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Identification of Mutations in SDR9C7 in 6 Families with Autosomal Recessive Congenital Ichthyosis

A. Hotz¹, C. Fagerberg², A. Vahlquist³, A. Bygum⁴, H. Törmä³, M-A. Rauschendorf⁴, H. Zhang³, L. Heinz¹⁵, E. Bourrat⁶, I. Hauser⁷, V. Vestergaard⁸, A. Dragomir⁹, A. D. Zimmer¹, J. Fischer¹

¹Institute of Human Genetics, Medical Center – University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany
²Department of Clinical Genetics, Odense University Hospital, Odense, Denmark
³Department of Medical Sciences, Section of Dermatology, University Hospital, Uppsala, Sweden
⁴Department of Dermatology and Allergy Centre, Odense University Hospital, Odense, Denmark
⁵Faculty of Biology, University of Freiburg, Freiburg, Germany
⁶Centre de Reference des Genodermatoses, Hôpital Saint-Louis, Paris, France
⁷Institute of Pathology, University Hospital Heidelberg, Heidelberg, Germany
⁸Department of Clinical Pathology, Odense University Hospital, Odense, Denmark
⁹Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden
Corresponding author:

Prof. Dr. med. Dr. Judith Fischer
Institute of Human Genetics
Medical Center – University of Freiburg
Faculty of Medicine
University of Freiburg
Breisacherstraße 33, 79106 Freiburg, GERMANY
Tel.: +49 (0)761-270-70510
Fax: +49 (0)761-270-70410
judith.fischer@uniklinik-freiburg.de

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Autosomal recessive congenital ichthyosis (ARCI) is a heterogeneous group of disorders of keratinization. To date, ARCI has been associated with following genes: ABCA12, ALOX12B, ALOXE3, CERS3, CYP4F22, NIPAL4, TGM1, PNPLA1 and recently SDR9C7 and SULT2B1.\(^1\text{-}^6\) Furthermore, seven patients from a large consanguineous family were described as ARCI due to a homozygous mutation in LIPN.\(^7\) However, the first symptoms appeared only from the age of 5 years and the criterion of a congenital form of ichthyosis is not fulfilled. In this study we report the clinical and molecular findings of seven ARCI patients.
who carried five previously unreported mutations in \textit{SDR9C7}. We could identify homozygous mutations in \textit{SDR9C7} using whole exome sequencing in three patients belonging to two families (P1-1, P1-2, P2). A subsequent screening of genetically unsolved ARCI cases revealed four additional patients (P3-P6) with mutations in \textit{SDR9C7} (reference sequence NM_148897.2).

All seven patients showed a relatively mild ichthyosis phenotype with generalized dry and scaly skin and a mild or local erythema (Fig. 1). With one exception (P2), the patients were not born as collodion babies (CB). In one case (P3), the ichthyosis phenotype firstly appeared at the age of 6 months. In three patients (P2, P3, P4) keratoderma of feet and/or hands was observed, P3 showed only a mild palmar hyperkeratosis. Three further patients (P1-1, P1-2 and P6) presented hyperlinearity of palms and soles. Erythema was present in most of our patients, but only at birth or in mild and local forms. Fungal infections were frequent in three patients (P1-1, P1-2, P3). Anhidrosis was observed in four patients (P2, P3, P4, P6), while two patients (P1-1, P1-2) had no problems with sweating. Two patients (P3, P6) had swollen hands, feet or legs.

The mutation c.112G>A (p.Gly38Arg, rs764593071), that we found in three families (homozygous in P1-1/P1-2, heterozygous in P4, P6), is located in the motif Thr-Gly-X-X-X-Gly-X-Gly in SDR9C7 and corresponds to the last glycine. This highly conserved region is important for the maintenance of the central $\beta$-sheet, which is essential for cofactor binding, suggesting a strong functional impairment of SDR9C7 in these patients. In P4 and P6, we identified the heterozygous mutation c.551A>G (p.Asp184Gly, rs138435128). The amino acid Asp at position 184 is highly conserved in mammals and birds and is located in the neighbouring region of the motif Tyr-X-X-X-Lys, which responds amino acids 172-176 in
human SDR9C7. This motif is important for the catalytic mechanism of the protein.\textsuperscript{8,9} The homozygous mutation c.562C>T (p.Arg188Cys, rs150520393) in P3 alters the second base of exon 3 and may affect the splicing. Furthermore, we found a homozygous nonsense mutation c.658C>T (p.Arg220*, rs774363396) in P2 and a homozygous frameshift mutation c.831_821delCA (p.Tyr277*) in P5.

Hematoxylin and eosin (HE) staining revealed acanthosis with thickening of the stratum spinosum and stratum granulosum in the skin of P1-2 and orthohyperkeratosis (Fig. 1). Ultrastructural analysis in P3 showed no regular aberrations of components involved in terminal differentiation and keratinization (Fig. 1). In P3 and P6 we performed immunofluorescence (IF) staining of SDR9C7 and TGM1 (Fig. 1, data shown for P6). As already shown for other ARCI-causing genes, SDR9C7 is mainly expressed in the stratum granulosum. IF staining revealed no significant difference in SDR9C7 expression in the investigated patients compared to controls. IF staining for TGM1 revealed a notably increased expression of TGM1 in both patients compared to controls. An increased TGM1 expression in the skin of patients with mutations in several ARCI genes has previously been described.\textsuperscript{10}

Our analysis of the phenotypes revealed that most of the patients with SDR9C7 mutations present a relatively mild ichthyosis. The presence of a collodion membrane at birth seems to be a rare feature. It is unclear, whether frameshift mutations lead to a more severe phenotype. P2 and the cases of Takeichi \textit{et al.}\textsuperscript{4} and Karim \textit{et al.}\textsuperscript{5} presented a CB phenotype at birth, all carrying frameshift mutations in SDR9C7. However, P5 and the patients of Mohamad \textit{et al.}\textsuperscript{3} also carry frameshift mutations, but were not born as CB. The patients of Karim \textit{et al.}\textsuperscript{5} present a comparatively more severe phenotype with dark brown scales and erythema. In
contrast, the patients of Mohammad et al.\textsuperscript{3} present a relatively mild phenotype with mild fine whitish scales. Furthermore it is noteworthy that many cases had complicating dermatophytic infections, which appears to be a frequent finding in patients with \textit{SDR9C7} mutations.

In summary, we identified five previously unreported pathogenic \textit{SDR9C7} mutations. The mutation c.112G>A seems to be a recurrent mutation that is predicted to impair a highly conserved region. Our results strongly support the findings of previous studies,\textsuperscript{2-5} and thus underlines that \textit{SDR9C7} is a novel causative gene for ARCI. Further analyses are necessary to understand the function of the SDR9C7 protein in the skin, the interaction with other proteins and the pathological pathway to develop the phenotype.

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\textbf{References}


**figure legend:**

Figure 1: Clinical and histological features in patients with *SDR9C7* mutations. (a-c) (P1-2, 35 years old man): Peculiar reticular scaly pattern on the trunk (a), plate-like scales with redness are observed on extremities (b), palmar hyperlinearity on the hand (c). (d-f) (P3, 72 years old woman): Left arm and upper back showing coarse scaling (d), lower arms with lamellar scaling (e), palms with mild hyperkeratosis (f). (g-i) (P6, 28 years old woman): coarse scaling on the back (g), hyperkeratosis on dorsal feet with mild pitting oedema (h), moderate palmoplantar keratoderma and palmar erythema (i). (j-k): HE staining revealed acanthosis with thickening of the stratum spinosum and stratum granulosum in the skin of P1-2 (k) compared to control (j). In P1-2, the number of horny lamellae is increased with absence of parakeratosis. Scale bar (j-k): 25µm. (l-m): Ultrastructure in P3 revealed no consistent aberrations of components involved in terminal differentiation and keratinization. Granular cells display normal morphology with keratin bundles (K), keratohyaline granules (KH), desmosomes (D) and lamellated lamellar bodies (LB). Scale bars 500nm (l) and 200nm (m). (n-o): IF staining of SDR9C7 (green) revealed an expression of SDR9C7 in the stratum granulosum and lower stratum corneum. There is no significant difference in the expression of SDR9C7 in P6 (o) compared to control (n). (p-q): IF staining of SDR9C7 (green) and TGM1 (red). The TGM1 expression is increased in P6 (q) compared to control (p). Scale bar (n-q): 20µm. We used the primary antibodies Goat polyclonal anti-SDR9C7 (SDR-O) (E-14, sc-169269, Santa Cruz) and Mouse monoclonal anti-TGM1 (TGase1) (E-6, sc-166467, Santa Cruz) and the Donkey anti-Goat IgG (H+L) Secondary Antibody, Alexa Fluor 488 (Thermo Fisher Scientific).