

SHORT COMMUNICATION

Target-sequence Capture and High Throughput Sequencing Identify a *De novo* *CARD14* Mutation in an Infant with Erythrodermic Pityriasis Rubra PilarisCristina Has¹, Agnes-Schwieger-Briel¹, Nina Schlipf², Ingrid Hausser³, Nadja Chmel¹, Bernd Rösler², Kristin Technau¹, Thilo Jakob^{1,4}, Andreas Zimmer² and Judith Fischer²Departments of ¹Dermatology and ²Human Genetics, Medical Center, University of Freiburg, Hauptstrasse 7, DE-79104 Freiburg, ³Department of Pathology, University of Heidelberg, Heidelberg, and ⁴Department of Dermatology and Allergology, University Medical Center Giessen (UKGM), Justus-Liebig University Giessen, Giessen, Germany. E-mail: cristina.has@uniklinik-freiburg.de

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Although relatively uncommon in paediatric patients, erythroderma may be the clinical presentation of a wide range of acquired and inherited diseases, including infections, inflammatory skin diseases, ichthyoses, and congenital immunodeficiency. There is a strong genetic background of the latter diseases, and an increasing number of genes have been involved in such phenotypes in recent years. For example, inherited, not syndromic, ichthyoses are due to mutations in approximately 12 distinct genes (1). Psoriasis and atopic dermatitis are even more heterogeneous conditions with polygenic inheritance, for which some dominant genetic factors have been identified (2). In particular, mutations in the *CARD14* gene, encoding the caspase recruitment domain family, member 14 (*CARD14*) have been associated with psoriasis vulgaris, generalized-pustular psoriasis (GPP) and pityriasis rubra pilaris (PRP) (3–6). *CARD14* is a known activator of the non-canonical nuclear factor-kappa B (NF- κ B) pathway, leading to activation of the inflammatory response.

Here, we have employed target-sequence capture and high throughput sequencing of genes associated with keratinization disorders in a sporadic case of infantile erythroderma with high levels of IgE.

CASE REPORT

The index case is a boy born to healthy non-related German parents, and has a healthy older sibling. His skin appeared normal at birth, but at the age of 6 weeks erythematous and squamous lesions appeared on his neck and ears, which resolved with mild topical steroids. From the age of 3 months, well-demarcated scaly and erythematous plaques developed on his cheeks and limbs (Fig. 1a, left). At the age of approximately 7 months, the scaly and erythematous plaques associated with pruritus extended progressively, losing the well-demarcated character (Fig. 1a, right). Ectropion and eclabion developed and the scalp was involved. There was no improvement after short-term application of topical steroids, but a slight amelioration after intensive use of moisturizers. At the age of 10 months, the child developed significant failure to thrive. He was only breast-fed as he refused any other oral food. With the help of a naso-gastric tube he gained weight again. At about the same time, cutaneous lesions spread out to erythroderma and generalized lamellar scaling, without any spared skin area (Fig. 1b). Itch was prominent leading to excoriations. Even though we did see spared areas in the initial phase, we never noticed keratotic follicular papules.

Histopathology of a skin specimen obtained at the age of 6 months, showed acanthosis with elongation of the rete ridges, patchy loss/reduction of stratum granulosum and alternations of hyper- and parakeratosis, dilated capillaries and moderate lymphocytic infiltrate (not shown). Transmission electron microscopy revealed disturbed terminal differentiation of the keratinocytes, but the findings were not characteristic for any specific disorder (not shown). Laboratory work-up at 9 months revealed increased IgE levels of 3,495 kU/l; there were elevated levels of specific IgE for egg white, soy bean, peanut, wheat and grass (ingestion of peanut had led to an allergic reaction with pruritus, suggesting a clinically relevant sensitization).

Genomic DNA from the index patient and his parents was extracted from blood (see Fig S1¹). First, *SPINK5* and *DSG1* were screened by Sanger sequencing, but no mutations were identified. To disclose the disease-causing genetic defect, we performed next generation sequencing (NGS) using the Agilent HaloPlex techno-



Fig. 1. Clinical and findings in the index case. (a) At the age of 6 months he presented facial erythema and scaling extending to the limbs (left panel). At the age of 7 months, the lesions disseminated and lost the well-demarcated appearance (right panel). (b) At age of 24 months: erythroderma with lamellar scales, large scales on palms and soles, thickened nails and malformed ears.

logy with a custom-designed gene panel containing 79 genes (Target Region Size: 224,563 kbp) associated in inherited skin diseases, especially keratinization disorders (available on request) (see Fig. S1¹). A heterozygous *CARD14* sequence variant was disclosed in the patient, c.412G>A, p.Glu138Lys (hg19 chr17:g.78157774G>A), which was excluded in the DNA of the parents (Fig. S1a¹). PolyPhen2 and Mutation Taster predicted the variant to be disease causing.

To get insight into the consequences of the mutation we also studied the cytokine profile in the skin of the patient, showing the inflammatory infiltrate enriched in CD3-positive cells (Fig. S1b¹). There was also a strongly increased proliferation rate of basal keratinocytes as demonstrated by Ki67 staining (clone MIB-1) (Fig. S1b¹). Interestingly, there was almost complete absence of filaggrin (Abcam, Cambridge, UK) protein (Fig. S2a¹), while the signal for LEKTI was abundantly present in the upper epidermal layers of the patient's skin (Fig. S2b¹). These latter changes are probably occurring secondary, since *FLG* and *SPINK5* mutations or variants were excluded in the genetic analysis.

Total RNA was extracted from paraffin skin sections of the index patient and from control normal skin using the RNAeasy[®] FFPE kit (QIAGEN, Hilden, Germany). We found increased *IL1B*, *IL22* and *TNFA* mRNA levels in the skin of the patient compared with the control skin (Fig. S2c¹).

DISCUSSION

In contrast to previously reported cases with *CARD14* mutations, in our patient, clear-cut classification of the disease based on phenotypic and histopathological findings was challenging at a young age. In the same codon, p.Glu138Ala was associated with GPP and p.Glu138del with PRP (3, 5). Recently, p.Glu138Lys was found in an adult with PRP type V (7) and in a patient with an additional *GJB4* mutation and a phenotype varying between PRP and erythrokeratoderma (8). In our case, we considered the diagnosis of PRP type V (juvenile atypical familial), based on the following arguments: (i) age of onset, (ii) clinical course, (iii) familial character (autosomal dominant inheritance starting with the index case), and (iv) previous report (7). This case clearly illustrates that the clinical spectrum associated with *CARD14* mutations covers a continuum, with rather uncharacteristic features in infants. Our findings validate the pathogenic role of the variant p.Glu138Lys and the genotype-phenotype correlations.

In addition, in our patient, severe itch and increased IgE levels were associated with reduction of filaggrin expression leading to altered skin barrier. This secondary modifying factor may be due to the upregulation of *IL22* (9).

Treatment and prognosis are challenging in our patient. Biologicals should be able to modulate the severity of the disorder. Ustekinumab is a fully human monoclonal antibody directed against the p40 subunit shared by interleukin (IL)-12 and IL-23, which are known to cause activation of the NF- κ B pathway, which is inappropriately active. Ustekinumab brought

a dramatic improvement in patients with PRP, with no adverse effects (10–12). Thus, it may also represent an option in our patient, but, so far, the parents were reluctant to agree to any systemic treatment, or topical therapies containing any active ingredients (e.g. corticosteroids, retinoids, and vitamin D analogues). Therefore skin care was restricted to the use of emollients, which were of limited efficacy.

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