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*Published in:*  
Acta Dermato-Venereologica

*DOI:*  
10.2340/00015555-2418

*Publication date:*  
2016

*Document version:*  
Final published version

*Document license:*  
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*Citation for pulished version (APA):*  
Hellström Pigg, M., Bygum, A., Gånemo, A., Virtanen, M., Brandrup, F., Zimmer, A. D., Hotz, A., Vahlquist, A., & Fischer, J. (2016). Spectrum of Autosomal Recessive Congenital Ichthyosis in Scandinavia: Clinical Characteristics and Novel and Recurrent Mutations in 132 Patients. *Acta Dermato-Venereologica*, 96(7), 932–937. <https://doi.org/10.2340/00015555-2418>

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## CLINICAL REPORT

# Spectrum of Autosomal Recessive Congenital Ichthyosis in Scandinavia: Clinical Characteristics and Novel and Recurrent Mutations in 132 Patients\*

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**Autosomal recessive congenital ichthyosis (ARCI) represents a heterogeneous group of rare disorders of cornification with 3 major subtypes: harlequin ichthyosis (HI), lamellar ichthyosis (LI) and congenital ichthyosiform erythroderma (CIE). A 4<sup>th</sup> subtype has also been proposed: pleomorphic ichthyosis (PI), characterized by marked skin changes at birth and subsequently mild symptoms. In nationwide screenings of suspected cases of ARCI in Denmark and Sweden, we identified 132 patients (age range 0.1–86 years) classified as HI ( $n=7$ ), LI ( $n=70$ ), CIE ( $n=17$ ) and PI ( $n=38$ ). At birth, a collodion membrane or similar severe hyperkeratosis was reported in almost all patients with HI and LI, and in nearly half of patients with CIE and PI. Persistent ectropion was more common in HI (85%) and LI (57%), than in CIE (35%) and PI (5%). Anhidrosis was a frequent problem in all 4 groups (58–100%). A scoring (0–4) of ichthyosis/erythema past infancy showed widely different mean values in the subgroups: HI (3.2/3.1), LI (2.4/0.6), CIE (1.8/1.6), PI (1.1/0.3). Novel or recurrent mutations were found in 113 patients: *TGM1* ( $n=56$ ), *NIPAL4* ( $n=15$ ), *ALOX12B* ( $n=15$ ), *ABCA12* ( $n=8$ ), *ALOXE3* ( $n=9$ ), *SLC27A4* ( $n=5$ ), *CYP4F22* ( $n=3$ ), *PNPLA1* ( $n=1$ ) and *ABHD5* ( $n=1$ ). In conclusion, by performing a deep phenotyping and gene screening, ARCI can be definitely diagnosed in 85% of cases in Scandinavia, with a prevalence of 1:100,000 and >8 different aetiologies. Key words: ARCI; congenital ichthyosiform erythroderma; harlequin ichthyosis; lamellar ichthyosis; pleomorphic ichthyosis; collodion baby.**

Accepted Mar 24, 2016; Epub ahead of print Mar 30, 2016

Acta Derm Venereol 2016; 96: 932–937.

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\*The Editor-in-Chief (AV) has not had responsibility for this article; it has been handled fully by the Co-Editor, who made the decision to accept it.

Autosomal recessive congenital ichthyosis (ARCI) comprises a group of rare genetic disorders of cornification separate from syndromic ichthyoses, epidermolytic ichthyosis and the more common ichthyosis vulgaris (IV) and X-linked ichthyosis (XLI) which only rarely appear at birth (1). The rarest and most severe form of ARCI is harlequin ichthyosis (HI) caused by truncating mutations in the *ABCA12* gene essential for normal functioning of the lamellar (Odland) bodies in the upper epidermis (2, 3). Lamellar ichthyosis (LI) and congenital ichthyosiform erythroderma (CIE) are moderately severe forms of ARCI with partially overlapping phenotypes, ranging from coarse to fine scaling and mild to severe erythema (1). Nine genes<sup>1</sup> have so far been implicated in the aetiology of LI and CIE, all encoding epidermal enzymes and transport proteins, such as transglutaminase 1, ichthyin and lipoxygenases E3/12B (4–14), essential for the formation of a normal stratum corneum (SC) (see e.g. 15, 16).

A fourth type of autosomal recessive ichthyosis, interchangeably called non-LI/non-CIE (17) or pleomorphic<sup>2</sup> ichthyosis (PI) (18), is characterized by marked cutaneous hyperkeratosis at birth followed by spontaneous improvement during infancy and subsequently mild skin symptoms. The suggested umbrella term PI encompasses several distinct conditions: self-improving collodion ichthyosis (SICI) (19), ichthyosis prematurity syndrome (IPS) (20), bathing-suit ichthyosis (BSI) (21), and congenital ichthyosis with fine/mild scaling (CIFS) (22). Many of these conditions have a known aetiology; for example: *TGM1*, *ALOX12B* and *ALOXE3* mutations in SICI and BSI (23, 24), and *SLC27A4* (9q34.11) mutations in IPS (25). Although the latter condition is frequently associated with prematurity, neonatal asphyxia and atopy, recent evidence suggest

<sup>1</sup>Genes known to cause ARCI are: *TGM1* (14q11.2), *ABCA12* (2q34), *ALOXE3* (17p13.1), *ALOX12B* (17p13.1), *NIPAL4* (5q33.3), *CYP4F22* (19p13.12), *PNPLA1* (6p21.31), *LIPN* (10q23.31) and *CERS3* (15q26.3).

<sup>2</sup>The term "pleomorphism" implies a condition in which an individual assumes a number of different forms during its life-cycle (Oxford Medical Dictionary).

that these features are only secondary to the cutaneous pathology<sup>3</sup>, implying that IPS is in fact non-syndromic and should be grouped together with other ARCI, i.e. contrary to its current classification (1).

Although there is some evidence for a genotype–phenotype correlation in ARCI (1, 27), there has been little research into the full spectrum of all clinical and genetic variants in patients from a defined geographic area. Our study was initiated over a decade ago with the explicit aim of examining as many clinically suspicious cases of ARCI as possible in 2 neighbouring Scandinavian countries, Sweden and Denmark, with a combined population of 15 million. In an attempt to provide a full overview of the genotypic and phenotypic spectra of ARCI in Scandinavia, this paper now presents a compilation of our new data, together with some previously published results on the same cohort of patients (20, 22, 25, 28–34).

## PATIENTS AND METHODS

### Patients

This study, which was approved by the ethics committees in Uppsala and Odense, involved patients with suspected ARCI and neonatal signs of ichthyosis as reported by the patients, parents or hospital files. Patients were referred to our diagnostic centres for genodermatoses established in the late 90ies at the university departments of Dermatology in Uppsala and Odense, respectively. All paediatric and dermatological departments in Denmark and Sweden, as well as 2 national patient organizations for ichthyosis, were informed about our study and invited to refer patients, who were at least one month old when investigated by us between 1997 and 2011. The inclusion criteria included ichthyosis symptoms at birth and no obvious signs of inherent systemic disease. Patients with neonatal erythroderma and signs of severe skin barrier failure (e.g. Netherton syndrome and epidermolytic ichthyosis) or extracutaneous symptoms consistent with a neuroectodermal syndrome (e.g. Sjögren-Larsson syndrome) were not included; neither were families with a typical dominant mode of inheritance over several generations. After initial clinical screening, 138 patients underwent more

extensive examinations, at which at least 2 of the authors (AG, AB, FB, MV, AV) participated and agreed on the diagnosis, the subtype of ARCI (HI, LI, CIE or PI) according to previously established criteria (1, 18), and a scoring of ichthyosis and erythema severity using a standardized protocol (22), whereby all parts of the body (trunk, arms, legs, face, scalp, hands, feet, elbows/knees and flexural areas) are first visually scored from 0 to 4 (none to very severe), followed by multiplying the score values of each area with its fractional contribution to the body surface using “the rule of 9” (from 0.01 for hands to 0.36 for trunk); the sum of these products represents the patient’s whole body score with a maximal value of 4.

Four patients (1 Danish and 3 Swedish) were eventually excluded from the ARCI group when typical features of ichthyosis with confetti (IWC) developed during adolescence and DNA analysis confirmed dominant *KRT10* mutations (Dr Keith Choate, personal communication). Two of these patients were previously published as CIE in childhood without known mutation (22, Figs 1e and f). A further 2 patients were excluded when DNA analysis unexpectedly confirmed common ichthyosis; one patient had recurrent homozygous *FLG* mutations, and another male had a recurrent *STS* mutation.

The final study group comprised 132 patients from 120 families. Seven patients originated from non-Scandinavian countries (Iceland, Poland, Middle-East, Cuba and India). Consanguinity in the last 3 generations was traced in 6 families. Many of the Swedish ARCI patients have been reported previously with respect to clinical and ultrastructural findings in the skin, and whether *TGM1* mutations were present, although without providing any mutation details (22). One Swedish family with mother and 2 daughters affected with LI was previously reported to have novel compound *TGM1* mutations, whereas the father was a heterozygote carrier (28). Mutation details in 3 of the patients with HI (32, 33), several of the patients with SICI (24), many of the Swedish patients with *NIPAL4* mutations (31), and all patients with IPS have also been published earlier (25, 34), but without providing any ichthyosis and erythema scores (now shown in Table S1<sup>4</sup>).

Venous blood samples were collected from all patients (except for the case of one baby with HI where blood was collected only from the parents, patient 97 in Table S1<sup>4</sup>, showing *ABCA12* mutations in both cases).

### Mutation analysis

Genomic DNA was extracted from white blood cells using standard procedures. The complete coding DNA including intron/exon boundaries of the following genes were sequenced either using Sanger sequencing or next-generation sequencing (NGS): *TGM1*, *ABCA12*, *ALOXE3*, *ALOX12B*, *NIPAL4*, *CYP4F22*, *PNPLA1*, *CERS3*, *SLC27A4* and *ABHD5*. Sequence variants found by next NGS were verified by the Sanger technique.

NGS was performed using Agilent HaloPlex (Agilent Technologies; Santa Clara, CA, USA) with a custom-designed multi-gene panel containing 79 genes (Target Region Size: 224,563 kbp) associated in inherited skin diseases especially keratinization disorders (available on request). The sequencing was performed on an Illumina MiSeq<sup>®</sup> sequencer (Illumina; Ann Diego, CA, USA) using MiSeq Reagent Kit v2 (2×150 bp). The fraction of target bases with at least 50 reads was ~97%. Variant calling was performed using GATK (ver. 3.1-1-g07a4bf8) and the variants were annotated using ANNOVAR (version date 2013-11-12).

<sup>3</sup>In the position paper by Oji et al. (1) the separation of syndromic and non-syndromic forms of ichthyosis is discussed; the conclusion was that when extracutaneous features, e.g. atopic diathesis, are secondary to a faulty skin barrier, such conditions should not be referred to as “syndromic ichthyosis”. However, this was not discussed for IPS, which at the time was considered a “true” syndrome and thus excluded from ARCI. In a recent study of 22 Norwegian patients with IPS, Khnykin et al. (26) conclude that all extracutaneous symptoms are probably secondary to a massive scaling *in utero* and a subsequent skin barrier defect. Another argument for including IPS among the ARCI is that at post-infancy the skin phenotype is almost indistinguishable from mild ichthyosis. Thus, when a patient with undiagnosed IPS is seen for the first time in late childhood (or adulthood) and no information is available about the neonatal events, this diagnosis can easily be overlooked. Indeed, 9 of the patients in Khnykin et al.’s study were not diagnosed until 2–40 years of age, and in an ongoing study of nearly 700 families with ichthyosis, including ~20 patients with IPS, one-third of patients with *SLC27A4* mutations were reportedly diagnosed earlier as having mild ARCI (JF, unpublished data).

<sup>4</sup><http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-2418>

## RESULTS

*Clinical subtyping and severity scoring*

Based on the investigators' consensus decisions, 132 patients were classified into 4 major clinical subtypes; a majority (55%) belonged to the LI group, 27% to PI, 13% to CIE, and 5% to HI (Table I). Median age at examination ranged from 2 years in the HI group to 36 years in the CIE group, which precluded proper statistical comparisons between the groups. Ichthyosis and erythema scores were close to maximum in 3 patients with HI with malformed fingers and toes, alopecia, tight-sitting ears and abnormal shape of the head. Slightly lower scores and less pronounced malformations were noted in 4 patients with HI-like features, 2 of whom were described in a previous publication (29). The patients with LI and CIE had more variable combinations of moderate-severe ichthyosis and none-moderate erythema scores, whereas those with PI had

generally low scores. The relationship between ichthyosis and erythema severity in the 4 groups of patients is schematically depicted in Fig. S1<sup>4</sup>, with mapping circles partially overlapping for LI, CIE and PI, as opposed to HI, which is clearly separated from the others.

Despite the generally low score values observed in patients with PI at post-infancy, at least 50% of them were born with a collodion or massive hyperkeratosis membrane, i.e. consistent with the PI subtypes known as SICI, BSI and IPS. A similar frequency of collodion at birth was noted in the CIE group (53%), whereas 75–100% of the LI and HI patients had this neonatal phenotype (see Table I). More persistent skin problems, such as ectropion (35–85%) and palmoplantar keratoderma (70–100%), were common in the HI, LI and CIE groups, but rarer and milder in the PI group (5% and 32%, respectively). Anhidrosis was a common problem in all 4 groups (58–100%).

Thirteen (10%) of the patients (HI=2; LI=7; CIE=4) used oral retinoids (acitretin or isotretinoin) at the time of examination; this probably somewhat reduced the mean ichthyosis scores, but is unlikely to have affected the subgrouping of ARCI.

Table I. *Clinical and genetic characteristics of 132 Scandinavian patients with ARCI classified as: harlequin ichthyosis (HI), lamellar ichthyosis (LI), congenital ichthyosiform erythroderma (CIE) or pleomorphic ichthyosis (PI). The mean scores of ichthyosis (IS) and erythema (ES), and the frequency of certain clinical features are shown. "Mutated gene" refers to mutations found in 113 cases; 112 of these were considered diagnostic (see Table SI<sup>4</sup>)*

ARCI subtype	HI <sup>a</sup>	LI	CIE	PI <sup>b</sup>
Total patients, <i>n</i>	7	70	17	38
Females, <i>n</i>	3	40	12	23
Danish patients, <i>n</i>	1	21	10	8
Families, <i>n</i>	7	61	17	35
Age, years, median (range)	2 (0.1–39)	31 (1–81)	36 (5–67)	15 (1–79)
Phenotypic score (0–4)				
IS, mean (SD)	3.23 (0.43)	2.43 (0.82)	1.83 (0.90)	1.05 (0.49)
ES, mean (SD)	3.10 (0.77)	0.60 (0.50)	1.55 (0.61)	0.26 (0.40)
Other features, <i>n</i> (%)				
Collodion or similar	7 (100)	52 (74)	9 (53)	19 (50)
Ectropion	6 (85)	40 (57)	6 (35)	2 (5)
Keratoderma	7 (100)	57 (81)	12 (70)	12 (32)
Anhidrosis	7 (100)	67 (96)	14 (82)	22 (58)
Retinoid therapy <sup>c</sup>	2 (29)	7 (10)	4 (23)	0
Mutated gene, <i>n</i>				
<i>TGMI</i>	0	48	3	5
<i>NIPAL4</i>	0	10	3	2
<i>ALOX12B</i>	0	3	2	10
<i>ALOXE3</i>	0	1	0	8
<i>ABCA12</i>	6	0	2	0
<i>SCL27A4</i>	0	0	0	5
<i>CYP4F22</i>	0	2	1	0
<i>PNPLA1</i>	0	1	0	0
<i>ABHD5</i>	0	0	(1) <sup>d</sup>	0

<sup>a</sup>Includes 3 typical HI and 4 HI-like cases. Two babies died within 5 weeks of age. <sup>b</sup>PI includes 18 patients with self-improving collodion ichthyosis (*TGMI* (*n*=4), *ALOX12B* (*n*=9) and *ALOXE3* (*n*=4)), 14 patients with congenital ichthyosis with fine scaling (*ALOX12B* (*n*=1), *ALOXE3* (*n*=4), *NIPAL4* (*n*=1) and *ABCA12* (*n*=1)), 5 patients with ichthyosis prematurity syndrome due to *SCL27A4* mutations, and one patient with bathing-suit ichthyosis due to *TGMI* mutations. <sup>c</sup>Refers to ongoing oral acitretin or isotretinoin therapy at the time of examination. <sup>d</sup>This patient was excluded from Table SI<sup>4</sup>, but is included in Table II (novel mutation). She has CIE but no extracutaneous signs of Chanarin-Dorfman syndrome (see text). Whether or not her mono-allelic *ABHD5* mutation contributes to the pathogenesis of CIE cannot yet be determined.

*Mutation analysis*

Novel or recurrent mutations were found in 113 (86%) patients; of these, 111 (84%) had bi-allelic mutations thus confirming the aetiology of ARCI (Tables I, II and Table SI<sup>4</sup>). Patient no. 45 in Table SI<sup>4</sup> had only one recurrent *TGMI* mutation, but complementary analysis disclosed a gene duplication as culprit. A further patient had a novel *ABHD5* mutation (see Table II), but no signs of liver, muscle or central nervous system (CNS) involvement characteristic of Chanarin-Dorfman syndrome (1), which probably excludes this gene as the cause of the patient's CIE.

The mutation details are highlighted in Tables II and SI<sup>4</sup>, and are discussed below and in a footnote to Table SI<sup>4</sup> in relation to previous reports (4, 5, 8, 9, 24, 28, 31–45).

Altogether, *TGMI* mutations clearly predominate as cause of ARCI (*n*=56), followed by *NIPAL4* (*n*=15), *ALOX12B* (*n*=15), *ALOXE3* (*n*=9), *ABCA12* (*n*=8), *SLC27A4* (*n*=5), *CYP4F22* (*n*=3), and *PNPLA1* (*n*=1). No *LIPN* and *CERS3* mutations were found.

As can be seen from Table I, the aetiology distribution differed among the subgroups: HI was genetically most homogenous, with *ABCA12* mutations found in 6 of 7 patients. LI was caused by mutations in 6 different genes, with *TGMI* as the leading cause (74%). CIE showed a more scattered aetiology, with *TGMI* and *NIPAL4* mutations each accounting for 1/4 of the molecularly established diagnoses. In contrast, *ALOX12B* and *ALOXE3* mutations predo-



Table II. Novel mutations detected in the investigated ARCI genes of the Scandinavian patients<sup>a</sup> (For further information about allele pairs, clinical details, etc., see Table S1<sup>a</sup>)

Mutation	Consequence
<i>TGMI</i>	
c.918C>G	p.Asp306Glu
c.1094A>G	p.Tyr365Cys
c.1163T>C	p.Leu388Pro
c.1389A>T	p.Gln463His
c.1438A>T	p.Ile480Phe
c.1686_1695delCCACGGCAGC	p.His563fs
c.1927+1G>A	splice site (intron 12)
c.2150T>G	p.Leu717Arg
<i>ALOXE3</i>	
c.353-1G>C	splice site (intron 3)
<i>ABCA12</i>	
c.1002_1004delAACinsT	p.Thr335fs
c.1782G>A	p.Glu594Glu <sup>b</sup>
c.4554G>A	p.Trp1518Term <sup>c</sup>
c.4896delG	p.Ser1633fs
c.6263T>C	p.Leu2088Pro
c.7137delG	p.Met2380fs
c.7412G>A	p.Gly2471Glu
<i>CYP4F22</i>	
c.59dupG	p.Ile21fs
c.667C>T	p.Gln223Term
c.727C>T	p.Arg243Cys
<i>ABHD5</i>	
c.341G>T (mono-allelic)	p.Arg114Leu (carrier?) <sup>d</sup>
<i>PNPLA1</i>	
c.775+3A>T	splice site (intron 5) <sup>e</sup>

<sup>a</sup>These mutations are either not in ExAC/dbSNP or have a minor allele frequency <0.008. <sup>b</sup>Silent mutation affecting a splice site in exon 14. <sup>c</sup>p.Trp1518Term as a consequence of c.4553G>A is previously reported. <sup>d</sup>No mutation was identified in the second allele by sequencing of the coding regions or by deletion/duplication analysis. <sup>e</sup>Immunohistochemistry showed deficient epidermal expression of the protein (result not shown).

minated the PI group, especially in patients subtyped as SICI or CIFS without proven birth as collodion baby, whereas *SCL27A4* mutations were restricted to patients with IPS. Lastly, mutations in *CYP4F22*, *PNPLA1* and (*ABHD5*) were associated with LI or CIE.

## DISCUSSION

This study, which describes the phenotypic and genotypic characteristics of an ethnically not completely homogenous cohort of 132 patients with ARCI living in 2 neighbouring countries (Sweden, population 9.5 million, and Denmark, population 5.5 million), represents one of the largest published so far. In our capacity as the national referral centres for ichthyosis in Denmark and Sweden, working in close alliance with existing patient organizations, we have reason to believe that our cohort represents >90% of all patients with ARCI living in this region, yielding a prevalence figure of approximately 1:100,000 in the period 1997 to 2010. This figure is close to the estimates in many other countries, including a recent study from Spain (46).

Some notable features of our study are that: (i) all patients underwent a deep phenotyping and clinical sub-

grouping of ARCI (see Table I); (ii) we used the same area-related scoring method for scaling and erythema as in a previous study of Swedish patients belonging to the same cohort (22); (iii) the scoring, although not yet validated, was performed by the same investigators and at an age (>1 year) when the patients' skin phenotype is mostly stable (many patients were followed by us for decades), and (iv) the genetic screening, which continued over a period when several new ARCI genes and improved DNA technologies appeared, unravelled the aetiology in approximately 85% of patients, i.e. higher than in many previous studies using a more restricted definition of ARCI (for review see (27)).

Some of the phenotypic data have already been discussed under Results. A pertinent finding is the high frequency of anhidrosis also in the PI group (58%), despite a barely visible ichthyosis in many cases. This may bring into question the current hypothesis about the pathogenesis of anhidrosis, i.e. that a marked ichthyosis will obstruct the sweat ducts and thus prevent sweat from reaching the skin surface.

Some of the DNA results deserve special comments. As in previous studies from other countries (e.g. 27, 41, 47), we found *TGMI* mutations to be the leading cause of ARCI in Scandinavia, with 29 different mutations represented, 8 of which are novel. The point mutation c.877-2A>G was identified in 14% of alleles associated with LI/CIE, mainly in the Swedish patients. This mutation has also been described from several other countries (37, 38, 40, 41, 48–53) and is reported as a founder mutation in Norway (49), located close to Sweden. The p.Ser358Arg mutation was originally identified in a Swedish family (28) and there is no report of this mutation outside Sweden and Norway (unpublished data), which suggests a local founder mutation. The reason for the lower frequency of *TGMI* mutations in Danish (22%) compared with Swedish (50%) patients is not known, but a lack of founder effect is a possible explanation.

Although *TGMI* mutations were primarily associated with the LI phenotype, their rare occurrence also in the CIE group and in occasional patients with BSI and SICI in the PI group is noteworthy. The association of *TGMI* mutations with many different skin phenotypes is further illustrated by the previous finding of 2 different electron microscopy (EM) patterns in SC; type 1 (lipid droplets) and type 2 (cholesterol clefts) (22, 35, 49, 54). In contrast, *NIPAL4* mutations are often associated with EM type 3 (bizarre membranes) (31) and can cause LI and CIE, but hardly any other ARCI phenotype. *NIPAL4* mutations were the second most common cause of ARCI in Denmark (16%) and the third most common cause in Sweden (8%).

*ALOX12B* mutations are predominantly associated with the SICI subtype of PI (24, 55) and were more frequent in Swedish (13%) than in Danish (8%) patients, whereas *ALOXE3* mutations were associated with both

LI and PI, and were altogether less common; although not as rare as for example in Asia (56).

*ABCA12* mutations, previously reported in 3 of the Swedish patients with HI (32, 33), were now identified in 3 additional HI-like patients carrying 3 novel mutations (see Table II). Our finding of *ABCA12* mutations also in 2 patients with CIE corroborates previous suggestions that different types of *ABCA12* mutations may produce widely different phenotypes (7, 57).

Three patients had mutations in *CYP4F22* and one in *PNLPA1*, in all cases associated with mild to moderate LI/CIE. No new *SCL27A4* mutations causing IPS were identified in this study. The mutation details of the 5 included patients from Sweden and Denmark were discussed previously (25, 34), albeit then without providing any score data. A clustering of IPS in northern Sweden and Norway motivates its inclusion in the differential diagnosis of ARCI in Scandinavia, especially when examining adult cases with mild ichthyosis and no knowledge is available about the perinatal events (see footnote 3). It is also important to recognize other types of ichthyosis, such as IWC, IV and XLI, which especially in children may mimic ARCI, as illustrated by 6 excluded cases in our study (see Materials and Methods).

In conclusion, this study highlights how a deep phenotyping (clinical subtyping plus severity scoring of ichthyosis and erythema) can help clinicians and geneticists to preliminarily classify a case of ARCI and hence to decide which candidate genes should be prioritized in the search for a molecular diagnosis, i.e. the very basis for a proper genetic counselling and, presumably in the future, for a correct therapy. It is noteworthy that, despite our extensive screening of 10 genes, we failed to establish a molecular diagnosis in approximately 15% of the patients (mainly in those with the CIFS subtype of PI). This strongly suggests that new aetiologies remain to be discovered<sup>5</sup>. It is hoped that recent progress in NGS methods and gene panels will open up the possibility of rapid diagnostic analysis of ichthyosis by including a large number of ARCI-related genes.

## ACKNOWLEDGEMENTS

The authors would like to thank the patients and families for their co-operation and the many Swedish and Danish dermatologists and paediatricians for referring new families. We thank Prof. David Kelsell, London for the initial *ABCA12* analyses, and Mrs Benita Andersson and Lena Claesson, Uppsala for technical assistance. We are especially grateful to Florence Jobard and Caroline Lefèvre from CNG/Evry for the technical realization of the sequencing, Dr Johannes Kjeldstrup Kristensen, Dr Mette Sommerlund, Dr Gitte Strauss, Dr Eva Benfeldt and Dr Tomas Norman Dam are kindly thanked for cooperation on tracing patients from the Danish departments of dermatology. This study was supported by grants from the Swedish Research Council, Kgl. Hofbuntmager Aage Bangs Fond, various funds at the Uppsala University, the H. and G. Ankarstrands Foundation, E. Jarls and M. Linds foundation, the Welander-Finsens foundation, the Nansen foundations, and Anders Jahres fund. The work of JF was supported in part by the Centre Nationale de Génotypage (CNG), Association Athina ichtyose Monaco (Athina), FIRST (Foundation for Ichthyosis and Related Skin Types) and by a grant from the German research foundation DFG (FI1767/3-1).

<sup>5</sup>Although large deletions or duplications and regulatory sequence variants cannot be ruled out as an alternative disease mechanism (such analyses were not routinely performed), this kind of mutations has so far not been reported to be common in ARCI, although there are some reports of these kind of mutations (3, 10, 14, 53, 58, 59), and one case of TGM1 duplication was indeed encountered in our study (pat. no. 45 in Table SI<sup>1</sup>). Furthermore, it was not the goal of our study to analyse consequences of the splice site mutations. However, these mutations have been described previously in many patients, as well as in our diagnosed patients, and they are considered as VUSS (disease-associated sequence variations).

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## REFERENCES

- Oji V, Tadani 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.
- Akiyama M, Sugiyama-Nakagiri Y, Sakai K, McMillan JR, Goto M, Arita K, et al. Mutations in lipid transporter *ABCA12* in harlequin ichthyosis and functional recovery by corrective gene transfer. *J Clin Invest* 2005; 115: 1777–1784.
- Kelsell DP, Norgett EE, Unsworth H, Teh MT, Cullup T, Mein CA, et al. Mutations in *ABCA12* underly the severe congenital skin disease harlequin ichthyosis. *Am J Hum Genet* 2005; 76: 794–803.
- Huber M, Rettler I, Bernasconi K, Frenk E, Lavrijsen SP, Ponc M, et al. Mutations of keratinocyte transglutaminase in lamellar ichthyosis. *Science* 1995; 267: 525–528.
- Russell LJ, DiGiovanna JJ, Rogers GR, Hashem N, Compton JG, Bale SJ. Mutations in the gene for transglutaminase 1 in autosomal recessive lamellar ichthyosis. *Nat Genet* 1995; 9: 279–283.
- Parmentier L, Blanchet-Bardon C, Nguyen S, Prud'homme J-F, Dubertret L, Weissenbach J. Autosomal recessive lamellar ichthyosis: identification of a new mutation in transglutaminase 1 and evidence for genetic heterogeneity. *Hum Mol Genet* 1995; 4: 1391–1395.
- Lefèvre C, Audebert S, Jobard F, Bouadjar B, Lakhdar H, Boughdene-Stambouli O, et al. Mutations in the transporter *ABCA12* are associated with lamellar ichthyosis type 2. *Hum Mol Genet* 2003; 12: 2369–2378.
- Jobard F, Lefèvre C, Karaduman A, Blanchet-Bardon C, Emre S, Weissenbach J, et al. Lipoxygenase-3 (*ALOXE3*) and 12(R)-lipoxygenase (*ALOX12B*) are mutated in non-bullous congenital ichthyosiform erythroderma (NCIE) linked to chromosome 17p13.1. *Hum Mol Genet* 2002; 11: 107–113.
- Lefèvre C, Bouadjar B, Karaduman A, Jobard F, Saker S, Özguc M, et al. Mutations in *ichthyin* a new gene on chromosome 5q33 in a new form of autosomal recessive congenital ichthyosis. *Hum Mol Genet* 2004; 13: 2369–2378.
- Lefèvre C, Bouadjar B, Ferrand V, Tadani G, Mégarbané A, Lathrop M, et al. Mutations in a new cytochrome P450 gene in lamellar ichthyosis type 3. *Hum Mol Genet* 2006; 15: 767–776.

11. 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.
12. 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.
13. Eckl KM, Tidhar R, Thiele H, Oji V, Hausser I, Brodessaer S, et al. Impaired epidermal ceramide synthesis causes autosomal recessive congenital ichthyosis and reveals the importance of ceramide acyl chain length. *J Invest Dermatol* 2013; 133: 2202–2211.
14. Radner FP, Marrakchi S, Kirchmeier P, Kim GJ, Ribierre F, Kamoun B, et al. Mutations in CERS3 cause autosomal recessive congenital ichthyosis in humans. *PLoS Genet* 2013; 9: e1003536.
15. Li H, Loriè EP, Fischer J, Vahlquist A, Törmä H. The expression of epidermal lipoxygenases and transglutaminase-1 is perturbed by NIPAL4 mutations: indications of a common metabolic pathway essential for skin barrier homeostasis. *J Invest Dermatol* 2012; 132: 2368–2375.
16. Li H, Vahlquist A, Törmä H. Interactions between FATP4 and ichthyin in epidermal lipid processing may provide clues to the pathogenesis of autosomal recessive congenital ichthyosis. *J Dermatol Sci* 2013; 69: 195–201.
17. Williams ML, Elias PM. Heterogeneity in autosomal recessive ichthyosis. Clinical and biochemical differentiation of lamellar ichthyosis and nonbullous congenital ichthyosiform erythroderma. *Arch Dermatol* 1985; 121: 47–88.
18. Vahlquist A. Pleomorphic ichthyosis: proposed name for a heterogeneous group of congenital ichthyoses with phenotypic shifting and mild residual scaling. *Acta Derm Venereol* 2010; 90: 454–460.
19. Van Gysel D, Lijnen RL, Moekti SS, de Laat PC, Oranje AP. Collodion baby: a follow-up study of 17 cases. *J Eur Acad Dermatol Venereol* 2002; 16: 472–475.
20. Bygum A, Westermark P, Brandrup F. Ichthyosis prematurity syndrome: a well-defined congenital ichthyosis subtype. *J Am Acad Dermatol* 2008; 59: S71–S74.
21. Bourrat E, Blanchet-Bardon C, Derbois C, Cure S, Fischer J. Specific TGM1 mutation profiles in bathing suit and self-improving collodion ichthyoses: phenotypic and genotypic data from 9 patients with dynamic phenotypes of autosomal recessive congenital ichthyosis. *Arch Dermatol* 2012; 148: 1191–1195.
22. Gånemo A, Pigg M, Virtanen M, Kukk T, Raudsepp H, Rossman-Ringdahl I, et al. Autosomal recessive congenital ichthyosis in Sweden and Estonia: clinical, genetic and ultrastructural findings in eighty-three patients. *Acta Derm Venereol* 2003; 83: 24–30.
23. Raghunath M, Hennies HC, Ahvazi B, Vogel M, Reis A, Steinert PM, et al. Self-healing collodion baby: a dynamic phenotype explained by a particular transglutaminase 1 mutation. *J Invest Dermatol* 2003; 120: 224–228.
24. Vahlquist A, Bygum A, Gånemo A, Virtanen M, Hellström-Pigg M, Strauss G, et al. Genotypic and clinical spectrum of self-improving collodion ichthyosis: ALOX12B, ALOXE3, and TGM1 mutations in Scandinavian patients. *J Invest Dermatol* 2010; 130: 438–443.
25. Klar J, Schweiger M, Zimmerman R, Zechner R, Li H, Törmä H, et al. Mutations in the fatty acid transport protein 4 gene cause the ichthyosis prematurity syndrome. *Am J Hum Genet* 2009; 85: 248–253.
26. Khnykin D, Ronnevig J, Johnsson M, Sitek JC, Blaas H-G, Hausser I, et al. Ichthyosis prematurity syndrome: Clinical evaluation of 17 families with a rare disorder of lipid metabolism. *J Am Acad Dermatol* 2012; 66: 606–616.
27. Fischer J. Autosomal recessive congenital ichthyosis. *J Invest Dermatol* 2009; 129: 1319–1321.
28. Huber M, Yee VC, Burri N, Vikerfors E, Lavrijnsen APM, Paller AS, et al. Consequences of seven novel mutations on the expression and structure of keratinocyte transglutaminase. *J Biol Chem* 1997; 272: 21018–21026.
29. Virolainen E, Niemi KM, Gånemo A, Kere J, Vahlquist A, Saarialho-Kere U. Ultrastructural features resembling those of harlequin ichthyosis in patients with severe congenital ichthyosiform erythroderma. *Br J Dermatol* 2001; 145: 480–483.
30. Vahlquist A, Gånemo A, Pigg M, Virtanen M, Westermark P. The clinical spectrum of congenital ichthyosis in Sweden: a review of 127 cases. *Acta Derm Venereol* 2003; Suppl 213: 34–47.
31. Dahlqvist J, Klar J, Hausser I, Anton-Lamprecht I, Pigg MH, Gedde-Dahl T Jr, et al. Congenital ichthyosis: mutations in ichthyin are associated with specific structural abnormalities in the granular layer of epidermis. *J Med Genet* 2007; 44: 615–620.
32. Thomas AC, Sinclair C, Mahmud N, Cullup T, Mellerio JE, Harper J, et al. Novel and recurring ABCA12 mutations associated with harlequin ichthyosis: implications for prenatal diagnosis. *Br J Dermatol* 2008; 158: 611–613.
33. Rajpopat S, Moss C, Mellerio J, Vahlquist A, Gånemo A, Hellstrom-Pigg M, et al. Harlequin ichthyosis: a review of clinical and molecular findings in 45 cases. *Arch Dermatol* 2011; 147: 681–686.
34. Sobol M, Dahl N, Klar J. FATP4 missense and nonsense mutations cause similar features in ichthyosis prematurity syndrome. *BMC Res Notes* 2011; 4: 90.
35. Laiho E, Ignatius J, Mikkola H, Yee VC, Teller DC, Niemi K-M, et al. Transglutaminase 1 mutations in autosomal recessive congenital ichthyosis: privat and recurrent mutations in an isolated population. *Am J Hum Genet* 1997; 61: 529–538.
36. Bichakjian CK, Nair RP, Welby WW, Goldberg S, Elder JT. Prenatal exclusion of lamellar ichthyosis based on identification of two new mutations in the transglutaminase 1 gene. *J Invest Dermatol* 1998; 110: 179–182.
37. Hennies HC, Raghunath M, Wiebe V, Vogel M, Velten F, Traupe H, et al. Genetic and immunohistochemical detection of mutations inactivating the keratinocyte transglutaminase in patients with lamellar ichthyosis. *Hum Genet* 1998; 102: 314–318.
38. Shevchenko YO, Compton JG, Toro JR, DiGiovanna JJ, Bale SJ. Splice-site mutation in TGM1 in congenital recessive ichthyosis in American families: molecular, genetic, genealogic, and clinical studies. *Hum Genet* 2000; 106: 492–499.
39. Akiyama M, Takizawa Y, Suzuki Y, Ishiko A, Matsuo I, Shimizu H. Compound heterozygous TGM1 mutations including a novel missense mutation L204Q in a mild form of lamellar ichthyosis. *J Invest Dermatol* 2001; 116: 992–995.
40. Oji V, Hautier JM, Ahvazi B, Hausser I, Aufenvenne K, Walker T, et al. Bathing suit ichthyosis is caused by transglutaminase-1 deficiency: evidence for a temperature-sensitive phenotype. *Hum Mol Genet* 2006; 15: 3083–3097.
41. Farasat S, Wei MH, Herman M, Liewehr DJ, Steinberg SM, Bale SJ, et al. Novel transglutaminase-1 mutations and genotype-phenotype investigations of 104 patients with autosomal recessive congenital ichthyosis in the USA. *J Med Genet* 2009; 46: 103–111.
42. Herman ML, Farasat S, Steinbach PJ, Wei MH, Toure O, Fleckman P, et al. Transglutaminase-1 gene mutations in



- autosomal recessive congenital ichthyosis: summary of mutations (including 23 novel) and modeling of TGase-1. *Hum Mutat* 2009; 30: 537–547.
43. Eckl KM, Krieg P, Küster W, Traupe H, André F, Wittstruck N, et al. Mutation spectrum and functional analysis of epidermis-type lipoxygenases in patients with autosomal recessive congenital ichthyosis. *Hum Mutat* 2005; 26: 351–361.
  44. Lesueur F, Bouadjar B, Lefèvre C, Jobard F, Audebert S, Lakhdar H, et al. Novel mutations in ALOX12B in patients with autosomal recessive congenital ichthyosis and evidence for genetic heterogeneity on chromosome 17p13. *J Invest Dermatol* 2007; 12: 829–834.
  45. Eckl KM, de Juanes S, Kurtenbach J, Nätebus M, Lugassy J, Oji V, et al. Molecular analysis of 250 patients with autosomal recessive congenital ichthyosis: evidence for mutation hotspots in ALOXE3 and allelic heterogeneity in ALOX12B. *J Invest Dermatol* 2009; 129: 1421–1428.
  46. Hernández-Martín A, García-Doval I, Aranegui B, de Unamuno P, Rodríguez-Pazos L, González-Enseñat MA, et al Vicente A. Prevalence of autosomal recessive congenital ichthyosis: a population-based study using the capture-recapture method in Spain. *J Am Acad Dermatol* 2012; 67: 240–244.
  47. Israeli S, Goldberg I, Fuchs-Telem D, Bergman R, Indelman M, Bitterman-Deutsch O, et al. Non-syndromic autosomal recessive congenital ichthyosis in the Israeli population. *Clin Exp Dermatol* 2013; 38: 911–916.
  48. Hennies HC, Küster W, Wiebe V, Krebsová A, Reis A. Genotype/phenotype correlation in autosomal recessive lamellar ichthyosis. *Am J Hum Genet* 1998; 62: 1052–1061.
  49. Pigg M, Gedde-Dahl Jr T, Cox D, Hausser I, Anton-Lamprecht I, Dahl N. Strong founder effect for a transglutaminase 1 gene mutation in lamellar ichthyosis and congenital ichthyosiform erythroderma from Norway. *Eur J Hum Genet* 1998; 6: 589–596.
  50. Pigg M, Gedde-Dahl T Jr, Cox DW, Haugen G, Dahl N. Haplotype association and mutation analysis of the transglutaminase 1 gene for prenatal exclusion of lamellar ichthyosis. *Prenatal Diagnosis* 2000; 20: 132–137.
  51. Esposito G, Auricchio L, Rescigno G, Paparo F, Rinaldi M, Salvatore F. Transglutaminase 1 gene mutations in Italian patients with autosomal recessive lamellar ichthyosis. *J Invest Dermatol* 2001; 116: 809–812.
  52. Shawky RM, Sayed NS, Elhawary NA. Mutations in transglutaminase 1 gene in autosomal recessive congenital ichthyosis in Egyptian families. *Dis Markers* 2004; 20: 325–332.
  53. Terrinoni A, Serra V, Codispoti A, Talamonti E, Bui L, Palombo R, et al. Novel transglutaminase 1 mutations in patients affected by lamellar ichthyosis. *Cell Death Dis* 2012; 3: e416.
  54. Akiyama M, Sawamura D, Shimizu H. The clinical spectrum of nonbullous congenital ichthyosiform erythroderma and lamellar ichthyosis. *Clin Exp Dermatol* 2003; 28: 235–240.
  55. Akiyama M, Sakai K, Yanagi T, Tabata N, Yamada M, Shimizu H. Partially disturbed lamellar granule secretion in mild congenital ichthyosiform erythroderma with ALOX12B mutations. *Br J Dermatol* 2010; 163: 201–204.
  56. Sugiura K, Akiyama M. Lamellar ichthyosis caused by a previously unreported homozygous ALOXE3 mutation in East Asia. *Acta Derm Venereol* 2015; 95: 858–859.
  57. Natsuga K, Akiyama M, Kato N, Sakai K, Sugiyama-Nakagiri Y, Nishimura M, et al. Novel ABCA12 mutations identified in two cases of non-bullous congenital ichthyosiform erythroderma associated with multiple skin malignant neoplasia. *J Invest Dermatol* 2007; 127: 2669–2673.
  58. Thomas AC, Cullup T, Norgett EE, Hill T, Barton S, Dale BA, et al. ABCA12 is the major harlequin ichthyosis gene. *J Invest Dermatol* 2006; 126: 2408–2413.
  59. Scott CA, Plagnol V, Nitoiu D, Bland PJ, Blaydon DC, Chronnell CM, et al. Targeted sequence capture and high-throughput sequencing in the molecular diagnosis of ichthyosis and other skin diseases. *J Invest Dermatol* 2013; 133: 573–576.