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Nanomechanical properties of collagen VII anchoring fibrils in recessive dystrophic epidermolysis bullosa patients

B Mayer, P Silló, M Mazán, D Haluszka, MZ Kellermayer and S Kárpáti *Semmelweis University, Budapest, Hungary*

Our aim was to apply novel nanomechanical tests to investigate the biophysical properties of collagen VII anchoring fibrils in skin sections in a healthy control and in two recessive dystrophic epidermolysis bullosa (RDEB) patients. One patient with localized RDEB was a compound heterozygote with a consequent R1957W change within the hinge region and a premature termination (Y2819*) within the acidic region of collagen VII a1 chain. The other was diagnosed with generalized severe RDEB, was homozygous and had truncated collagen VII a1 chains in the NC-1 domain (K142R). Sections of skin biopsies from patient and control were investigated with atomic force microscopy (AFM) combined with fluorescence imaging. In the localized RDEB patient and healthy control, the anchoring fibrils were first immunostained, identified and scanned using AFM. In the generalized severe RDEB patient (no collagen VII staining), the dermal surface of the basement membrane zone (BMZ) adjacent to bulla formation was scanned. Nanoindentation was used to obtain force-volume maps and to characterize local viscoelasticity. Force-displacement curves were fitted with the Hertz model to calculate the local Young modulus. The control skin specimen could be partitioned into high-, intermediate- and low-Young-modulus areas that correspond to the dermis, BMZ and the epidermis, respectively. Remarkably, the dermis was stiffer than the epidermis by an order of magnitude. The region with intermediate Young modulus could not be clearly identified in the RDEB samples. The healthy skin may thus be seen as a highly compliant epidermis woven onto a stiff dermis. Hence, collagen VII plays an important role in coupling the mechanically distinct epidermis and dermis. The applied biophysical measurements enabled us to obtain clinically relevant data and may be used in the future to evaluate protein or gene therapies in RDEB patients.

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Improving diagnostic yield for filaggrin; hidden mutations in the Dutch population

M van Geel[1,2], CN van Oosterhoud[2], SA Ghesquiere[2], RR Brandts[2] and PM Steijlen[1] *1 Dermatology, Maastricht University Medical Centre, Maastricht, Netherlands and 2 Clinical Genetics, Maastricht University Medical Centre, Maastricht, Netherlands*

Molecular diagnostics with conventional Sanger sequencing for ichthyosis vulgaris (IV) has been hampered by the notoriously difficult to analyse filaggrin (*FLG*) gene, caused by its homologous and polymorphic repeated units. By implementation of single molecule molecular inversion probes (smMIPs) and next generation sequencing (NGS), an alternative screening strategy for analysis of the entire coding region of the *FLG* gene becomes feasible. Genetic analysis of the whole gene instead of screening for only population-specific mutations, would improve diagnostic yield by scrutinizing also for rare family-specific mutations. The smMIP-NGS strategy is easy to implement, affordable and since exclusion of NGS-duplicate-reads is possible, mutation-percentages can be related and assigned to polymorphic duplicated filaggrin-repeat-unit 8 and 10. In a cohort of previously screened Dutch patients (N=70) for only the population-specific mutations, retrospectively the whole *FLG* gene was analysed. Since all known mutations result in premature protein termination, focus of attention was on identifying nonsense and small insertion or deletion mutations. In several (8/70) of the screened patients additional novel truncating mutations were identified, elucidating their previously unexplained (more severe) clinical presentations. This study emphasises the need for screening the entire *FLG* gene for mutations, to improve the diagnostic yield in IV and identify hidden variants in the homologous repeated units of the gene. Herein, the smMIP-NGS method proves to be a reliable straightforward strategy to boost clinical diagnostics for IV.

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Targeting plasma pyrophosphate deficiency in mouse models of heritable ectopic mineralization disorders – PXE and GACI

Q Li and J Uitto *Dermatology and Cutaneuous Biology, Thomas Jefferson University, Philadelphia, PA*

Pathologic mineralization of the skin and vascular connective tissues is the hallmark of certain single gene heritable disorders. Pseudoxanthoma elasticum (PXE), caused in most cases by loss-of-function mutations in the *ABCC6* gene, serves as a prototype of heritable multi-system ectopic mineralization disorders with normal calcium and phosphate homeostasis. Generalized arterial calcification of infancy (GACI), caused in most cases by mutations in the *ENPP1* gene, has overlapping phenotypic features with PXE. However, it was recently shown that PXE and GACI in some cases can also be caused by mutations in either *ENPP1* or *ABCC6*, respectively. These observations suggested the possibility of shared pathomechanistic pathways for these conditions, and specifically, studies in these diseases and their corresponding mouse models revealed reduced plasma levels of inorganic pyrophosphate, a powerful inhibitor of mineralization. Consequently, the results suggested that insufficient inorganic pyrophosphate in the blood circulation underlies ectopic mineralization in both conditions. Towards development of treatment for these ectopic mineralization disorders, we have performed studies targeting plasma pyrophosphate deficiency through direct supplementation of inorganic pyrophosphate, or administration of stable, nonhydrolyzable pyrophosphate analogues, bisphosphonates, for treatment of these ectopic mineralization disorders in mouse models of PXE and GACI, *Abcc6-/-* and *Enpp1asj*. These approaches resulted in reduced ectopic mineralization in these mice, suggesting novel treatment modalities for these currently intractable diseases.

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Establishing a cost-effective strategy for diagnosis of inherited epidermolysis bullosa in resource-limited settings

VK Yenamandra[1,2] *1 Dermatology, All India Institute of Medical Sciences, New Delhi, India and 2 Dermatology, University Medical Centre Groningen, Groningen, Netherlands*

Accurately diagnosing the subtype of Epidermolysis Bullosa (EB) is critical for management and genetic counselling. Modern laboratory techniques are largely inaccessible in developing countries, where the diagnosis remains clinical and often inaccurate. In order to simplify the diagnostic strategy, we developed a novel diagnostic electronic tool using only clinical features and explored the potential of whole exome sequencing (WES) in identifying the mutational spectrum of EB. Thirty eight EB patients, aged 2 days to 24 years were included in this study. Whole-exome capture was performed using genomic DNA from each case of EB, followed by massively parallel sequencing. We also developed a novel diagnostic matrix indicating presence or absence of a set of distinctive clinical features that are diagnostically informative and covered the more prevalent EB subtypes. To test an individual patient, the presence or absence of these features was compared to the findings expected in each of the subtypes to see which corresponded the best. Finally matrix diagnoses were compared with genetic diagnoses for concordance. Overall, WES confirmed the genetic diagnosis in 30 patients (78.9%) that included mutations in KRT5 (1), KRT14 (3), LAMA3 (1), LAMB3 (4), ITGB4 (2), COL17A1 (1) and COL7A1 (18) genes. None of the variations were recurrent and 8 cases remained inconclusive. On comparison with the genetic diagnosis, the matrix has accurately identified the major type of EB in 92.5% cases ($p<0.001$) and sub-type in 75% cases ($p<0.001$). Discordance occurred in cases where diagnostic difficulty is well recognised. The developed electronic version was simple, quick and more user-friendly. In conclusion, WES is beneficial for identifying pathogenic variants in genetically heterogeneous diseases like EB for its cost effectiveness, quick turn-around times and better phenotype-genotype correlations. The clinical diagnostic matrix appears to be practical, valid and useful in diagnosing the type and sub-type of EB.

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Mechanistic insight into the repigmentation of piebaldism: Functional characterization of a mutant KIT in melanocyte regeneration

A Shimizu[1], M Hattori[1], D Oikawa[2], H Amano[1], A Ishida-Yamamoto[3], H Nakano[4], D Sawamura[4], K Wakamatsu[5], F Tokunaga[2] and O Ishikawa[1] *1 Dermatology, Gunma University, Maebashi, Japan, 2 Pathobiochemistry, Osaka City University, Osaka, Japan, 3 Dermatology, Asahikawa Medical University, Asahikawa, Japan, 4 Dermatology, Hirosaki University Graduate School of Medicine, Hirosaki, Japan and 5 Graduate School of Science and Technology, Gunma University, Kiryu, Japan*

Piebaldism is a pigmentary disorder characterized by white forelock and depigmented patches. Mutations of the *KIT* gene underlie the autosomal dominant trait of the disease. Piebaldism is generally stable throughout life, however, repigmentation occurs in some patients. We report a Japanese family with piebaldism in which the affected members showed spontaneous repigmentation. A genetic analysis revealed a heterozygous mutation, c.645_650delTGTGTC, in the *KIT* gene, which resulted in the deletion of Val216 and Ser217 in the extracellular region of KIT. Although melanocytes was absent in the depigmented skin of the proband's mother who had piebald skin, MITF-positive melanocyte stem cells were observed in the bulge region of the hairs in the mother's depigmented skin. The *KIT* mRNA extracted from leg hairs of mother's normal skin was decreased, however, both wild type (Wt) and mutant *KIT* mRNA were expressed. Immunofluorescence analyses demonstrated that the mutant KIT had accumulated in the endoplasmic reticulum, and was sparsely expressed on the cell surface when HEK293T cells were transfected with both Wt and mutant KIT. An immunoprecipitation analyses showed that the Wt and mutant KIT formed a heterodimer and was phosphorylated by SCF stimulation. Collectively, these results suggest that melanocyte stem cells were present in the white patches and that the mutant-KIT was expressed on the cell membrane and formed a heterodimer receptor with the Wt KIT that mediated the SCF signals for repigmentation.

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The effect of human mesenchymal stem cell therapy on in vitro model of alopecia areata

J Kim, Y Woo, J Oh, K Jeong and H Kang *Department of Dermatology, The Catholic University of Korea, St. Paul's Hospital, Seoul, Korea (the Republic of)*

Mesenchymal stem cell therapy has been illuminated as a new therapeutic strategy for immunologic disorders. Alopecia areata is a representative hair loss disorder by autoimmune mechanism. Patients with refractory alopecia areata have poor prognosis and there have been no proven effective treatment so far. Recently, there have been only few attempts to treat alopecia areata with mesenchymal stem cell therapy. However, their efficacy and mechanisms in treating alopecia areata is not known. We sought to investigate the therapeutic efficacy of human hematopoietic MSCs (hHMSCs) on in vitro model of alopecia areata and to explore relevant mechanisms that regulate their efficacy. Alopecia areata-like environment was induced in the human dermal papilla cells by pretreatment of IFN-γ. hHMSCs were administered to the human dermal papilla cells, and cell viability assay was determined. The change of expression of Wnt/β-catenin pathway and JAK-STAT pathway-related molecules, cytokines and growth factors in hHMSCs-treated dermal papilla cells was also examined by reverse transcription-PCR and western blot assay. Cell therapy with hHMSCs enhanced the cell viability of the human dermal papilla cells dose-dependently. hHMSCs activated several molecules in the Wnt/β-catenin signaling pathway including ß-catenin and phosphorylated GSK3b and decreased the expression of DKK1 in human dermal papilla cells. hHMSCs suppressed IFN-γ-induced expressions of caspase-1, 3, and IFN-γ receptor and reversed the phosphorylation of STAT1 and STAT5 increased by IFN-γ pretreatment in human dermal papilla cells. hHMSCs-treated dermal papilla cells also showed increased levels of several growth factors, especially FGF2. These data suggest that the cell therapy with hHMSCs may promote hair growth and recover the hair follicle-immune privilege by modulating Wnt/β-catenin pathway and JAK-STAT pathway and could be a new therapeutic option in treating alopecia areata.