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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 coz1rnification with 3 major subtypes: harlequin ichthyosis (HI), lamellar ichthyosis (LI) and congenital ichthyosiform erythroderma (CIE). A 4th 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*.

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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[1] 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[2] 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

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[1]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).

[2]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[3], implying that IPS is in fact non-syndromic and should be grouped together with other ARCIs, 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 SI[4]).

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 SI[4], 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® 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).

[3]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 ARCIs 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).

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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 comparisions 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[4], 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[4])*

ARCI subtype	HI[a]	LI	CIE	PI[b]
Total patients, *n*	7	70	17	38
Females, *n*	3	40	12	23
Danish patients, *n*	1	21	10	8
Families, *n*	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, *n* (%)				
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[c]	2 (29)	7 (10)	4 (23)	0
Mutated gene, *n*				
TGM1	0	48	3	5
NIPAL4	0	10	3	2
ALOX12B	0	3	2	10
ALOXE3	0	1	0	8
ABCA12	6	0	2	0
SCL27A4	0	0	0	5
CYP4F22	0	2	1	0
PNPLA1	0	1	0	0
ABHD5	0	0	(1)[d]	0

[a]Includes 3 typical HI and 4 HI-like cases. Two babies died within 5 weeks of age. [b]PI includes 18 patients with self-improving collodion ichthyosis (*TGM1* (*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 *TGM1* mutations. [c]Refers to ongoing oral acitretin or isotretinoin therapy at the time of examination. [d]This patient was excluded from Table SI[4], 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 pathogenisis 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[4]). Patient no. 45 in Table SI[4] had only one recurrent *TGM1* 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[4], and are discussed below and in a footnote to Table SI[4] in relation to previous reports (4, 5, 8, 9, 24, 28, 31–45).

Altogether, *TGM1* 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 *TGM1* as the leading cause (74%). CIE showed a more scattered aetiology, with *TGM1* 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*[a] *(For further information about allele pairs, clinical details, etc., see Table SI*[4]*)*

Mutation	Consequence
TGM1	
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
ALOXE3	
c.353-1G>C	splice site (intron 3)
ABCA12	
c.1002_1004delAACinsT	p.Thr335fs
c.1782G>A	p.Glu594Glu[b]
c.4554G>A	p.Trp1518Term[c]
c.4896del G	p.Ser1633fs
c.6263T>C	p.Leu2088Pro
c.7137delG	p.Met2380fs
c.7412G>A	p.Gly2471Glu
CYP4F22	
c.59dupG	p.Ile21fs
c.667C>T	p.Gln223Term
c.727C>T	p.Arg243Cys
ABHD5	
c.341G>T (mono-alleic)	p.Arg114Leu (carrier?)[d]
PNPLA1	
c.775+3A>T	splice site (intron 5)[e]

[a]These mutations are either not in ExAC/dbSNP or have a minor allele frequency <0.008. [b]Silent mutation affecting a splice site in exon 14. [c]p.Trp1518Term as a consequence of c.4553G>A is previously reported. [d]No mutation was identified in the second allele by sequencing of the coding regions or by deletion/duplication analysis. [e]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 subgrouping 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 *TGM1* 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 *TGM1* mutations in Danish (22%) compared with Swedish (50%) patients is not known, but a lack of founder effect is a possible explanation.

Although *TGM1* 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 *TGM1* 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[5]. 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.

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[5]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[1]). 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 VUS5 (disease-associated sequence variations).

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