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Epidermal barrier impairment associated with *CYP4F22* mutations in autosomal recessive congenital ichthyosis

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Running head: Barrier impairment in ARCI with *CYP4F22* mutations

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What's already known about this topic?

- Autosomal recessive congenital ichthyosis (ARCI) caused by *CYP4F22* mutations is very rare.
- There is evidence that *CYP4F22* plays a role in the 12(R)-lipoxygenase pathway and therefore is important for skin barrier function.

What does this study add?

- We report a novel homozygous splice site mutation c.549+5G>C in *CYP4F22* in two sisters with ARCI, presenting with and without a collodion membrane at birth.
- Transmission electron microscopy reveals epidermal barrier abnormalities in ARCI with *CYP4F22* mutations.

Abstract (244 words)

Autosomal recessive congenital ichthyosis (ARCI) caused by mutations in *CYP4F22* is very rare. *CYP4F22*, a protein of the cytochrome-P450 family 4, encodes an epidermal ω -hydroxylase decisive for the formation of acylceramides, which is hypothesised to be crucial for skin barrier function. We report a girl with consanguineous parents presenting as collodion baby with contractures of the great joints and palmoplantar hyperlinearity. In the course of the disease she developed fine scaling of the skin with erythroderma, the latter disappearing until the age of 6 months. Her sister showed a generalised fine scaling phenotype, and interestingly, was born without a collodion membrane. The analysis of all known candidate genes for ARCI in parallel with a next generation sequencing approach using a newly designed dermatogenetics gene panel revealed a previously unknown homozygous splice site mutation c.549+5G>C in *CYP4F22* in both girls, confirming the diagnosis of ARCI, type lamellar ichthyosis. Ultrastructural analysis by transmission electron microscopy in both patients showed epidermal hyperplasia, orthohyperkeratosis with persistence of corneodesmosomes into outer stratum corneum layers, fragmented and disorganised lamellar lipid bilayers, which could be ascribed to inhomogeneous lamellar body secretion, as well as lamellar body and lipid entombment in the corneocytes. These findings correlated with increased transepidermal water loss on the functional level. For the first time, this work reports a collodion baby phenotype and epidermal barrier impairment in *CYP4F22*-deficient epidermis on both the ultrastructural and functional level and corroborates the importance of *CYP4F22* for epidermal maturation and barrier activity.

Introduction

Mendelian disorders of cornification are a clinically heterogeneous group of skin diseases, characterised by disturbed terminal differentiation of keratinocytes. Most of these disorders belong to the group of rare diseases and are monogenic.^{1,2} At present, mutations in nine genes are known to be causal for the subgroup of autosomal recessive congenital ichthyosis (ARCI). Most mutations are found in *TGM1*, coding for transglutaminase-1, which is involved in the formation of the cornified envelope; 12R-LOX and eLOX-3, encoded by *ALOX12B* and *ALOXE3*, contribute to the terminal processing of epidermal ceramides; ceramide synthase-3 (CerS3), encoded by *CERS3*, and CyP4F22, a protein of the cytochrome-P450 family 4 subfamily F encoded by *CYP4F22*, play a role in the formation of acylceramides in the epidermis;³⁻⁵ *ABCA12* encodes a lipid transporter involved in packing lipids into lamellar bodies;⁶ the importance of *NIPAL4*, *PNPLA1* and *LIPN* is not known in detail so far.

Lamellar ichthyosis caused by mutations in *CYP4F22* is very rare and only seven reports are available in the literature.⁷⁻¹³ The cytochrome P450 proteins are of extensive importance in several metabolic processes and catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other skin lipids. Very long-chain ceramides can be ω -hydroxylated by CyP4F22 in the epidermis. Following the transfer of linoleoyl-hydroxyceramides to the upper granular layers linoleate is hydrolysed and ω -hydroxyceramides are covalently attached to proteins of the cornified cell envelope. Similar to CerS3, the function of CyP4F22 is supposed to be of direct importance for skin barrier function. Here, we have analysed a case of ARCI with mutations in *CYP4F22* and for the first time studied the epidermal barrier both on the ultrastructural and functional level.

Case reports

A girl was delivered by Caesarean section at 35 weeks of gestation because of premature detachment of the placenta. The newborn presented as collodion baby (Fig. 1a) with an acute respiratory distress syndrome and was too small for date (below third percentile). There was no eclabion and ectropion. Supportive care was provided in a humidified and temperature-regulated incubator with appropriate nutritional measures. Interestingly, the baby showed contractures of

the great joints due to tightened skin of the extremities (Fig. 1b, c). Palms and soles revealed hyperlinearity, fingers were moderately edematous (Fig. 1d), but nails appeared normal. Ophthalmologic and ENT examination, echocardiography as well as routine and metabolic blood tests did not show any abnormalities. Within the first week of life the parchment-like membranes disintegrated with fragments peeling off, leaving transient erosions at the abdomen. Afterwards the baby developed erythroderma with fine white scaling of the skin (Fig. 1e). Erythroderma gradually disappeared until the age of 6 months, but fine greyish scaling remained (Fig. 1f, g). Due to the early initiation of physical therapy, contractures improved within the first three months of life, and completely resolved by the age of 9 months. Topical treatment was carried out with emollient ointments.

The girl was the third child of healthy consanguineous parents (Fig. 1i). The older brother was healthy, the older sister was diagnosed with congenital ichthyosis. She was born with dry skin, however, without a collodion membrane and developed a generalised fine scaling phenotype by the age of 6 months, which is persisting (Fig. 1h). The extended family history did not reveal other family members with skin diseases (Fig. 1i). For both sisters, a suspected diagnosis of ARCI was made. Following informed consent and in accordance with the Declaration of Helsinki principles, peripheral blood for genetic analysis was drawn, and skin biopsies of the trunk for H&E histology and transmission electron microscopy (OsO₄ and RuO₄ postfixation) were taken.

Histopathology of both patients revealed orthohyperkeratosis, keratotic plugging of follicular orifices, a prominent granular layer with occasional small cytoplasmic vacuoles in the cells, but no dermal inflammation on the light microscopic level (Fig. 1j), which was compatible with ARCI.

Genetic analysis

Candidate genes for ARCI were analysed in parallel with next generation sequencing using our dermatogenetics gene panel (Supplemental methods). We have developed an algorithm for the differential analysis of sequenced genes based on the clinical diagnosis (Fig. S1). All target regions were covered more than 30x, the total mean coverage was 581x. Analysis of nine candidate genes for ARCI revealed only one variant, the previously unknown homozygous splice site mutation c.549+5G>C in *CYP4F22* (Fig. 2) in the patient. The index patient's sister was also homozygous for

this mutation, both parents were heterozygous mutation carriers. Unfortunately, RNA samples from the family members were not available to analyse the consequences of the expected splice error, however, defective splicing was clearly predicted by various algorithms. Therefore, the final diagnosis of ARCI type lamellar ichthyosis, caused by a homozygous mutation in *CYP4F22*, was made.

Ultrastructural analysis

As *CYP4F22* encodes an ω -hydroxylase important for the synthesis of ω -hydroxyceramides in the epidermis, we further assessed the ultrastructure of the epidermis and in particular the lipid bilayers of the stratum corneum (SC) in our index patient and her sister. In comparison to four age-matched normal controls, ultrastructural features revealed epidermal hyperplasia, orthohyperkeratosis with persistence of corneodesmosomes into outer SC layers, fragmented and disorganised lamellar lipid bilayers, as well as lamellar body and lipid entombment in the corneocytes (Fig. 3). Abnormal lamellar bilayers could be ascribed to inhomogeneous and premature lamellar body secretion (Fig. 3). Whereas lamellar bodies appeared smaller and occasionally showed partially empty internal structures, the density of organelles was increased, suggesting a compensating mechanism (Fig. 3). Assessment of cornified envelopes, corneocyte lipid envelopes, cytoplasmic keratohyalin and desmosomes was normal in our patients.

Impaired epidermal permeability barrier function correlated with increased levels of transepidermal water loss, with average values on the extensor surface of the upper and lower extremities of 25 and 42 g/m²·h, respectively, compared to four normal controls showing 11 and 14 g/m²·h.

Discussion

Here we report a case of ARCI associated with a homozygous splice site mutation in *CYP4F22*. Of note from the clinical point of view are the variable phenotypic features of collodion membrane and joint contractures attributed to higher skin tension in lamellar ichthyosis associated with *CYP4F22* mutations. As contractures can dramatically complicate the child's mobility and flexibility, it is important to start physical therapy in newborns, thus assisting regular sensomotor development. Because of these complications this form of lamellar ichthyosis may be mistaken

for syndromic ichthyosis such as ARC (arthrogryposis-renal dysfunction-cholestasis) syndrome, which could require surgical treatment. These clinical ambiguities again underline the importance of fast and efficient molecular analysis especially for these heterogeneous disorders.

In the literature, clear genotype/phenotype correlations for ARCI with *CYP4F22* mutations have rarely been reported. It has been described that patients who were homozygous for *CYP4F22* missense mutations did not present as collodion babies, while patients harboring one or two truncating mutations affecting a substrate-binding region were born with a collodion membrane, suggesting a genotype/phenotype correlation.^{7,8,11} However, we cannot confirm this correlation in our case report where a homozygous splice site mutation, predicted to lead to loss of the protein, was associated with a collodion membrane in one child and dry skin without collodion at birth in her sister. Unfortunately, we cannot answer the question whether or not a residual amount of functional transcript is present in our case.

CYP4F22 has recently been identified as a fatty acid ω -hydroxylase necessary for the synthesis of long-chain acylceramides,⁵ which are further processed to linoleoyl-hydroxyceramides. This finding suggests a direct importance of the enzyme for the formation of the epidermal permeability barrier, which relies on the integration of ω -hydroxyceramides into the cell envelopes. Our ultrastructural and functional analysis of the barrier confirmed its impairment accompanied by premature epidermal maturation and secretion of lamellar body contents in the granular layer and remnants of non-secreted components in the corneocytes. CYP4F22 acts in line with ELOVL4, the fatty acid elongase that generates very long-chain acyl groups, and CerS3, which synthesises long-chain ceramides from acyl CoA and a sphingoid base. While mutations in *ELOVL4* lead to a syndromic ichthyosis associated with spastic quadriplegia and mental retardation that is lethal in early childhood,¹⁴ mutations in *CERS3* result in ARCI associated with ultrastructural features comparable with those we have seen in our patient with *CYP4F22* mutations.^{4,15} These results corroborate the importance of CYP4F22 in the early steps of cutaneous acylceramide metabolism in the suprabasal layers of the epidermis.

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Figures

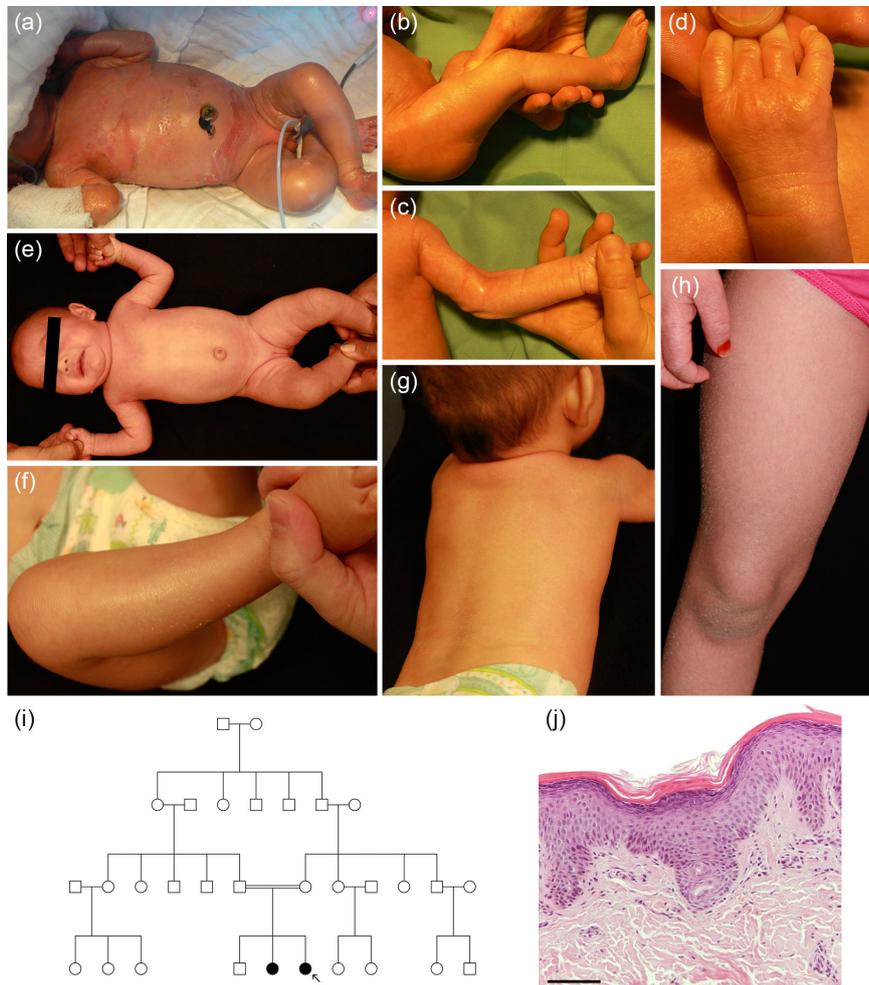


Fig. 1: Phenotypical features, pedigree and histology. (a) Collodion membrane in the index patient. (b, c) Contractures of the knee joint and cubital joint due to tightened skin. (d) Moderately edema of the fingers. (e) Erythroderma with fine white scaling of the skin. (f, g) Fine greyish scaling of the skin on the extensor surfaces of the extremities and the trunk. (h) Generalised fine scaling phenotype of the index patient's sister. (i) Consanguinity of the sisters' parents. (j) Orthohyperkeratosis, prominent granular layer with small cytoplasmic vacuoles in the cells and no dermal inflammation. Bar = 200µm.

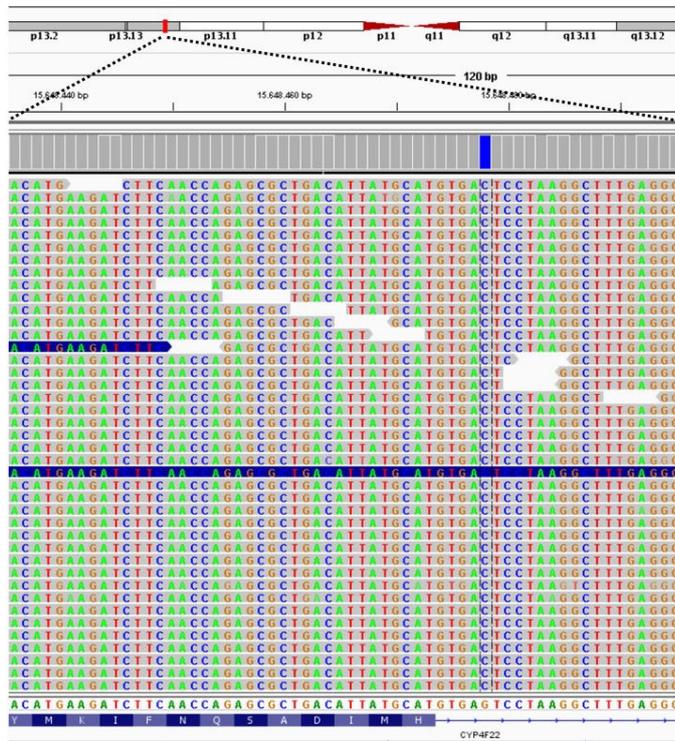
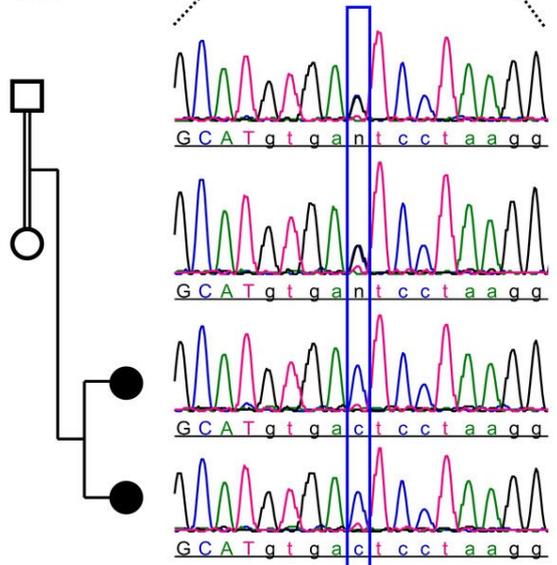


Fig. 2: Mutation analysis. Next generation sequencing using our dermatogenetics gene panel revealed the homozygous splice site mutation c.549+5G>C in *CYP4F22* in both the index patient and her sister. The parents are heterozygous for this mutation.

CYP4F22



c.549+5G>C

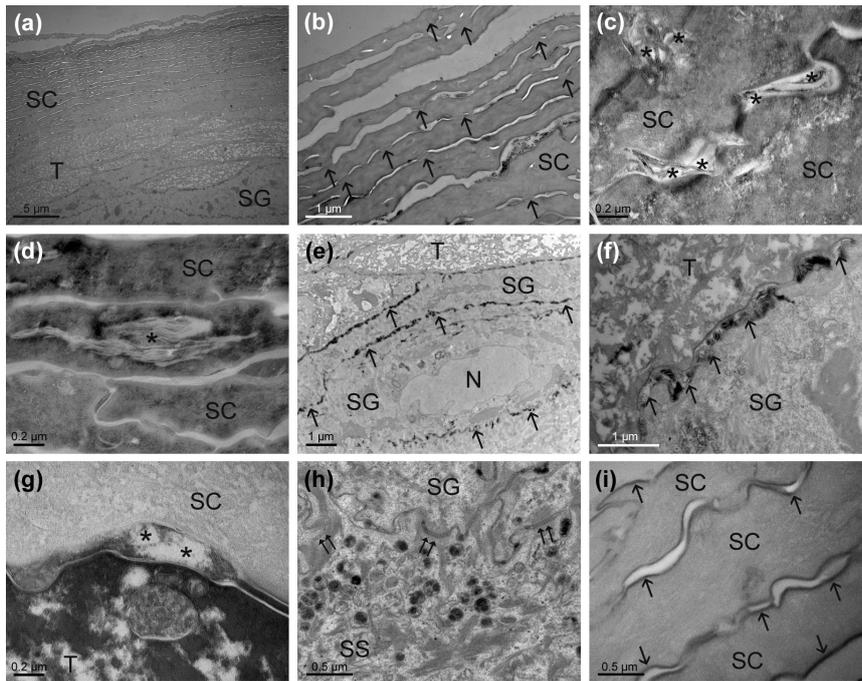


Fig. 3: Ultrastructural features of *CYP4F22*-deficient epidermis. (a) Othohyperkeratosis. (b) Persistence of corneodesmosomes (arrows) into outer SC layers. (c) Fragmentation and disorganisation of extracellular lamellar lipid bilayers with non-lamellar vesicular contents (asterisks) in the SC. (d) Entrapment of non-secreted lamellar body contents and lipids within corneocytes. (e) Premature lamellar body secretion (arrows), with lamellar body contents in the intercellular spaces of the SG. (f) Inhomogeneous lamellar body secretion (arrows) at the SG-T interface. (g) Lamellar membrane arrays are disrupted by non-lamellar domains (asterisks), suggesting impaired processing of secreted lipids. (h) Increased density of lamellar bodies, which occasionally show partially empty internal structures. Normal appearing desmosomes (double arrows). (i) Normal cornified envelopes (arrows). Transmission electron microscopy, osmium tetroxide postfixation (a, b, e, f, h, i), ruthenium tetroxide postfixation (c, d, g). N, nucleus; SC, stratum corneum; SG, stratum granulosum; SS, stratum spinosum; T, transition cell layer.

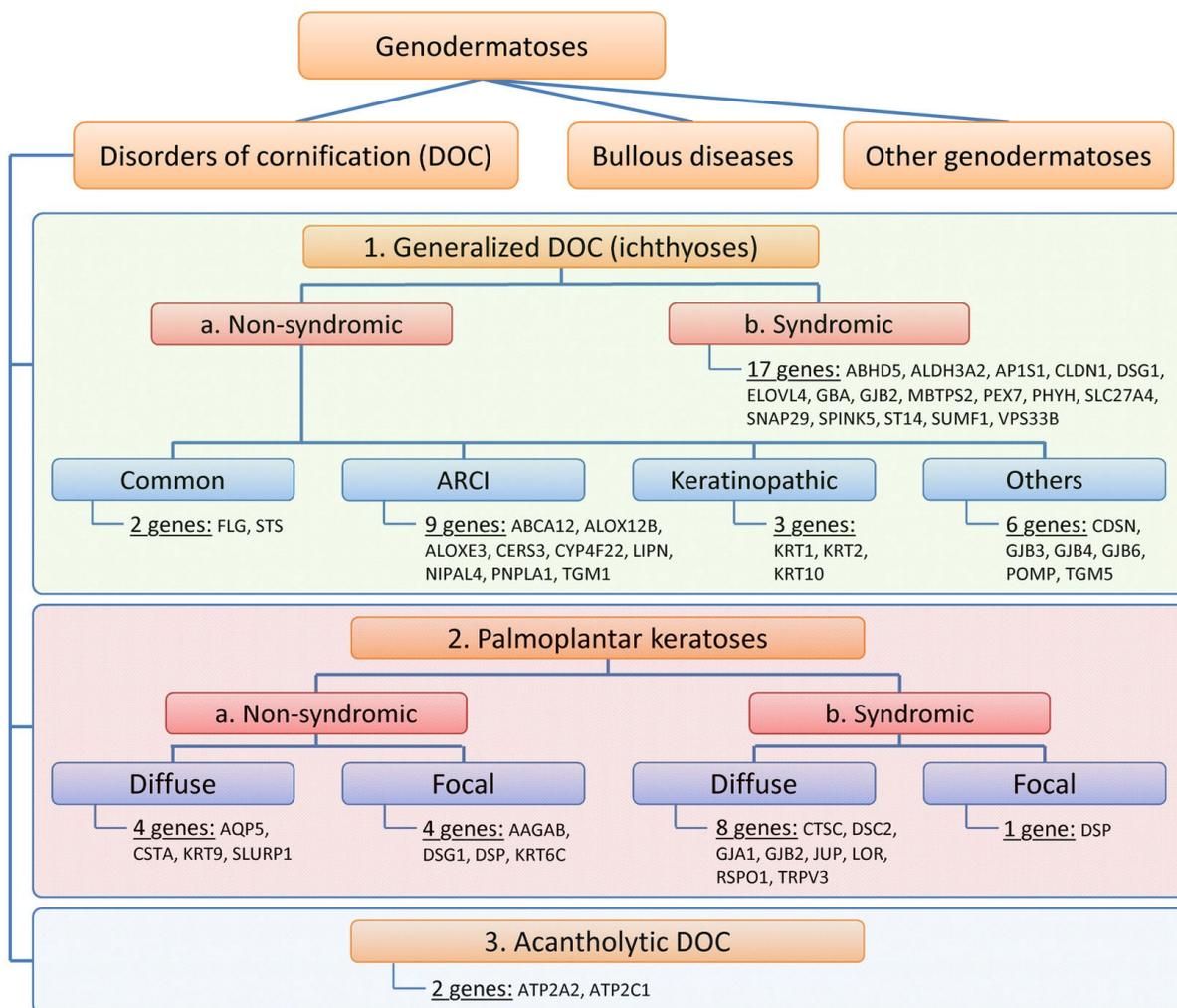


Fig. S1: Scheme of the analysis algorithm of our dermatogenetics gene panel. The gene panel comprises genes for various groups of genetic skin disorders. Only included genes for disorders of cornification (DOC) as of July 2015 are depicted here. Subgroups of genes are analysed based on the clinical diagnosis of nonsyndromic/syndromic generalised ichthyosis, nonsyndromic/syndromic palmoplantar keratoderma, or acantholytic DOC.