

Oral Presentations

O1: Disruption of LIPH/LPA/P2Y5 Pathway in Autosomal Recessive Woolly Hair/Hypotrichosis

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Key words: Woolly hair, hypotrichosis, P2RY5, LIPH

Autosomal recessive woolly hair (ARWH)/hypotrichosis is a hereditary hair disorder which is characterized by tightly curled hair, which usually progresses to sparse or absent hair. ARWH can be caused by mutations in either the *P2RY5* or lipase H (*LIPH*) gene. Here, we show extensive genetic evidence that disruption of either gene results in phenotypes with features of both WH and hypotrichosis. In this study, we identified two Guyanese families with ARWH, both of which are of recent Indian descent. Mutation analysis resulted in the identification of pathogenic mutations in the *LIPH* gene in both families. These mutations were previously identified in several Pakistani families with ARWH. Haplotype analysis using microsatellite markers close to the *LIPH* gene defined a founder haplotype shared in families from Pakistan and Guyana. In addition, we identified 8 consanguineous Pakistani families with ARWH, and found pathogenic mutations in the *P2RY5* gene in all 8 families. Finally, we analyzed a Japanese family with ARWH, and identified compound heterozygous mutations in the *LIPH* gene. The *LIPH* gene encodes a phospholipase A1 family member and is a key enzyme in the synthesis of lysophosphatidic acid (LPA) which is an extracellular mediator of many biological functions. The *P2RY5* gene encodes a G protein-coupled receptor known as P2Y5, which was recently shown to be a LPA receptor. Our results underscore the crucial role of the LIPH/LPA/P2Y5 signaling pathway in hair growth in humans.

O2: A New Mechanism for Genetic Hair Loss

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Key words: Marie Unna hereditary hypotrichosis; HR; uORF

Loss-of-function mutations in the hairless (*HR*) gene cause an autosomal recessive form of hair loss called atrichia with papular lesions. Marie Unna hereditary hypotrichosis (MUHH), an autosomal dominant form of genetic hair loss, has been mapped to 8p21, the chromosomal region harboring *HR*. In a large Chinese family carrying MUHH, we confirmed

the genetic linkage to 8p21 and identified a pathogenic initiation codon mutation in U2HR, the second upstream open reading frame (uORF) in the 5' UTR of the human *HR* gene. U2HR is predicted to encode a 34-amino acid peptide that is highly conserved among mammals. In 18 more families from different ancestral groups, we identified a range of defects in U2HR, including loss of initiation, delayed termination codon and nonsense and missense mutations. Functional analysis showed that these classes of mutations all resulted in increased translation of the main HR physiological ORF, suggesting that the molecular mechanism underlying MUHH is gain of function of the *HR* gene. Our results establish the link between MUHH and U2HR, demonstrate that fine-tuning of HR protein levels is important in control of hair growth, and identify a potential mechanism for preventing hair loss or promoting hair removal.

O3: Genetics of Male Pattern Androgenetic Alopecia: Androgen Receptor and Recent Findings on Chromosome 20p11

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Key words: Androgenetic alopecia, male-pattern baldness, androgen receptor

The pathogenesis of androgenetic alopecia (AGA) is androgen dependent and a genetic predisposition is the major requirement for the phenotype. The androgen receptor gene (*AR*) on the X-chromosome is the first gene to have been identified as contributing to the development of AGA, and has been reported by several studies (e.g. Hillmer *et al.*, *Am J Hum Genet*, 2005). The AR is a member of the nuclear receptor superfamily and mediates the effects of androgens. A functional AR and circulating androgens are essential requirements for the development of AGA. We and others have confirmed the important role of the AR locus through use of genome-wide association studies, and have also identified a further important genetic locus on chromosome 20p11 (Hillmer *et al.*, *Nat Genet* 40:1279-1281; Richards *et al.*, *Nat Genet* 40:1282-1284). The etiological fraction of the 20p11 locus is estimated to be 0.32, underlining its importance in the development of AGA. Risk allele frequency varies between 0.03 to 0.86 worldwide. This may partly account for population-specific differences in AGA prevalence, though it

does not explain the lower prevalence reported in Southeast Asian populations. The biological role of this new locus has not yet been clarified, and there is no obvious connection with the androgen pathway. A large international meta-analysis is currently underway to identify further risk-associated loci in the human genome. Discovering the functional consequences of the 20p11 locus and other, yet to be identified, loci will increase our understanding of the molecular and cellular basis of scalp hair development.

O4: The Genetic Basis of Female Pattern Hair Loss

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Female Pattern hair Loss (FPHL) has substantial phenotypic variation, as indicated by wide distributions of age at onset, rate of progression, and severity of hair loss. This suggests a polygenic mode of inheritance as this wide phenotypic range is likely to be due to the actions of multiple genes that collectively contribute to the manifested phenotype. Contributions from individual genes to polygenic conditions are usually small, but the identification of key genes can provide potentially novel and important therapeutic targets. The discovery of the association between genetic variation in the androgen receptor gene, *AR*, on the X-chromosome and male androgenetic alopecia (AGA) has been replicated by three independent groups and is consistent with the well-established androgen-dependent nature of this condition. In contrast, a role for androgens in FPHL is far less clear as the examination of *AR* in females is not straight-forward. Females have two copies of *AR* and one copy is subjected to inactivation during Lyonization so that only the active copy is expressed. Since Lyonization occurs at random, the active and inactive copies of *AR* are likely to be different in heterozygous females.

In addition genes regulating synthesis and the metabolism of oestrogen and its' receptors might influence the risk of developing FPHL. We have investigated associations between variants of a number of candidate genes by single nucleotide polymorphisms (tag SNPs) in 484 Caucasian women with grades 3-5 FPHL on the Sinclair scale, and 471 Caucasian women with no evidence of hair loss. Although we could not provide definitive evidence for the role of either *AR* or estrogens in FPHL, our findings support the view that estrogens are detrimental to hair growth.

O5: Neuroendocrine Regulation of Keratin Expression in the Skin

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Key words: Prolactin, TSH, TRH, keratin, hair follicle

Keratins serve crucial roles in hair follicle (HF) physiology and pathology, by providing mechanical support and regulating HF cycling and cell growth. While some information is known on the regulation of keratin expression by steroid hormones, neuroendocrine controls of keratin expression have been sparsely investigated. Since human HFs express functional receptors for prolactin (PRL), thyrotropin releasing hormone (TRH) and thyroid stimulating hormone (TSH), and may even express all three neuroendocrine modulators, we have studied whether TRH, TSH, and/or PRL exert any effect on keratin expression in normal HFs. We treated human scalp microdissected HFs for 5 or 6 days under serum-free organ culture conditions with TSH (10 or 100 mU/ml), TRH (1-100 ng/ml) or PRL (400 ng/ml). Microarray analysis revealed a large subset of keratins to be regulated by these neuroendocrine mediators. By immunohistochemistry, TRH downregulated K6 immunoreactivity (IR), while TSH upregulated K5 IR, in HF keratinocytes *in situ*. PRL upregulated K15 and K19 IR, two proposed markers of HF progenitor cells, and additionally upregulated K5 and K14 IR. K6 IR was downregulated. All the effects of PRL were blocked by a pure competitive PRL receptor antagonist. Using human K15-promoter-driven GFP labelling, we could demonstrate that GFP-specific IR was upregulated following PRL treatment. Changes in keratin expression were confirmed by qPCR analyses of ORS keratinocytes in culture. In this study we provide first evidence for neuroendocrine control of keratin expression in the HF. This lends further support to the importance of these hormones in HF growth and function.

O6: Seasonality of Hair Growth and Shedding in Otherwise Healthy Women Complaining of Hair Loss

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Frequently encountered in dermatologic practice is the otherwise healthy woman who is complaining of hair loss. Many factors can lead to pathologically increased hair loss. Once a definitive diagnosis is established, treatment appropriate for that diagnosis is likely to control hair loss. Nevertheless, a number of women complain of recurrent hair loss, despite at times successful

therapy. The condition tends to run a fluctuating course. To test the hypothesis that periodicity in shedding of hairs reflects seasonal changes in human hair growth, we performed over a period of 6 years a retrospective study of telogen rates as assessed by the trichogram technique in 823 otherwise healthy women complaining of hair loss in relation to the season. Analysis of trichograms demonstrated annual periodicity in the growth and shedding of hair, manifested by a maximal proportion of telogen hairs in summer, while telogen rates were lowest in late winter. This analysis confirms the findings of former authors, though this is the first study performed systematically in a representative number of women. It seems likely that human hair follicles undergo cyclical activity and are influenced by environmental factor, such as the photoperiod. The existence of seasonal fluctuations in hair growth and shedding complicates the assessment of pharmacological effects. Awareness of these fluctuations is prerequisite to providing the correct cause and prognosis to the patient, ensuring patient compliance with therapy, but also has potentially serious implications for investigations with hair growth promoting agents: Depending on the stage of periodicity in growth and shedding of hair for a particular subject, the heterogeneity of included subjects may be enough to distort the clinical efficacy results and the perceived benefit of an investigational agent.

O7: How Does the Hair Growth Stimulator, Minoxidil, Actually Act?

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Minoxidil is the most common treatment for hair loss. Originally an oral antihypertensive drug with unacceptable hair growth side-effects, minoxidil was adopted as a topical hair promoter without understanding its mechanism over 20 years ago. Stimulating local vasculature was proposed, but other potassium channel opening drugs (PCOs) also promote hair growth implicating ATP-sensitive potassium (K_{ATP}) channels in cell membranes.

If acting via follicular K_{ATP} channels, stimulation should occur in organ cultured follicles without a blood supply. Minoxidil and other PCOs stimulated deer follicle growth *in vitro*, an effect prevented by K_{ATP} channel blockers (Davies *et al.*, 2005) indicating follicular channels.

Previous human studies with minoxidil in insulin-supplemented media were inconclusive. In insulin-free medium, minoxidil stimulated anagen in human follicles, an effect inhibited by K_{ATP} channel blocker, tolbutamide (Shorter *et al.*, 2008) indicating a biological response in organ culture and concurring with the deer results.

We now know that PCOs bind to the regulatory sulfonyleurea

receptors (SUR) of K_{ATP} channels causing potassium ions to enter cells through their pores (Kir sub-units). Isolated human follicles expressed genes for SUR1, SUR2B, Kir6.1 and Kir6.2, but not SUR2A, indicating they contained at least 2 forms of K_{ATP} channels; only SUR2 channels respond to minoxidil. When hair bulb tissues were examined separately, epithelial matrix expressed SUR1 & Kir6.2, while both dermal papilla and dermal sheath exhibited SUR2B and Kir6.1. Immunohistochemistry demonstrated similar protein distributions (Shorter *et al.*, 2008).

These results suggest minoxidil acts directly on human follicles via Kir6.1/SUR2B K_{ATP} channels in the dermal papilla.

Reference: Davies GC, Thornton MJ, Jenner TJ, Chen YC, Hansen JB, Carr RD, Randall VA (2005) *J Invest Dermatol* 125: 686-694. Shorter K, Farjo NP, Picksley SM & Randall VA (2008). *FASEB Journal* 22, 1725-1736.

O8: Analysis of Family Factors According to Each Pattern Type of BASP Classification in Korean AGA Patients

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Key words: Androgenetic alopecia, BASP classification, family factors

Androgenetic alopecia (AGA) has a widely different prevalence rate based on races and individuals. The exact pathophysiological mechanism and genetic cause has not been elucidated yet, however, AGA is marked by autosomal-dominant inheritance. The influence from the paternal and maternal genes as well as gene transmissions in X and/or Y chromosomes is also suggested. According to the previous studies, Korean with male and female pattern hair loss (MPHL/FPHL) had 62.9% and 48.8% correlation with family members on paternal side with AGA, respectively. On the contrary, patients with MPHL have paternal gene influences while patients with FPHL receive maternal gene influences in Singapore. These results show that various types of genetic transmission methods may involve. In 2007, we presented Basic and specific (BASP) classification via collaboration of the Korean Hair Research Society, which is a completely different approach from AGA pattern. In contrast with Caucasian, Korean MPHL patients have hair loss that is similar to female patterns. BASP classification not only represents balding patterns of Oriental MPHL but is also applicable for both males and females. This study applies BASP classification on 1,220 AGA patients (998 male, 222 females) from 13 university hospitals in Korea to analyze the influence of family factors in

the patterns of hair loss. Henceforth, the result can be used to determine the difference of genes among the different patterns of balding. Furthermore, if the family factors exist, we can easily speculate the shape of baldness and the period when hair will start to fall off.

O9: Characterisation of hTERT Immortalised Balding Dermal Papilla Fibroblast Cell Lines

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Key words: Androgenetic alopecia, dermal papilla, immortalisation

Androgenetic alopecia (AGA), a hereditary disorder that involves the progressive thinning of hair in a defined pattern is the most common type of hairloss in men & is driven by androgens. The hair follicle (HF) dermal papilla (DP) expresses androgen receptors (AR) and in AGA it has been proposed that the inhibitory actions of androgens are mediated via the DP although the underlying molecular mechanisms remain unknown. DP cells derived from frontal (balding) human scalp HF (BDPC) can be used to study AGA however, cultured BDPC have a limited life span of 2-6 passages and we recently have shown, undergo premature senescence. This represents a substantial obstacle for subsequent biochemical analysis, genetic manipulation and development of in vitro models. To circumvent this we generated immortalized balding human DP cells (IBDPC) by ectopic expression of human telomerase. IBDPC have undergone over 100 passages without showing any phenotypic changes and cellular transformation. As with primary BDPC, they showed expression of *wnt7a*, *wnt3a*, AR and 5α reductase type 2 and expressed the stem cell markers CD44, oct3/4 and Nanog. IBDPC underwent adipogenic and osteogenic differentiation when cultured in medium known to induce adipocyte and osteocyte lineages. As IBDPC maintain similar characteristics to primary DPC they represent a useful model with which to investigate the actions of androgens on hair growth. Moreover maintenance of stem cell markers expression in the IBDPC and their ability to differentiate along different lineages, suggest they may represent a useful model with which to investigate stem cell fate.

O10: Responsiveness of Ex Vivo Human AGA Male Hair Follicles to CRH-induced Stress and its Modulation by Caffeine

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Key words: Stress, CRH, hair growth, caffeine

It was hypothesized that stress may deteriorate human hair growth in men with androgenetic alopecia (AGA). Since corticotropin-releasing hormone (CRH) is a strong stress-mediator in the human organism which acts along the systemic hypothalamic-pituitary-adrenal (HPA) axis and, additionally, a peripheral HPA-axis equivalent has been identified within the human hair follicle, we investigated whether CRH would induce a local stress response in human organ cultured hair follicles obtained from men expressing AGA. Moreover, we tested for protective effects of caffeine, a well-known phosphodiesterase-inhibitor and energy promoting substance which has recently shown to exert specific effects on human hair growth in vitro. Human anagen hair follicles (HFs) from the balding vertex area of men with AGA were organ-cultured over 120hrs in Williams E medium (control) or CRH (10⁻⁷ M) or CRH (10⁻⁷ M) in combination with caffeine (0,001% or 0,005%). CRH mediated decrease of hair shaft elongation, matrix keratinocyte proliferation and expression of IGF-1, as well as induction of catagen and TGF- β 2 expression. CRH significantly induced also HPA-axis related expression of CRH-R1/R2, inositol-1,4,5-triphosphate-receptor (IP3-R) and MC-R2. Additionally, the HPA-axis independent stress mediator substance P was significantly induced. Addition of caffeine to CRH-stressed HFs reversed all CRH-mediated stress events in the follicle shown above and even induced expression of the NGF high affinity receptor TrkA exerting anti-apoptotic activity. Thus, the data provide strong evidence for a CRH-inducible stress response in human HFs in vitro that is significantly modified by caffeine. Perspectively, this might have further clinical implications for androgenetic alopecia in vivo.

O11: The Human Hair Follicle: An innovative Tool for Therapeutic Delivery

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The importance of selective follicular targeting is increasingly evident in the development of future therapeutic strategies, especially for disorders such as seborrhoea, seborrhoeic dermatitis, acne, hypertrichosis and laser epilation. In the past, the role of the hair follicle for skin penetration and barrier function was considered relatively minor. However, in the light of recent findings and considerably improved understanding of skin physiology, advances have been made in the delivery to specific sites of the hair follicle. Hair follicles are the preferred pathways for specific molecules and formulations, which pass faster into these shunts than through the stratum corneum. Hair follicle density, follicular orifice size, surface of the infundibulum, and the relation between hair follicle growth activity and sebum

secretion rate (“open and closed follicles”), in combination with follicular targeting using microspheres, permit us to selectively penetrate distinct structures of the hair follicle

The transfollicular administration of pharmacologically active molecules is of current therapeutic interest, not only with regard to delivery to specific sites but also to different types of hair follicles (terminal and vellus HF). In vellus hair follicles topically applied 1.5 μm and 0.75 μm microparticles aggregated in the follicular infundibulum without penetration into the skin (“Macro-Targeting” of the human vellus hair follicle). Microparticles of this size may be used as drug carrier systems to create depots in the follicular duct (“Macro-Targeting” of the human vellus hair follicle). Nanoparticles e.g. 40 nm particles, in contrast, penetrate deep into human vellus hair follicles (“Micro-Targeting” of the human vellus hair follicle). Thus, nanoparticles are able to aggregate in hair follicle openings and to penetrate along the follicular duct; they are promising carrier systems for interfollicular application and transfollicular drug delivery.

In a large series of measurements on anagen scalp terminal hair follicles and retroauricular vellus hair follicles, we were able to demonstrate, that the dimensions of defined hair follicle types including overall length, size and position of infundibulum and bulge region, width of the hair follicle openings and thickness of the epithelium differ significantly. The concept of targeting drug delivery systems specifically to the different hair follicle types is a feasible approach, also in larger numbers of patients. Therefore future research projects regarding follicular targeting must consider these new recent developments.

O12: The Effects of Dihydrotestosterone on the Dermal Papilla Cells of Balding, Non-Balding and Beard

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Key words: Alopecia, DHT, dermal papilla, beard

Dihydrotestosterone (DHT) is famous as the key regulator of expression in human hair growth. They also plays an important role in male-pattern baldness. In human body, circulating DHT enters the hair follicle dermal papilla cells (DPCs) via the capillaries located at the bottom of the follicles. After puberty, frontal (balding) area is miniaturizing, in the other hands, Occipital area is not affected at all. Interestingly beard hair and pubic hair are growing much more after adolescence. Each DPCs may have their distinct properties. To investigate this paradoxical character of hair, we cultured each areas in same individual (Frontal, Occipital, Beard). After that, We treated DHT on each DPCs. Then we isolated RNA of DPCs. We used Human Genome U133A 2.0 gene chips (Affymetrix) and Hugen ST 1.0

chips (Affymetrix) to check gene expression levels. For systems biological approach to analyze them, we used “R” program and R-packages as analysis tool as well as matlab programs. We found some signaling pathway molecules are differentially involved. Some genes are paradoxically affected by DHT. High-throughput data approaches to androgenic alopecia and hair research would widen our knowledge of disease as well as pathophysiologic pathway of male-pattern baldness.

O13: Hair Follicle Melanocytes – Sensors of (Neuro) Hormonal Stimulation

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Key words: Hair follicle, melanocytes, neuroendocrine, sensors

We are currently experiencing a spectacular surge in our knowledge of skin and skin appendage function both at the organ and organism levels. Much of this is due to a flurry of cutaneous neuroendocrinologic data that positions the skin as a major sensor at the periphery. As our body’s most expansive organ the skin incorporates all major support systems including blood, muscle and innervation as well as its role in immunocompetence, psycho-emotion, ultraviolet radiation sensing, endocrine function etc. increasing evidence suggests that the skin may also be important for the maintenance of overall mammalian homeostasis as it utilizes locally-produced neuroendocrine hormones to neutralize noxious stimuli.

In particular, the cutaneous pigmentary system is an important stress response element of the skin’s sensing apparatus; where intersecting stimuli involving corticotrophin releasing factor (CRF) and pro-opiomelanocortin (POMC) peptides of the Hypothalamus-Pituitary-Adrenal axis, thyroid hormones of the Hypothalamus-Pituitary-Thyroid axis and the melatonin/serotonin system may all help to regulate pigmentation in the hair follicle and the epidermis. These pigmentary units appear to be organized into symmetrical functional units composed of multiple neuroendocrine hormones (e.g., CRF, POMC peptides etc), and so has led to the concept of “self-similarity” of neuroendocrine systems (e.g., melanocortin) based on their expression both at the local (skin) and systemic (CNS) levels. Indeed, the only apparent difference appears to be one of scale.

This overview talk will explore this concept and will describe how the components of neuroendocrine systems may help regulate the human hair follicle pigmentary unit.

O14: Stress, Hormones and the Hair Follicle

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The hair follicle is a key endocrine organ producing and responding all relevant factors of the classical stress-axis, the hypothalamus-pituitary-adrenal axis and the sympathetic axis. It was therefore proposed to view the hair follicle in the center of a local equivalent of the central stress response. In addition the hair follicle is intimately connected to the central nervous system through its dense and complex innervation. Through this connection a third stress-axis employing neuropeptides and neurotrophins can mediate centrally perceived stress-stimuli and directly affect hair growth and cutaneous immunity. The net effect results in increased hair loss after acute stress-exposure. Thus the hair follicle is a true mirror of our emotional well being, the amount of experienced stress and our capacity to cope with them. Future research efforts will focus on the analysis of stress-habituation and “anti-stress” hormones and their role in hair follicle biology and treatment of hair disease.

O15: European Consensus on the Evaluation of Women Presenting with Excessive Hair Growth

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Key words: Hirsutism, diagnosis, evaluation, consensus

Hirsutism is defined as excessive terminal hair that appears in a male (androgenic) pattern in women. It is difficult to diagnose because women present in a myriad of different ways and various countries have differing routes and strategies for their diagnostic work up and management. The Skin Academy is led by an interdisciplinary group of leading dermatological and endocrinological experts who use the latest scientific and clinical data to promote awareness, education and best clinical practice. They met to develop guidance for the evaluation of hirsutism in premenopausal women. Consensus was guided by systematic review and discussion of current clinical practice across Europe during several group meetings, supported by conference calls, and e-mail communications. The result is a Diagnostic Evaluation Form – a series of questions aimed at helping the clinician to evaluate a woman presenting with excessive facial hair. The Evaluation Form is comprised of three sections: (i) History (age, speed of onset, medication, ethnicity, family history, menstrual cycle, hyperandrogenism, galactorrhoea), (ii) clinical examination (distribution of hair, dermatological examination, acanthosis nigricans, polycystic ovary disease, BMI, Ferriman-Gallwey score), and (iii) investigations (thyroid function, testosterone and Free Androgen Index, prolactin level, ultrasound of pelvis). Each section is split into sub-sections, allowing the clinician to address each factor in turn. The rationale for the sections and

sub-sections of the Diagnostic Evaluation Form is explained and allows the clinician to address each factor in turn.

O16: Regulation of Melanogenesis by GDNF and Neurturin as New Candidates in Human Follicular DPC

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Key words: Hair, dermal papilla cells, glial cell line-derived neurotrophic factor, melanogenesis

Hair and skin colour mostly depend on the proper activity of melanogenic melanocytes. New candidates were reported from both black and white haired dermal papilla cells (DPCs) of the human scalp by cytokine array. These are glial cell line-derived neurotrophic factor (GDNF), and its family member, neurturin (NTN). These are detected more in DPCs from black haired follicles versus white haired follicles. We discovered that the comparative expressions of GDNF and NTN and their cognate receptors were decreased in white haired follicles *in vitro*. We examined a pivotal role of melanogenesis by GDNF and their family members, neurturin (NTN) in murine melanoma cell line, B16F10. Our study confirmed that GDNF and NTN have a great melanogenic potential. Cells were serum-starved for 24 hr, and replenished with GDNF or NTN for indicated times. The samples were harvested and analyzed by zymography for tyrosinase activity and by spectrophotometry for melanin content measurement. The expressions of melanocyte-specific proteins including MITF (microphthalmia transcription factor), TYR (tyrosinase), and TRP1 (tyrosinase-related protein 1) increased by GDNF and NTN treatment. By contrast, there was no change in the expression of DCT (tyrosinase-related protein 2) by treating both molecules. The proliferation of melanocyte was also augmented by GDNF and NTN. Our preliminary findings suggest that hair follicle melanogenesis may be regulated by GDNF and NTN acting via MITF and TYR promoter.

O17: Human Hair Growth Modulation by Erythropoietin

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Key words: Erythropoietin, erythropoietin receptor, hair follicle, dermal papilla cell

The glycoprotein hormone erythropoietin (EPO) is known as a key regulator for erythrocyte precursor cells by stimulating the

proliferation and differentiation. Recently, it is reported that human hair follicles are an extrarenal source and a nonhematopoietic target of EPO. It is also demonstrated that dermal papilla cells (DPC) in hair follicle respond to EPO as examined by Stat5 activation status using *in vivo* response-based identification of direct EPO target cell populations. However, expression of EPO-R in human DPC has not been reported and role of EPO in hair growth is not clear. Therefore, we here examined the expression of EPO-R in human hair follicles and investigated the effects of EPO on human hair growth. Immunofluorescence staining showed EPO-R expression in DP of human hair follicles. We also detected EPO-R expression in cultured DPC by RT-PCR analysis and immunoblot. In addition, EPO promoted elongation of hair shafts with increased the proliferation of matrix keratinocytes in cultured hair follicles. Our data suggest that EPO promotes human hair growth by regulating activity of DPC.

O18: Psychosocial Aspects of Hairloss

Uwe Gieler, Germany

O19: Psychocutaneous Disorders of the Hair and Scalp

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Many patients with a hair or scalp disorder have psychological issues associated with their chief complaint. To handle cases of disproportionate emotional overtones or imaginary hair loss, abnormal scalp sensations, and self-induced injury to the hair or scalp, the following steps are substantial: (1) Learn to classify and diagnose psychocutaneous disorders of the hair and scalp. Most can be grouped into: *psychophysiological disorders*, in which the scalp disorder is exacerbated by emotional factors, *primary psychiatric disorders*, in which there is no real skin condition, but all symptoms are self-induced or delusional, *cutaneous sensory disorders*, in which the patient has abnormal sensations of the scalp with no primary dermatologic lesions or internal medical condition responsible for the sensations, and *secondary psychiatric disorders*, in which patients develop emotional problems as a result of hair loss. (2) Become familiar with the therapeutic options available, non-pharmacologic and psychopharmacologic. Moreover, the best way to alleviate the emotional distress caused by a hair disorder is to effectively treat it. The intensity of the distress that the patient expresses should influence the clinician's decision to treat the hair disorder. (3) Recognize the limits of what can be accomplished in a dermatologic practice. If treatment with psychopharmacologic agents is considered, the selection of appropriate agents is dictated by the nature of the underlying psychopathologies. (4) Finally, optimize the working relationship with the psychiatrist, since dermatologists and psychiatrists tend to have different perspectives in analysing a clinical situation,

different styles of communication, and different approaches to management.

O20: Psychosocial Stress and Alopecia Areata: A Survey in 45 Alopecia Areata Patients

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Psychosocial stress may be able to trigger and maintain episodes of Alopecia Areata (AA) via psychoneuroimmunological pathways. In this study stress perception, stress regulation, body image, and coping strategies used in AA were assessed to derive psychological interventions.

Half-standardized interviews were performed among 45 AA patients, covering stress experiences before the onset of AA and at the time of the interview, stress-reactivity of AA, subjective disease models, consequences of AA and illness-related coping strategies. Demographic and AA-specific data, measurements of general coping strategies and body image were conducted additionally. Six months later the status of disease was assessed via telephone interviews.

AA patients did not differ from the normative sample in their general coping and body image. However, beneficial coping strategies and a positive body image could be linked to less stress-reactivity and better coping with AA. Participants experienced high stress levels before the onset of disease. They described their lives less positively and in terms of more illness, daily hassels, life changes, and emotional distress. Psychosocial factors were described as triggers in the onset and course of AA by 73% of patients. Their disease models influenced coping and were highly correlated with stress-reactivity. Study results also demonstrate the high impact of AA on patients' lives.

Results point at several psychological aspects in AA that should be addressed by medical professionals. Even when psychosocial stressors play a minor role in triggering AA, patients could benefit from psychological interventions comprising general and AA-specific coping strategies and techniques of regulating their emotions.

O21: Psychological State and Quality of Life in Patients with Hair Loss

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Key words: Hair loss, androgenetic alopecia, alopecia areata, anxiety, depression, quality of life

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Hair loss is a common disorder, and affects the image, social intercourse and psychological experience. To investigate the anxiety, depression and quality of life (QoL) in patients with hair loss, we compared 437 patients (331 males and 106 females) with androgenetic alopecia (AGA), 178 patients (85 males and 93 females) with alopecia areata (AA), and 434 healthy subjects (327 males and 107 females), and the degree of hair loss was mainly moderate. The forms of Self-Rating Anxiety Scale (SAS), Self-Rating Depression Scale (SDS) and Dermatology Life Quality Index (DLQI) were finished. The scores of SAS in AGA and AA groups were higher than that in healthy subjects ($P < 0.05$), however, there was no significant difference between AGA and AA ($P > 0.05$). There were no significant differences of the scores of SDS between three groups ($P > 0.05$). The scores of DLQI in AGA and AA groups were higher than that in healthy subjects ($P < 0.05$), and the scores of DLQI in AA group was higher than that in AGA group ($P < 0.05$). All the scores of female patients were higher than that of male patients. In conclusion, patients with hair loss could be in anxious state, the depression maybe slight. The QoL in patients with hair loss was affected, which was worse in AA patients than that in AGA patients. Female patients were affected more severely than male patients in anxiety, depression and QoL.

O22: Classification Criteria of Cicatricial Alopecia

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The cicatricial alopecias represent both a diagnostic and a therapeutic challenge to the practitioner. They encompass a diverse group of disorders characterized by irreversible hair loss due to permanent destruction of the hair follicle. The loss of follicular orifices in an area of alopecia points to a permanent loss of hair. Where there is no obvious physical/chemical injury or acute infectious etiology, clinical differential diagnosis is often difficult. In all cases microbiological studies and a scalp biopsy are indicated. Microbiological studies will identify fungal (tinea capitis), superficial (ostiofolliculitis) or persistent deep bacterial infections (folliculitis decalvans). In all other cases, the cause remains largely unknown. On the basis of histopathology, primary and secondary scarring alopecia are differentiated: While the former is due to preferential destruction of the follicle, the latter results from events outside the follicle, which eventually impinge upon and eradicate the follicle. These include infiltrative processes such as granulomatous inflammation or neoplastic disease. In the group of primary scarring alopecia, well-defined chronic-inflammatory diseases of the scalp are differentiated microscopically on the basis of the type of inflammatory cell that predominates (lymphocytic in lupus erythematosus, lichen planopilaris, and pseudopelade Brocq; neutrophilic in folliculitis decalvans, dissecting cellulitis, and

tinea capitis; or mixed in acne miliaris necrotica, acne keloidalis, and erosive pustular dermatosis) and the pattern of inflammation. Although clinicopathologic features allow for accurate diagnosis in many cases, etiopathologic certainty is often elusive and therapeutic limits reflect the boundaries of our present understanding. With the expanding knowledge of the immunology and molecular biology of the hair follicle, there is hope for a better understanding of the underlying etiologies and pathogenesis especially of the primary scarring alopecias and therapeutic interventions that interfere early in the course of the pathogenic processes ultimately leading to the permanent loss of hair.

O23: Management of Scarring Alopecia

Pascal Reygane, France

O24: Microarray Analysis of Lichen Planopilaris and Pseudopelade of Brocq Suggest they are Distinct Entities

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Key words: Scarring alopecia, lichen planopilaris, pseudopelade of brocq

Pseudopelade of Brocq (PPB) and lichen planopilaris (LPP) are the most common primary cicatricial (scarring) alopecias encountered. Because these diagnoses share so many signs and symptoms, their distinct nosological identities have been questioned. The purpose of this study was to identify how similar PPB and LPP are by gene expression profiling. Alopecia affected, and clinically normal haired, scalp tissue biopsies were obtained from 8 untreated patients diagnosed clinically and histologically with PPB or LPP. Microarray analysis, using a 21K expanded sequence verified cDNA set was performed and analysed by Significance Analysis of Microarrays (SAM). Selected genes were further examined using quantitative PCR (qPCR) and immunohistology. Both LPP and PPB exhibited significant differential expression profiles compared to intra-control scalp skin. Genes associated with negative regulation of cellular and physiological processes, response to DNA damage stimulus, and cell death were activated in both LPP and PPB. Genes involved in chemotaxis and inflammatory response, were uniquely identified in LPP as compared to PPB. The qPCR results from 3 selected genes (MMP11, TNFSF13B, APOL2) showed increased expression in LPP compared to PPB and MMP11 expression was confirmed in disease affected hair follicles by immunohistology. Though PPB is regarded by some as the end stage of LPP, we found relatively little evidence to support this view. While common gene function categories are represented in LPP and PPB, the

results indicate they each exhibit distinct, active gene expression profiles suggesting they have unique biological identities. This information may help to establish treatment protocols for these diseases.

O25: Alopecia Challenging Clinicians and Dermatopathologists: Alopecia Under TNF- alpha blockers: The Spectrum of Changes

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The TNF-alpha blockers infliximab and adalimumab have gained wide acceptance in the treatment of inflammatory bowel's disease, rheumatoid and psoriatic arthritis, active ankylosing spondylitis and moderate to severe psoriasis.

We describe five women with an age range from 19 to 37 years. Three patients had Crohn's disease and one was suffering from ankylosing spondylitis. TNF-alpha blockers were administered because previous therapy regimens did not show significant clinical results of the underlying disorder. Four patients developed scalp psoriasis being associated with a dramatic effluvium involving most parts of the scalp in three cases. The fourth case had concomitant alopecia areata and scalp psoriasis. Psoriasiform scalp alopecia developed de-novo in three patients and was associated with palmoplantar pustulosis in two cases and psoriasis of the trunk in one. One patient suffered from an acute exacerbation of known scalp psoriasis. After withdrawal of the biologic there was complete regrowth of hairs in three patients. One case, however, developed a scarring alopecia. The last patient revealed a diffuse non-scarring alopecia, being consistent with a diagnosis of telogen effluvium.

Apart from new-onset psoriasis infliximab and adalimumab may induce dramatic hair loss with severe, potentially permanent alopecia. We observed a spectrum of changes, including alopecia areata, telogen effluvium and psoriasiform scalp alopecia. Because hair loss was permanent and scarring in one patient we advocate rapid withdrawal of TNF-alpha blockers in case of alopecia.

O26: The Spectrum of Alopecia Areata

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Alopecia areata (AA) has always been considered a distinctive

homogeneous clinical entity with a classical presentation and autoimmune pathogenesis. There is, however, the definite possibility that other conditions of hair loss are either pathogenetically related to AA or even different clinical presentations of the same disorder, which, therefore, would carry its name illegitimately. In fact, why AA is "areata", that is patchy, is unclear, and, on this basis, one may wonder why it cannot present clinically in a different way.

1. Alopecia areata incognita (AAI)
2. Marie Antoinette syndrome (MAS)
3. Telogen effluvium (TE)
4. Loose anagen syndrome (LAS)

LAS, TE and AA present in a very different way. while TE and AAI are very similar in that the latter appears clinically as a severe form of TE. MAS is so rare as to prevent a thorough investigation. In addition, the age of onset is different. Young children in LAS, youngsters in AA and adults in TE and AAI. The classical marker of AA, dystrophic anagen hairs, can be found mostly at the periphery of the area. In TE and AAI, they can be found occasionally and only if they are looked for. It is practically impossible to come across one of them with methods other than MWT, in particular with trichogram or scalp biopsy. Histopathology as well, is unlikely to give more information for the same reason. If guided by dermoscopy, it may prove to be more helpful. The nature of the four conditions remains unclear, but several elements point to an inflammatory one, in particular, trichodynia (possibly due to peribulbar inflammation), yellow dots and dystrophic anagen hairs. In addition, the relevance of stressful episodes preceding most of the diseases and most of autoimmune diseases, and the common finding of anti-thyroperoxidase antibodies in TE all suggests that the inflammation is autoimmune in nature.

Additional studies are clearly needed to understand whether at least TE, AAI and MAS belong to the same autoimmune disease we dub, in such a case illegitimately, AA, just because of its patchy presentation.

O27: A Case Report of Alopecia Universalis with Co-existence of a Scarring Alopecia

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Key words: Alopecia universalis, scarring alopecia, xerosis, AD-like skin lesion.

We report a 35-year-old woman with a history of hair loss for 3 years and multiple nodules in the crown area for the last 3 months before visited us in September 2008. The hair loss

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started patchy and progressed gradually into alopecia totalis in a period of two years, twice recovered by steroid administration. In the last 3 months, she had painless nodules on scalp in the crown area. Lesions increased with time and some of them erupt spontaneously with discharge of blood. She had regular menses and otherwise healthy. The patient never grows axillar and pubic hair since puberty.

Physical examination revealed no significant positive findings in her body systems. The hair loss involves scalp and the whole body area. Multiple purplish colored soft nodules were found in the crown area. In addition, xerosis involving most of her body was noticed, especially on her back and flexor aspect of limbs, with multiple scaly erythema and AD-like lesions.

Lab tests were unremarkable except for a high eosinophils count. Histopathologic findings of a scalp nodule revealed epidermal atrophy, follicular plug and marked dense perifollicular and perivascular infiltration with mainly CD3+ lymphocytes and eosinophils, in the upper dermis. Density of follicle unit was slightly decreased and most of hair follicles were in telogen stage.

The tentative diagnosis is alopecia universalis with a coexistence of scarring alopecia, possibly folliculitis decalvans. Graham-Little syndrome and Keratosis follicularis spinulosa decalvans were also considered for differential diagnosis. A treatment regime of anti-bacterial and retinoid was initiated with a positive feedback, but the patient was soon lost contact in follow-up.

O28: Alopecic and Aseptic Nodules of the Scalp: A Clinicopathological Study of 10 Cases

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Key words: Alopecic nodule scalp, nonscarring alopecia

Alopecic and aseptic nodules of the scalp (AANS) is a new entity described in Japan in 1992 as pseudocyst of the scalp. We report 10 cases of AANS observed at our hair clinic in Paris in 2008.

The main age was 32 years. The majority (9/10) were male. 8 were Caucasians and 2 blacks. The symptoms when present (8/10) were mild: pain, discomfort. The number of nodules ranged from 1 to 3. The sized varied from 5 to 40 mm. The main site (8/10) was the upper part of occipital area. The alopecia associated with the nodules was nonscarring in all the patients. 9 patients had a puncture, always negative. The histopathological features were characterized by inflammatory infiltrates located in reticular and deep dermis. 4 patients had a non specific lymphohistiocytic inflammation. In one patient we found a picture of an inflamed infundibular cyst. We never found pseudocyst formation. Nine

patients were treated with doxycycline (100 mg/day). After one month 6 out of 8 patients became asymptomatic. The nodules resolved partially in all the patients. A partial regrowth occurred in 5 patients.

AANS is a rare and probably unrecognized entity reported only in Japan and France (1, 2). In our patients, doxycycline (100mg/day) was effective. The etiology of AANS is unknown. It is probably a particular form of deep folliculitis. Future research studies are needed to determine whether AANS is caused by local factors (follicular occlusion,^[1] foreign body) or is mediated by immune process.^[2]

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O29: Finasteride (1 mg/d) Induced Gynecomastia

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Key words: Finasteride, androgenetic alopecia, gynecomastia

Finasteride, 1 mg/d (Propecia[®]; MSD), a type-II 5 α -reductase inhibitor, is the only approved treatment prescribed at present for androgenetic alopecia. Although enjoying a relatively good safety profile, several sex-related adverse effects have been reported with this drug, e.g. decreased libido, erectile dysfunction and decreased ejaculation volume. In addition, gynecomastia is a well recognized side effect of finasteride in the dose of 5 mg/d. Here, we report two cases of gynecomastia caused by Propecia[®]. A 21-year-old male developed bilateral gynecomastia four months after finasteride initiation, with no apparent improvement ten months after drug cessation. The second patient is a 65-year-old male who developed unilateral gynecomastia following two months of treatment. Six years after treatment termination residual breast swelling was still present. While being a benign condition, gynecomastia may cause substantial embarrassment as well as anxiety and discomfort in the affected patient. This side effect of finasteride can present either as unilateral or bilateral gynecomastia, and usually develops relatively late after treatment initiation. Improvement may be prolonged, and residual symptoms may still be apparent years after stopping the drug. This side effect seems more common and meaningful than previously reported, and should be emphasized when administering this drug for the treatment of androgenetic alopecia. New studies are warranted in order to assess the real incidence of this side effect.

O30: Targeting Follicular Antigen Presenting Cells With Nanoparticles

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Key words: Follicular targeting, langerhans cell, vaccine

Skin antigen presenting cells (APCs) play an important role in the context of transepidermal vaccination strategies. Recently, we have shown that these cells can be selectively targeted using nanoparticles (NPs). In this study we investigated the penetration of NPs in vellus hair follicles (VHFs) of human skin explants and their uptake by Langerhans cells (LCs). Two types of fluorescent NPs have been compared: poly-styrene (PS) particles and biodegradable poly-lactic-acid (PLA) particles loaded with the HIV p24 peptide. The follicular penetration profiles showed that both particle types penetrate and accumulate in more than 50% of all observed VHFs. In a significant percentage of VHF (15-30%) they were observed up to the entrance of the sebaceous gland. Both nanoparticles could be taken-up by isolated LCs in *in vitro* conditions. However, only PS nanoparticles were detected in LCs after topical application of NPs on freshly excised human skin. The different uptake of NPs in our ex-vivo experiments is possibly due to the fact that PLA NPs had the tendency to form irreversible aggregates upon contact with the lipophilic environment of the skin while PS NPs showed to be more stable, probably forming only reversible aggregates. These results show that the physicochemical nature of nanoparticles plays an important role with regard to their stability in physiological non-aqueous compartments like skin. The understanding of the principle governing the stability of NPs upon contact with the skin will open the possibility to design efficient and selective carriers systems for follicular targeting and transepidermal drug delivery.

O31: Dermoscopy in Hair Diseases

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Recently, the use of dermoscopy has demonstrated to be a novel and important tool that improves diagnostic accuracy in the evaluation of patients with scalp and hair disorders.

For scalp examination, dermatologists can use a manual dermoscope ($\times 10$ magnification) or a videodermoscope equipped with various lenses (from $\times 20$ to $\times 1000$ magnification). Dermoscopy findings include vascular patterns, follicular and perifollicular signs and hair shaft characteristics.

Examination of the normal scalp can show simple fine red loops that represent capillary loops in the dermal papilla. In dark skinned individuals, a perifollicular pigmented network (honeycomb pattern) is well appreciated.

In scalp psoriasis, within the typical scaly plaques, twisted red loops are observed. Twisted loops are also seen to a limited extent in unaffected psoriatic scalp as well as in newly treated psoriatic scalp. In seborrheic dermatitis, arborizing red lines, which have a wider caliber than the loops, can be observed.

In alopecia areata, yellow dots are very characteristic. These dots represent follicular openings filled with keratinous debris mixed with sebum. They are often associated with classic findings of active disease as dystrophic hair, exclamation mark hairs and cadaverized hairs. Dermoscopy findings are important to differentiate alopecia areata from trichotillomania where hairs are broken at different distances from the scalp and longitudinal splitting of hair shafts can also be seen.

Androgenetic alopecia is characterized by hair diameter diversity due to miniaturization of the hair follicles. Variability in hair shafts diameter of more than 20% is diagnostic of this condition.

O32: Reproducibility of Hamilton-Norwood Classification

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Hamilton-Norwood scale (HNS) has been largely used to assess clinically the severity of androgenetic alopecia (AGA) especially in clinical trials and in epidemiological studies for the association with other diseases. We studied HNS reproducibility showing to dermatologists and dermatology residents photographs of male heads with different degrees of AGA. In an other study, some appraisers examined the same pictures of male heads with AGA in two different repeated occasions. In the first study, the concordance of appraisers vs external expert was fair as it was among appraisers. The concordance of dermatologists vs the external expert was also fair as it was the concordance of the residents vs the external expert. In the second study, the first and the second observations were concordant in only half of the appraisers. The reliability of HNS is only fair. To obtain a good reliability, the number of classes of the HNS may be reduced to less figures.

O33: The Use of TrichoScan in a CRO Setting: Some Practical Aspects and Experiences

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Key words: Androgenetic alopecia, methodology, TrichoScan, clinical trial

During recent years we have conducted 6 efficacy trials in approximately 600 male and female subjects with androgenetic alopecia using TrichoScan, a fully-automated method for measurement of hair growth parameters such as density, diameter and linear growth rate. All studies were either single- or two-center and were conducted by our clinical staff in our own clinical units.

In clinical investigations of hair growth it is important that the study procedures do not provide unnecessary hardships for the subjects. We have not experienced difficulties with recruitment due to the TrichoScan procedure, even shaving of a small area (approximately 2 cm²) is acceptable.

As with any photo analysis method, only technically correct images can deliver good results. Approximately three hours training were required per investigator/technician for the TrichoScan procedure, including instruction in quality control of images. Staff must be able to recognize dye remnants, unfocused images and air bubbles in the image. Importantly, there is a high reproducibility of images between trained investigators.

Practicality is important in routine use of a method by a CRO. In our experience TrichoScan images from up to 20 subjects/day can be comfortably made by one investigator/technician. The relatively small size and low weight of the TrichoScan camera are significant factors allowing frequent use without excessive tiring.

Most importantly, the results obtained with TrichoScan have proved reliable. E.g., using Minoxidil as positive control it was possible to show benefits already following 8 weeks of treatment.

O34: Shedding Light on Exogen

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Key words: Exogen, hair cycle

A decade ago, club fibre shedding was introduced as its own distinct phase into the hair cycle literature. Given the term 'exogen', it was proposed to be an independent phase of the hair cycle, with its own set of molecular and cellular cues controlling retention of the club fibre and active release. As a fundamental component of the hair cycle, exogen not only marks the termination of the life of a hair fibre, but also encompasses signaling and structural changes preceding the shedding of the fibre. However, relatively few studies have been carried out which focus on this phase of the hair cycle, and there is little insight into the signals which are

believed to regulate this process. Here, we use the vibrissa follicle as a model system in which to analyse the cycle phase exogen. Follicle cycling of vibrissae follicles is well defined, and the timing of hair shedding can be accurately predicted. We exploited this observation and carried out profiling of the outer root sheath cells surrounding the club fibre at different stages as the club fibre approached shedding. We observed a progressive decrease in cellular adhesion surrounding the club fibre as it approached final release. In addition, transcriptional profiling enabled us to compile a molecular signature of exogen, which we believe will shed light on the processes regulating this cycle phase.

O35: Don't Look for the Hair Cycle, It Doesn't Exist

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Key words: Hair cycle, bistable equilibrium

For decades, the hair cycle and its control have been an object of debate. Clearly, the hair follicle is the only organ in mammals, which "cyclically" degenerates and regenerates from stem cells, and the understanding of such a unique behavior would certainly give clues to tissue homeostasis and regeneration. A number of factors have been identified which can modulate this process, but its choreography remains elusive. For years, the hunt for the conductor has been on, but nobody ever caught him. Intuitively, the process being considered as cyclic, an automaton controlling this cycle should be looked for, by analogy with a clock. A famous paper evidenced the failure in this task^[1] and the question remains: Why is-it so difficult to identify and characterize this oscillator? My answer is simple: It simply does not exist. We have revealed that each follicle has an autonomous stochastic behavior, the probability of duration of each phase fitting with a lognormal equation.^[2] From this analysis, one can conclude that instead of a cyclical behavior with an intrinsic automaton, the hair follicle has a chaotic behavior. This new concept postulates the existence of a bi-stable equilibrium which controls hair follicle behavior, which under a stochastic way jumps from the dormant to the active branch of the equilibrium and vice-versa.

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O36: The Hair Growth-Promoting Effects of Adipose Tissue-Derived Stem Cells

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Key words: Adipose tissue-derived stem cells, hair growth

Mesenchymal stem cells within the stromal-vascular fraction of the subcutaneous adipose tissue (adipose tissue-derived stem cells [ADSCs]), display multi-lineage developmental plasticity and share similar characteristics with bone marrow-derived stem cells (BM-MSCs). In addition, ADSCs are known to have various cytokine-secreting properties and to have beneficial paracrine effects on surrounding cells or tissues. To determine whether these paracrine effects of ADSCs can promote hair growth, we investigated the effects of ADSC-conditioned media (CM) on hair growth. The ADSC-CM was harvested and evaluated for the stimulatory effects on hair growth. ADSC-CM increased the proliferation of dermal papilla cells (DPCs) and HaCaT cells. In a cell cycle analysis, ADSC-CM shortened G1 arrest and prolonged the synthesis and mitosis phases in DPCs. ADSCCM induced the anagen phase and promoted hair growth in a C3H mouse model and enhanced the elongation of hair shafts in ex vivo human organ cultures. The results presented in this study suggest that ADSCs promote hair growth by increasing the proliferation of DPCs, and possibly epithelial cells, through modulation of the cell cycle, and activating the anagen phase in hair cycles. Therefore, the rational manipulation of ADSCs might be a promising tool for hair growth promotion.

O37: Regulatory T cells and Th17 Might Have a Role in the Pathogenesis of Chronic Alopecia Areata

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Key words: Alopecia areata, regulatory T, T helper 17

AA is characterized by a peri- and intrafollicular infiltration of CD4+ and CD8+ T lymphocytes. There is also a suggested role for cytokines in the hair loss observed in AA. However, little is known about the role of Th17 and regulatory T cells in the pathogenesis of chronic AA. The objectives were to investigate the role of the major cytokines produced by Th1, Th2, Th17 and regulatory T cells in the pathogenesis of chronic AA. Seven patients who suffered from AA over 2 years and 7 age- and sex matched

healthy control subjects were enrolled. Serum and lesional tissue samples were obtained and prepared for real time RT-PCR. INF- γ , TNF- β , IL-4, IL-5, IL-13, IL-6, IL-17, IL-10 and TGF- β were compared according to sample origin. A distinct pattern of gene expression was seen from chronic AA tissue compared to those that of normal healthy controls. The mRNA expressions of lesional and serum TGF- β were highly down-regulated (13.71 fold). INF- γ was dramatically up-regulated in the AA lesion compared to controls, conversely, that of serum sample was down-regulated. Lesional and serum IL-17 mRNA expression levels were highly upregulated (7.43 and 6.23, respectively) and its expression patterns were positively correlated with the extent of AA. These results reveal some putative candidates that participate in the cellular immunity of AA. Diminished TGF- β may contribute to the impairment of regT cell function in AA. Increased expression of IL-17 proposes some pivotal role of Th17 cell in the pathogenesis of AA.

O38: A Role of HGF and MSP in Human Hair Growth (Are HGF & MSP and their receptors (c-Met & RON) expressed in human hair follicles)

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Key words: Human hair follicles, androgens, androgenetic alopecia, HGF, MSP, c-Met, RON, RT-PCR, dermal papilla, dermal sheath, hair matrix.

Androgens are main modulator of human hair growth. Paradoxically, androgens can stimulate hair growth in the beard and in hirsutism and can also inhibit scalp follicles causing androgenetic alopecia. Androgens exert their effects on the follicular dermal papilla via the altering the regulatory factors produced by the dermal papilla. Since members of the hepatocyte growth factor family, hepatocyte growth factor (HGF) and macrophage stimulating protein (MSP) are implicated in hair growth, these paracrine factors are candidates for androgen regulated expression. The aims of this study were to identify whether these factors were expressed by components of human hair follicles and whether their expression was altered in balding. Scalp skin from healthy individuals undergoing cosmetic surgery was transported in RNA stabilization reagent RNA later for RT-PCR and media for cell culture. Gene expression of MSP and its receptor RON, and HGF and its receptor c-Met were investigated in cDNA from individual hair follicle, hair follicle bulb components and balding scalp hair follicles. Results showed that scalp hair follicles from 5 individuals expressed the genes for MSP, RON, HGF and c-Met. Gene identity was confirmed by sequencing. When bulb components were isolated from 3 individuals separately; dermal papilla and dermal sheath, both expressed MSP, RON, HGF, c-Met. In contrast, the epithelial

bulb matrix only expressed MSP and RON. In isolated hair follicles from balding, no MSP could be detected. HGF was also expressed by cultured dermal papilla cells (n=5) but those from balding scalp only exhibited low expression. Thus, human hair follicles express the genes and receptors for 2 members of the HGF family. The dermal papilla and sheath of normal follicles express both the paracrine factors and their receptors, while the matrix cells only produce MSP and RON. Balding follicles dermal papilla cells express less MSP and HGF suggesting these paracrine factors are involved in producing large hair follicles. Increasing the levels of these factors could stimulate hair growth in androgenetic alopecia.

O39: The Nature, Extent and Boundaries of Trichology

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Key words: Institute of Trichologists, hair loss cures, exploitation, diagnosis patient care

The Institute of Trichologists (IOT) was founded in 1902 by a group of Doctors and Scientists who realised the omission in the medical curriculum of the study of the scalp and hair.

Trichology, the science of the scalp and hair, has been taught by the Institute of Trichologists since 1902. We are recognised as the authoritative awarding body.

Latterly, various commercially minded people and companies have proliferated offering scalp and hair treatments with little or no knowledge of the subject, thus causing a deluge of “hair loss cures” to flood the market.

Those extravagant claims may possibly cause misunderstanding of the role, aims and objectives of the IOT and its’ Members within the medical profession.

We are not medically qualified, however our comprehensive training includes anatomy and physiology and other essential related sciences. We practice within clearly defined parameters.

The IOT is consulted and asked for help on a daily basis to deal with issues of disturbing exploitation of the public by the unscrupulous and untrained.

It is the IOT’s wish to explain our standpoint against the jungle of confusing and extravagant information and misleading advertising and explain how we protect the public.

Diagnosis is the key word in trichology, patient care is our

objective. Updating ourselves with current research and our liaison with medical and other health care professionals is essential to the Trichologist and the care of the patient.

O40: The Wilhelmy Force Profile of Human Hair as a Chronobiological Record

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Key words: Human hair, chronobiology, circadian rhythms, Wilhelmy force, wettability, Fourier analysis, principal components analysis

The natural surface of human hair is hydrophobic, consisting of a bilayer of heavily cross-linked proteins towards the cell inside combined with a layer of fatty acid (F-layer) to the outside. The properties of this surface were investigated by determining the Wilhelmy wetting force along hairs from adult females in water for segments, equivalent to about one month of growth (approx. 1cm). Using linear regression and data smoothing the Wilhelmy-force profile for a segment is isolated. Fourier analysis for sub-segments, representing about 1 week’s growth (2mm), showed in the individual periodograms cyclic events, which were consistent with multiple circadian rhythms, such as a wake- and a sleep-cycle. However, the differentiation of the effects vanished when averaging periodograms, thus showing the presence of a strong stochastic component in the signal. To further explore components underlying the Fourier spectrum, they were subjected to Principal Components Analysis (PCA). Three factors were consistently identified as underlying the Wilhelmy-profiles, proving the pronounced non-stochastic nature of the curves. The factor value curves showed clear maxima for spatial wavelengths associated with the expected diurnal events. Though the current data are limited, we furthermore observed consistently, largely bimodal, monthly changes that can be linked to the menstrual cycle. We hence put forward the hypothesis that the hair surface preserves a rather detailed, individual chronobiological record “written” by the status of the cell membrane between cuticle and inner-root sheath prior to apoptosis.

O41: Molecular Cloning of Cytochrome P450 Genes from *Malassezia globosa*, a Dandruff-Causing Yeast

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Key words: *Malassezia globosa*, cytochrome P450 genes, molecular cloning

The genus *Malassezia* are ubiquitous skin residents of humans and other warm-blooded animals. They are responsible for many skin disorders including dandruff, seborrheic dermatitis, malassezia folliculitis and pityriasis versicolor. Despite their widespread occurrence and association with multiple common skin disorders, remarkably little is known about these fungi.

More than 7 species of *Malassezia* are known -and among them, *Malassezia globosa* is one of the most common in isolated yeasts from human scalp. Recently, total genomic studies of *M. globosa* CBS 7966 were completed and the full genomic information

was revealed in 2007. To understand the pathophysiology of *Malassezia* in dandruff, we have focused on *M. globosa* P450s which may be involved in biosynthesis of ergosterols. To determine activities and functions of *Malassezia* P450 enzymes, we isolated four P450 putative genes from *M. globosa* genomic DNA for the first time.

This analysis may be helpful to understand pathophysiology of *Malassezia* species in the human skin diseases and valuable to develop specific antifungal agents against *Malassezia*. This study was supported by a grant of the Korea Health technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (A080065).