FAST TRACK

SHORT COMMUNICATION

Whole-Exome-Sequencing Reveals Small Deletions in *CASP14* in Patients with Autosomal Recessive Inherited Ichthyosis

Peter KIRCHMEIER[1,2], Andreas ZIMMER[1], Bakar BOUADJAR[3], Bernd RÖSLER[1] and Judith FISCHER[1]*
*[1]Institute for Human Genetics, University Medical Center Freiburg, Freiburg DE-79106, [2]Faculty of Biology, University of Freiburg, Freiburg, Germany, and [3]Department of Dermatology, CHU Bab El Oued, Alger, Algeria. *E-mail: judith.fischer@uniklinik-freiburg.de*
Accepted Jul 26, 2016; Epub ahead of print Aug 5, 2016

Ichthyoses are a genetically heterogeneous group of skin disorders characterized by a disturbed skin permeability barrier leading to an abnormal desquamation over the whole body. One of the major functions of the human skin is to protect the body against dehydration; therefore the water barrier of the skin strictly regulates transepidermal water loss (TEWL). The latest nomenclature of Oji et al. (1) distinguishes between syndromic and non-syndromic forms of ichthyoses; syndromic ichthyoses are relatively easy to diagnose due to their association with typical additional symptoms like hair abnormalities or neurological defects. The group of non-syndromic ichthyoses is divided into 3 subgroups: (*i*) common ichthyosis, such as ichthyosis vulgaris and X-linked recessive ichthyoses, which are mostly not present at birth; (*ii*) keratinopathic ichthyosis, which are caused by mutations in the genes *KRT1*, *KRT2* or *KRT10*; and (*iii*) autosomal congenital recessive ichthyosis (ARCI), for which mutations in 8 disease-associated genes are known to date: *TGM1, ABCA12, ALOXE3, ALOX12B, CYP4F22, NIPAL4* (reviewed in Traupe et al. 2014 (2)), *PNPLA1* (3) and *CERS3* (4). Additionally mutations in *LIPN* were described for a clinically similar non-congenital ichthyosis by Israeli et al. (5).

Using single nucleotide polymorphism (SNP)-genotyping and whole-exome-sequencing (WES) we identified a deletion of 2 base pairs (bp) (c.462_463delCA) in the gene Caspase 14 (*CASP14*) in 3 patients with a mild form of generalized ichthyosis from 2 Algerian families (Fig. S1a, b[1]). The mutation is predicted to lead to a frame shift, resulting in a truncated protein.

To date, 11 functional human caspases (**c**ysteinyl-**a**spartate specific **p**rote**ase**) are known (caspase 1–10 and caspase14). Most of them play a central role in apoptosis or inflammation and are ubiquitously expressed. They are synthesized as inactive zymogens and have to be processed to become activated. The zymogens consist of an N-terminal pro-domain, a large catalytic (p17) subunit and a small non-catalytic (p11) subunit. In contrast with other caspases CASP14 is not involved in apoptosis or inflammation and is mainly expressed in all suprabasal layers of the epidermis (6). A strong increase in CASP14 expression and activation has been observed during keratinocyte differentiation (7). After proteolytic maturation in the *stratum granulosum*, CASP14 degrades filaggrin (FLG) monomers in the *stratum corneum* to free hygroscopic amino acids (aa), the natural moisturizing factors (NMF) of the skin (8). *FLG* mutations are known to cause ichthyosis vulgaris (IV) (9). An additional function of CASP14 is the activation of mesotrypsin, which is necessary for the maturation of saposin A, a sphingolipid activator involved in the formation of the permeability barrier of the skin (10). Recently, Jung et al. (11) described a decreased CASP14 expression in patients with atopic dermatitis (AD) that correlates with an impaired skin barrier function.

METHODS

Blood samples were obtained from the 2 families with ichthyosis, and DNA was extracted from whole blood according to standard procedures, after obtaining written informed consent from all participants in the study. The study was approved by the medical ethics committee of AFM/Généthon. To identify causative mutations we performed homozygosity mapping by genome-wide SNP genotyping with a human SNP array (Illumina 370k Quad, San Diego, CA, USA) and additionally in selected cases, whole exome sequencing (WES) with a SureSelectHuman All Exon 50Mb Exome Enrichment Kit (Agilent, Santa Clara, CA, USA) on an Illumina Hiseq instrument (Illumina, San Diego, CA, USA). Sanger sequencing primers for *CASP14* (ENST00000427043) were designed with primer3 50 bp upstream and downstream of the coding exons (http://primer3.ut.ee/). Both DNA strands from all subjects and controls were sequenced using standard protocols.

RESULTS AND DISCUSSION

In a consanguineous Algerian family with 2 affected and 2 unaffected daughters (family A, Fig. S1a[1]) WES of 2 affected daughters (patient 1, patient 2) and their parents revealed 13 variants that are heterozygous in the parents and homozygous in the patients. Of these, only 6 are located in homozygous regions on chromosome 22 (22 Mb) and chromosome 19 (2.8 Mb). Only one variant lies in a gene listed in the ORESTES database of genes that are expressed in human granular keratinocytes (12). We therefore considered this gene, *CASP14*, as the most promising candidate gene. In both patients of family A we identified a deletion of 2 bp in exon 5 (c.462_463delCA) (Fig. S1d[1]), leading to a frame shift and a premature stop codon at aa position 180 (p.Asp154GlufsTer27) (Fig. S1c[1]). Sanger sequencing of all family members revealed segregation of the mutation with the disease in a recessive manner.

[1]https://doi.org/10.2340/00015555-2510

doi: 10.2340/00015555-2510
Acta Derm Venereol 2017; 97: 102–104

Re-analysis of the genotyping data revealed 11 patients with homozygous haplotypes around *CASP14* with a size from 180 kb to 31 Mb. Sanger sequencing of these patients identified an additional Algerian patient (patient 3) with the same mutation that also segregates with the disease in this family without known consanguinity. Patient 3 is the only affected individual in this family and was born without a collodion membrane (family B; Fig. S1b[1]). He showed fine whitish scales over the whole body without erythema. No other symptoms, such as palmoplantar keratoderma, nail-, hair-, or teeth- anomalies, were observed. The mild phenotype of patient 3 was primarily diagnosed as ichthyosis vulgaris without the mention of AD-like lesions and treated with Vaseline containing 5% acetylsalicylate. As both families are from the same geographical region and share the same haplotype and mutation, a common origin is very likely. Unfortunately no further clinical data, biopsies or photos are available from these Algerian families. Interestingly, patient 3 defined the smallest common homozygous interval of the 3 patients by a region of 0.6 Mb. This small region was not detected by homozygosity mapping. Obviously there was only a distant kinship degree between the parents, since they did not indicate a known consanguinity.

The deletion leads to a frame shift located at the second aa of the p11 subunit (Fig. S1c[1]), which is highly conserved in mammals (homology 70.9–98.7%) and essential for the formation of active CASP14 (6). In combination with the resulting premature stop codon at aa 180 the caspase activity is most likely strongly disturbed or completely abolished. The premature stop codon would result in a mRNA predicted to be degraded by nonsense mediated decay (13). "MutationTaster" calculated pathogenicity with a result score of 1.0 (www.mutationtaster.org/ accessed 10 November 2015). The ExAC-database lists this variation 25 times, but exclusively in a heterozygous status (allele frequency: 0.0002059). No other homozygous or compound heterozygous stop or frame shift mutations in *CASP14* are reported in the ExAC-database or in the literature (http://exac.broadinstitute.org/about). Furthermore, procaspase-14 has to be processed by the chymotrypsin-like serine protease kallikrein-related peptidase-7 (KLK7) to produce an intermediate form that is essential for the activation of procaspase-14. The lack of the KLK7 recognition and cleaving site in our patients precludes this process (14). Summing up a complete lack of CASP14-activity is very likely. The functions of CASP14 in the skin and the consequences of its deficiency in mice are well known and described in detail in the literature (15–18). The resulting disturbance of the skin permeability barrier, xerosis, an increased TEWL and hyperlinearity are symptomatic in patients with autosomal recessive ichthyoses (2), IV and patients with AD (11) and are also present in caspase-14-knockout mice (17, 18). Caspase-14 activated mesotrypsin processes prosaposin to produce saposin A, which is important for maintenance of the skin barrier. The lack of active mesotrypsin due to a CASP14 deficiency would result in an impaired permeability barrier (10), a main feature of autosomal recessive ichthyoses and AD. The mild phenotype of patient 3, which was first diagnosed as IV, allowed no distinct clinical classification. It demonstrates a clinical grey-zone of phenotypes between patients with lipid-processing disturbances, as seen in autosomal recessive congenital ichthyoses and patients with FLG defects observed in IV and AD, and in our patients with an intermediate phenotype with CASP14 defects. The involvement of FLG and CASP14 in the NMF-pathway may explain the very similar phenotypes of our patients and subjects with ichthyosis vulgaris.

In conclusion we suppose that mutations in human *CASP14* lead to a disturbed skin barrier and are causative for the skin phenotype in our 3 patients.

ACKNOWLEDGEMENTS

The authors would like to thank the families and patients involved in this study. Part of the study was supported by a grant from the German research foundation (DFG)

The authors have no conflicts of interest to declare.

REFERENCES

1. Oji V, Tadini G, Akiyama M, Blanchet Bardon C, Bodemer C, Bourrat E, et al. Revised nomenclature and classification of inherited ichthyoses: results of the First Ichthyosis Consensus Conference in Sorèze 2009. J Am Acad Dermatol 2010; 63: 607–641.
2. Traupe H, Fischer J, Oji V. Nonsyndromic types of ichthyoses – an update. J Dtsch Dermatol Ges 2014; 12: 109–121.
3. Grall A, Guaguère E, Planchais S, Grond S, Bourrat E, Hausser I, et al. PNPLA1 mutations cause autosomal recessive congenital ichthyosis in golden retriever dogs and humans. Nat Genet 2012; 44: 140–147.
4. Radner FPW, Marrakchi S, Kirchmeier P, Kim G-J, Ribierre F, Kamoun B, et al. Mutations in CERS3 cause autosomal recessive congenital ichthyosis in humans. PLoS Genet 2013; 9: e1003536.
5. Israeli S, Khamaysi Z, Fuchs-Telem D, Nousbeck J, Bergman R, Sarig O, et al. A mutation in LIPN, encoding epidermal lipase N, causes a late-onset form of autosomal-recessive congenital ichthyosis. Am J Hum Genet 2011; 88: 482–487.
6. Earnshaw WC, Martins LM, Kaufmann SH. Mammalian caspases: structure, activation, substrates, and functions during apoptosis. Annu Rev Biochem 1999; 68: 383–424.
7. Eckhart L, Declercq W, Ban J, Rendl M, Lengauer B, Mayer C, et al. Terminal differentiation of human keratinocytes and stratum corneum formation is associated with caspase-14 activation. J Invest Dermatol 2000; 115: 1148–1151.
8. Eckhart L, Tschachler E. Cuts by caspase-14 control the proteolysis of filaggrin. J Invest Dermatol 2011; 131: 2173–2175.
9. Smith FJD, Irvine AD, Terron-Kwiatkowski A, Sandilands A, Campbell LE, Zhao Y, et al. Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. Nat Genet 2006; 38: 337–342.
10. Yamamoto-Tanaka M, Motoyama A, Miyai M, Matsunaga Y, Matsuda J, Tsuboi R, et al. Mesotrypsin and caspase-14 participate in prosaposin processing: potential relevance to epidermal permeability barrier formation. J Biol Chem 2014; 289: 20026–20038.

11. Jung M, Choi J, Lee S-A, Kim H, Hwang J, Choi EH. Pyrrolidone carboxylic acid levels or caspase-14 expression in the corneocytes of lesional skin correlates with clinical severity, skin barrier function and lesional inflammation in atopic dermatitis. J Dermatol Sci 2014;76: 231–239.
12. Toulza E, Mattiuzzo NR, Galliano M-F, Jonca N, Dossat C, Jacob D, et al. Large-scale identification of human genes implicated in epidermal barrier function. Genome Biol 2007; 8: R107.
13. Schwarz JM, Rödelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. Nat Methods [Internet]. Nature Publishing Group; 2010; 7: 575–576.
14. Yamamoto M, Miyai M, Matsumoto Y, Tsuboi R, Hibino T. Kallikrein-related peptidase-7 regulates caspase-14 maturation during keratinocyte terminal differentiation by generating an intermediate form. J Biol Chem 2012; 287: 32825–32834.
15. Demerjian M, Hachem J-P, Tschachler E, Denecker G, Declercq W, Vandenabeele P, et al. Acute modulations in permeability barrier function regulate epidermal cornification: role of caspase-14 and the protease-activated receptor type 2. Am J Pathol 2008; 172: 86–97.
16. Hoste E, Kemperman P, Devos M, Denecker G, Kezic S, Yau N, et al. Caspase-14 is required for filaggrin degradation to natural moisturizing factors in the skin. J Invest Dermatol 2011; 131: 2233–2241.
17. Denecker G, Hoste E, Gilbert B, Hochepied T, Ovaere P, Lippens S, et al. Caspase-14 protects against epidermal UVB photodamage and water loss. Nat Cell Biol 2007; 9: 666–674.
18. Denecker G, Ovaere P, Vandenabeele P, Declercq W. Caspase-14 reveals its secrets. J Cell Biol 2008; 180: 451–458.