapies, we sought to reprogram regular fibroblasts into DP cells. The overexpression of previously identified DP signature transcription factors (TFs) in freshly isolated fibroblasts, in combination with inhibitors of histone modifiers, significantly upregulated several DP signature genes. Furthermore, 3D aggregation clustering of TF-overexpressing fibroblast lines isolated from double-transgenic Sox2-GFP/Lef1-RFP reporter mice activated reporter activity and induced the DP molecular identity. Our preliminary data suggest that the right combination of DP TFs can reprogram DP niche fate in regular fibroblasts that can potentially be utilized in future hair restoration efforts.

P154

Development of the mouse dermal fat layer is linked to hair follicle development, occurs independently of subcutaneous fat, and is marked by restricted early expression of FABP4

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The laboratory mouse is a key animal model for studies of fat biology, metabolism, and disease; yet the developmental changes that occur in tissues and cells that become the fat layer in mouse skin have received little attention. Moreover, the terminology around this body of fat is often confusing, as no distinction is made between fat within the skin and subcutaneous fat. Here the development of fat in mouse dorsal skin was investigated from before birth to the end of the first hair follicle growth cycle. Using Oil Red O staining, immunohistochemical, qRT-PCR, and TUNEL staining, we confirmed previous observations of a close spatio-temporal link between hair follicle development, vasculogenesis, and the start of adipogenesis. However, unlike previous studies, we observed that the skin fat layer was created from cells within the lower dermis. By day 16 of the embryonic development (e16), the lower dermis was demarcated from the upper dermal layer, and its commitment to adipogenesis was signaled by the expression of FABP4, a marker of adipocyte differentiation. Fabp4 expression was overwhelming in the lower dermis of the fetal skin. In mature mice, the skin fat layer is separated from underlying subcutaneous fat by the panniculus carnosus. We observed that the skin fat did not combine or intermix with subcutaneous fat at any developmental time point. By transplanting skin isolated from e14.5 mice (before the start of adipogenesis), under the kidney capsule of adult mice, we showed that skin fat develops independently and without influence from subcutaneous depots. This study has reinforced the developmental link between hair follicles and fat. We argue that because skin fat develops from cells of the dermis and independently from subcutaneous fat, it is accurately termed dermal fat, and that in laboratory mice at least that dermal fat could represent a separate fat depot.

P155

ATM expression in hair bulb melanocytes is a marker of oxidative stress in canities-prone scalp

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The *ATM* (ataxia-telangiectasia-mutated) gene encodes a kinase that acts as a cellular sensor and provides a checkpoint for DNA damage repair. Evidence suggests a role for ATM in protecting cells from damage caused by oxidative stress, and may also protect melanocyte stem cells from premature differentiation in mouse hair follicles. We have earlier shown that ATM is expressed exclusively in melanocytes within the hair follicle and that loss of ATM expression is associated with canities (hair graying) within the human scalp tissue. Here we examined the expression of ATM in hair follicle melanocytes (HFM) cultivated from human anagen scalp follicles (20-year-old male and 56-year-old female). Basal levels of ATM were expressed in the nuclei of melanocytes grown under routine culture conditions. The incubation of cells with 40 μM hydrogen peroxide (H_2O_2) to induce oxidative stress produced a twofold increase in ATM expression (over basal levels) as detected by western blotting and densitometry in HFM isolated from the young donor, compared with a fourfold increase in cells from the older donor. Increased ATM expression was returned to basal levels by coincubation in an antioxidant mixture of Vitamin E (10^{-5} M) and Quercetin (10^{-6} M). Selective siRNA knockdown of ATM gene expression in HFM before H_2O_2 incubation sensitized cells to treatment and produced visible areas of cell detachment and death compared with controls. The latter was more prevalent in hair melanocytes established from the older versus the younger donor. In conclusion, we show that ATM (a canities-associated hair bulb biomarker) is also a marker of oxidative stress in hair follicle melanocytes. ATM appears to have an important role in protecting human hair follicle melanocytes from oxidative stress/damage, and maintenance of its expression may be important for hair bulb melanocyte survival in canities-prone scalp hair follicles.

P156

Stem cell factor/c-kit signaling in the hair bulb is essential to maintain hair pigmentation in adult human hair follicles *in vivo* and in organ culture

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Changes in hair pigmentation are associated with hormonal changes and increased age. As stem cell factor (SCF)/c-kit signaling is implicated in hair melanocyte development and androgen-stimulated changes in hair pigmentation, molecular biological and organ culture methods were used to investigate whether this is also involved in normal human hair graying. Individual anagen follicles were microdissected from human scalp samples from 10 people aged 45–68 years. Gene expression of SCF (soluble and membrane-bound) and c-kit was compared in pigmented and unpigmented lower hair follicles from the same individuals. To clarify which cell types expressed these genes, further follicles were microdissected to isolate bulb matrix components. To establish whether local SCF is necessary for adult hair pigmentation in a dynamic situation, the effect of anti-c-kit antibodies on human anagen follicles in organ culture was examined. Cryosections of cultured follicles and scalp skin were examined immunohistologically to localize melanocytes. Pigmented anagen human hair follicles expressed the genes for soluble and membrane-bound SCF and c-kit with soluble SCF having the highest levels. Unpigmented hair follicles expressed significantly less SCF and c-kit than pigmented follicles. Pigmented hair matrix cells always expressed *c-kit* unlike those from unpigmented follicles (only two out of six). There were also differences in follicular behavior in organ culture. Unpigmented hair follicles maintained anagen longer and grew faster than matched pigmented follicles ($n = 6$). Anti-c-kit antibody reduced hair pigmentation and increased growth; melanocytes became undetectable by immunohistochemistry. In scalp sections, unpigmented follicles showed significantly reduced expression of melanocyte antigens and c-kit in the hair bulb. These results indicate that loss of hair melanocytes and/or melanin facilitates increased cell division/differentiation. They also confirm that SCF/c-kit signaling within anagen hair bulbs is necessary to maintain bulb melanocytes and hair pigmentation in normal adult scalp follicles in organ culture and *in vivo*.

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Effect of the catagen transition induced by epidermal growth factor on a mouse model of chemotherapy-induced alopecia

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Chemotherapeutic agents primarily damage highly proliferative anagen hair follicles, and thus it would be worthwhile to investigate whether the transition of hair follicle to less proliferative stage (i.e. catagen) may have beneficial effects on the prevention of chemotherapy-induced alopecia (CIA). The aim of the present study was to investigate the effect of the catagen transition induced by epidermal growth factor (EGF) on C57BL/6 mouse model of CIA. We pretreated hair follicles with a vehicle or EGF encapsulated with nano-liposome system (50 mg/ml or 100 mg/ml), which has been reported to enhance the localization of EGF in skin, before treatment with a chemotherapeutic agent. To validate the catagen-inducing property of EGF and damage response after chemotherapy, we evaluated the hair cycle score in histological sections and the number of TdT-mediated dUTP nick-end labeling (TUNEL)-positive cells in the hair bulbs of control and EGF-treated mice. It was noted that topical EGF application induced early catagen-like stage, representing with a narrower dermal papilla compared with anagen VI hair and increased apoptotic cells as observed by TUNEL staining. In addition, after the injection of a chemotherapeutic agent (cyclophosphamide 120 mg/kg), hair follicles treated with EGF entered the less severe chemotherapeutic insult of the dystrophic anagen pathway, followed by primary recovery, whereas hair follicle treated with vehicle entered the more severe chemotherapeutic insult of the dystrophic catagen pathway, followed by secondary recovery. Thus, the results of this study suggest that catagen inducers could be useful as a new alopecia protection strategy, especially in the context of CIA.

P158

IL-1β-mediated protection from chemotherapy-induced alopecia in the wound-healing milieu

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Wound healing is an intricately orchestrated cascade of events relying heavily on cellular crosstalk. Among the chief effectors of these interactions are cytokines, chemokines, growth factors, and degradation proteases. In addition to these molecules, hair follicles aid in the process of epithelialization by way of providing stem cells. Although much is known about the role of the hair follicle in wound healing, the effect of the wound healing milieu itself on the hair follicle remains obscure. In this regard, we observed that excisional wounding results in phenotypic changes in the neighboring hair follicles that render them resistant to chemotherapy. This effect is observed when 3-day-old rat pups are wounded and chemotherapy is administered on day 11. The driving hypothesis behind this study was that selective players of the wound healing milieu confer protection against chemotherapy-induced alopecia. Therefore, we screened the levels of cytokine expression post wounding by DNA array analysis. As it has been well documented that IL1-β, TNF-α, IFN-γ, EGF, FGF, and TGF-β are released early in the wound healing process, we proceeded to systematically inject all the aforementioned cytokines intracutaneously into the left flank of 3-day-old rats to mimic the wound healing milieu and injected etoposide, cyclophosphamide, or doxorubicin/cyclophosphamide on day 11. Of all the cytokines tested, IL1-β was the only one that induced protection from alopecia against all the chemotherapies. Indeed, it was observed that there was a 27-fold increase in IL-1β over other cytokines at this stage. Taken together, these results strongly indicate that IL-1β is a key regulator of the hair follicle cycle alone or in the wound healing milieu itself.

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Topical botanical extract for the management of chemotherapy-induced alopecia (CIA)

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Chemotherapy-induced alopecia (CIA) is considered the most visible and emotionally distressing side effect of cancer therapy. However, there are no approved satisfactory pharmacological treatments available. The chemotoxic agents damage the rapidly dividing anagen cells that undergo premature apoptosis inducing the early onset of catagen. The intrinsic pathway of apoptosis is essentially mitochondria-dependent and executed by members of anti-apoptotic Bcl-2. Pharmacological inhibition of apoptosis pathways has been proposed to prevent CIA. Considering the sensitivity of cancer patients, AGA subjects could potentially be recruited for proof of concept study. We present our study of a GMP-grade botanical extract (hereafter ''the Product'') on AGA subjects, using Bcl-2 as a biomarker. Out of safety concerns, Bcl-2 levels in non-alopecia and AGA subjects were checked and compared.

Study design: Use a phototrichogram to calculate *A/T* ratio of 19 AGA male subjects before and after topical application of the product. Use immunohistochemistry to examine scalp biopsies from 25 male non-alopecia subjects and 15 AGA sufferers (before and after application of the Product).

Results: The product raised the *A/T* ratio from 2.96 (day 1) to 4.30 within 44 days, resulting in an increase of 8200 new anagen hair in 86 days. The product prevents premature apoptosis (by reestablishing intracellular Bcl-2 from its markedly low level) and dampens the microinflammatory status in the scalp (by acting through Langerhans cells), two key aspects causing AGA. After the product application, the raised Bcl-2 level (from 1.72 to 3.24; $P = 0.001$, paired *t*-test) remained below the normal Bcl-2 level of non-alopecia subjects (4.73). No adverse events were observed.

Conclusion: The anti-hair loss product is a safe agent that stops premature apoptosis (early onset of catagen) in AGA subjects. It may provide with a promising strategy to manage CIA in cancer patients receiving chemotherapy or radiotherapy, in a dosage and formulation to be investigated.

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Inhibition of proteases involved in the exogen process by Zinc gluconate

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Hair loss is a common problem and is suffered by much of the world population in both women and men. The hair follicle (HF) renews itself in a cyclic manner, undergoing periods of growth (anagen), regression (catagen), and rest (telogen) before restarting the cycle. More recently, a fourth phase termed 'exogen' in the hair cycle was proposed by Stenn *et al.* Our studies have identified proteases localized in the human hair follicle. Proteases have been implicated in the exogen process of hair shedding, and we have searched for a cosmetically acceptable active system that would be capable of inhibiting the proteases found in the hair follicle with the potential to reduce hair shedding. We have demonstrated earlier that a combination of Trichogen and climbazole is capable of inhibiting protease activity in hair fiber club root extracts. Trichogen is a complex mixture of ingredients, including natural plant extracts. We have carried out an investigation to identify the protease inhibitory component(s) of Trichogen and investigated the mode of enzyme inhibition. Acetyl tyrosine and zinc gluconate were found to be the major protease inhibitory components of Trichogen. The mode of inhibition was identified to be noncompetitive. The combination of zinc gluconate and climbazole was found to have a synergistic inhibitory effect on a serine protease, trypsin. We also conducted studies to measure the force required to remove hair using a pig skin model. Results indicate that zinc gluconate produced an increase in the force required to remove the fibers. These findings indicate that zinc gluconate +/- climbazole may be useful in delaying hair fall in consumers by inhibiting the enzymatic processes contributing to exogen.

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A method to accurately evaluate hair growth in organ-cultured hair follicles

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Background: Hair follicle (HF) organ culture techniques are widely used in hair biology research. In HF organ culture, hair growth is commonly used as a measurement of follicular activity. For the measurement of hair growth, the microscope fitted with an eyepiece graticule is routinely used. However, using this method, it is difficult to measure the crooked hair growth, which is always showing in the cultured HFs. Furthermore, even in the same anagen phase, individual HFs are not consistent and show different hair growth ability, dependent on the hair bulb size.

Objectives and methods: In this study, we propose an improved method that is designed to accurately measure the HF length in organ-cultured HFs. The improvement focused on the measurement of crooked hair growth and the detection of effective hair growth relative parameters to minimize the difference in hair length of individual HFs in same phase.

Results: We showed that there is a significant difference in HF length between linear length, which is measured by routine method, and crooked length, which is measured by improved method used in this study. Auber's line was the most effective hair growth-relative parameters to minimize the difference in hair length of individual HFs in the same phase. With this improvement, the discrete trend of individual HFs was significantly reduced.

Conclusion: Altogether, these results indicate that the current novel method should be a more accurate measurement of hair growth.

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A comparison of methods of anagen synchronization in the chemotherapy-induced alopecia adult rat model

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Chemotherapy-induced alopecia (CIA) is a psychologically devastating side effect experienced by 65% of cancer patients receiving anti-neoplastic therapy. To date, there is no effective treatment curbing this unwanted hair loss. However, several reliable animal models have been developed for the purpose of screening various prophylactic agents. The adult mouse and rat models are often utilized for their extrapolative properties to the human condition. To induce CIA in the adult murine models, hair follicles (HFs) must be synchronized into the anagen before the administration of chemotherapeutic agents. Clipping and waxing have previously been described as effective modes of HF synchronization. However, waxing was shown to result in the regeneration of thinner hair fibers. Furthermore, traumatic removal of the hair shaft by waxing induces wounding. Consequently, we sought to compare the histological profiles of adult rat skin treated with one of four methods of depilation: shaving, clipping, waxing, or plucking to determine whether they induce similar HF changes. Biopsies were taken at the time of chemotherapy administration, 15 days after depilation. There were significant differences in the histological profiles of shaving and clipping versus waxing and plucking. Both waxing and plucking resulted in severe HF distortion and noticeable trauma. Moreover, the superficial portions of the HF appeared to be cystic in nature. In contrast, skin biopsies from the shaving or clipping methods demonstrated atraumatic, unaffected-looking follicles. Taken together, forcibly removing the hair shafts by either waxing or plucking traumatizes the hair follicle and surrounding structures, consequently affecting hair regrowth and possibly subsequent experimental results.

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Biomarker analysis of hair follicles in canities-affected human scalp

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Canities (or graying) is an age-linked loss of pigment from hair. Although the specific cause(s) of the associated deletion of melanogenically active melanocytes from affected human scalp anagen hair bulbs is unclear, oxidative stress appears to be involved. We have examined, by immunohistochemistry, the follicular melanin unit in variously pigmented follicles from the aging human scalp of 22 healthy individuals (22–70 years). Over 20 markers were selected within the following categories: melanocyte-specific, apoptosis, cell cycle, DNA repair/damage, senescence, and oxidative stress. Immunohistochemistry revealed a reduction in melanocyte-specific markers in proportion to the extent of canities. A major finding of this study was the strong and exclusive expression of ATM (a protein that phosphorylates several key proteins to activate the DNA damage checkpoint, leading to cell cycle arrest, DNA repair, or apoptosis) within surviving melanocytes in canities-affected hair bulbs, the frequency of which correlated with pigmentation status. Increased levels of 8-OhDG (a major product of DNA oxidation with links to oxidative stress), GADD45 (a multi-protein interactive stress sensor modulates the cellular response to genotoxic/ physiological stress), and GP-1 (an enzyme that protects cells from oxidative damage) were detected within some bulbar melanocytes, although they lacked a clear association with the age of the donor or the extent of canities. Surprisingly, we found no specific evidence of increased expression of other studied markers of oxidative stress, senescence, or DNA damage/repair in the canities-affected melanocytes compared with surrounding bulbar keratinocytes, although some markers did show basal expression in other parts of the hair follicle. In conclusion, we identify here ATM, GADD45, and GP-1 immunoreactivity as important biomarkers of melanocyte status in canities-affected human scalp hair follicles. These markers may provide protection against canities through the DNA/Damage repair pathway.

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Evaluation of hair follicle regeneration ability using hair patch assay

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With the introduction of hair regeneration techniques, hair follicle regeneration became much easier. However, current success in *de novo* hair regeneration has been dependent on the use of cells from newborn or embryonic mice. The aim of this study was to find key genes that determine hair follicle inductivity in newborn. ''DPC (dermal papilla cell)-rich dermal cells'' from newborn (p0), postnatal day 7(p7) and postnatal day 14 (p14) C57BL6 mice were transplanted with epidermal cells from adult C57BL6 mice. Dermal cells from p0 mice successfully induced new hair follicles with adult epidermal cells. However, hair-inducing ability decreased rapidly thereafter; dermal cells from p7 mice induced less hair follicles and dermal cells from p14 mice failed to induce hair follicles. Gene expression of dermal cells from p0 and p7 mice was compared with cDNA microarray, and a marked decrease of histidine decarboxylase (HDC) expression was observed. RT-PCR analysis also showed that mRNA level of HDC is much lower in p7 mice compared with p0 mice. To assess the role of HDC in folliculogenesis, HDC expression in p0 dermal cells was suppressed with small interfering RNA. Hair patch assay showed that the HDC siRNA-treated p0 dermal cells induced less hair follicle and shorter and thinner hair shafts than mock-treated cells. We also found that the expression of adiponectin was suppressed. It is reported that adiponectin showed hair growth-promoting effects. However, in our study, hair patch assay demonstrated that the number of hair is increased after small interfering RNA (siRNA to adiponectin) treatment. These findings suggested that the same factors can have diverse effects according to the different developmental stages. Finally, hair patch assay can be a useful tool to investigate the role of each gene in neofolliculogenesis.

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The role of *Sox2* in mouse hair-type specification and pigmentation

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Signals from the dermal papilla (DP) are essential in regulating hair morphogenesis. *Sox2* is specifically expressed in the DP of primary and secondary hair, and a recent study has shown that the loss of *Sox2* in the DP affects hair growth and the migration of progenitor cells. However, the functions of *Sox2* in hair-type specification and pigmentation remain to be understood. To analyze the function of *Sox2* in hair-type specification, we utilized $Sox2^{Ysb/Ysb}$ and $Sox2^{Lcc/Lcc}$ allelic mutants, which the cis-acting regulatory elements of *Sox2* were disrupted because of chromosomal rearrangements. *Sox2* was downregulated in the DP during embryonic development. They displayed lighter hair coat color because of a change in hair-type specification, with increased number of tertiary hair and reduced secondary hair without a change in total density. The band of pheomelanin was extended in the mutants as well. These data suggest an additional role of *Sox2* in specifying hair types and pigmentation, apart from regulating hair growth during morphogenesis.

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Leontopodium alpinum (Edelweiss) extract activates wnt/β-catenin signaling, induces anagen, and increases hair follicle formation in skin reconstitution assays

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Wnt/β-catenin signaling has an important role in many developmental processes, including hair follicle morphogenesis and stem cell differentiation. Recently, wnt/β-catenin signaling has also been revealed as a crucial factor of hair follicle regeneration in adult skin. In search of novel hair growth-stimulating agents, hundreds of plant extracts were screened for wnt/β-catenin signaling activation using a TOP Flash reporter assay. The generated candidates were also examined on the expression and translocation of β-catenin and phosphorylation of GSK3β using immunocytochemical and immunoblot analyses. We finally observed the anagen induction at the dorsal skins of 7-week-old C57BL/6 mice with topical application, and hair follicle formation in skin reconstitution assays with silicone chamber. We found that the extract of *Leontopodium alpinum* (Edelweiss) with cold-water extraction method significantly stimulated the transcriptional activity of TOP Flash. Edelweiss also induced the expression and nuclear translocation of β-catenin in cultured human dermal papilla cells, and enhanced the phosphorylation of GSK3β. We further observed earlier conversion of telogen-to-anagen phase on the dorsal skins of C57BL/6 mice applied with Edelweiss extract compared with nontreated controls ($n=9$). Furthermore, in preliminary studies ($n=4$) using reconstitution assays, we demonstrated that the Edelweiss extract added to isolated neonatal mouse dermal and epidermal cell mixtures, which were then injected into the back skin of nude mouse in a silicone chamber, resulting in skin with significantly greater numbers of hair follicles compared with control nontreated mixtures. Our results suggest that the Edelweiss extract activates the wnt/β-catenin signaling pathway and induces hair growth and new hair follicle generation in reconstitution assays. These preliminary findings suggest that additional experiments are warranted to test the effects of the Edelweiss extract on hair growth.

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P63 and TGF-β1 and -β2 can be important markers in the differentiation degree and malignancy potential of sebaceous and follicular tumors

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Among the epidermal appendage tumors, certain tumors may show any stages of differentiation such as hyperplasia (hamartoma), adenoma, benign epithelioma, primordial epithelioma, and malignant tumor. The p63 gene is expressed in normal human epidermal appendages, and its correlation with tumor grading and/or aggressive behavior in epithelial tumors was well studied. During catagen development in ORS and epithelial strands of the hair, TGF-β1 is increased. However, during the carcinogenesis process, TGF-β1 and -β2 as tumor suppressors may also have pro-oncogenic activities in a complex manner. We planned to elucidate the expressions of TGF-β1 and -β2 and p63 in benign and malignant tumors toward hair and sebaceous gland directions and their importance in differentiation degrees of the corresponding tumors. Therefore, we conducted immunohistochemical studies with some antibodies such as Gli1 (a marker for sebaceous tumors), PHLDA-1 (a marker for hair follicle ORS cells), p63, TGF-β1, and TGF-β2 in trichofolliculoma ($n=1$), desmoplastic trichoepithelioma ($n=3$), trichilemmal carcinoma ($n=3$), morpheaform BCC ($n=3$), nevus sebaceous ($n=4$), sebaceous hyperplasia ($n=3$), sebaceous adenoma ($n=3$), sebaceous epithelioma ($n=3$), and sebaceous carcinoma ($n=4$). Gli1 proteins were expressed in the basaloid cells, sebocytes, sebaceous carcinoma cells, and were decreased with the higher differentiation. PHLDA-1 was expressed in the ORS cells and some cells of follicular tumors. The p63 was expressed in the nuclei of the outermost basaloid cells (seboblasts) and poorly differentiated sebaceous carcinoma cells, and was also seen in tumor cells toward hair direction. Remarkably, TGF-β1 was expressed exclusively in the nuclei of benign and malignant (hair) follicular tumors in correlation with the differentiation degree, but not so in sebaceous tumors. Moreover, TGF-β2 was seen focally in the structures of dermal papilla. In conclusion, we propose the usefulness of p63 and/or TGF-β1 in the differentiation degree and malignancy potential of sebaceous and follicular tumors and in discerning between trichilemmal carcinoma and sebaceous carcinoma, for example.

P168

Size of the human hair follicles

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Objectives: To know the size of different hair follicles in Pakistani population

Materials and methods: The study was conducted on 15 patients undergoing first session of the hair transplantation selected randomly. The donor area was marked in the sitting position. About 40 ml of tumescent solution (xylocaine, epinephrine, and normal saline) were used in the donor area. The strip was harvested in prone position. The donor site was closed using 3-0 nonabsorbable monofilament suture. The strip removed was kept in the normal saline at room temperature. The strip was thoroughly washed and any trace of blood was removed. The strip was divided into three equal parts. The slivering and cutting of the follicles was done using the microscope at 10X magnification. From the patient, 10 follicles each of one-hair, two-hair, three-hair, and four-hair were selected randomly. The size of each follicle was measure and noted.

Results: The size of one-hair FU ranged from 0.mm to 0.9 mm (average 0.44 mm). Majority of the follicles had 0.3 mm (40%) and 0.4 mm (24%). The two-hair FU had an average size of 0.72 mm (ranging from 0.4 mm to 1.3 mm), majority having 0.4 mm to 0.6 mm (52%). The average size of three-hair FU was 1.30 mm (ranging 0.9 mm to 1.7 mm) and four-hair FU was 1.50 mm (ranging from 0.9 mm to 2.4 mm).

Conclusion: The one-hair FUs are the smallest-sized FUs, being 0.44 mm in diameter. The instruments for making the recipient sites should be equal or larger than the average follicle size.

HAIR RESTORATION

P169

New antioxidants, the vitamin E derivatives, improve anti-cancer drug-induced alopecia and inhibit various kinds of cancer growth

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Objectives: The oxidative stress and active oxygen are strongly associated with the development of inflammation and malignant tumor. The vitamin E derivatives, which are known as antioxidants, have anti-inflammatory and/or anti-cancer actions. We coupled amino acids to vitamin E and developed various kinds of vitamin E derivatives that strengthened the stability and antioxidant effects. We investigated the effects of new antioxidants on anti-cancer drug-induced alopecia and cancer cell growth.

Methods: Wistar rats (8 days old) were cotreated with cytosine arabinoside (AraC; 20 mg/kg by daily intraperitoneal injection; day 0–day 6) and ESeroS-GS (0, 0.5, 01, 2, or 4% topically applied in a white petrolatum base; day 0–day 12). A control group received daily saline injections (day 0–day 6) and topical application of white petrolatum (day 0–day 12). At day 12, we evaluated hair loss and histologic changes to scalp tissue for each group ($n=10$). We measured caspase 3/7 activities and performed malondialdehyde assay in the skin tissue. The proliferation rates of various cancer cell lines were determined by trypan blue exclusion assay.

Results: Rats treated with AraC and 0% ESeroS-GS cream exhibited complete hair loss; however, cotreatment with 1%, 2% ESeroS-GS significantly reduced chemotherapy-induced hair loss. A histopathological study showed an accumulation of inflammatory cells around the root of hair in the control group, whereas these histological findings were reduced in ESeroS-GS groups. In the ESeroS-GS group, caspase 3/7 activities and MDA levels were significantly reduced than those in the control group. Vitamin E derivatives inhibited the growth of various kinds of cancer cell lines, but did not inhibit growth to skin fibroblasts.

Conclusions: Our findings demonstrate that ESeroS-GS reduces chemotherapy-induced alopecia, indicating the potential use of ESeroS-GS as a therapeutic tool against this common side effect of chemotherapy.

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The alpha-lipoic acid derivative, sodium zinc dihydrolipoylhistidinate (DHLHZn), reduces chemotherapy-induced alopecia in a rat model and inhibits various kinds of cancer growth

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Objectives: Alopecia is one of the most common side effects of chemotherapy, for which treatments have not been developed. In the present study, we evaluated the effects of sodium zinc dihydrolipoylhistidinate (DHLHZn), a new derivative of the multifunctional antioxidant alpha-lipoic acid, to treat chemotherapy-induced alopecia.

Methods: Wistar rats (8 days old) were cotreated with cytosine arabinoside (AraC; 20 mg/kg by daily intraperitoneal injection; day 0-day 6) and DHLHZn (0, 0.5, or 5% topically applied in a white petrolatum base; day 0–day 12). A control group received daily saline injections (day 0–day 6) and topical application of white petrolatum (day 0–day 12). At day 12, we evaluated hair loss and histologic changes to scalp tissue for each group ($n=10$). Caspase 3/7 activity and Malondialdehyde (MDA) level were measured using ELISA. Furthermore, effects of new antioxidants on cancer cell growth, and the expression of cell cycle-related proteins and their phosphorylation were investigated using bio-plex method.

Results: Rats treated with AraC and 0% DHLHZn cream exhibited complete hair loss; however, cotreatment with 0.5% or 5% DHLHZn significantly reduced chemotherapy-induced hair loss. Histologic analysis revealed that AraC treatment promoted inflammatory cell infiltration of hair follicles, but this inflammatory response was attenuated by DHLHZn. DHLHZn inhibited the growth of various kinds of cancer cell lines, but did not inhibit the growth to skin fibroblasts. DHLHZn arrested the cell cycle of colorectal cancer cells, and the apoptosis did not induce it.

Conclusions: New antioxidants, α-lipoic acid derivative, improved anticancer drug-induced alopecia with reduced inflammation, and inhibited the cancer cell growth with arrested cell cycle. This study indicates the potential use of this alpha-lipoic acid derivative as a therapeutic tool against this common side effect of chemotherapy.

P171

LED irradiation stimulates hair growth through activated ERK and Akt from human dermal papilla

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Aims: This study was aimed to investigate the effects of light-emitting diode (LED) irradiation on hair growth promotion *in vitro*.

Methods: We assessed cell proliferation in cultured human DPCs by MTT assay and determined the level of KGF by RT-PCR and measured the expression levels of extracellular signal-regulated kinase (ERK), Akt, GSK-3β, and β-catenin by Western blot analysis. (This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science, and Technology (2011-0008687)).

Results: LED irradiation did not influence the proliferation of cultured human DPCs in various wavelengths (415, 530, 630, 660, 850, 940), but increased the proliferation of cultured DPCs at 830 nm irradiation. Similarly, RT-PCR analysis revealed the increase in the amount of mRNA for KGF after LED irradiation at 830 and 940 nm in human DPCs. In addition, LED irradiation (830, 850, and 940 nm) enhanced the expression levels of phosphorylated ERK and phosphorylated Akt, and increased β-catenin expression.

Conclusion: LED irradiation enhances the survival of human DPCs by stimulating together ERK, Akt, and β-catenin at 830, 850, and 940 nm. In addition, LED irradiation at 830 nm induced the proliferation of human DPCs by the activation of β-catenin and release of KGF. Thus, we suggest that LED irradiation promotes human hair growth.

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Low-level light enhances hair follicle epithelial–mesenchymal interaction and anagen entry

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The low-level light irradiation has gained popularity in the treatment of various types of alopecia in recent years, but the underlying mechanism is unknown. In this research, we aim to investigate the mechanisms. Primary dermal papilla cells and *epithelial cells* were irradiated with low-level light of wavelength 630 nm. We found a higher proliferation rate of either dermal papilla cells or keratinocytes that was associated with a higher proportion of cells in S/G2M phase in the irradiated group. We also found that the stimulative effect was mediated through the ERK and Akt/PI3K pathways. Keratinocytes showed a higher cell proliferation rates when cocultured with low-level light-irradiated dermal papilla cells than irradiated keratinocytes that were not cocultured with dermal papilla cells. In the *in vivo* condition, we found low-level light-shortened telogen in the irradiated mice. Hence, low-level light not only promoted cell growth in each cell compartment but also enhanced the epithelial–mesenchymal interaction, thereby leading to earlier anagen entry and reduced alopecia.

P173

Hairline preference among various layers of Korean population

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When planning hair transplantation, degree of alopecia and esthetic preference should be considered for hairline design. There are various methods and criteria for hairline design. However, the techniques introduced in previous reports are subjective and ethnic, and racial differences result in different hairline preference, making it difficult to apply to various races. The aim of this study was to determine hairline preference among four different hairline shapes in Korean population. We investigated the preference of height of hairline because hair transplantation to a round-type hairline is commonly carried out in female patients in Korea, and determination of height of hairline is very important. In addition, we investigated whether hairline preference is affected by sex, age, education, social status, location, marital status, and history of hair transplantation. A total of 609 raters were asked to evaluate and rate the hairline profile through online questionnaire using eight photographs showing different hairlines. M-type hairline was selected as the most preferred hairline, whereas round-type hairline was selected the least. In contrast, round-type hairline was selected as the most preferred hairline in the group with a history of hair transplantation. Apart from the history of hair transplantation, several factors such as sex, age, education, social status, location, and marital status also affected the hairline preference.

P174

Symptoms of scalp burning and pain help identify patients with a potential contraindication to hair transplant surgery

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Introduction: Many scalp diseases present a contraindication to hair transplant surgery, including active scarring alopecias, alopecia areata, and hair-shedding disorders. Symptoms of scalp itching, burning, or tenderness should raise the hair transplant surgeon's suspicion for another diagnosis, in addition to androgenetic alopecia. In my practice, patients with significant symptoms of scalp burning or tenderness typically receive a scalp biopsy to exclude hair loss conditions that cannot be transplanted.

Objective: To review the sensitivity of the symptoms of scalp burning and pain in identifying patients with a possible contraindication to hair transplant surgery.

Materials and methods: A retrospective review of the records of 523 consecutive patients (348 men and 175 women) seeking advice on hair restoration was conducted. Information regarding current symptoms of itching, burning, and tenderness was evaluated and compared with the final diagnosis rendered.

Discussion: Occasional itching was documented in 109 of 523 (21 %) of patients and was more likely in those currently using minoxidil and those with pityriasis capitis and seborrheic dermatitis ($P<0.05$). A total of 47 patients (9 %) reported having burning and/or tenderness in the scalp, and 29 of these patients (62 %) were ultimately diagnosed with a condition that presented a contraindication to hair transplant surgery. These included diagnoses of either active cicatricial alopecia (lichen planopilaris, folliculitis decalvans, and central centrifugal cicatricial alopecia) or alopecia areata diffusa. In 12 of the 47 (26 %) patients with burning and/or tenderness, the diagnosis was not clinically obvious and the clinical tool served as a helpful method to exclude patients with contraindications to surgery.

Conclusion: Burning and tenderness are important scalp symptoms that should not be ignored by the hair transplant surgeon. A quick screening of scalp symptoms should always be sought.

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Hair transplantation in androgenetic and scarring alopecia: differences in techniques

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Modern hair transplantation involves microsurgical follicular unit transplantation (FUT), performed in multiple delicate steps using local tumescent anesthesia. The main indication is mid-stage androgenetic alopecia in men and women. Hair is harvested from the donor area in an elliptic strip using trichophytic closure to minimize the linear scar, followed by microscopic dissection of natural follicular units of one-hair to four-hair (FUT). This technique can be repeated, leading to high graft numbers. The other option is follicular unit extraction (FUE) using very small punches leaving only pinpoint scars. The overall graft yield is lower, and overharvesting may result in visible occipital thinning. To create recipient sites, small slits are made in high densities, also in thinned areas to increase hair density. The esthetic outcome is determined by a long-term plan, an authentic hairline design, as well as distribution, angle, and direction of the slits and careful graft handling. Medical treatments should be combined with hair transplantation, but not alter the surgical plan. In secondary scarring alopecias, such as after surgery or trauma, the technique is adjusted depending on scar quality. An initial scar reduction or conditioning is possible. Larger grafts with more perifollicular tissue may have higher survival rates. In hypertrophic scars, the use of small punches to create the recipient size may improve the blood supply and decrease pressure on the grafts. In atrophic scars, preoperative injections of saline may improve tissue quality. Usually, multiple sessions are required, gradually increasing graft density and skin quality. In primary scarring alopecias, hair transplantation should be limited to burnt-out stages to decrease the risk of graft failure and reactivation. Biopsies or test areas may be helpful, and the interfollicular tissue is usually normal. Current areas of research in hair restoration include graft-holding solutions, wound healing, tissue engineering, and robotic automation.

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Micrograft indications for eyebrows, eyelashes, beard, and mustache alopecia

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Introduction: Definitive female eyebrows thinning and eyelashes alopecia can be esthetically reconstructed with hair implants ''one by one''. Men with facial hair have various connotations such as wisdom and knowledge, sexual virility, masculinity, or high social status. The beard and mustache transplantation has roles mostly in the correction of cicatricial or traumatic alopecia, cleft lip scar, or small area of missing hair. A minority of cases is for the enhancement of virility in men. A schematic pattern for drawing the outline and understanding of the hair direction has been purposed.

Objective: To purpose the easy schematic pattern of drawing the beard, the mustache, the eyebrows, and the eyelashes; to describe the use of two procedures of follicular units: follicular unit extraction (FUE) and follicular unit long hair (FUL).

Methods: (1) Design of the area to be transplanted according to a schematic pattern and the patient's esthetic desire; (2) nerve blocks and local cream anesthesia; (3) 500 to 1500 one-hair follicular unit; (4) follicular unit long hair (FUL)—no previous shaving of donor area; (5) follicular unit extraction (FUE)—previous shaving of donor area; (6) hair harvested from the scalp will grow at the same rate as scalp hair.

Results: (1) Choose the hair color, thickness, and orientation; (2) implantation parallel to the skin; (3) no bandage; (4) crusts for 7 days; (5) sometimes swelling for 3–4 days.

Conclusion: The reconstruction of eyebrows and eyelashes allows an esthetic and definitive result. The beard and mustache pattern varies among the ethnic group. The Arabians and Caucasians have a more extensive area and higher density, whereas the Asians have the least presentation of facial hair. High patient satisfaction is achievable if patients are well selected and, particularly, among the psychological profile.

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Estimation of pre-operative scalp mobility for strip harvesting

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Objectives: A very simple method has been developed to assess the scalp mobility.

Materials and methods: The study was conducted on 50 patients who underwent surgery by strip method. Only the patients undergoing the first session were included. Patients undergoing the second or third session or those undergoing FUE (follicular unit extraction) were excluded. A scale was devised (grade 0–5).

TECHNIQUE: First of all, the upper line was marked in the donor area, and the movement of the skin was noted above and below the reference line, then the lower line was marked according to the surgical plan, and the movement above and below the line was marked. The degree of the overlap was noted.

Results: The mean age of the patients was 35.12 years and majority of them (52%) belonged to the younger age group (<35 years). Majority of the patients (36%) belonged to group III and 24% belonged to group IV of the vertical elasticity scale. Only 6% of the patients belonged to group I (Table 1). A 10-mm overlap was noted in 20% of the patients, followed by a 9-mm overlap in 10%.

Conclusion: It is an inexpensive method to measure pre-operative scalp mobility for strip harvesting.

PSYCHOLOGY AND HAIR DISORDERS

P178

Familial trichotillomania in three generations

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Trichotillomania (TTM) is a type of impulsive control disorder, characterized by recurrent pulling of hair, which leads to pleasure and relief of tension. It is estimated that 1–3% of the general population suffers from this condition. The disease leads to hair loss, which may sometimes be severe and lead to significant social and functional impairment. The etiology of this disorder is still unknown, although some have suggested a genetic component, based on several reports of familial hair pulling. Here we report a case of familial TTM in three generations. A 13-year-old male presented with a 1-year history of focal hair loss in the mid-frontal scalp. He was earlier treated with local steroids for presumed alopecia areata, with no improvement. Examination revealed a patchy area of hair loss, with several short broken hair of varying lengths. Dermoscopy and pathology examinations were consistent with TTM. Upon further questioning, the father admitted repeated pulling of his beard hair since puberty. The paternal grandfather also suffers from severe recurrent hair pulling of his beard since puberty, which sometimes precludes him from leaving home. Several reports, including a recent twin concordance study, have suggested a higher incidence of TTM and other obsessive-compulsive disorders in family members of TTM patients. However, to our knowledge, this is the first report of TTM in a three-generation family. This report strengthens the possibility that TTM is a genetic disease, probably with a complex inheritance pattern. It also underlines the importance of proper taking of family history when examining a TTM patient.

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Ex vivo hair follicle model to evaluate damage induced by photoepilation

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Photoepilation, based on absorption of light by melanin pigment, is a commonly used method to remove unwanted body hair using short pulses of laser or intense pulsed light (IPL). To improve the efficacy of photoepilation treatment, physiological understanding of the effects of photoepilation is crucial. We developed an *ex vivo* hair follicle model to evaluate the damage induced by various photoepilation treatment parameters. We evaluated the effects of multiple flashes with a consumer IPL device, as an alternative to pulse trains and stacking used in professional systems. Human skin samples obtained few hours post surgery were treated with a single flash or three consecutive flashes of a consumer device for photoepilation. Both control and treated follicles ($n=10$) were extracted and placed in culture for the evaluation of their growth rate during 10 days. At day 2, follicles were killed for histological cell viability analysis using H&E staining and TUNEL method. Control follicles retained normal morphology and a linear growth rate of $\sim$250 μm/day during culture. Treated follicles generally showed an anagen-to-catagen transition, observed as a retraction of the hair fiber from the dermal papilla (DP). No macroscopic differences were observed between single and triple flashes. Histology demonstrated three types of damage: (1) detachment of DP from a damaged hair fiber (occasionally with damaged DP); (2) damaged DP moving upward with damaged hair fiber; and (3) matrix disruption. Follicles treated with one flash generally demonstrated damage type 1, whereas three consecutive flashes resulted in type 2 damage, occasionally along with type 3 damage. Overall, we developed a physiological *ex vivo* hair follicle model to monitor follicular damage induced by different treatment parameters. The model is used for pre-clinical tests and validation of an opto-thermal hair follicle model. This fundamental knowledge will provide further understanding, and thereby possible improvement of photoepilation treatment efficacy.

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Female hair loss dysmorphic disorder vs female pattern hair loss: how do the quality of life deficits compare?

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Hair is a significant component of the importance of physical attractiveness and body image, and therefore losing hair often affects an individual's physical appearance, self-image, self-esteem, and overall quality of life. Previous studies on the psychological and quality of life effects of female pattern hair loss have shown that women with genetic hair loss are more ashamed, distressed, anxious, and concerned about their hair loss, have lower self-evaluation and self-esteem scores, and have more psychosocial problems than men with genetic alopecia or women with normal amounts of hair. A woman's genetic alopecia is likely to lead to greater social problems, as well as having a negative effect on her general daily life and causing her to feel more uncomfortable in the presence of others. However, not every woman who complains of hair loss actually has a hair loss problem. A small percentage of women with no obvious hair thinning are extremely concerned about losing their hair. Few studies have been conducted on these women who present with complaints of hair loss yet exhibit normal amounts of hair loss. The studies that have mentioned this condition have described it as symbolic for obtaining help for other underlying psychological or personal problems, marital or work problems, or from anxiety, depression, and obsessive-compulsive disorder. This phenomenon has been called Hair Loss Dysmorphic Disorder (HLDD). This presentation will compare the quality of life effects of hair loss on women with HLDD (that is, women without a hair loss problem) with the quality of life effects of hair loss on women with female pattern hair loss. It will also discuss specific case histories of women with HLDD, as well as potential subsets of HLDD.

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Alopecic models down the ages

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From time immemorial, artists have endeavored to depict beauty in their masterpieces. Since the Renaissance, the more astute observers noted that even pathological changes in the human body (including hair disorders) have been exhibited in priceless works of Art. This review analyses a few examples of male and female alopecia in paintings between the 16th and 19th centuries. Male alopecia is probably less often represented in Art compared to its frequency in real life. ''On the threshold of Eternity'' is a Van Gough masterpiece painted in 1890. It depicts a bald man about to commit suicide. The artist seems to have instinctively felt the emotional pain experienced by man with androgenetic alopecia. ''Aigueperse St. Sebastian'', drawn nearly five centuries ago by Andrea Mantegna (1431–1506), hangs in the Louvre. The central figure of the martyred St. Sebastian is flanked by two villainous archers who have assassinated him. One of the assassins is clearly shown to have round patches of alopecia in the beard area, possibly indicating alopecia areata. It may be an expression of the artist's moral condemnation of the killer. Illustrations of female alopecia are less frequent as women generally want to look beautiful in their paintings. Jan Vermeer (1632–75), a Dutch artist, is well known for his series of paintings of a young woman. A closer examination shows frontal recession in almost every picture. It is variably attributed to postpartum hair loss, traction, circumferential pressure by tight head dresses, or plucking of hairs by the woman in the paintings. La Mujer Barbuda, painted by Ribera in 1631, is a further example of female alopecia, possibly androgenetic. The subject also shows signs of virilization, suggesting that an androblastoma (androgen producing tumour) may have been responsible.

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Hair loss as an expression of stress—psychosomatic concepts applied to trichology

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Studies of Hans Seyle, in the 1950s, stated that many clinical problems have their origin in stressful events. On the basis of these concepts and knowing that, within the principles of embryology, nervous and skin systems are derived from the same embryonic tissue (ectoderm), we decided to investigate how the relationship between the two tissues proceeds from the psychic aspects and results in clinical signs of hair loss. The evaluation of patients who suffer from hair loss shows very strongly how this clinical sign may be the result of stressors. Furthermore, the stress resulting from the perception of hair loss feeds back the clinical sign making it even more severe as it modifies the chemistry involved in the hair cycle and causes hair loss. A frequent example of such cases is alopecia areata, an autoimmune disease that affects around 0.5 to 2% of the population in various age groups. This condition is usually triggered by stressful or traumatic events. Studies using laboratory animals conducted by the team of researcher Ralf Paus proved that events that generate stress induce anagen follicle to enter into catagen phase, causing an effluvium that tends to decrease when the stressors cease. Our group based this study on analytical psychology especially with the concepts of archetype, complex and symbol, as well as the structure and dynamics of personality relates to the phenomenon of stress-triggering diseases whose clinical sign is hair loss.

STEM CELLS AND EPIGENETICS

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microRNAs are important players controlling hair follicle development and regeneration

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Hair follicle development is governed by complex programs of gene activation and silencing, including microRNA-dependent modulation of gene expression. We show that miR-214 is expressed in the hair follicle (HF) during skin morphogenesis and its cycling. To explore its role in the control of skin development, we generated doxycycline-inducible miR-214 (K14-rtTA/TRE-miR-214) transgenic mice (TG). Activation of transgene expression during skin embryogenesis resulted in the development of thinner epidermis, reduced keratinocyte proliferation, and appearance of a ''rough'' coat postnatally as a result of the development of about 40% fewer HFs in back skin. The hair bulbs in TG mice were markedly reduced in size, which was associated with decreased cell proliferation in the hair matrix and significantly thinner hair shaft production. However, the ratio between different HF types (guard, awl, auchene, or zig-zag) in TG and WT mice was not changed. The inhibitory effects of miR-214 on skin and HF development were associated with decreased expression of the key components of the Wnt signaling pathway (beta-catenin, Lef-1), as well as with activation of BMP signaling in the epidermis and HFs as was documented by increased pSmad1/5 expression. A luciferase reporter assay confirmed bioinformatic prediction that miR-214 directly targets beta-catenin in keratinocytes. In primary keratinocytes, miR-214 mimic prevented nuclear translocation of beta-catenin in response to Wnt activator, lithium chloride, abrogated lithium chloride-induced expression of Axin2, and significantly diminished TOPflash activity induced by the Wnt activator BIO. Taken together, these data reveal an essential role of miR-214 in the control of skin and HF development and suggest miR-214 as a key regulator of the activity of the Wnt/beta-catenin signaling pathway in the keratinocytes.

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Roles of MED1 in quiescence of hair follicle stem cells and maintenance of normal hair cycling

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MED1 (Mediator complex subunit 1) is expressed by human epidermal keratinocytes and functions as a coactivator of several transcription factors, including nuclear receptors. To elucidate the role of MED1 in keratinocytes, we established keratinocyte-specific *Med1*-null (*Med1*$^{epi-/-}$) mice using the *K5Cre-LoxP* system. Development of the epidermis and appendages of *Med1*$^{epi-/-}$ mice was macroscopically and microscopically normal until the second catagen stage of the hair cycle. However, the hair cycle of *Med1*$^{epi-/-}$ mice was spontaneously repeated after the second telogen stage and the characteristic hair cycle-domain pattern was observed on *Med1*$^{epi-/-}$ back skin. These features do not occur in wild-type mice. Although the hair cycle of *Med1*$^{epi-/-}$ mice was frequently repeated until 6 month of age, hair follicles of *Med1*$^{epi-/-}$ mice could not enter the anagen thereafter, resulting in sparse pelage hair in older *Med1*$^{epi-/-}$ mice. Interfollicular epidermis of *Med1*$^{epi-/-}$ mice was acanthotic and more proliferative than that of wild-type mice, whereas these findings were less evident in older *Med1*$^{epi-/-}$ mice. Flow cytometric analysis revealed that the numbers of hair follicle bulge stem cells were reduced in *Med1*$^{epi-/-}$ mice from a few months after birth. The SOX9 expression in *Med1*$^{epi-/-}$-derived cultured keratinocytes was downregulated, and the SOX9 expression was reduced in some 6-month-old *Med1*$^{epi-/-}$ hair follicles by immunohistochemical studies. These results suggested that MED1 has roles in maintaining quiescence of keratinocytes and preventing depletion of the follicular stem cells.

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The role of nestin-expressing stem cells in formation of the hair follicle sensory nerve

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Vibrissa hair follicles including their sensory nerve stump were excised from transgenic mice in which the nestin promoter drives green fluorescent protein (ND-GFP) and were placed in 3D culture supported by Gelfoam. Confocal microscopy showed trafficking of the nestin-expressing cells from the bulge to the whisker sensory nerve stump over a 21-day period of Gelfoam culture. ND-GFP expressing cord-like structures extended from the nerve stump by day 10. The cord-like structures consisted of ND-GFP-expressing spindle-shaped cells, which coexpressed the neuron markers β-III tubulin, the immature Schwann-cell marker p75^{NTR}, and TrkB which is associated with neurons. β-III tubulin-positive fibers, consisting of ND-GFP-expressing cells were observed to extend 500 μm from the whisker nerve stump. The fibers had growth cones on their tips expressing F-actin, demonstrating that they were growing axons. The extending whisker sensory nerve was highly enriched in ND-GFP cells, which appeared to have a major role in its elongation, as well as joining with other nerves in the 3D culture, including the sciatic nerve, the trigeminal nerve, and the trigeminal nerve ganglion. A major role of the nestin-expressing cells in the hair follicle is to extend the follicle sensory nerve and join with other nerves.

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Topology-dependent plucking leads to more regenerating hairs than plucked via extrafollicular macroenvironment modulation

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Recent work showed that hair stem cell activity is controlled by both intrafollicular and extrafollicular dermal factors. On the basis of this, regeneration following intrafollicular hair plucking can elicit signaling changes in the adjacent dermal environment and activate neighboring unplucked follicles in the region. To test this, 200 hairs were plucked with different spacing. Interestingly, high-density hair plucking can initiate the regeneration of up to 1000 hairs by activating adjacent unplucked telogen follicles. A gene profiling-based molecular comparison of plucked / unplucked / induced follicles and their surroundings combined with bead-mediated molecular misexpression and genetic mutant analyses demonstrated that this novel extrafollicular hair induction process involves apoptosis and Tnf-a signaling. A new tissue interaction mechanism was discovered in which an injured organ can not only activate its own stem cells but also spread activation signals to other organs through extrafollicular signaling cascade. This work provides a new possibility for enhancing hair regeneration and lays down the basis for future translational application.

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Competitive balance of intrabulge BMP/WNT signaling reveals a robust gene network governing stem cell homeostasis and cyclic activation

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Hair follicles facilitate the study of stem cell behavior because stem cells in progressive activation stages are ordered within the follicle architecture, and they are capable of cyclic regeneration. To study the gene network governing the homeostasis of hair bulge stem cells, we developed a Keratin 15-driven genetic model to directly perturb molecular signaling in the stem cells. We visualize the behavior of these modified stem cells, evaluating their hair-regenerating ability and profiling their molecular expression. Bone morphogenetic protein (BMP)–inactivated stem cells exhibit molecular profiles resembling those of hair germs, yet still possess multipotentiality *in vivo*. These cells also exhibit upregulation of Wnt7a, Wnt7b, and Wnt16 ligands and Frizzled (Fzd) 10 receptor. We demonstrate direct transcriptional modulation of the *Wnt7a* promoter. These results highlight a previously unknown intra-stem cell antagonistic competition, between the BMP and Wnt signaling pathways, to balance stem cell activity. Reduced BMP signaling and increased Wnt signaling tilt each stem cell toward a hair germ fate and, vice versa, on the basis of a continuous scale dependent on the ratio of BMP/Wnt activity. This work reveals one more hierarchical layer regulating stem cell homeostasis beneath the stem cell dermal papilla–based epithelial–mesenchymal interaction layer and the hair follicle intradermal adipocyte–based tissue interaction layer. Although hierarchical layers are all based on BMP/Wnt signaling, the multilayered control ensures that all information is taken into consideration and allows hair stem cells to sum up the total activators/inhibitors involved in making the decision of activation.

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Global genome analysis of Lhx2 target genes in hair follicle stem cells and their progenies reveals the nonrandom distribution and topological associations with functionally related gene loci

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Lhx2 transcription factor operates as a central link in the genetic networks that coordinate multiple signaling pathways controlling organ development and stem cell maintenance and differentiation. Lhx2 also controls hair follicle development, cycling, and differentially regulates Sox9, Tcf4, and Lgr5 in hair follicle stem cells during skin regeneration after injury. Integration of global microarray data obtained from Lhx2-null mice and genome-wide ChIP data revealed that majority of the Lhx2 target genes were upregulated in the Lhx2 null mice versus WT controls, thus suggesting that Lhx2 operates predominantly as transcriptional repressor. Allocation of Lhx2 target genes to distinct chromosomes showed their nonrandom distribution, and topological analysis revealed that 123 Lhx2 targets belong to keratinocyte-specific gene loci, such as Epidermal Differentiation Complex or keratin-associated protein locus, as well as to smaller genomic loci containing functionally related genes (e.g., encoding Wnt ligands, serpin protease inhibitors, distinct adhesion molecules, and so on.). Expression changes of the functionally related non-Lhx2 targets in such loci in Lhx2-null versus WT mice were similar compared with the expression dynamics of the direct Lhx2 target genes. Analyses of the regulatory regions of these non-Lhx2 target genes showed significant ($P<0.01$) enrichment of the binding sites for Sox9 and Tcf4 transcription factors, serving as direct Lhx2 targets in hair follicle stem cells. These data suggest existence of positive regulatory loops between Lhx2 and Sox9/Tcf4 that might provide coordinated control of expression of functionally related genes in distinct genomic loci. Thus, Lhx2 regulates gene expression in hair follicle stem cells through targeting not only single genes but also functionally related gene groups in distinct loci, it and might also form regulatory networks with other transcription factors.

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Direct differentiation of hair follicle stem cells into dopaminergic neurons without reprogramming

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Cell-based therapy is considered a promising mean for restoration in neurodegenerative disorders. Transplantation of dopaminergic neurons generated from embryonic neural precursor cells (NPCs) or induced pluripotent stem cells shows functional recovery in animal models of patients with Parkinson's disease. One of the major challenges remains to define a cellular source for neurons, which is ideally safe, effective, and easily accessible. Hair follicle stem cells (HFSCs) are pluripotent and may spontaneously differentiate into various cells including neurons. HFSCs are of ectodermal origin. In addition, a population of HFSC readily expresses the NPC marker Nestin. Therefore, we hypothesized that HFSCs may differentiate into dopaminergic neurons without viral vector–induced reprogramming, ectoderm induction, and neural induction, which are usually necessary for non-neural cells to differentiate into neurons *in vitro*. Whisker hair follicles from male C57BL/6 mice were dissected and single-cell suspension from the bulge region was obtained by enzymatic digestion. After 2–3 weeks of culturing under proneurogenic conditions, we obtained slow-growing, sphere-forming, NPC-like cells. RT-PCR analyses revealed that the HFSCs express many marker genes for NPCs and embryonic stem cells. Important genes for the differentiation of NPCs into dopaminergic neurons (e.g. Nurr1) were already expressed in HFSCs. Immunostaining confirmed that HFSCs expressed Nestin, Sox2, and GFAP. Subsequently, directed differentiation was attempted to generate dopaminergic neurons from the HFSCs. After the differentiation, HFSCs expressed markers of mature neurons (e.g. MAP2) and enzymes for dopamine synthesis/degradation (e.g. TH) both in mRNA and protein level. Finally, dopamine production and release was confirmed in the medium by means of HPLC. Taken together, our study demonstrated that HFSCs have the potential to differentiate into dopaminergic neurons without reprograming. HFSCs may be able to serve as a safe, effective, and easily accessible source for dopaminergic neurons for research and/or therapeutic purposes.

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Human hair follicle dermal cells provide enhanced support for both epithelial and embryonic stem cell culture proliferation and maintenance

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Both human hair follicle dermal papilla (DP) and dermal sheath (DS) cells support epithelial cells responsible for hair fiber production and maintenance, indicating that they could be effective supports for epithelial cell populations *in vitro*. Furthermore, we hypothesized that both human DP and DS cells could maintain pluripotent stem cells in an undifferentiated state. Cultures of adult human DP, DS, and outer root sheath (ORS) were isolated through microdissection from donor hair follicles, with dermal fibroblasts (DF)-isolated and keratinocytes-isolated cultures from donor adult glabrous tissue. For epithelial support, DP, DS, and DF, cultured in dermal favoring MEM, were compared with the gold standard of 3T3 mouse fibroblasts and epithelia favoring the Rheinwald and Green medium. DP, DS, and DF were compared with mouse embryonic fibroblasts (MEFs) for their ability to maintain the pluripotency of the human H9 ES line, and human induced pluripotent stem cells (iPSCs). Keratinocytes formed significantly more colonies when cocultured with DP (30.9 ± 4.2) and DS cells (35.7 ± 5.1) when compared with keratinocytes cocultured with DF cells (15.1 ± 6.2, all $n=3$). Levels of rhodamine-B eluted from keratinocytes cocultured with DS and DP cells in MEM were comparable and similar to those obtained from keratinocytes cultured using 3T3 cells and R&G medium. Both groups displayed significantly ($n=4$, $P \leqslant 0.001$) increased proliferation compared with DF and 3T3 cocultures in MEM. ES cells cocultured with MEF, DP, or DS cells showed comparable levels of expression of SSEA-4, TRA-1-60, Oct4, and Nanog. Similarly, human iPSCs cocultured with MEF, DP, or DS cells maintained normal ES morphology, similar levels of alkaline phosphatase activity, and TRA-1-60 and TRA-1-81 expression levels. ES and iPS colonies cocultured with DF lost expression of the aforementioned markers. These data suggest that follicular dermal cells could provide a xeno-free alternative to current support cells for stem and epithelial cell culture.

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Upper part of hair follicle can produce large numbers of cultured nestin-expressing stem cells for nerve repair demonstrating clinical potential

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We have previously demonstrated that the stem-cell marker nestin is expressed in hair follicle stem cells. Nestin-expressing cells were initially identified in the hair follicle bulge area (BA) using a transgenic mouse model in which the nestin promoter drives the green fluorescent protein (ND-GFP). The hair follicle ND-GFP–expressing cells are keratin 15 negative and CD34 positive and could differentiate into neurons, glia, keratinocytes, smooth muscle cells, and melanocytes *in vitro*. Subsequently, we showed that the nestin-expressing stem cells could affect nerve and spinal cord regeneration after injection into mouse models. We previously separated the mouse vibrissa hair follicle into three parts (upper, middle, and lower) and cultured each part and showed that the upper part produced large numbers of multipotent stem cells. In the present study, the upper part of the follicle produced sufficient cultured nestin-expressing stem cells for transplantation between severed sciatic nerve fragments of the mouse. The transplanted cell differentiated into GFAP-positive Schwann cells and promoted the recovery of pre-existing axons. The method described here is appropriate for future use with human hair follicles producing nestin-expressing hair follicle stem cells in sufficient quantities for nerve and spinal cord regeneration in the clinic.

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Acceleration of skin wound healing in keratinocyte-specific mediator complex subunit 1 null mice

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MED1 (Mediator complex subunit 1) is a coactivator of several nuclear receptors and functions in multiple transcription signaling pathways. We have already established keratinocyte-specific MED1 null mice (Med1epi−/−) using K5-Cre-LoxP system and found epidermal hyperplasia in Med1epi−/− mice. In this study, to investigate the function of MED1 in skin wound healing, full-thickness wounds were generated on the backs of Med1epi−/− mice and the healing process was analyzed. Macroscopic wound closure and microscopic rate of re-epithelialization were accelerated in 8-week-old Med1epi−/− mice compared with age-matched wild-type mice. The number of Ki67-positive cells at leading edges of the wounds was higher in 8-week-old Med1epi−/− mice compared with age-matched wild-type mice, whereas the migration of keratinocytes, dermal contraction, and the area of α-SMA-positive myofibroblasts in the granulation tissue were unaffected. Immunoblotting revealed that the expression of follistatin was decreased and JNK was phosphorylated in Med1epi−/− keratinocytes *in vitro*. Furthermore, we demonstrated that skin wound healing in 6-month-old Med1epi−/− mice was significantly delayed compared with age-matched wild-type mice, corresponding to our previous observation that the numbers of hair follicle bulge stem cells were reduced in older Med1epi−/− mice. These results provide a novel insight into MED1 function, as well as a possible new therapeutic approach for skin wound healing and aging.

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Targeting of antigen presenting cells by HIV-1 virus-like particles for transcutaneous vaccination

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The idea of easy accessibility of cutaneous antigen presenting cells (APC) fostered the development of various transcutaneous vaccination strategies. In intact skin, the hair follicles are key structures, because deposition of vaccine in hair follicle openings and delivery to the skin APC across such barrier discontinuities have been shown to be highly promising approaches. In human explants, we investigated the effect of three different administration routes for transcutaneous vaccine delivery on skin penetration and cellular uptake of fluorescently labeled virus-like particles (VLPs) carrying HIV-1 antigen. The effect of Cyanoacrylate Skin Surface Stripping (CSSS), which induces mild barrier disruption and opens hair follicles, was compared with the effects of pricking of epidermis and dermal injection. Skin penetration was assessed microscopically on cryosections. CD1c-positive APCs were isolated from human skin by magnetic cell separation, as well as HLA-DR-positive migratory cells, and were analyzed by flow cytometry and fluorescence microscopy. We observed VLP uptake after CSSS and pricking by Langerhans cells (relative mean fluorescence intensity (rMFI): 1.36; 1.05) and also by dermal CD1a-positive dendritic cells (DCs) (rMFI: 1.12; 1.11). CD1a^{high} DCs showed particle uptake after CSSS, pricking, and ID (rMFI: 1.16; 1.11; 1.17). Uptake was also detected in migrated HLA-DR-positive DCs after CSSS, as well as after pricking (rMFI: 1.11; 2.56). Absolute numbers of particle-positive cells varied among the probes. However, in each individual sample, reduction of particle-positive epidermal cells was accompanied by an increase in particle-positive dermal and migratory cells, suggesting that particle uptake by APC occurred in all samples and induced APC migration. In conclusion, different administration modes resulted in particle uptake by different APC populations and different migration patterns. These results open perspectives for specific targeting of skin APC subsets. In fact, recent studies suggest that targeting of different skin APC subsets may affect the quality of immune response.

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Optimization of intercellular communication inside the human hair follicle

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The hair follicle (HF) is a highly organized and regulated organ that undergoes a cyclic activity. Successive phases of regeneration and regression give rhythm to the hair follicle life. Because of its unique organization and functioning, the HF is the seat of numerous interactions that take place inside the HF itself and between the HF and its cutaneous environment. In the present study, we were interested in studying the interactions implicating laminin-511 (LN-511), its integrin receptor, and β-catenin. Indeed, the LN-511 pathway was previously associated with hair follicle maintenance in the active phase of the hair cycle (anagen). For this purpose, we developed a specific compound that targets LN-511. The first part of the study involved characterizing the expression levels of LN-511 and α3- and β1-integrins in cultured normal human keratinocytes (NHKs) and dermal papilla cells (DPCs). Thereafter, in order to study communication between these two cell types, we applied conditioned medium of NHKs treated with the selected compound on DPCs. We observed an increase in β-catenin staining in DPCs treated with NHK-conditioned medium, suggesting an effect on intercellular communication. The outcomes of the treatment were then studied at the level of the hair follicle by using two models: *ex vivo* scalp biopsies and isolated hair follicles. A 24 h application of the compound led to an increase in the staining intensities of LN-511 and α3- and β1-integrins in the hair follicles of both models. Moreover, in the hair follicles maintained in culture for a longer time, the compound helped in preserving LN-511 expression and HF structure. Maintaining intercellular communication inside the hair follicle, by targeting LN-511, could be considered as a potential way to preserve optimal hair-growth environment.

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Structural analysis of a hair cuticle using TOF-SIMS and AFM

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A hair cuticle, which consists of flat overlapping cells that surround the hair fiber, protects inner tissues against external stimuli. In this study, the composition and the physical properties of the hair cuticle were directly analyzed by two different methods. First, depth profiling of the amino acid composition was conducted by using time-of-flight secondary ion mass spectrometry (TOF-SIMS) combined with the C_{60} sputtering technique. Second, the elastic modulus was measured by atomic force microscopy (AFM). By applying these two methods, the correlations between the composition of each tissue layer in a single cuticle cell and the physical properties were investigated. For healthy hair fibers, it was revealed that the elastic modulus of each tissue increases as the cysteine/cystine content increases. In addition, the existence of a thin layer that has a characteristic amino acid composition on the outermost hair surface is considered to be the epicuticle. It was concluded that significant information about the nature of the hair cuticle can be obtained by the methods presented in this paper.

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Imaging the penetration and distribution of small molecules in hair fibers

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Biophysical applications of Fourier transform infrared (FTIR) imaging microspectroscopy and confocal Raman spectroscopy techniques have been developed, which permit the collection of hundreds of spatially resolved spectra from biological samples and substrates. These arrays of spectra can then be interrogated to produce two-dimensional and three-dimensional images describing any feature of the biological sample's composition, conformation, and organization inherent within the infrared and Raman spectra. A significant advantage in interpreting the results of these ''molecular histology'' images is that they are generated without the addition of exogenous fluorescent probes or chemical stains being added to the sample. Thus, they directly describe the distribution and organization of the hair's endogenous molecules. We have recently shown that these biophysical FTIR imaging and confocal Raman techniques can be applied to cross sections of human hair and intact hair fibers, respectively, to image the changes associated with environmental and chemical stress. In the current work, we have sectioned human hair and collected large arrays of spatially resolved FTIR spectra across many hair sections exposed for increasing time periods to solutions containing small molecules such as resorcinol believed to penetrate the fiber. Hair fibers from the same experiments were analyzed with confocal Raman spectroscopy. Spectra were collected from the hair surface to a depth of approximately 20 microns into the fiber. Both experimental methods successfully demonstrated that they can directly image the penetration of small molecules into hair and visual both the concentration and distribution of the penetrating molecule as a function of exposure time. This presentation will present the specific results from FTIR and Raman imaging of resorcinol penetration into hair while also discussing this as a general approach to measure small molecule penetration into hair fibers including imaging subsequent changes in hair protein chemistry.

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How does lifestyle stress affect the hair follicle? Development of *ex vivo* and *in vitro* approaches

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The hair follicle (HF) is constantly exposed to multiple stressors, resulting in modifications of hair final appearance. The cumulative effects of these daily aggressions were reported to contribute to hair graying and reduction of hair diameter, leading to thinner hair. In the present study, different approaches were developed to evaluate the effect of lifestyle stress on HF, from the most complete full-thickness scalp biopsies to isolated HF and cultured HF cells. The first model developed consisted in full-thickness biopsies of scalp skin, maintained in an air-liquid interface culture system. This model preserved the interactions between the HF and its cutaneous environment and allowed topical application of potential stressors or formulated products. By performing serial sections at the level of the HF, followed by hematoxylin-eosin staining, we observed the damaging effects in the hair bulb, resulting from the topical application of a detergent solution. Moreover, this *ex vivo* model was also appropriate for the evaluation of damage induced by UVA/UVB irradiation and oxidative stress. The second model used was the HF isolated from scalp skin by microdissection. When exposed to H_2O_2 stress, a reduction of melanin content was observed in the hair bulb, as revealed by performing the Fontana-Masson staining. Finally, using fibroblasts from dermal papilla cells, we developed a model of *in vitro*-aged human dermal papilla cells (HDPC) by applying methylglyoxal (MGO). The aged phenotype of MGO-stressed HDPC was confirmed by altered cell morphology, increased beta-galactosidase staining, reduced alkaline phosphatase activity, and disorganized fibronectin network. Furthermore, electron microscopy revealed vacuolization and decrease in intracellular organelles in stress-induced senescent cells. Combinations of these different methods could help understand HF response to different stressors affecting its functioning, and propose solutions for HF protection.

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Mild hydration effect on bound-water dynamics in human hair monitored by ^{1}H-NMR

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Gaseous-phase hydration significantly modifies the biological and physical properties of many biological systems and biopolymers, including the hair fibre. By using hydration kinetics, sorption isotherm, ^{1}H-NMR spectroscopy, and relaxometry, we analyzed a number of water-binding sites on human hair, the sequence and kinetics of their saturation, and formation of tightly and loosely bound water fractions. Terminal Dark Hair came from 21-year-old Caucasian female. Hair color was defined as W (brown black) according to the Fisher-Saller scale, terminal Red Hair came from 25-year-old Caucasian female (IV according to the Fisher-Saller scale), and terminal Gray Hair came from 57-year-old Caucasian male. Proton free induction decays (FIDs) were recorded using a high-power relaxometer. The resonance frequency was 30 MHz. All measurements were performed at room temperature ($t = 22^0$C). The hydration kinetics shows (i) a very tightly bound water fraction, (ii) a tightly bound water, and (iii) a loosely bound water pool. The sorption isotherm is sigmoidal in form reasonably well-fitted using Dent model. The relative mass of water saturating primary binding sites is average equal to $\Delta M/m_0 = 0.07$. ^{1}H-NMR signals show a Gaussian component ($T_2{}^* \approx 18\,\mu s$), coming from solid matrix of hair, and two exponential components ($T_{2L1}{}^* \approx 60$–$250\,\mu s$ and $T_{2L2}{}^* \approx 300$–$580\,\mu s$, respectively) from water tightly and loosely bound. The liquid to solid signal ratio L/S increases linearly with the increased hydration level.

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Correlating confocal Raman and FTIR spectroscopic imaging of hair with changes in fiber mechanical properties as a function of chemical stress

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Confocal Raman and Fourier transform infrared (FTIR) imaging spectroscopy methods are used to characterize biological substrates including bone, teeth, and skin by producing spatially resolved images describing any feature of the sample composition and structure inherently characterized in the spectra, thereby generating images without the use of probes or stains. Recently, we have extended IR imaging microspectroscopy and confocal Raman microscopy to the study of human hair fibers, generating images that characterize the spatial distribution of protein secondary structures (alpha-helix, beta sheet), lipid chain conformational order, and the distribution of disulfide cross-links in hair fibers. In the current work, changes in the organization and chemistry of hair fiber components as a function of chemical and physical stress, including pH and UV radiation, have been imaged with confocal Raman and FTIR spectroscopy. These new data will be presented to illustrate the general utility of IR and Raman for characterizing the molecular impact of external stresses upon hair structure. Results from corresponding mechanical studies of the bulk and surface mechanical properties of fibers, and fiber assemblies, will be presented to provide a direct link between the changes in the molecular biophysics and chemistry of the hair fiber to changes in the fiber material properties.

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Straight hair as a phenomenon of reality distortion in South America

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Brazilian and Argentine clinical centers studied over 200 patients with digital tricoschopy, micro and macro photography, hygroscopicity, sebumetry, and electron microscopy. The results obtained quantify the hair fiber damage in patients relating to some degree of chronic alopecia. After a detailed anamnesis and physical/trichological exams, the diagnosis is diffuse alopecia caused by repetition of chemical and physical aggressors, which is difficult to treat because of the severity of the damage and psychological impact. We found that this type of alopecia caused by hair-straightening products is an increasing South American issue that finally results in hair damage:

(1) Women face tremendous sociocultural pressure and succumb to commercials and fashion trends, causing them to undergo hair-straightening procedures.

(2) After the fourth straightening session, the increase in fiber weakness is a common manifestation, trichorexis nodosa can be a clinical feature, and the intended results of the procedures are not as good as expected.

(3) Patients even change their hairstylist/hairdresser to hide the fact that they have undergone previous straightening procedures, because ethical professionals refuse to perform the procedure if there is some evidence of risk to hair health.

(4) Patients continue to undergo the straightening procedure hoping, at each new procedure, for better results than those obtained after the previous straightening sessions.

(5) The hair fiber is overexposed to the straightening methods, increasing the risk of trichorexis nodosa and the patient complains of no hair growth.

(6) The need for good results from the straightening method increases after the first few sessions and raises the anxiety of the patient, thereby worsening the clinical features.

Although regulations in Brazil and Argentina prohibit the use of formaldehyde-containing straightening products, their widespread use is a striking phenomenon. Such is the degree of perceived need and sociocultural requirements for straight hair, which leads women to be exposed to health- and hair health–related risks.

P201

Quantitative risk assessment for contact allergens: a simplified approach for hair dye ingredients

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The majority of consumers can use hair colorants without any worry. However, hair dyes can cause allergic skin reactions in a small number of individuals, like other ingredients in common use products, such as certain foods or drugs. Here, we discuss the Quantitative Risk Assessment (QRA) for contact allergens, which is a risk-assessment approach allowing to assess the actual allergy (induction) risk of products containing ingredients with a given allergy potential. The QRA comprises a number of key steps, mainly determination of a no-expected-sensitisation-induction-level (NESIL), followed by the application of sensitization assessment factors (SAFs), reflecting uncertainties regarding variations between the subjects, different product use patterns, and matrix effects. Both allow the determination of an acceptable exposure level (AEL) to be then compared with the consumer exposure level (CEL) resulting from product use. This approach has been successfully applied to a variety of product types and ingredients, like perfume ingredients or preservatives. Here, we propose an alternative approach within the QRA, to be used for hair dye ingredients featuring precise product exposure data generated in support of the EU commission hair dye safety strategy. This data set provides firmly established dose/unit area concentrations under relevant consumer use conditions, referred to as the Measured Exposure Level (MEL). The MEL includes product exposure scenarios usually addressed by SAFs. In this instance, it is therefore possible to make a direct comparison between the NESIL with the MEL, as a proof-of-concept quantification of the risk of skin sensitization under product-in-use conditions. This is illustrated by two specific hair dye ingredients, PPD, and resorcinol. Comparison of the robust and toxicologically relevant NESIL and MEL data is therefore considered an improvement versus a hazard-based classification of hair dye ingredients.

P202

Quantitative analysis of hair surface properties using ATR-FTIR spectroscopy and high-throughput microfluorometry

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Hair fiber surface properties are influenced by several factors including chemical treatments and external environment factors such as ultraviolet (UV) radiation exposure. In this study, control and chemically bleached hair samples were analyzed by attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy and microfluorometry. The information inherent in the ATR-FTIR spectra of hair provides chemical information on the outer few microns of the hair surface and semiquantitatively can track the formation of cysteic acid following oxidative treatment of the hair. By using cationic fluorescent labels, the micro-fluorometry measurement quantitatively detects electrical charge modifications at the hair surface after chemical oxidation. The two measurement methods are complimentary in providing direct characterization of surface chemistry changes, as well as changes in surface energy and charge properties. The extent of change in both hair fiber surface parameters is shown to increase with longer peroxide treatment times and longer UV exposure times. These measurement techniques, and the results of the specific chemical and UV stresses used in the current work, will be discussed with a focus on their general application to provide quantitative surface information related to hair fiber damage, protection, and repair.

P203

The influence of keratin actives on the internal structure and thermal stability of hair

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Consumers regularly subject their hair to intensive treatment, care, and styling routines to help themselves achieve desired look. The actions performed by the consumer introduce changes to the chemistry and physics of hair fibers and the consequence of these changes are generally perceived by the consumer as damage. Combing and brushing of hair mechanically abrades the surface of hair and making them rougher and increasing their frictional characteristics. Hair lightening or coloring treatments generally involve an oxidative step to break down melanin and to develop the new hair color, but the hair fiber protein can also be oxidized that results in changes in the number and types of covalent and noncovalent bonds and interactions in the fiber influencing structural integrity, thermal stability, and mechanical properties. It has been shown that certain organic acids, carbohydrates, and salts can penetrate into hair and influence the secondary structural characteristics, which in turn change the hair's thermal stability. Transmission Fourier Transform Infra-Red (FTIR) has been used to demonstrate the chemical and structural changes arising from damage treatments and has subsequently shown the location and impact of a number of active materials on the fiber structure. Differential scanning calorimetry (DSC) has been used to show a decrease in the thermal stability resulting from damage and has subsequently shown how the stability reduction can be ameliorated through application of the aforementioned active materials. DSC results have shown that the magnitude of the thermal stability change can be increased by the repeated application of the active materials from a hair care product system and have been shown to increase the thermal stability of damaged hair to a level equal to or greater than that of undamaged hair.

P204

Long-term effect of anti-dandruff shampoo on scalp condition

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Background & Objective: This study was designed to build the understanding of how scalp condition changes with antidandruff shampoo treatment, as well as to give insights into the natural variability in scalp condition, over a 12-month period.

Methods: This is an UK-based study of a double-blind, parallel, randomized design. Subjects were recruited on the basis of both self-perception of dandruff (Y/N) and visual technical assessment of scalp (TWHS) to provide a range of scalp conditions. Subjects underwent a run-in phase (2–4 weeks) on beauty shampoo and were then randomized to one of the three shampoos (1% zinc pyrithione shampoo, prototype antidandruff shampoo, and placebo shampoo). The allocated shampoo was used at home as a part of their normal hair care routine for 12 months. Dandruff was assessed at baseline (Feb) and after 4, 12, 26, 39, and 52 weeks. Data were analyzed using analysis of covariance with product, week and TWHS as main fixed effect.

Results: For the whole population, both antidandruff shampoos gave significantly lower scores compared with the placebo shampoo at all time points ($P<0.05$). Both active shampoos were also superior to the beauty shampoo in the lower flake population (TWHS<28, $n=229$). In the populations with higher flakes (TWHS≥ 28, $n=78$), the zinc pyrithione shampoo was superior to placebo at all time points, whereas the prototype shampoo was not significantly different from the placebo at 12 and 39 weeks. Further analysis of the data suggested that there are seasonal changes in scalp flake levels.

Conclusions: The data demonstrate a sustained anti-dandruff benefit for dandruff sufferers with continued zinc pyrithione shampoo use. In addition, those subjects with low levels of scalp flakes also showed benefit from the use of the anti-dandruff shampoos.

P205

Electrophoretical comparison of individual and commercial hair strands and the effects of internal and external influences

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The knowledge about changes of human hair proteins introduced by aging, environmental influences, and cosmetic treatments is an important requirement for the development of innovative cosmetic products. The electrophoretical fractionation of hair cortex proteins is a well-established tool for analyzing such changes. The analysis of electrophoretical protein patterns (SDS-PAGE) gives detailed information about chemical changes of the intermediate filament proteins, as well as of the various groups of intermediate filament–associated proteins. For the electrophoretical analysis, cortex proteins have to be reductively extracted from hair. The amount of extractable cortex proteins varies from person to person, as well as along hair length. A progressive decrease in the amount of extracted proteins can be observed along hair fibers due to weathering-induced cross-linking and protein loss. A large variation in protein extractability can be observed between the individuals, which is assumed to be caused by different diffusion rates for reagents into the fiber. These variations can clearly be distinguished from genetic changes in protein band formation in the pattern. Commercially available hair strands generally show a reduced extractability of cortex proteins. Changes in the extractability are not influenced by hair color and age of donors. Statistical evaluation of electrophoretical protein patterns is able to give detailed information about weathering along hair, as well as individual extractability, separately for intermediate filament proteins and intermediate filament–associated proteins. This approach shows specific promise for the detailed analysis of chemical processes of cosmetic relevance based, namely, on reduction and oxidation.

P206

Hair and scalp surface properties, and formulation design, influence the selective delivery and deposition of actives

J Coret, S Gourion-Arsiquaud and DJ Moore *TRI/Princeton, Princeton, New Jersey, USA*

Inducing the targeted delivery of actives to scalp compared with hair would greatly enhance the efficacy of actives that treat scalp disorders. In addition, the selective deposition of actives onto the surface of hair fibers, but not on to scalp, would reduce unwanted staining and/or irritation risks associated with certain types of hair products. The deposition of model actives onto biological substrates such as hair and skin was studied using a variety of Fourier transform infrared (FTIR) spectroscopy and confocal Raman spectroscopy methods in combination with analytical methods such as tape stripping. The influence of pH, a critical formulation solution parameter, was studied for two water-soluble actives with very different pKa values such that the actives have opposite ionic charges at a specific pH. These initial studies will describe the deposition of each active as measured in separate *ex vivo* hair and skin experimental models. The results are discussed in the context of inducing and controlling selective deposition of specific actives or drugs on hair versus scalp skin, or vice-versa. The influence of chemical treatment of hair on active delivery and deposition was also studied by comparing deposition results on undamaged hair to that of bleached hair. Our spectroscopic results indicate that active deposition is heavily influenced by long-range electrostatic factors and is dependent on the nature of the ionic groups that are to be found on each biological surface.

P207

The design of superior emulsions in hair care: a model of deposition and thin-film lubricant performance

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The use of silicones and oils in personal products is well known. In the case of hair care products, such as shampoos and conditioners, silicones and oils are commonly used to reduce the damaging friction encountered during daily hair care routines, and they impart a smooth and superior feel to the substrate. Silicones in particular are remarkable in being able to deliver highly significant lubricant effects from very thin films. Detailed studies of molecular dynamics in these films have therefore been an area of significant industrial and academic interest in recent years. These studies have highlighted the importance of molecular considerations in the design of superior lubricant systems. In aqueous products, such as hair shampoos, however, these materials are often incorporated as emulsions. In this case, the emulsion characteristics such can heavily influence the performance of the final film. In this paper, we present a physical model of emulsion deposition and final film performance. This model correlates extremely well with experimental data and demonstrates the significant influence of properties such as emulsion particle size and spreading behavior. In this manner, the lubricant film may be designed to deliver superior lubrication while minimizing the amount of material required. In applications such as the design of superior hair products, therefore, this may be used to deliver benefits such as hair smoothness without incurring consumer-perceived negatives such as heaviness and greasiness.

P208

Scalp and hair health—interrelationships and molecular insights

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Scalp and hair problems have high global prevalence spanning across genders and ethnicities. We will review the current scientific knowledge in this area and then present new hair and scalp research, both at the micro and molecular levels, which has led to a new, detailed understanding of these problems that will help in the advancement of their effective treatment and prevention. The scalp surface features a unique microenvironment comprising of the superficial surface and a substantial portion of the follicular infundibulum. The surfaces in all areas of this microenvironment are sebaceous, lipid-rich, relatively dry, and physically protected by hair. For the most common scalp condition, dandruff, the presence of hair is a prerequisite. Conversely, the hair fiber is negatively affected by the physiological changes associated with this very scalp condition. We have complemented molecular methods for assessing the scalp condition with new approaches toward assessing the microenvironment of the follicular infundibulum. These methods have enabled a detailed understanding of the scalp conditions and have led to the development of superior technologies for treating and preventing these conditions, as well as providing a favorable environment for healthy hair to grow. Beyond the scalp, the hair fiber can degrade as a function of external stress, one type being oxidative stress. Reliable measures to understand what is happening to the hair fiber undergoing oxidative stress can provide invaluable data to understand new mechanisms and treatment approaches. The field of proteomics has enabled us to examine how oxidative stress impacts on the integrity of a hair fiber's protein structure. To that end, we have previously shared biomarkers linked to oxidative coloring. Recently, we have identified peptide biomarkers that differentiate the oxidative damage done to hair's protein composition via UV-light from that of oxidative coloring. These fundamental protein changes have been linked to the perception of hair quality.

P209

Effects of basic amino acids on hair

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The hair fiber is particularly rich in cysteine, serine, and glutamic acid, and the properties of the constituent amino acids confer functionality on the hair fiber due to the prospective interactions that take place. The hydroxyl, amide, and basic functional groups can interact via hydrogen bonding and ionic interactions. Hair proteins are composed of a greater proportion of negatively charged amino acids, which can encourage the uptake of positively charged species. Dibasic amino acids such as arginine and lysine have been examined to assess their effect on hair fiber properties using a number of techniques. Uptake and penetration into the fiber has been confirmed using radio labeling and fluorescent detection. The impact of these actives has also been studied using a number of physicochemical techniques and the increase in the protein denaturation temperature has been measured by differential scanning calorimetry. This work has shown that these amino acids are taken up by the hair fiber and the impact on damage has been demonstrated.

P210

Comparison Study between the tribological and nanomechanical properties of two ethnic groups of hair fibers

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In the past, the chemical and physical properties of the hair fibers, like chemical composition, microstructure, and hair growth, were extensively studied. It was found that human hair is a biological nanocomposite fiber with well-characterized microstructures. In this work, the tribological and nanomechanical properties, such as coefficient of friction, hardness, and Young's modulus, of different hair fibers were measured using the nanoscratch and nanoindentation techniques. The nanoindentations tests were performed on both the transverse and the longitudinal surfaces of each hair fiber. These properties, as a function of hair structure (hair of different ethnicity), were discussed.

P211

Evaluation of postoperative edema in patients having hair transplant surgery

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Swelling or edema of forehead or eyelids is a common consequence of hair transplant surgery. The study was conducted to evaluate the status of postoperative edema after hair transplant. All the patients who underwent hair transplantation surgery from January 2007 to December 2009 were included. All the procedures were performed under local anesthesia. About 100 ml of tumescent anesthesia was used for the recipient areas using 1% xylocaine with 1:100,000 epinephrine and 40 mg of Triamcinolone acetate. After the surgery, an elastic bandage was used, which was removed after 16 to 20 hours. Patients were advised to apply ice packs and were instructed to lie in the supine position for 48 hours. No oral / intramuscular / intravenous steroids were advised. Any swelling/edema was assessed and classified according to different grades (grade '0' to 'IV'). A total of 300 male patients were included in the study. The average age of the patients was 36.1 years. Majority of the patients belonged to middle age group (31 to 40 years). Majority of the patients (95.7%) developed 'no edema' or 'swelling' postoperatively. Few patients (4%) developed edema in the forehead but none of them developed a black eye. Only one patient developed periorbital edema. Thus, we see that local use of steroid prevents edema formation.

P212

Clinical and biochemical evaluation of the effectiveness of shampoo RV3438D in chronic recurrent squamous states -Interest of a maintenance treatment

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Background: Relapses are always observed after stopping scalp anti-dandruff treatment.
Shampoo RV3438D (cyclopiroxolamine, zinc, acid Pirythione beta-glycyrrhetinic acid) was developed to treat chronic recurrent squamous states (CRSS).
We wanted to evaluate the effect of a short attack phase on the improvement of CRSS, its persistence and interest in a maintenance phase in the prevention of recurrences.
Materials and methods: A total of 67 subjects with CRSS of moderate-to-severe scalp erythema and itching have been included in this study conducted in 2 phases: phase 1 (attack 2 weeks), noncomparative and phase 2 (8 weeks), comparative, divided in 2 parallel groups, namely, maintenance or discontinuation ('stop') group. RV3438D shampoo was used 3 times /week during phase1 and then 1 time /week during phase2 only in the maintenance group, alternating with a neutral shampoo. In the 'stop' group, only the neutral shampoo was used. The efficacy of the shampoo was assessed as follows: clinically, on the basis of the Overall Clinical Dandruff Score, erythema, global efficacy, self-assessment (efficacy, dandruff, discomfort, and itching), and time to first recurrence; biochemically (scale samples), on the basis of Malassezia species (qPCR) and inflammation markers (ELISA analysis). Tolerance was observed.
Results: During phase 1, clinical scores, the quantity of malassezia species, and the levels of inflammation markers significantly decreased ($P<0.0001$). During phase 2, in the 'maintenance' group, the improvement was maintained with a decrease from baseline remaining significant ($P<0.05$). In the group 'stop', improvement regressed significantly for all the parameters studied. Intergroup difference was significant, and the time to relapse was found to be significantly higher in the 'maintenance' group ($P=0.0001$). Transient reactions to shampoo have been observed in 8.6% of the cases, mainly after the first use.
Conclusion: The effectiveness of shampoo RV3438D on clinical and biochemical signs of CRSS was demonstrated after a short attack phase. In a period of 8 weeks after discontinuing the treatment, this improvement regressed significantly. This decline could be avoided by introducing a maintenance treatment (1/sem), allowing the maintenance of treatment effect, and spacing significant recurrences.

P213

Microstructural re-engineering of conditioners for better performance

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Hair conditioners are currently a major source of growth within the hair products category. A growth, which is partly driven by an insatiable consumer demand for improvements in the look and feel of hair, and a demand, which is further exacerbated by the rise in hair chemical treatments, which while improving and changing the look of hair do cause significant deterioration to the health and tactile properties. Hair conditioners help to ameliorate dryness and/or damage arising from environmental exposure (sun, UV), use of devices (hair dryers and hair straighteners), or through chemical treatments (bleach and perming). In particular, conditioners lubricate the hair and aid in detangling to prevent the various types of damages, such as breakage and split ends, which is most commonly caused when the hair is wet. Performance of products during the wet/rinsing stage is also critical in driving an enhancement in the look and shape of the hair at the end. Hair that is more easily detangled and aligned during the wet condition will be more manageable and styled once dried. Hair conditioners contain a number of different components; however, it is the lamellar gel phase in the hair conditioner, which is critical for developing the wet-conditioning benefits of detangling and alignment. Presented in this poster will be new insights on how the microstructure of a lamellar gel phase can be engineered in order to deliver the optimum hair conditioner gel phase. Moreover, presented in this poster will be the technical and consumer benefits of engineered gel phases, including friction/lubrication, breakage, detangling, and manageability benefits.

P214

Trials and tribulations of aging hair—It is not only how it grows but also what we do to it!

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As you age, one of the first signs is the loss of pigmentation, resulting in grey hair. However, there are other important changes leading to issues with manageability. These can be attributed to two main areas: (a) diameter changes and (b) sebum level reduction. Diameter reduction together with changes in the shape of the fiber, results in lack of volume and thin appearance. It has been shown that the strength of the hair fiber is relative to its thickness, thinner fibers require less energy to break. Sebum levels rise up to the age of 30 and then fall, reduction in sebum results in hair and scalp being drier leading to misalignment and reduction in shine. These changes in hair cause women to alter their routine: wash frequency decreases and styling product use, perming, and coloring increase. These habitual changes could result in hair being damaged. For example, chemical treatment may result in wearing away or lifting of the cuticles leaving hair rough, with coarser feel and less shine. Bleached and damaged hair is weaker and therefore would break easily. These will be discussed in this poster.

P215

Hair care benefits arising from the application of hair oils

F Bell *Unilever R&D, Bebington, UK*

The application of oils to hair is a cultural norm in India and provides a number of care, appearance, and style benefits. Traditionally, naturally derived oils such as those from coconut, sesame, and mustard have been a commonplace but a number of alternative oils are now available to the consumers. This work describes the observations from a number of experiments in which the delivery and benefits of a natural oil and an oil blend are compared. It has been shown that the oil blend can penetrate the hair more readily than the natural oil, while providing similar or larger magnitude physical property benefits but with improved feel characteristics.

TISSUE ENGINEERING/REGENERATION

P216

Involvement of the immune response in regeneration of experimentally amputated whisker follicles *in vivo* and in a novel culture model of regeneration using newborn follicles

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Follicles have a unique capacity to regenerate a functional end bulb after experimental amputation. Previous work suggests that regeneration entails an initial phase resembling skin wound healing, followed by specific remodeling of the bulb involving epithelialmesenchymal interactions. A key feature of the phenomenon is that the mesenchymal (dermal) components of the follicle (the dermal papilla and dermal sheath) reconstitute without scarring (fibrosis). Macrophages, as well as TGFβ family members, have been strongly linked with fibrosis; therefore one hypothesis is that lower follicle regeneration involves a reduced or modified immune response. This study aimed to investigate this question and to develop a novel culture model of follicle regeneration. For *in vivo* studies mouse and rat whisker follicles were experimentally amputated and follicles recovered between 24 hours and 3 weeks postoperatively. For *in vitro* work, follicles were isolated from newborn mice, bases amputated, and the follicles cultured on filters for up to 4 days. All specimens were cryofrozen or wax processed for immunohistochemistry/immunofluorescence. Early changes to the epithelium mirrored that of skin wound healing with migration of the follicle epithelium to fill the inner follicle silo, upregulation of cytokeratin15 near the follicle base, and strong expression of fibronectin. The *in vitro* model was able to undergo this first phase of regeneration. Labeling of *in vivo* specimens with a CD68 antibody (rat) and CD11b, F4/80 antibodies (mouse) identified highest concentrations of macrophages around the site of amputation at 48 hours, these cells being lost by 7 days. Interestingly, macrophages were also detected in the culture model. TGFβ1 was strongly expressed in follicle dermis, although, interestingly, not at the immediate site of dermal papilla regeneration. We suggest that lack of scarring in regenerated follicles cannot be attributed to lack of an immune response, although subtleties involving macrophage subpopulations are currently being investigated.

P217

Full-thickness skin with mature and cycling hair follicles using tissue culture expanded human cells

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Regeneration of hair follicles using tissue cultureexpanded human dermal and epidermal cells has been a long-term challenge. Although a recent report described human hair follicle formation within a skin equivalent containing TSCII mutant skin fibroblast cells, the hair shafts formed were abnormal. Here, we report the formation of skin with robust hair follicle growth using dissociated and cultured human scalp dermal cells combined with cultured neonatal foreskin keratinocytes. Our results show a full-thickness skin containing mature epidermis, dermis, and subcutis. The regenerated hair follicles show normally layered sheaths with sebaceous glands, arrector pili muscles, dermal papilla, and mature hair shafts. The follicles show anagen, catagen, and telogen forms. We demonstrated that the regenerated skin and the hair follicles therein (including interfollicular and follicular epidermis, dermal fibroblasts, papilla, and dermal sheath) are of human origin. Staining with differentiation markers documented good follicular and interfollicular epidermal maturation and regenerated interfollicular and follicular epidermis with distinct differentiated layers. Interestingly, human antigen-positive sweat glands, vessels, and neurons were present in the skin grafts, a novel finding. Time-course analysis shows that neogenesis of human hair follicles recapitulates embryonic morphogenesis. Finally, we show that the reconstituted skin can maintain human features for at least 1 year after grafting and that it is able to heal with human cells after injury. In summary, our findings have four implications:

1. Tissue culture expanded human cells can indeed produce mature skin with subcutis and fully developed cycling, shaft-forming hair follicles, and eccrine glands.
2. Closer study of this system will allow mechanistic insights into human skin and appendage physiology and morphogenesis.
3. This system will allow for new laboratory models of skin disorders using patient or mutated cells.
4. The method will accelerate the generation of a complete skin for clinical applications.

P218

Enhancing hair follicle neogenesis in bioengineered human skin substitutes

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Bioengineered dermalepidermal composites (DECs) restore barrier function but not hair follicles (HFs) to the skin. Our initial objective was to demonstrate *de novo* human HF neogenesis in grafted DECs. Human Dermal Papilla (DP) cells isolated from lateral scalp dermis from three female donors (HDP43, HDP47, and HDP52, named according to donor age in years) were propagated *in vitro*. Reconstitution assays using HDP47 and HDP52 grown as spheroids were positive for HF induction only when coinjected with mouse epidermal aggregates but not with human neonatal foreskin keratinocytes. Moreover, HFs that were formed were chimeric with mouse-derived cells comprising the epithelial component and human-derived cells dominating the region adjacent to the epithelium. Remarkably, we achieved human HF neogenesis in grafted DECs made using HDP47 and foreskin keratinocytes. Interestingly, the percentage of cells staining positive for alkaline phosphatase activity, a DP marker, which correlates with trichogenicity, was significantly different among the samples ($P<0.001$), with the majority of HDP47cells showing activity. Our next step was to determine the effect of keratinocyte passage on HF neogenesis. DECs were created with HDP47 and either primary or passaged keratinocytes; these were then grafted onto immunodeficient mice and were harvested after 8 weeks. All of the grafts using primary keratinocytes ($n=6$) had HFs, 83% of grafts using passage 1 keratinocytes ($n=6$) had HFs, and only 71% of grafts using passage 3 keratinocytes ($n=7$) had HFs. Enhanced HF neogenesis using early keratinocyte cultures may be the result of greater abundance and/or responsiveness of progenitor cells with a capacity for HF formation. These studies demonstrate the feasibility of using normal human cells to create superior skin substitutes that develop skin appendages. Furthermore, the studies provide a model system to optimize autologous tissue engineered skin substitutes for clinical applications.

P219

Interleukin 6 promotes adult *de novo* hair follicle organogenesis through STAT3 phosphorylation

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Adult *de novo* organogenesis holds great promise in regenerative medicine. As a potential therapy for many types of alopecias, we study Wound-Induced Hair follicle Neogenesis (WIHN), where embryogenesis is recapitulated after full-thickness excisional wounding and *de novo* hair follicles are counted within the healed wound. To identify causes of natural variation, we screened gene expression differences between mice with high and low WIHN levels. Interleukin 6 (IL-6) was identified as prominently upregulated in mice with increased WIHN. We hypothesized that IL-6 and subsequent STAT3 phosphorylation promotes WIHN. Both IL-6 and STAT3 phosphorylation are increased immediately after wounding and in the re-epithelialized keratinocytes. Levels of IL-6 mRNA ($n=4$; $P<0.05$) and protein ($n=3$; $P<0.05$) positively correlated with regeneration ability among mouse strains. Exogenous addition of recombinant IL-6 during wound healing significantly increased WIHN in C57BL/6J mice ($n=20$; $P<0.01$). Paradoxically, WIHN is also significantly increased in IL-6 null mice, compared with strain-matched (C57BL/6J) controls ($n=45$; $P<0.01$), highlighting the complexity associated with tissue regeneration. However, phosphorylation of STAT3, a downstream mediator of IL-6 signaling, is also significantly increased in IL-6 null mice compared with controls ($n=4$; $p=0.02$), suggesting that other IL-6 superfamily members that also activate STAT3 compensate for IL-6. Indeed, the IL-6 family member oncostatin M (OsM) is significantly increased ($n=4$, $P<0.01$) in IL-6 null mice compared with strain-matched controls. Underscoring its importance, pharmacological inhibition of STAT3 phosphorylation inhibits WIHN in both wild-type ($n=6$–9; $p=0.01$) and IL-6 null mice ($n=7$–9; $p=0.03$). Overall, these findings demonstrate that IL-6 and STAT3 phosphorylation trigger hair follicle regeneration. These results suggest targets to promote *de novo* hair morphogenesis in human clinical trials for alopecias and also argue against generalizations about inflammatory mediators negatively impacting regeneration.

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Human hair follicle neogenesis using microenvironmentally reprogrammed dermal papilla cells

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Hair follicle (HF) neogenesis refers to the generation of an entirely new HF in recipient skin using HF dermal papilla (DP) cells. This has been extensively demonstrated in rodent skin, either using intact DP or using cultured DP cells. In contrast, HF neogenesis in human skin has not previously been achieved using human cells. We performed global transcriptional profiling of both intact and cultured DP cells using the Affymetrix U133Plus2.0 array, which revealed several pathways expressed in intact DP, which are capable of neogenesis, but absent in cultured cells, that lack the potential to induce *de novo* HF growth. We postulated that one approach to restoring the microenvironmental and anatomical context of intact DP is to grow the cells in hanging drops, which results in the formation of DP spheroids. We then profiled DP spheroids for changes in gene expression and determined that the average correlation coefficient between the transcriptomes of intact DP and the cultured cells is 0.42, whereas that between the intact DP and the spheroids it is 0.56, which equates to a significant restoration of an intact DP signature by 3D culture. To evaluate whether recapitulation of the DP signature equated to a restored inductive potential, we established a contextual human-to-human HF neogenesis assay that could be used to assess the inductive capacity of human DP cells in human skin. When we microimplanted DP spheroids into recombined foreskins placed onto the back of SCID mice, we observed marked HF neogenesis by 6 weeks, showing for the first time that intact human DP can induce *de novo* human HFs. We conclude that the partial restoration of the transcriptional profile in human DP cells, achieved simply by growing the cells in a 3D spherical microenvironment, is sufficient in some instances to restore the inductive capacity of DP cell cultures and elicit human HF neogenesis.

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Calcium microcapsule: a possible scaffold for building artificial dermal papillae

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Although dermal papilla (DP) transplantation is one of the most promising treatments for baldness, lack of DP with hair follicle inductive properties still remains a major obstacle. Previously, we developed artificial DP by enclosing DP cells (DPCs) within microcapsules. In this study, we used barium (Ba) and calcium (Ca) microcapsules as candidates for a scaffold for artificial DP. We compared both microcapsules in terms of biocompatibility, structural stability, and permeability, as well as cell viability and hair follicle inductive properties in the short term. Both Ba and Ca microcapsules maintained xenogenic DPCs in an immunoisolate environment and induced the formation of hair follicle structures. Biocompatibility, permeability and cell viability were better with Ca than Ba microcapsule scaffolds. Especially, biocompatibility was better with Ca than Ba microcapsules when transplanted into the peritoneal cavity of mice. Ca microcapsules were assessed for long-term inductive properties with xenotransplantion into Sprague Dawley rat ears. Before 20 weeks, Ca microcapsules gathered together, with no substantial immune response. After 32 weeks, some microcapsules were near inflammation cells and were wrapped with fiber. A few large hair follicles were found. Control samples showed no marked changes in the implantation site. Ba microcapsules are advantageous for structural stability. Even after microcapsules are broken, the inside cell can be isolated from immune cells. Ba microcapsules may be of use in short-term transplantation study. Ca microcapsules may provide an effective scaffold for building artificial DP.

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Human Scalp-derived fibroblasts are multipotent and trichogenic

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Along with the hematopoietic and intestinal systems, the hair follicle has become one of the three current laboratory models for studying stem cells. Because of its cyclic reformation, the hair follicle also serves as a powerful tool for studying tissue regeneration. By using a mouse system, many laboratories have isolated hair folliclederived cells that can regenerate into skin and its appendages. In this study, we report the isolation and tissue culture expansion of human adult scalp fibroblasts, which have mesenchymal stem cell (MSC) and trichogenic properties. Fibroblasts were isolated from fragments of human adult occipital full-thickness scalp skin (IRB approved protocol) and used at passage 2. Adipose derived MSCs were purchased (Thermo Scientific). Trichogenicity was measured using the Aderans HPA (Hair Patch Assay) (Zheng *et al.* 2005). Expression of progenitor and dermal papilla cell genes were probed using real time RT-PCR. The properties (differentiation potential, trichogenicity, and gene profile) of stem cells derived from the scalp were compared with stem cells derived from adipose. By using the bioassay, we found human scalp-derived fibroblasts induce and are incorporated into hair follicles. When placed into differentiation medium the same population of cells has the ability to differentiate into adipocyte, osteocyte, smooth muscle, glial, and neural cell lineages. These cells displayed high levels of progenitor cell and papilla genes. In comparison, although adipose derived MSCs form somatic lineages (adipocyte and osteocyte), they are not trichogenic in the bioassay and express lower levels of progenitor and hair follicle associated genes. Our studies suggest that MSCs isolated from human scalp skin make up the trichogenic population in the dermis. These cells are unique, when compared with adipose derived MSC, having differentiated into additional lineages and in their trichogenic properties.

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Hair follicle restoration by intradermal microinjection of trichogenous dermal cells in a humanized-scalp model mouse

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Hair follicles (HFs) are generated in the embryonic skin development and, once established, are retained through life by a unique self-renewal activity termed recycling. Hitherto, accumulated studies have revealed basic mechanisms underlying these processes, and the development of modern technologies has enabled us to manipulate the cells that have major roles in these processes. However, therapeutic technology has not been established by which clinicians can restore weakened or miniaturized hairs by autotransplanting healthy trichogenous dermal cells. The absence of a suitable rodent model for evaluating the therapeutic activities of the respective hair follicle cells in regeneration and restoration of damaged human HFs (h-HFs) has been one of the said obstacles. In the present study, we first generated a rodent model that has the humanized skin composed of h-HFs, which we call ''a humanized scalpmodel mouse''. Second, we damaged the model's h-HFs in their lower half parts by inserting an ophthalmologist's scalpel. Third, we microinjected into the amputated area the NEO-STEMlabeled human trichogenous dermal cells, which had been propagated in serial cultivation, and we followed the development and growth of the damaged HFs up to 8 weeks posttransplantation. These transplanted cells were incorporated into the damaged regions of the HFs by 2 weeks after transplantation and participated in restoring hair bulbs. These HFs composed of host cells and transplanted cells, which we call chimeric HFs, continued to develop into normal hairs by 8 weeks posttransplantation. In conclusion, HFs whose lower half parts had been injured were restorable to normal HFs by receiving exogenous trichogeneous dermal cells into the damaged regions. The presently developed humanized scalpmodel mice are expected to be an appropriate and useful *in vivo* model to explore a clinically effective technology for hair restoration therapy by autologous cell transplantation to patients with weakened or miniaturized hairs such as androgenic alopecia.

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Long-term organ culture of intact hair follicle units in a chip-based perfusion system

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Since decades, the hair follicle organ culture model developed by Philpott and colleagues has been the gold standard when it comes to compound testing on human hair growth *in vitro*. Here, truncated human hair follicles were cultured with a potential hair growth promoting or inhibiting agent by measuring the length of the emerging hair shaft with the adjacent inner and outer root sheath. One limiting factor of this system is the reduced time of culture before showing massive cellular and matrix protein destruction. Although signs of catagen progression can been observed, other stages of the hair follicle cycle cannot be investigated. This includes miniaturized or dormant follicles. Here, we report on a chip-based perfusion system capable of culturing complete philosebaceous units freshly taken during a hair transplantation procedure using Follicular Unit Extraction (FUE). In comparison with truncated hair follicles and follicles cultured with conventional static conditions, perfused follicular units showed remarkable consistency of the follicular structure and vitality revealed by matrix protein expression and TUNEL/ Ki67 double labeling after 14 days of culture. After clipping, hair shaft growth beyond the epidermis could easily be measured online. Keeping the follicular unit structure intact, also sebocyte and melanocyte functions could be studied. These results render the used chip-based perfusion system to be a useful tool for long-term culture of hair follicles, keeping most of their structure undamaged. Additional hair follicle stages like telogen, early Anagen, and late catagen or even miniaturized hair follicles seen in androgenetic alopecia can easily be placed and observed within the chip-System.

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Effect of stretching force on human hair dermal papilla cells: possibility of manipulating mechanobiology to induce hair regeneration

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Introduction: Mechanical forces have an important role in regeneration of tissues, such as bone, cartilage, blood vessels, and myocardium. For hair regeneration, interaction of hair dermal papilla cells (HDPCs) with hair matrix cells is essential. Our hypothesis is that appropriate mechanical stimulation on the HDPCs may enhance its interaction with hair matrix cells to accelerate hair regrowth. As our first step, we evaluated the effect of a stretching force on HDPCs by analyzing the gene expression of stretch-stimulated HDPCs.

Methods: A commercialized cell line of HDPCs was cultured with 10% fetal bovine serumcontaining Dulbecco's Eagle medium. Thereafter, $1x10^5$ of three-passaged cells were seeded on a silicone chamber that can be stretched cyclically by computer modulation. The stretching stimulation was set as 20% stretch, 10 cycles/min for 24 and 72 hours. Thereafter, the cells were harvested and their gene expression was analyzed using cDNA microarray methods.

Results: The HDPCs aligned themselves perpendicularly to the stretching direction 72 hours after stretching, whereas the non-stretched cells showed a random distribution. After 24 hours of stretching, 373 and 407 genes were upregulated and downregulated, respectively ($n=5$). After 72 hours of stretching, 2655 and 2823 genes were upregulated and downregulated, respectively ($n=5$). The upregulated genes included hair growth genes such as VEGF, WNT, BMP, and PDGF, and the downregulated genes included hair-removing genes such as IL-6 and TNF.

Conclusion: An optimal stretching force applied for a suitably long period can upregulate hair growth genes and downregulate hair removal genes in HDPCs. Thus, mechanical force may be useful in hair tissue engineering. In addition, mechanical stimulation of the scalp may be a natural, easy, and economical way to stimulate hair growth. Further studies will be required to understand the effect of mechanical force in hair regeneration.

P226

Transplantation of hair follicle dermal cells as a therapeutic approach for recessive dystrophic epidermolysis bullosa

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Intravenous (IV) injection of bone marrow stem cells and direct injection of dermal fibroblasts to skin wounds are being investigated as cell therapies for the treatment of recessive dystrophic epidermolysis bullosa (RDEB). Both approaches have resulted in the improvements in skin integrity; however, our hypothesis is that other cell types may be capable of doing the same thing more efficiently. Dermal cells from the hair follicle, which are derived from skin, may also be good candidates for a cell therapy for RDEB as they produce type VII collagen, have natural wound healing and stem celllike properties, and are capable of supporting normal epidermal keratinocytes in a three-dimensional skin culture model. We are currently investigating in normal mice the wound homing response and durability of murine dermal sheath cells after IV administration, and a novel method of direct transplantation of these cells into skin using three-dimensional cell aggregate. At present, our data demonstrate that murine dermal sheath cells can indeed home to skin wounds. Moreover, these cells persist in skin for a number of weeks while eliciting only a minimal immune response. We are currently also testing the capacity of murine dermal sheath cells to restore basement membrane function in collagen VII hypomorphic mice and human dermal sheath cells to support human keratinocytes from RDEB patients in three-dimensional skin culture models.

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New development in unlimited hair follicle transplant

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Hair transplantation has had an important role in the treatment of hair loss. However, hair transplantation in fact represents hair redistribution. Thus, traditional hair transplantation is greatly restricted by insufficient transplants. Some studies have demonstrated that HF (hair follicle) upper fragments can regrow HFs. Despite the low success rate, hair graft number can be increased by hair follicle dissection. Here, we established a method to increase the regrowth rate of ''single HF upper fragments.'' HF upper fragments were obtained from the vibrissa of SpragueDawley rats at the level of hair bulb. Segments were transplanted underneath the skin of nude mice (6 segments per mouse, 1.5-cm distance between segments). Then culture medium of vibrissa HF was injected into the transplanted sites every 2 days, and grafts were excised at days 3, 7, and 9 and weeks 2 and 4. The control group received DMEM + 10% fetal calf serum instead of HF culture medium. In all, 91.7% of the fragments (66/72) were able to regrow into intact HF from 7 days to 4 weeks. Only 2/38 of control mice fragments showed intact HF regrowth after 4 weeks of transplantation. We traced the expression of Wnt5a during the regrowth of HF: at 3 days after transplantation, the experimental group showed Wnt5a expression at the newly formed connective sheets. At 9 days, Wnt5a was expressed strongly at the typical newly formed DPs. We also designed a novel HF transplantation device that includes scissors at the bottom to dissect the HF bulb when obtaining HF from the donor. The remaining HF bulbs were able to regrow intact HFs in the donor sites. As well, the upper site of HFs can be used as hair graft transplants by the method above. Thus, our HF fragment transplantation method is a new development in unlimited hair follicle transplant.

P228

Scalable production of controllable dermal papilla spheroids on polyvinyl alcohol surface: effect of spheroid size on efficiency and efficacy of hair follicle regeneration

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Organ size and numbers are vital issues in bioengineering for hair follicle (HF) regeneration. Murine HF dermal papilla (DP) cells are able to induce HF neogenesis when transplanted as aggregates. However, how the preparation of murine and human DP aggregates affects HF inductivity and the size of regenerated HF is yet to be determined. Here, we report a scalable method for the production of controllable human and rat DP spheroids in general labs for reproducible experiments. Compared with more hydrophobic polyethylene and poly(ethylene-co-vinyl alcohol), DP cells are poorly adhesive to hydrophilic polyvinyl alcohol (PVA). Seeded in the PVA-coated 96-welled commercial PCR tube arrays, DP cells quickly aggregate into single spheroids with progressive compaction. Varying seeded cell numbers and culture periods enable us to control the size and cell number of the spheroids. The spheroids obtained have high viability and preserve DP characters. A proof of principle experiment was conducted to examine the size effect on the efficiency and efficacy of HF regeneration. We found that both human and rat DP spheroids are able to induce HF neogenesis and larger DP spheroids exhibit higher HF inductivity. However, the average diameter of regenerated hair fiber did not significantly change with the increasing size of transplanted DP spheroids. The result suggests that an appropriate size of DP spheroid is essential for HF inductivity, but its size cannot be directly translated to a thicker regenerated hair. Our results also have implications on the efficiency and efficacy in the regeneration of other epithelial organs.

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Follicular epithelium prone nature distinguishes a human induced pluripotent stem cell line with hair forming capacity from others lines

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We previously generated human induced pluripotent stem cellderived ectodermal precursors (hiPSC-EPCs) using three hiPSC lines. 201B7 hiPSC-EPCs better communicated with human dermal papilla (DP) cells than WD39 and WDT2 lines in coculture and contributed to hair morphogenesis *in vivo*. The aim of the current study is to investigate what properties endowed 201B7 hiPSC-EPCs such functional privileges. To probe their natural fate, each hiPSC line was converted into embryo bodies and allowed to differentiate without induction. 201B7 line more readily expressed ectoderm and epithelial markers, whereas WD39 and WDT2 lines were prone to express endoderm and mesoderm markers. To further assess the epithelial/ ectodermal prone nature intrinsic to 201B7, additional hiPSC lines (414C2, 253G2 and 409B2) were tested. Interestingly, 414C2 line demonstrated lineage marker expression similar to that of 201B7. However, when converted to EPCs and cocultured with human DP cells, 201B7, but not 414C2 line, upregulated all follicular keratinocyte genes (KRT75, MSX2, LEF1, and TRPS1) and DP biomarkers (ALPL, BMP4, and LEF1) examined, suggesting that ectodermal / epithelial prone nature alone was not a mechanism enabling 201B7 hiPSC-EPCs to interact well with trichogenic mesenchyme. WNT and SHH signaling are essential in hair follicle development. In particular, WNT activation predisposes keratinocytes to a follicular fate. All hiPSC-EPCs expressed higher levels of WNT signaling genes than normal human keratinocytes (NHKCs). Interestingly, 201B7 hiPSC-EPCs expressed lower level of WNT-negative regulator AXIN2 but higher levels of LEF1 and WNT downstream target CCND1 compared with other hiPSC-EPC lines, implying that 201B7 hiPSC-EPCs might be in higher WNT-activated state than other lines. SHH gene expression levels were higher in all hiPSC-EPCs than in NHKCs; however, line-specific upregulation was not observed. Accordingly, 201B7 hiPSC-EPCs were distinguished by follicular epithelium prone nature via a possible WNT-activated state, which may be essential to achieve efficient HF regeneration using hiPSC-EPCs.

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Optimized preparation of dermal papilla cell (DPC) construct for hair regeneration therapy

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Introduction: To develop an efficient and practical hair regeneration therapy, we have sought to optimize DPC culture method (Aoi N, *et al.* Stem Cells Transl Med, 2012) and DPC transplantation method, which we call ''hemi-vascularized sandwich (HVS) method'' (Aoi N, *et al.* J Tissue Eng Regen Med, 2012). In this study, we sought to optimize preparation of transplantation construct of cultured DPCs.

Methods: We prepared three different DPC transplants (cell suspension, cell sheet and cell aggregation) using cultivated rat DPCs (rDPCs), rDSCs (dermal sheeth cells), rDFs (dermal fibroblasts), h (human) DSCs, hDPCs, and hASCs (adipose-derived stem cells). Expression of hair inductivity-associated genes was examined with real time RT-PCR. Each cell construct was transplanted into the sole skin of rat with HVS method. In addition, some constructs were combined with aggregation, sheet, or collagen scaffold. Eight weeks after transplantation, harvested samples were histologically evaluated.

Results: Electron microscope showed that a large aggregate made of 15 small DPC aggregations had a proper space to allow plasmatic diffusion and avoid central necrosis. In both rat and human samples, TGFβ2 mRNA expression was the highest in cell sheet than other constructs in both DPCs and DSCs. Wnt10b was higher in aggregation than in suspension and sheet in rDSCs and hDSCs. ALP was higher in aggregation than in suspension and sheet in human DSCs and DPCs. In animal experiments, visible hairs were regenerated only in DPC sheet transplantation, while immature hair follicles were histologically observed in aggregation and combinations with aggregation.

Discussions/Conclusions: The construct form substantially changed DPC function such as hair inductivity. TGFβ2 expression appeared to be a reliable index predicting hair inducing capacity of DPCs. A sheet was the most efficient form with functioning DPCs, but a combination with undifferentiated aggregates may work better for hair regeneration with cycling.

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Laser-assisted rapid patterning of epithelial-mesenchymal interaction for skin and hair follicle regeneration

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Bioartificial skin containing keratinocytes and fibroblasts has been used to accelerate wound healing and reduce scar contracture. However, the skin appendages including hair follicles cannot be regenerated. Reconstitution of appropriate epithelial-mesenchymal interactions that can elicit regeneration of arrays of hair follicles with desired distribution is essential for regeneration of fully functional skin. Here, we developed a method for fast patterning of keratinocytes, fibroblasts and trichogenic dermal papilla cells into desired topological arrangement on a glass chip. The cell adhesivity of poly-(vinyl alcohol) (PVA) dynamically varies in the presence of serum. Freshly PVA-coated glass is non-adherent to all three cells, but it becomes adherent to fibroblasts and DP cells later in culture. We take this advantage as a surface barrier for cell patterning through laser-assisted surface microfabrication. By varying carbon dioxide laser parameters for ablation, we are able to create arrays of micro-domains of controllable size, depth and spacing on PVA-coated glass chips. With the adjustment of cell seeding, DP cells attach to these micro-domains, forming corresponding arrays of multicellular aggregates of controllable size and cell number. Fibroblasts seeded later adhere to the cell-free PVA domain as the substratum adhesivity increases. The finally seeded keratinocytes attach either to fibroblasts or DP cells depending on the cell types they contact. The final organization is an epithelial sheet on top of a mesenchymal layer of fibroblasts doped with arrays of DP islets. Functionally, keratinocytes on top of DP islets, but not those on top of fibroblasts, show differentiation toward HF. This method con be applied in the research of interactions between heterotypic cells, and it also shows potential for generation of bioartificial skin with patternable epithelial-mesenchymal interaction.

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Maintenance of hair-inducing capacity of cultured dermal cells by addition of cell culture medium

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The neogenesis of the hair follicle through cultured cell implantation is a promising alternative for the treatment of hair loss. Success of neogenesis of the hair follicle greatly depends on the ability to expand hair-inductive dermal cells *in vitro*. However, cultured dermal cells lose their hair follicle-inducing capacity (trichogenicity) during subculture. Therefore, to achieve successful hair follicle neogenesis, identification of cell culture conditions that enable dermal cells to maintain trichogenicity is an important matter. In this study, we show our data of maintenance of trichogenicity of dermal cells by treatment with human follicular keratinocyte-conditioned media. We also show enhancement of trichogenicity of dermal cells by UVB-irradiated adipose-derived stem cell-conditioned media.

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Differences in the upregulation of inhibitor of apoptosis proteins (IAPs) in human dermal fibroblasts derived from hair-bearing and non hair-bearing skin in a scratch wound assay

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Observational studies describe rapid donor site healing when scalp skin is used for split-thickness skin grafting, suggesting that wounds heal better from hair-bearing skin. This has mainly been attributed to the hair follicle, but studies also suggest that dermal fibroblasts from different regions exhibit phenotypic differences. Recently the role of IAPs, a family of antiapoptotic proteins that inhibit the activity of caspase proteins, in wound repair has been highlighted. Using matched patient skin samples ($n=3$), we cultured dermal fibroblasts from hair-bearing scalp skin and adjacent skin that does not contain terminal follicles. In a comparison of fibroblasts derived from the same patients, at the same passage number, those derived from hair-bearing skin grew at a much slower rate over a 12 day period, compared to their adjacent counterparts. Using a scratch wound assay we compared the expression of a panel of IAPs (NAIP, c-IAP2, XIAP and Apollon) and their antagonists (XAF1 and SMAC/DIABLO) immediately after scratching and 24 hours later using immunocytochemistry quantitated by ImageJ. Fibroblasts from hair-bearing skin demonstrated an increase in the expression of NAIP, Apollon and SMAC/DIABLO immediately after scratching; all returned to control levels after 24 h. In contrast, only an upregulation in the expression of SMAC/DIABLO was observed in matching fibroblasts from non hair-bearing skin. These results provide further evidence for important phenotypic differences in human dermal fibroblasts from skin containing either terminal or vellus hair follicles. An immediate upregulation of the expression of IAPs in fibroblasts from hair-bearing skin suggests that they have better mechanisms in place for cell survival following wounding, which may contribute to the superior wound healing observed in hairy skin. Further studies are required to determine if fibroblasts from hair bearing skin significantly contribute to the wound healing response, which in turn may lead to improved therapies for chronic non-healing wounds.

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SOX2 expression in human scalp derived follicular progenitor cells correlates with trichogenicity

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Hair follicle formation and growth is driven by stem cell populations in both the epidermal and dermal compartments. SOX2 gene expression in mouse has been found in the dermal papilla of guard, awl, and auchene, but not zig-zag, hairs. Moreover, SOX2 identifies skin derived precursor cells, cells which have stem cell, trichogenic and homing properties. We have been interested in defining the trichogenic dermal stem cell population of human scalp. In this study we focused on the properties of SOX2 using immunohistochemistry, a triochogenic bioassay (Aderans HPA, Hair Patch Assay, [Zheng *et al.* 2005]), enhanced culture conditions, and RT-PCR techniques. SOX2 is expressed throughout morphogenesis starting with the dermal condensate. In adult occipital skin follicles, SOX2 is expressed in the dermal papilla and sheath of all follicles and in all stages of follicle cycling. To test the correlation between SOX2 expression and trichogenic potential, we isolated dermal cells from adult occipital scalp and assessed cellular trichogenicity with a bioassay (Aderans HPA). We found a correlation of bioassay response to level of SOX2 expression. To directly test the role of SOX2 in trichogenic potency of the cells we used siRNA to knock down SOX2 expression in cultured cells. siRNA not only reduced SOX2 expression of the transfected dermal cells (greater than two fold reduction) but also abrogated their trichogenicity. This work convincingly links SOX2 gene expression to the dermal papilla and sheath cells of human scalp follicles. SOX2 expression of scalp derived cells is maintained in culture and correlates with trichogenicity.

TRICHOSCOPY

P235

Follicular red dots: a normal trichoscopy feature in patients with pigmentary disorders?

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Follicular red dots (FRD) are erythematous structures regularly distributed around follicular ostia. They were first described in patients with active discoid lupus erythematosus (DLE) of the scalp and considered a specific trichoscopic feature of the disease. To our knowledge, no other scalp disorders have been associated with FRD, since the initial description. We report four patients with pigmentary disorders in whom FRD were detected during scalp examination. In two patients with albinism and one patient with extensive vitiligo and no previous history of hair disorders, FRD were identified in a homogenous pattern of distribution. In one patient with extensive vitiligo and female pattern hair loss (FPHL), FRD were irregularly distributed. FRD were linked to the presence of dilated vessels and red blood cell extravasation around the isthmus associated with an atrophic epidermis, in active DLE lesions. Since three of our patients had no previous history of hair loss and none of them had history of cicatricial alopecia, we believe that the red color observed under trichoscopy is possibly related to the rich vasculature that naturally envelops the normal hair follicle. FRD in these cases were probably easily identified due to the lack of pigmentation of the overlying skin. Furthermore, we speculate that the irregular distribution pattern of FRD in the patient with FPHL is a reflection of the disease process, with alopecic areas where FRD are absent representing long-standing disease.

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Computer assisted image analysis after manual processing (CAIAMP) generates more precise and less variable body hair counts than Trichoscan

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Body hair counts by Trichoscan and CAIAMP were compared to detect possible influences of *in-vivo* procedures on naturally dark body hair. Six sites on the forearms of a Caucasian male individual were imaged three times on the same day before and after application of a hair dye and immediately after hair-length reduction. Trichoscan was used first and the automatically defined target site in each individual image was subsequently used after randomization for CAIAMP (blinding of technician as to site, time, and procedure). CAIAMP has been validated (accuracy and precision after hair dye), and it detected a total of six thin ($20 \leqslant \varnothing < 40\,\mu m$) and 331 thick ($\varnothing \geqslant 40\,\mu m$) hair strands, whereas Trichoscan® counted 63 thin and 1066 thick hair strands (ANOVA, $P < 0.01$). There was also a wider variation depending on the procedures with Trichoscan (ANOVA, $P < 0.0001$). The thick hair counts (averages of the 6 target sites) were 44 before dye, 73 after dye and 61 after hair length reduction with Trichoscan as opposed to 18, 18 and 19 with CAIAMP. The range of variation was -4 to $+6$ thick hair with CAIAMP as opposed to -51 to $+24.5$ with Trichoscan. Thin hair counts were lower and less variable with CAIAMP (range -1 to $+1$) as compared with Trichoscan (range -5.5 to $+3$; ANOVA, $P < 0.0001$). Furthermore, with CAIAMP it was possible to explain two aspects in relation with variability:

(1) obviously hair dye did not improve hair detection, confirming the good natural contrast, and (2) skin elasticity with minimal shifts in or out of the target site may result in CAIAMP variation of a small number of hairs, unlike Trichoscan.

The present findings confirm previously published data (*J Eur Acad Dermatol Venereol* 2006;**20**:578–583) and suggest that the use of CAIAMP by a calibrated qualified technician is preferable for body hair measurement, its reduction, or its removal.

P237

New trichoscopy findings in trichotillomania

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Aims: To determine if differential diagnosis between trichotillomania and other focal alopecias can be performed by trichoscopy. To determine the trichoscopic features of trichotillomania.

Materials and methods: Trichoscopy was performed in 370 patients with focal alopecia. Forty-four patients were diagnosed with the trichotillomania, 314 with alopecia areata, and 12 with tinea capitis. Trichoscopic images were searched for specific trichoscopic features from hair shafts and from interfollicular surface.

Results: The main and specific trichoscopic findings in trichotillomania patients were: irregularly broken hairs (100% vs. 67% in alopecia areata vs. 83% in tinea capitis), v-sign (57% vs. 0.6% in alopecia areata vs. 0% in tinea capitis), flame hairs (25% vs. 0% in alopecia areata vs. 0% in tinea capitis), hook-hairs (25% vs. 0% in alopecia areata vs. 0% in tinea capitis), hair powder (16% vs. 0% in alopecia areata vs. 0% in tinea capitis), and coiled hairs (39% vs. 0.6% in alopecia aretata vs. 0% in tinea capitis). All these differences were statistically significant.

Conclusions: Trichoscopy allows clinicians to easily diagnose trichotillomania. We describe new trichoscopy findings, characteristic for trichotillomania, the flame hairs, and the V-sign.

P238

Broom hairs in trichoscopy provide different histological findings. What is the meaning of broom hairs?

I Czuwara, A Rakowska, M Slowinska, M Jasinska and L Rudnicka *Department of Dermatology, CSK MSW, Warsaw, Poland*

Dermoscopy of the hairs and scalp (trichoscopy) is a useful non-invasive technique facilitating the diagnosis of alopecia, hair, and scalp disorders. Trichoscopic evaluation of the short, disrupted hairs easily accessible to dermoscopy is characteristic for many diseases. Examples include comma hairs in tinea capitis, microexclamation, and tulip hairs in alopecia areata, and Tosti hairs in androgenetic alopecia (AGA) among others. Recently we noticed a peculiar type of short, dark hairs protruding like a brush from one follicle opening on the scalp in alopecia cases. We called them ''broom hairs''. Broom hairs were noticed on seven female patients referring to the clinic with visible alopecia complaint. In all patients frontotemporal hairline recession with diffuse hair loss in the central part of the scalp was observed with clinical and dermoscopic presentation characteristic for AGA. One patient was suspected of overlapping scarring alopecia because of characteristic dermoscopic findings. Frontal fibrosing alopecia was in differentiation. Trichoscopy revealed two types of brooms, one of short, thick and frayed hairs, the second of thin, longer, lighter, densely bundled hairs. Clinically their appearance was similar to pili multigemini and trichostasis spinulosa. Biopsy oriented on broom hair was performed in four cases followed by histological evaluation. The biopsies showed AGA features with abnormal infundibular keratinization. Two cases presented features of scarring alopecia without inflammation; longstanding AGA in such a scenario cannot be ruled out. In one case a typical picture of trichostasis spinulosa was seen. In reflectance confocal microscopy compressed, abnormal shapes of hair shafts and follicles were visible. In conclusion, broom hairs do not seem to be a pathognomonic sign of particular entity. They may represent the dystrophic stage of terminal hairs in diffuse, longstanding AGA, or in non-active scarring alopecia such as frontal fibrosing alopecia overlapping with AGA.

P239

"Normal" limits for caucasian scalp hair with contrast-enhanced-phototrichogram method with exogen collection (CE-PTG-EC)

DJJ Van Neste[1,2] [1]*Skinterface, Tournai, Belgium and* [2]*Brussels' Hair Clinic, Brussels, Belgium*

There is no information about normal values for scalp hair growth using CE-PTG-EC. Normal hair values were evaluated on the top of the head during four repeat observations (two during summer and two during winter) in young volunteers (16–20 years). Ethical approval was obtained before enrollment and all participants declared to be in good health without excess hair shedding. Data from 52 CE-PTG-EC on 14 males and 10 females are reported. Besides nano hair ($\varnothing \leqslant 20\,\mu m$) that were not included in total hair counts (THC), THC was split into thin ($20 < \varnothing \leqslant 40\,\mu m$) and thick ($\varnothing > 40\,\mu m$) hair and anagen hair only if elongation exceeded 150 μm/24 h. Extensive descriptive statistics reported no significant influence of session and season, but there were statistically significant gender differences. Herein we report values of the most "robust" (applicable for M and F) criteria that could serve to describe "extreme normal limits" of CE-PTG-EC data (either P5 or P95).

Accordingly, in 1 cm^2 scalp of a healthy subject one should find:

(1) less than 17 nano hair;
(2) more than 210 hair in total (THC);
(3) more than 170 thick hair (73% of THC) of which at least 76% are anagen;
(4) less than 88 thin hair (27% of THC);
(5) less than 7 exogen hair.

In our panel, 1 male and 1 female showed three parameters on one CE-PTG-EC session outside the limits and it is unclear whether this might already reflect the incipient deficiency of hair growth. Our preliminary data could help individual patients complaining about increased hair shedding, insufficient growth, or incipient hair loss. Also, when three criteria at least would be outside those limits, subjects might be given treatment at an early "preclinical stage" of the condition.

P240

Usefulness of a handheld dermoscope for clinical diagnosis of alopecia in Koreans

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Trichoscopy, hair, and scalp dermoscopy is a noninvasive diagnostic technique performed with a handheld dermoscope or videodermoscope focused on follicular and perifollicular pattern, vascular pattern, and hair shaft characteristics. There have been many reports regarding the value of the videodermoscope in clinical evaluation of alopecia; however, studies performed with a more convenient handheld dermoscope are scarce and limited to a few disease entities. Moreover, previous reports were mostly based on Caucasians and studies regarding Korean alopecia patients, whose skin color as well as characteristics of hair is different from Caucasians, have rarely been reported. We studied the characteristic trichoscopic features of normal scalp and distinct types of alopecia in Koreans to assess the potential usefulness of a handheld dermoscope in clinical diagnosis of alopecia. In all, 338 alopecia patients and 160 unaffected control subjects of Koreans who visited the Department of Dermatology in Chonbuk National University Hospital were enrolled in the study. Dermoscopic examination was performed by a polarized-light handheld dermoscope with a 10-fold magnification. The images were obtained by a digital camera with a 3-fold optical zoom.

P241

Contrast-enhanced phototrichogram with exogen collection (CE-PTG-EC) documents 0% anagen: who has alopecia areata, telogen effluvium, or trichotillomania? A challenging "biopsy-proven" (?) study!

DJJ Van Neste[1,2] and N Renard[3] [1]*Skinterface, Tournai, Belgium;* [2]*Brussels' Hair Clinic, Brussels, Belgium and* [3]*CHWAPI, Tournai, Belgium*

CE-PTG-EC was recorded in three subjects with clinically severe acute hair loss. All three had medical history, extensive blood sampling, and two 4-mm punch scalp biopsies (double-check reports with second opinion). A female nurse showed no improvement during "mesotherapy" and we unravelled 0% anagen predicting total hair loss within the next 3 months. Biopsy confirmed clinical hypothesis of alopecia areata (AA). An Indonesian female with a history of severe weight loss, chronic cough, and moderate chronic fever had 0% anagen on CE-PTG-EC pointing to total hair loss in the near future. Global health status with severe anemia and bronchial mycotic contamination appeared to be related to an active HIV infection. The biopsy reported "acute telogen effluvium". Follow-up indicated total hair loss at 3 months with satisfactory scalp hair regrowth as observed at 6 months after initial diagnosis in the absence of the usual treatments used for AA. A 12-year-old boy experienced sudden diffuse hair loss during the period of school tests. CE-PTG-EC showed 0% anagen. Biopsy reported aspects compatible with "trichotillomania". Thorough explanation of the natural course of the process, including total hair loss in the next 3 months, was understood and accepted by the young patient. Daily application of topical steroid lotion resulted in total "vellus-like" hair regrowth by the fifth month. Together with the clinical course favoring the diagnosis of AA totalis, the CE-PTG-EC was against the histopathological diagnosis of trichotillomania. Information generated by the CE-PTG-EC and histopathological diagnosis may be "convergent" (cases 1 and 2) or "divergent" (case 3). The initial diagnosis may be modified according to the clinical course and the follow-up, but CE-PTG-EC objectivates the seriousness of the hair loss process such that the patients can adjust to a rapidly changing and dramatic situation.

P242

A multisite, double-blind, placebo-controlled clinical trial of a dietary supplement containing marine proteins shows statistically significant benefits in reducing hair shedding and an increase in vellus hair diameter in those with subclinical hair thinning/loss

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Scalp hair growth is a very powerful social signal in humans. Thus, hair thinning and loss, even at subclinical levels, can provoke profound psychoemotional anxiety. Poor nutrition has been implicated in suboptimal hair growth, and although drugs are available to treat recognized clinical causes of hair loss, those with subclinical hair thinning may seek benefits through nutritional approaches. In order to formally evaluate a dietary supplement containing marine proteins, a multisite, double-blind, placebo-controlled clinical study was conducted by the global research organization, Stephens & Associates. A total of 72 adult women (age range 24–55 years, mean; 44 years) were randomly and equally assigned to the test and placebo groups and a 6-month-period study involving six evaluations was completed. Study participants underwent physical and scalp assessment of their general health status to exclude pre-existing scalp conditions. Hair growth was evaluated using a phototrichogram (tattooed test site 0.25 cm^2; vellus hair defined as $\leqslant 40\,\mu m$; terminal hair defined $> 40\,\mu m$ with) and hair shedding was assessed using a validated shampooing protocol. Subjects also completed a quality-of-life questionnaire and maintained a daily diary. Phototrichogram data revealed a statistically significant increase in mean vellus hair caliber after 6 months in those subjects taking the marine peptide complex when compared with those in the placebo control group. Moreover, hair counts revealed a statistically significant lower shedding rate at month 3 in those in the oral supplement group compared with those taking the placebo tablets. These data are, to the best of our knowledge, the first from a multisite, double-blind, placebo-controlled clinical study to reveal statistically significant benefits from an oral dietary supplement by decreasing hair shedding rate and by increasing mean vellus hair fiber diameter, making these hairs more pronounced. Although the reduction in hair shedding peaked at 3 months of supplementation, significant vellus hair fiber diameter increase was seen after 6 months and could be envisaged to continue at least during the same anagen VI stage of the hair cycle.

7th World Congress for Hair Research
Invited Speaker Abstracts

PLENARY SESSION 1
PS1.1

Hair follicle stem cells in alopecia

L Garza[1], G Cotsarelis[2] [1]*Johns Hopkins Medical School, Baltimore, Maryland, USA and* [2]*Department of Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, USA*

We originally localized hair follicle stem cells to the mouse and human hair follicle bulge. Much progress has been made in understanding of mouse bulge cells, but the relevance of this information to human hair follicle biology often is not studied. We evaluated the status of hair follicle stem cells in androgenetic alopecia (AGA). We analyzed bald and non-bald scalp from men with AGA for the presence of hair follicle stem and progenitor cells. Cells expressing cytokeratin15 (KRT15), CD200, CD34, and á6-integrin (ITGA6) were quantitated via flow cytometry. High levels of KRT15 expression correlated with stem cell properties of small cell size and quiescence. These KRT15hi stem cells were maintained in bald scalp samples. However, CD200hi/ITGA6hi and CD34hi cell populations—both of which possessed a progenitor phenotype, in that they localized closely to the stem cell-rich bulge area but were larger and more proliferative than the KRT15hi stem cells—were markedly diminished. These findings support the notion that a defect in conversion of hair follicle stem cells to progenitor cells plays a role in the pathogenesis of AGA. We then showed that prostaglandin D2 synthase (PTGDS) is elevated at the mRNA and protein levels in bald scalp compared to haired scalp of men with AGA. The product of PTGDS enzyme activity, prostaglandin D2 (PGD2), is similarly elevated in bald scalp. PGD2 inhibits hair growth in explanted human hair follicles and when applied topically to mice. Hair growth inhibition requires the PGD2 receptor (GPR44). Furthermore, we find that a transgenic mouse, K14-Ptgs2, demonstrates elevated levels of PGD2 in the skin and develops alopecia, follicular miniaturization, and sebaceous gland hyperplasia, which are all hallmarks of human AGA. These results define PGD2 as an inhibitor of hair growth in AGA and suggest the PGD2–GPR44 pathway as a potential target for treatment.

PS1.2

Mechanisms of hair follicle aging and stem cell regulation

EK Nishimura *Department of Stem Cell Biology, Medical Research Institute, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan*

Multicellular organisms senesce with the expression of various aging phenotypes characterized by functional tissue decline and organismal changes with decreased regenerating capabilities. Hair loss and hair graying are typical aging phenotypes in mammals, but the underlying mechanisms of aging are still largely elusive in most tissues. In recent decades, some signaling pathways that determine organismal lifespan and molecules responsible for progeroid syndromes have been identified in some organisms, but the underlying cellular mechanisms of aging-associated tissue decline and diseases are still largely unknown. We have studied the mechanisms of aging-associated hair graying and hair loss by focusing on adult stem cells. We previously identified melanocyte stem cells (McSC) within the bulge–subbulge area of mouse hair follicles. The population is cyclically activated to self-renew and to provide mature melanocytes for hair pigmentation. Our chronological analysis of McSCs and hair follicle stem cells (HFSCs), the niche for McSCs, demonstrated that mouse hair follicles age through defective renewal of McSCs and HFSCs. McSCs differentiate into pigment-producing melanocytes in the niche without renewing themselves under excessive genomic stress or with aging. Strikingly, HFSCs with a sustained DNA damage response also showed characteristic fate changes that are clearly distinguished from cellular senescence. The stem cell fate changes with aging and the underlying mechanisms of tissue aging will be illustrated and discussed.

PS1.3

Biological and translational potential of hair follicle mesenchymal cells

CAB Jahoda *School of Biological and Biomedical Sciences, Durham University, Durham, UK*

The recognition that hair follicle mesenchymal cells have potential stem cell or progenitor activities now extends well beyond the original observations of regeneration within the follicle. Both hair follicle dermal papilla (DP) and dermal sheath (DS) cells resemble other mesenchymal stem cell populations in that they can be directed to differentiate down new lineages, and indeed DP cells have been completely reprogrammed into iPS cells. Notwithstanding these findings, in the broader translational sphere hair follicle mesenchymal cells may ultimately have a greater role in maintaining other stem cell populations. In this and other contexts it is important to consider DP and DS separately, since there are notable differences between them. One example of the wider stem cell potential of hair follicle mesenchymal cells is exemplified by their capacity to form skin-derived precursors (SKPs), where the dermal papilla has shown to be an enriched niche. Intriguingly, this SKP-forming capacity is lost by DP cells after culture, and this may be a result of the cells undergoing accelerated environmental reprogramming. In the context of skin, we, like others, have postulated that hair follicle mesenchymal cells might serve as a superior source of donor cells during skin wound healing. To investigate this question we have modified and developed a number of three-dimensional skin models to incorporate cultured follicle dermal cells in place of conventional dermal skin fibroblasts. We have shown that these models elicit normal epidermal differentiation and that the hair follicle cells have potential for skin replacement therapy. Three-dimensional reconstituted cell aggregations that incorporate follicle mesenchymal cells may also represent a useful means of introducing and maintaining small pieces of tissue in discrete locations for therapeutic applications.

PS1.4

Cellular and signaling mechanisms that regulate hair follicle stem cells by live imaging

V Greco[1,2] [1]*Department of Genetics, Yale, New Haven, USA and* [2]*Department of Dermatology, Yale, New Haven, USA*

Stem cells and their niches are critical for tissue development and regeneration. Yet, we still lack knowledge of the sequential steps stem cells undertake to sustain regeneration. Outstanding unanswered questions remain: (1) what is the behavior of stem cells?, (2) which signals control stem cell behaviours?, and (3) what is the functional role of the niche during physiological regeneration?. My laboratory has recently established an *in vivo* strategy to visualize the components of the hair follicle stem cell niche, track them over time, and manipulate them by two-photon microscopy in live mice. By these means, we have uncovered that hair follicle growth relies on both spatial organization of cell divisions as well as directional cell movements within the stem cell/progeny compartments. In order to address which signaling controls the identified cellular behaviors, we have utilized genetic approaches to perturb key regenerative signaling pathways that control hair follicle regeneration and dissect in real time the mode of action of evolutionally conserved pathways such as Wnt. Thus, we have established an *in vivo* approach that has led to the discovery of unpredicted cellular mechanisms of growth regulation, and enabled us to precisely investigate the functional requirements of stem cell niche components along with key signaling pathways during the process of physiological regeneration. This work is supported in part by NIH-NIAMS and ACS.

PLENARY SESSION 2
PS2.1

Genetics and immunology of alopecia areata

AM Christiano *Department of Dermatology and Genetics, Columbia University, New York, USA*

We recently reported the identification of several susceptibility loci in alopecia areata (AA) using genomewide association studies (GWAS), which have since been independently confirmed in other cohorts. These studies identified the NKG2D axis as a central player in AA pathogenesis, and invited therapeutic interventions along these targets. Using the C3H/HeJ mouse model, we have shown that several rationally selected drugs can both prevent disease onset as well as reverse established disease. These studies illustrate the rapid pace at which GWAS studies can uncover new genetic pathways that are critical to a complex disease, and lead to unanticipated therapeutic approaches guided by the underlying genetics.

PS2.2

Xenobiotic receptors in cicatricial alopecia: linking environment to disease pathogenesis

P Karnik *Department of Dermatology, Case Western Reserve University, Cleveland, Ohio, USA*

The aryl hydrocarbon receptor (AhR) is a transcription factor activated by xenobiotic ligands that induce enzymes which metabolize environmental toxins such as dioxins and polycyclic aromatic hydrocarbons. AhR is known to regulate diverse physiological processes and cellular functions in the pathogenesis of cancer, cardiovascular disease, metabolic syndrome, and diabetes. The role of AhR in the skin is not known, but the activation of AhR by xenobiotics or endogenous ligands could interfere with normal skin functions, thereby triggering disease. We discovered that the expression of AhR-target genes is increased in human primary cicatricial alopecia (PCA). This suggests a correlation between AhR activation and PCA pathogenesis. Treatment of hair follicle cells with xenobiotics showed increased expression of AhR-target genes, suggesting the activation of this receptor *in vitro*. Based on this evidence, we hypothesized that overexpression of AhR in the skin causes scarring alopecia. Indeed, keratinocyte-targeted overexpression of AhR in mice is reported to cause hair loss due to unknown mechanisms. We have shown that the upregulation of AhR-target genes causes progressive alopecia and whole-skin lesions including hyperkeratosis, loss of sebaceous glands, dystrophic hair follicles, follicular plugging, inflammation, and scarring. The loss of stem cells and sebaceous glands precedes the inflammatory response in this mouse model. These data suggest that the AhR transgenic mouse is an excellent experimental model for scarring alopecia. The human and animal studies taken together implicate AhR in the onset and progression of PCA. Thus, targeting AhR activity with naturally occurring or synthetic antagonists may provide an innovative therapeutic strategy for PCA. Our studies provide a novel link between environmental factors and the etiology of primary cicatricial alopecia.

PS2.3

The latest updates of the management of pattern hair loss

W-S Lee *Department of Dermatology, Institute of Hair & Cosmetic Medicine, Yonsei University Wonju College of Medicine, Wonju, Republic of Korea*

Because of the social and psychological impact of pattern hair loss (PHL, androgenetic alopecia), patients may seek inappropriate and unproven therapies that are available in nonmedical settings, often at great expense to the consumer. The aim of treatment of PHL is to increase scalp coverage or to retard the progression of hair thinning, or both. There are effective medical treatments available currently for some men and women with PHL, but clearly further treatment options are desired, particularly for women with FPHL. Agents used to treat PHL may be nonspecific biologic response modifiers that enlarge suboptimal hair follicles regardless of the underlying pathophysiology, androgen blockers to interrupt the 5a-reductase enzymes, or androgen receptor protein inhibitors to specifically block the binding and transport of androgens to the cell nucleus. The purpose of this lecture is to provide current information on an approach to the evaluation and medical treatment of pattern hair loss. This lecture discusses therapeutic options with current and emerging new treatment modalities. Also, management strategies for PHL will be covered in the setting of an algorithmic evaluation of pattern hair loss based on a newly designed classification system, named the Basic and Specific (BASP) classification.

PS2.4

The surgical management of cicatricial alopecias

RG Knudsen *The Knudsen Clinic, Double Bay, Australia*

It is well known that active cicatricial alopecias are unsuitable for surgical hair grafting or excision. There is some debate, however, about the correct management of cicatricial alopecias that no longer appear active. The current approaches relate to presumed ''stability'' of the condition. One approach is to wait for 12–24 months of stability post cessation of medication management. In this approach there is no medication ''cover'' during the surgical process. The alternative approach is to proceed to surgery during medication management if the patient has completed a successful ''stable'' period of 12 months on medication. Previous reports of re-activation of some cicatricial alopecias have suggested there is some risk in pursuing surgery, particularly if the patient is not currently using medication. My personal experience suggests there is greater risk of re-activation in patients with frontal fibrosing alopecia (FFA) compared to other cicatricial alopecias. With this in mind, it seems prudent to both warn all patients with cicatricial alopecia of the risk and suggest some period of medication cover during the surgery and for at least 12 months after. In patients with FFA, I would recommend that patients pursue lifetime medication, as the re-activation risk is high and often considerably delayed. I will present case studies to illustrate this problem.

KEYNOTE SPEAKERS

K1

Skin stem cells: in silence and in action

E Fuchs *Howard Hughes Medical Institute, Rockefeller University, New York, USA*

How stem cells balance self-renewal and differentiation is of fundamental importance to our understanding of normal tissue maintenance and wound repair. Moreover, increasing evidence suggests that the regulatory circuitry governing this balancing act is at the root of some types of tumors both in mice and in humans. The hair follicle is an ideal model system for exploring how stem cell behavior is controlled and how this process goes awry in tumor progression. In the adult, hair follicles undergo cyclical bouts of tissue regeneration, destruction, and rest. The hair cycle is fueled by stem cells located within a niche referred to as the bulge. In the mouse, hair cycles are synchronous, making them an especially attractive model to explore how quiescent stem cells become mobilized to actively regenerate tissue, how they self-renew to maintain a pool of stem cells, and how they return to quiescence following tissue production. This process is related to a challenge faced by many adult stem cells, namely, to be able to respond quickly to injury, repair tissue, and then return again to a quiescent state. Using the hair follicle as our paradigm, we've been dissecting the crosstalk that takes place between a stem cell and its microenvironment (stem cell niche) that governs when stem cells will be activated to make tissue in normal homeostasis and wound repair. Our findings provide us with an understanding of how the hair cycle works, why the resting phase gets longer as we age, and how hair follicle and melanocyte stem cells coordinate their behavior and how this can be uncoupled in disease states. Our studies also provide new insights into the process of stem cell activation, and in so doing have revealed mechanisms that are also deregulated in squamous cell carcinomas (SCCs), among the most prevalent and life-threatening cancers world-wide.

K2

Hair follicle: the root of the future. Clinical research, implications, and advances

U Blume-Peytavi *Department of Dermatology and Allergy, Charité-Universitätsmedizin Berlin, Berlin, Germany*

The human hair follicle, a highly developed biological autonomous machinery with individual self-renewing capacities, produces keratin fibers possessing an incredible symbolic power for attractiveness, strength, gender definition, as well as emotional and physical well-being. Creating and maintaining life-long beautiful hair, which is easy to grow or eliminate, pigment or depigment, newly create or replace, is the key interest to anyone interested and involved in hair research and management of patients with hair loss, alopecia, or excessive hair growth. Advances in understanding hair follicle physiology, hair growth and cycling, hair genetics, innovative diagnostic techniques, hair care, hair removal, and restoration have significantly contributed to an improvement in patient care and management. Today, the pilosebaceous unit is exemplary in successful translational research, with the human being still remaining the best model to study and investigate hair cycling and growth behavior. Advances in the field of stem cell biology have led to direct clinical impact in projects of hair follicle neogenesis and hair transplantation. Targeted follicular delivery, using the unique role of hair follicle pathways in percutaneous penetration with drug delivery systems, has opened a new dimension in the development of hair growth therapeutics and adjacent indications such as transfollicular vaccination strategies. Advances in experimental and clinical research enable us today to offer our patients innovative diagnostic tools and new testing devices and procedures. These in turn enable us to validate and quantify efficiently new candidate molecules for treating hair disorders. Trichology today incorporates evidence-based guidelines established for the management of androgenetic alopecia, alopecia areata, and hirsutism.

CONCLUDING PLENARY

CP1

What's new in clinical hair research?

A Zlotogorski *Department of Dermatology, Hadassah - Hebrew University Medical Center and The Center for Genetic Diseases of the Skin and Hair, Jerusalem, Israel*

This lecture will give a brief overview of the recent advances in clinical hair research. It will cover the progress in the diagnosis and treatment of common hair disorders such as androgenetic alopecia, alopecia areata, and scarring alopecia. Additionally, innovations in a range of inherited, infectious, and structural disorders, as well as hirsutism, will be discussed. Potential medical and surgical treatments will be reviewed too.

CP2

What's new in basic science aspects of hair research?

SE Millar *Departments of Dermatology and Cell and Developmental Biology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, USA*

In this talk I will review recent exciting findings in basic hair research. I will cover the latest studies of cell–cell signaling mechanisms; describe new approaches examining the functions of regulatory factors at the genomic level; explore the roles of global chromatin regulators in the hair follicle; present exciting new developments in live imaging techniques that permit visualization of hair follicle formation and stem cell activity; and provide an update on approaches to hair follicle regeneration. These striking advances have revealed new molecular and cellular mechanisms that will impact therapeutic strategies.

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