ORIGINAL ARTICLE

Biallelic Mutations in *KDSR* Disrupt Ceramide Synthesis and Result in a Spectrum of Keratinization Disorders Associated with Thrombocytopenia

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Mutations in ceramide biosynthesis pathways have been implicated in a few Mendelian disorders of keratinization, although ceramides are known to have key roles in several biological processes in skin and other tissues. Using whole-exome sequencing in four probands with undiagnosed skin hyperkeratosis/ichthyosis, we identified compound heterozygosity for mutations in *KDSR*, encoding an enzyme in the de novo synthesis pathway of ceramides. Two individuals had hyperkeratosis confined to palms, soles, and anogenital skin, whereas the other two had more severe, generalized harlequin ichthyosis-like skin. Thrombocytopenia was present in all patients. The mutations in *KDSR* were associated with reduced ceramide levels in skin and impaired platelet function. KDSR enzymatic activity was variably reduced in all patients, resulting in defective acylceramide synthesis. Mutations in *KDSR* have recently been reported in inherited recessive forms of progressive symmetric erythrokeratoderma, but our study shows that biallelic mutations in *KDSR* are implicated in an extended spectrum of disorders of keratinization in which thrombocytopenia is also part of the phenotype. Mutations in *KDSR* cause defective ceramide biosynthesis, underscoring the importance of ceramide and sphingosine synthesis pathways in skin and platelet biology.

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INTRODUCTION

The hereditary palmoplantar keratodermas and ichthyoses comprise a heterogeneous collection of genodermatoses caused by mutations in more than 100 genes involved in a multitude of biologic pathways and processes (Oji et al., 2010; Sakiyama and Kubo, 2016). Despite major advances in discovering the underlying molecular genetic basis of many of these disorders, several cases remain unresolved, indicating the likely contribution of further gene pathology (Fischer, 2009).

One very recent discovery that expands the molecular pathology of ichthyosis has been the identification of mutations in *KDSR* in four individuals with clinical phenotypes of progressive symmetric erythrokeratoderma (Boyden et al., 2017). *KDSR* encodes 3-ketodihydrosphingosine reductase, which catalyzes the reduction of 3-ketodihydrosphingosine

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Abbreviations: DHS, dihydrosphingosine; KDS, 3-ketodihydrosphingosine; S1P, sphingosine-1-phosphate

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(KDS) to dihydrosphingosine (DHS), a key step in the de novo ceramide synthesis pathway (Linn et al., 2001). Previously, mutations in a different gene in this pathway, *CERS3,* have also been implicated in autosomal recessive congenital ichthyosis, emphasizing the clinical relevance of ceramide pathology in inherited disorders of cornification (Eckl et al., 2013; Radner et al., 2013). Ceramides also have key physiological roles in other organs: mutations in *ELOVL4*, encoding an enzyme necessary for the production of ultra-long chain ceramides in the skin, brain, and retina, lead to a recessive disorder characterized by ichthyosis, intellectual disability, and spastic quadriplegia (Aldahmesh et al., 2011).

In this study, we investigated four individuals from Spain, Japan, and the United Kingdom who presented with inherited disorders of keratinization but had clinical features different from those presented by Boyden et al. (2017). Two patients displayed a milder phenotype of palmoplantar and anogenital hyperkeratosis, whereas the other two patients had a more severe phenotype resembling harlequin ichthyosis. An additional finding, present in all our subjects, but not featured in the Boyden et al. study, was a reduction in the number of blood platelets (thrombocytopenia).

Using whole-exome sequencing, functional studies on skin and platelets, and in vitro analyses, we identified autosomal recessive mutations in *KDSR* in all four subjects, with only one heterozygous mutation overlapping with published findings (Boyden et al., 2017). Our findings expand the molecular and clinical pathology associated with *KDSR* mutations and show that this ceramide biosynthesis pathway has important roles in both skin and platelets.

RESULTS

Clinical features of individuals with *KDSR* mutations

Permission to report medical details and include clinical illustrations was obtained for all patients (from guardians for patients 1, 3, and 4 and from patient 2 himself).

Patient 1 is a 15-year-old male and the only child of unrelated healthy parents (family 1, Figure 1a). His parents are originally from the same geographic area in mid-southeast Spain. At the age of 12 months, he developed palmoplantar hyperkeratosis with extension to the dorsae of the hands and feet, wrists, and ankles, as well as anogenital hyperkeratosis and erythema (Figure 2a–c). At age 2 years, a blood count was performed because of mucocutaneous bleeding, which showed a severe, isolated thrombocytopenia (platelet count $< 30 \times 10^9$/L; bone marrow biopsy sample showed a normal to increased number of megakaryocytes only). A diagnosis of primary immune thrombocytopenia was made, but treatment with oral corticosteroids was suboptimal. Splenectomy at age 11 years led to a slight increase in platelets ($\sim 40 \times 10^9$/L), although clinically he continues to suffer recurrent nose bleeds. Light microscopy of lesional skin showed nonspecific findings of psoriasiform acanthosis, parakeratosis, and focal hypergranulosis but no epidermolytic changes (Figure 2d and e). Oral acitretin (0.5 mg/kg) prescribed for several months did not lead to any improvement in his skin.

Patient 2 is a 21-year-old male and the older of two brothers born to healthy unrelated parents (family 2, Figure 1b). He is the only affected individual among his relatives. This family originates from the same geographic region in Spain as family 1. At age 15 months, he developed diffuse hyperkeratosis on the palms and soles, without progression to the dorsae of the hands or feet (i.e., less severe than patient 1). He also developed perianal erythema and hyperkeratosis. As for patient 1, oral acitretin did not improve the hyperkeratosis. In addition, he suffered episodes of bruising with evidence of isolated thrombocytopenia. Bone marrow studies showed normal hematologic morphology. At present, he has not manifested clinically relevant signs of bleeding despite persistently low platelets ($\sim 20 \times 10^9$/L).

Patient 3 was the second child born to unrelated white parents from the United Kingdom (family 3, Figure 1c). His parents, older brother, and all other relatives were healthy. His mother's pregnancy was uneventful until the last trimester, when oligohydramnios was noted. She had spontaneous rupture of membranes at 33+5 weeks and underwent elective cesarian section at 35+2 weeks with an infant birth weight of 2.74 kg. At birth, the patient was covered in thick adherent plate-like scales, with prominent ectropion and eclabium, and pinching of all digits, collectively consistent with harlequin ichthyosis. He was treated in a humidified incubator with hourly greasy emollients and lubricating eye drops. Acitretin was started, which led to some reduction in adherent scaling, although he developed pseudomonas septicemia at age 15 days and further sepsis thereafter. At birth, platelet count was 120×10^9/L, but within 2 weeks this dropped to 50×10^9/L, and by the 3rd week to approximately $20–30 \times 10^9$/L and remained at this level. At day 36, he deteriorated clinically with tachypnea and hypotension associated with a profound metabolic acidosis. Despite efforts to resuscitate him, he died age 37 days.

Patient 4 is a 6-year-old Japanese male and is the younger of two brothers born to unrelated parents (family 4, Figure 1d). His mother and brother have atopic dermatitis, but there is no other noteworthy family history. He was delivered at 35+3 weeks by normal spontaneous vaginal birth with a birth weight of 1.9 kg. At birth, he had thick plate-like scales with deep fissuring overlying erythrodermic skin. Severe eclabium and ectropion were also observed. Skin biopsy showed marked hyperkeratosis with parakeratosis (see Supplementary Figure S1 online). These features were consistent with Harlequin ichthyosis. He was treated in the neonatal intensive care unit but did not receive systemic retinoids. Over the first 2 months of life, the thick scales desquamated gradually, resulting in generalized erythroderma and fine scaling. Platelet count was normal at birth ($140–150 \times 10^9$/L), but since the age of 2 months this progressively decreased, and at 3 years of age he had severe thrombocytopenia ($4–11 \times 10^9$/L).

Identification of compound heterozygous mutations in *KDSR* in all affected individuals

After ethics committee approval and written informed consent, whole-exome sequencing was performed using DNA from all affected probands. Candidate gene mutations were prioritized by filtering for variants with a frequency of less than 0.1% in public databases such as the Exome Aggregation Consortium (ExAC), Exome Variant Server, 1000 Genomes Project, and an in-house repository. Whole-exome sequencing