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Unravelling the pathophysiology of PXE: Insights into ABCC6-mediated cellular ATP release

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Pseudoxanthoma elasticum (PXE) is an autosomal recessive disease characterized by progressive ectopic mineralization of the skin, eyes and arteries. Inactivating mutations in the gene encoding the hepatic efflux transporter ABCC6 underlie PXE. For long it was unknown why the absence of an efflux transporter in hepatocytes results in peripheral soft tissue calcification. We have recently demonstrated that ABCC6 is involved in the release of nucleoside triphosphates (NTPs), predominantly ATP, from hepatocytes. Outside the hepatocytes, but still within the vasculature of the liver, the ectonucleotidase ENPP1 converts the released ATP into AMP and inorganic pyrophosphate (PPi), a crucial inhibitor of soft tissue calcification. ABCC6 is responsible for the bulk of all PPi present in the blood circulation. PXE patients therefore have plasma PPi concentrations that are only 30-40% of those found in healthy individuals. These data now explain why absence of ABCC6 in the liver results in the calcification of soft peripheral tissues. Our data firmly link ABCC6 to the release of ATP from (liver) cells. This is an unusual function for an ABC transporter like ABCC6: Most ABC transporters use the energy released by intracellular ATP hydrolysis to transport specific substrates across membranes, often against steep concentration gradients. Several ABC proteins have been shown to transport cyclic nucleotides and nucleoside analogues, but not ATP. It is currently unclear how ABCC6 mediates NTP release, but we have developed a robust assay to follow ABCC6-mediated ATP release from cells in real time a process with intriguing (electrophysiological) aspects.

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Autosomal recessive congenital ichthyosis: *CERS3* mutations identified by a next generation sequencing array targeting ichthyosis genes

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There are 38 mutant genes known to be associated with the ichthyosis phenotypes, and autosomal recessive congenital ichthyosis (ARCI) is a specific subgroup caused by mutations in 9 different genes. Mutations in some of these genes, as for example TGM1, are relatively common while mutations in other genes are rare, including CERS3 reported previously only in 2families. We have developed a next generation sequencing (NGS) array that incorporates 38 ichthyosis associated genes. We applied this sequencing array to DNA from 92 ichthyosis families with high prevalence of consanguinity, and identified 5 distinct mutations in CERS3. The affected family members demonstrated collodion membrane at birth, fine generalized scaling, hyperlinearity of the palms and soles, and ectropion. These mutations in each family co-segregated with the ichthyosis phenotype, the clinically unaffected parents being heterozygous carriers. None of these mutations have been reported previously, and were absent in 119,654 alleles in a control population. CERS3 encodes ceramide synthase 3, an enzyme critical for synthesis of very long-chain ceramides in the epidermis. Loss of this enzyme activity perturbs terminal differentiation of keratinocytes leading to impaired epidermal barrier function. Thus, application of a NGS array targeting 38 ichthyosis associated genes allowed us to identify novel mutations in CERS3. All families demonstrated consanguinity which clearly impacts on the types of mutations, CERS3 mutations so far described being homozygous. The presence of specific mutations in Iranian families will provide the basis to identify carriers in extended families allowing appropriate genetic counseling.

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Determining the genetic basis of epidermolysis bullosa symptoms through genotype-phenotype associations and NGS

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Among rare diseases, one of the most dramatic examples is Epidermolysis bullosa (EB), due to the extreme skin fragility these patients have. This disorder its characterized by its large genetic and clinical heterogeneity, caused by mutations in 18 genes and resulting in more than 30 different clinical subtypes, which enormously difficult its diagnosis and prognosis specially at the neonatal period where they all look very similar. We hypothesize there is a genetic basis for some-if not all-phenotypic variation observed in these patients. In this study, we have used next generation sequencing (NGS) technologies together with a high quality, detailed and extensive clinical evaluation to explore into the genetic basis of EB symptoms. Our cohort expanded to more than 100 Chilean patients from all 4 EB types, and clinical evaluations in 4 different health specialty areas: dermatology, pediatrics, ophthalmology and dentistry. Results from the first two years of the project have already demonstrated population-specific genetic variation with clinical significance. Furthermore, preliminary associations have shown a significant increase of eye involvement and hand deformity severity in patients with specific mutations in the *COL7A1* gene. Finally, these results will be collected as a comprehensive genotype-phenotype database that will be available for clinicians all over the world, helping them for making a correct diagnosis, deciding how to treat and counsel patients and giving EB patients the chance to aim for a personalized treatment in the future.

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GNA14 somatic mutation causes congenital and sporadic vascular tumors by MAPK activation

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Vascular tumors are among the most common neoplasms in infants and children, with up to 10% of newborns presenting with or developing lesions within the first 3 months of life. Most are benign infantile hemangiomas that spontaneously regress; others include tufted angiomas (TA), kaposiform hemangioendotheliomas (KHE), and lobular capillary hemangiomas (LCH). Some are locally invasive and unresponsive to pharmacologic intervention, and can rarely be associated with the Kasabach-Merritt syndrome, a potentially fatal consumptive thrombocytopenia and coagulopathy. Recently, activating mutations in *H-, K-, NRAS, BRAF, GNAQ,* and *GNA11* were found to cause certain types of childhood vascular tumors, and we now identify recurrent somatic activating mutations in *GNA14* by whole exome and targeted sequencing. We found somatic *GNA14* p.Q205L mutation in one TA, KHE, and LCH, and *GNA11* p.R183C mutation in two LCH. *GNA14* mutation has not been previously associated with human disease. We examined pathobiology via expression of mutant *GNA14* and *GNA11* in primary human endothelial cells and melanocytes, and found these mutations induce changes in cellular morphology and render cells growth factor independent by upregulating the MAPK pathway. Our findings identify *GNA14* mutation as a novel cause of childhood vascular tumors, offer insight into mechanisms of oncogenic transformation by Gaq family mutations, and suggest inhibition of the MAPK pathway as a potential therapeutic target for childhood vascular lesions unresponsive to conventional therapy.

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Somatic mutations in nevus comedonicus identify *nek9* as a determinant of follicular keratinocyte cell fate

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Genetic study of rare disorders permits discovery of genes with critical biologic importance and potential therapeutic relevance in common disease. We employed whole exome sequencing (WES) to study nevus comedonicus, which features Blaschko-linear stripes of comedones and inflammatory acne cysts. In paired WES of affected tissue and blood in 4 NC subjects, we identified somatic mutations in *NEK9*, including one patient with nevus comedonicus syndrome who presented with widespread skin lesions and cataracts. Each mutation affects highly conserved residues within the kinase or RCC1 domains of NEK9. We further found these mutations to increase phosphorylation at Thr210, a hallmark of NEK9 kinase activation, indicating gain-of-function. Examination of lesional tissue found loss of canonical follicular markers, and expansion of the keratin 15 expressing cell population in lesional follicles and cysts with paradoxical expression of interfollicular epidermis markers including keratin 10. While interfollicular and follicular keratinocytes differentiate in distinct patterns, but the factors governing their unique trajectories are not fully understood. Our findings in NC identify NEK9 as a novel regulator of keratinocyte cell fate.

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Mutations in *KLHL24* add to the molecular heterogeneity of epidermolysis bullosa simplex

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Epidermolysis bullosa simplex (EBS) is genetically heterogeneous with mutations in 11 different genes. Recently, one more gene, *KLHL24*, has been added with mutations in 19 individuals all affecting the methionine initiation codon. KLHL24 forms part of a ubiquitin-ligase complex, with mutations resulting in a stable truncated protein that promotes excessive ubiquitination and degradation of keratin 14. Within our DNA archive at the UK Diagnostic EB Laboratory, there are 183 EBS cases for which no mutation has been identified (~20% of all EBS referrals). Within this cohort, we identified 7 cases (6 families) with 4 different heterozygous mutations in *KLHL24*, all in the first methionine codon, including a new change (c.1A>T). The phenotype comprised quite marked birth trauma – especially on the lower legs, but which healed quickly with some atrophic scarring although generalized blistering persisted throughout childhood. Nail defects and oral ulceration were common and transient milia occurred. With age, blistering severity tended to lessen. Skin immunohistochemistry showed normal intensity keratin 14 immunolabeling but ultrastructurally basal keratinocytes were pale with a paucity of intermediate filaments, and numerous autophagosomes and autolysosomes were noted in basal cells. Our study underscores the impact of *KLHL24* mutations in expanding the clinicopathologic and molecular basis of EBS and demonstrates skin pathology consistent with dysregulation of autoubiquitination underpinning a new mechanism in inherited skin fragility.