ORIGINAL ARTICLE

Congenital ichthyosis: mutations in *ichthyin* are associated with specific structural abnormalities in the granular layer of epidermis

J Dahlqvist, J Klar, I Hausser, I Anton-Lamprecht, M Hellström Pigg, T Gedde-Dahl Jr, A Gånemo, A Vahlquist, N Dahl

J Med Genet 2007;**44**:615–620. doi: 10.1136/jmg.2007.050542

See end of article for authors' affiliations

Correspondence to: Professor Niklas Dahl, Department of Genetics and Pathology, Uppsala University, Rudbeck Laboratory, SE-751 85 Uppsala, Sweden; niklas.dahl@genpat.uu.se

Received 23 March 2007
Revised 11 May 2007
Accepted 16 May 2007
Published Online First 8 June 2007

Background: Autosomal recessive congenital ichthyosis (ARCI) is a heterogeneous group of skin disorders. Several mutant genes have been identified in ARCI, but the association between genotype and phenotype is poorly understood.
Methods: To investigate genotype–phenotype correlations in ARCI, we selected 27 patients from 18 families with specific ultrastructural features of the epidermis. The characteristic findings using electron microscopy (EM) were abnormal lamellar bodies and elongated membranes in the stratum granulosum, classified as ARCI EM type III. DNA samples from a subset of affected individuals were screened for homozygous genomic regions, and a candidate gene region was identified on chromosome 5q33. The region coincides with the *ichthyin* gene, previously reported as mutated in ARCI.
Results: Mutation screening of *ichthyin* revealed missense or splice-site mutations in affected members from 16 of 18 (89%) families with characteristics of ARCI EM type III. In a control group of 18 patients with ARCI without EM findings consistent with type III, we identified one patient homozygous for a missense mutation in *ichthyin*.
Discussion: Our findings indicate a strong association between ultrastructural abnormalities in the granular layer of epidermis and *ichthyin* mutations. The results also suggest that EM provides a tool for specific diagnosis in a genetically homogenous subgroup of patients with ARCI.

Autosomal recessive congenital ichthyosis (ARCI) is a clinically and genetically heterogenous group of disorders with abnormal differentiation and desquamation of the epidermis.[1] The main symptoms are widespread scaling of the skin and various degrees of erythema.[2] Six mutant genes, *TGM1* (OMIM 190195), *ABCA12* (OMIM 607800), *ALOXE3* (OMIM 607206), *ALOX12B* (OMIM 603741), *CYP4F22* and *ichthyin* (OMIM 609383), have to date been identified in non-syndromic ARCI.[1 3–8] Each of the corresponding proteins has been proposed to be involved in the formation of the permeability barrier of the skin, an important protection against water loss and physical injury.[9–12] Attempts have been made to link the various mutant genes to specific clinical characteristics in ARCI, but no strong genotype–phenotype correlations have been found.[13 14] The clinical symptoms of lamellar ichthyosis (LI; large brown scales covering the body) and non-bullous congenital ichthyosiform erythroderma (NCIE; fine scaling on an erythematous background) are associated with mutations in all the genes that have to date been connected to ARCI.[3 4 6–8 15]

Patients with ARCI have structural changes affecting terminal differentiation and keratinisation within the epidermis, which can be seen using electron microscopy (EM). About half of patients with ARCI investigated by EM lack unique characteristics of the epidermis but the remaining patients show defined ultrastructural markers that have been categorised into four main patterns: type I is characterised by broad stratum granulosum and numerous lipid vacuoles in corneocytes; type II shows clefts of former cholesterol crystals in stratum corneum; type III is characterised by abnormal lamellar bodies in stratum granulosum and perinuclear, elongated membranes; and type IV shows lipid membrane aggregations in upper epidermal cells.[16–19] The ultrastructural abnormalities are, however, not consistent with either the clinical presentation or the genetic lesions. Considerable clinical variation is associated with each of the different EM types although correlations have been reported between NCIE and EM type I, between LI and EM type II, and between ichthyosis prematurity syndrome (IPS; OMIM 608649) and EM type IV.[18 20–22]

In the present study, we performed a clinical examination of patients with ARCI and EM investigation on specimens from their epidermis. Patients with the distinct ultrastructural markers defined as ARCI EM type III underwent genetic analysis using single nucleotide polymorphism (SNP) chip technology and segregation analysis. A candidate region spanning the *ichthyin* gene was identified, and DNA sequencing of the gene revealed known and novel mutations in 93% of the patients with ARCI type III. Our findings suggest a strong correlation between ultrastructural characteristics of the epidermis and mutant *ichthyin* in this subgroup of patients with ARCI.

METHODS

Electron microscopy

The patients included in the study were selected on basis of ultrastructural criteria of the epidermis from Scandinavian patients with ichthyosis attending dermatology clinics. The characteristic EM picture (a hyperkeratotic stratum corneum with elongated membranes and a stratum granulosum with

Abbreviations: ARCI, autosomal recessive congenital ichthyosis; EM, electron microscopy; HI, harlequin ichthyosis; IPS, ichthyosis prematurity syndrome; LI, lamellar ichthyosis; LOX, lipoxygenase; NCBI, National Center for Biotechnology Information; NCIE, non-bullous congenital ichthyosiform erythroderma; OMIM, Online Mendelian Inheritance in Man; qPCR, quantitative PCR; SNP, single nucleotide polymorphism

perinuclear membranes, abnormal lamellar bodies and vacuoles) was designated as ARCI EM type III.

The EM procedure was performed as follows. Skin biopsy specimens were fixed for at least 2 h at room temperature in 3% glutaraldehyde solution in 0.1 mol/l cacodylate buffer pH 7.4. The specimens were cut into pieces approximately 1 mm^3 in size and washed in the same buffer. Biopsies were then postfixed for 1 h at 4°C in 1% osmium tetroxide, rinsed in water, dehydrated through graded ethanol solutions, transferred into propylene oxide and embedded in epoxy resin (Glycidether 100; Merck, Darmstadt, Germany). Semithin and ultrathin sections were cut on an ultramicrotome (Ultracut E; Reichert, Depew, New York, USA). Semi-thin sections were stained with methylene blue; ultrathin sections were treated with uranyl acetate and lead citrate and examined under an electron microscope (Philips EM 400, Best, The Netherlands). Patient specimens were classified according to the ultrastructural findings.

Subjects and samples

Affected patients from 18 families fulfilled the criteria of EM type III, and they and their affected siblings (n = 27) were included in the study. Two families were consanguineous. The patients comprised 20 female and 7 male patients aged 1–73 years at the time of examination. There were 24 patients who had been born at term; 3 patients were born 3–4 weeks prematurely, with a dry and scaly skin. One patient had been a collodion baby. After birth, all patients had normal psychomotor development except one, who had microcephaly, short stature and spasticity in the extremities. Since infancy, the patients had shown moderate to severe generalised ichthyosis with no, mild or moderate erythema. A few cases of ectropion were seen, and keratoderma on the soles and hypohidrosis had developed in most of the patients. In several of the patients, areas of reticulate ichthyosis with brownish hyperkeratosis and scaling could be observed on the trunk and in skin folds (fig 1). The severity of the skin symptoms varied within families, and some patients reported cyclical changes in the severity of their symptoms. No nail or hair abnormalities were found.

In total, 18 patients with ARCI (from 16 families) who were negative for *TGM1* mutation after screening were used as a control group. These patients had clinical presentations ranging between LI and NCIE and the ultrastructural findings were not consistent with ARCI EM type III criteria. The patients with ARCI EM type III and the control group originated from Norway, Sweden, and in one case, from the Faroe Islands. One healthy parent had a South American origin.

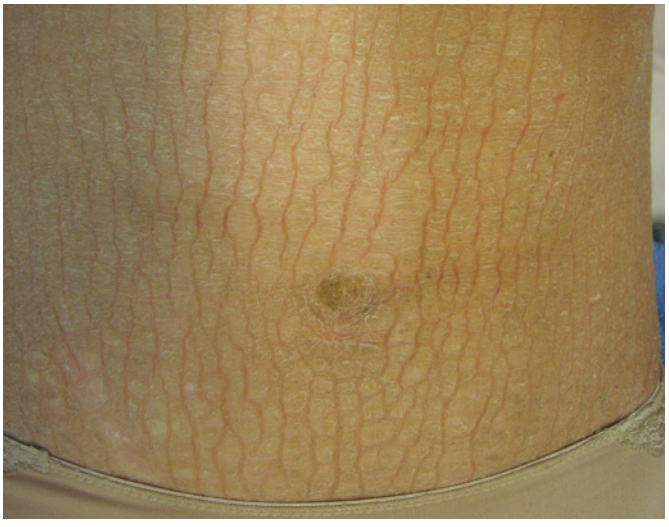

Figure 1 Reticulate ichthyosis with brownish hyperkeratosis and scaling on the trunk of an adult woman, a common clinical feature of patients with ARCI EM type III. Informed consent was obtained for publication of this figure.

Genetic mapping and sequencing

Blood samples were collected from probands, healthy siblings and parents after informed consent. DNA was extracted from peripheral blood leukocytes according to standard methods. In search for homozygous regions we used a chip array (SNP GeneChip Mapping 10 k array; Affymetrix, Santa Clara, California, USA) and genotyped 10 affected members of six families (IR17, IR23, IR84, IR99, EB32, IR56), all of whom were classified as having ARCI EM type III. The patients were selected by their clustered geographical origin, which suggested a founder effect. Two of the families were consanguineous (IR23, IR56). The DNA was processed according to the manufacturer's protocol (GeneChip Mapping 10 k *Xba*; Affymetrix®). The SNP-chip data output was exported to Microsoft Excel software for analysis and was adapted for autozygosity mapping using the EXCLUDEAR spreadsheet.[23] A limit for significant homozygosity was calculated in the families so that a number of consecutive homozygous SNPs should appear <1 in 1000 times by chance.[23] This limit was used because it equals a LOD score of >3, regarded as significant in conventional linkage analysis.

A candidate homozygous region on chromosome 5q33 was further analysed by microsatellite markers (D5S820, D5S2112, D5S378, D5S1403, D5S2049, D5S1955, D5S1394) on samples from the 10 patients and from healthy family members of families IR23 and IR56.

For the microsatellite marker analyses, we amplified genomic DNA by PCR using fluorescently labelled primer pairs (Sigma-Proligo, St Louis, Missouri, USA) and Taq DNA polymerase (Platinum Taq DNA Polymerase; Invitrogen, San Diego, USA) as described elsewhere.[22]

All six exons, the 5′ and 3′ untranslated regions, and the exon–intron boundaries of the *ichthyin* gene on chromosome 5q33 were analysed by DNA sequencing in samples from affected individuals and the parents of one proband who was not available for sampling. The primer sequences (available upon request) were designed according to the genomic sequence AC008676 (NCBI Map viewer) using the Primer3 program (http://frodo.wi.mit.edu) and ordered from Sigma-Proligo. Sequencing was performed using a commercial kit (BigDye Terminator v3.1 Cycle Sequencing Kit (Invitrogen,) and an automated sequencer (ABI Prism 3700 sequencer; Applied Biosystems, Foster City, CA, USA). The chromatograms were analysed using Sequencher V.4.1.2 (Gene Codes Corp., Ann Arbor, Michigan, USA).

Quantitative real-time PCR on keratinocyte-derived mRNA

Skin punch biopsies for cell culture were obtained from six affected patients diagnosed with ARCI EM type III and three healthy controls. Primary keratinocytes were obtained from the biopsies and were cultured in cell medium (65.6% Dulbecco minimal essential medium, 22% F12, 9.5% fetal calf serum, 0.1% hydrocortisone, 0.4% cholera toxin, 0.1% epidermal growth factor, 0.1% insulin, 1% Penieillin-Streptomyein, 1% non-essential amino acids, 0.2% adenine) with a feeder layer of radiated 3T3 cells. After 14 days, the keratinocytes were split and grown in Epilife with HKGS-v2 and PSA (Cascade Biologics, Portland, Oregon, USA) for an additional 5–7 days. The cells were maintained at 37°C in a humidified atmosphere with 5% CO_2. RNA was prepared using TRIzol (Invitrogen) according to the manufacturer's protocol. Reverse transcription

PCR and quantitative real-time PCR (qPCR) (Platinum SYBR Green qPCR supermix-UGD kit, Invitrogen) were performed as described previously.[34] For qPCR of the *ichthyin* cDNA we used a primer pair with the forward primer in exon 5 and the reverse primer in exon 6, generating an amplicon of 126 bp. cDNA levels of *β-actin* were used for normalisation. PCR and detection were performed (ABI Prism 7000 sequence detection system; Applied Biosystems) and the results analysed (ABI Prism 7000 SDS software; Applied Biosystems). Statistical calculations were executed with Microsoft Excel using unpaired *t* test, and significance set at $p<0.05$.

RESULTS

Electron microscopy

In total, 27 patients with ARCI (from 18 families) fulfilled the EM criteria of ARCI EM type III with the characteristics of hyperkeratotic stratum corneum with lamellae that contained low or minimal amounts of lipid droplets (fig 2). The same layer was characterised by regularly elongated membranes and vesicular complexes interpreted as irregularly processed content of lamellar bodies. The granular cells were characterised by normal keratohyaline, normal lamellar bodies and regularly elongated perinuclear membranes. In addition, specific vesicular complexes were present, as well as electron-lucent vacuoles sometimes containing one or a few smaller vesicles thought to represent abnormal lamellar bodies. The size of the vesicular structures made them possible to be recognised by light microscopy in the specimens of some patients. Specimens from different patients showed a predominance of either membranes or vesicular complexes (fig 2).

A group of 18 patients with ARCI (from 16 families) with various ultrastructural findings, not consistent with EM type III, were used as a control group for DNA analysis. *TGM1* mutations had been excluded in this group.

Genetic mapping

The 10 K SNP array analysis on 10 patients with ARCI EM type III gave an average call rate of 90.21% (range 85.78–97.21%). A genomic region of on chromosome 5q33 was found to be homozygous in six probands from four families (IR17, IR23, IR84, IR99; fig 3). The patients were homozygous for either of two haplotypes over a region that spanned a minimum physical distance of 873 kb flanked by the SNPs rs452223 and rs1957561 (positions 156 544 071 and 157 416 584 respectively; National Center for Biotechnology Information (NCBI) build 35.1). Four additional patients from two families (EB32 and IR56) were compound heterozygous for the two haplotypes. The candidate region spans 12 predicted genes (NCBI database) including the *ichthyin* gene, previously reported as associated with ARCI.[7] Association with the candidate region was confirmed by segregation analysis using samples from parents and siblings from families IR23 and IR56.

Mutation analysis

The *ichthyin* gene (accession number GeneID 348938, NCBI GenBank) was sequenced on DNA from all the 27 ARCI EM type III patients. Two missense mutations, p.A176D c.527C→A in exon 4, and p.G230R c.688G→A in exon 5, were identified in 25 of the 27 ARCI EM type III patients. The probands of families IR17, IR23 and IR84 were found to be homozygous for

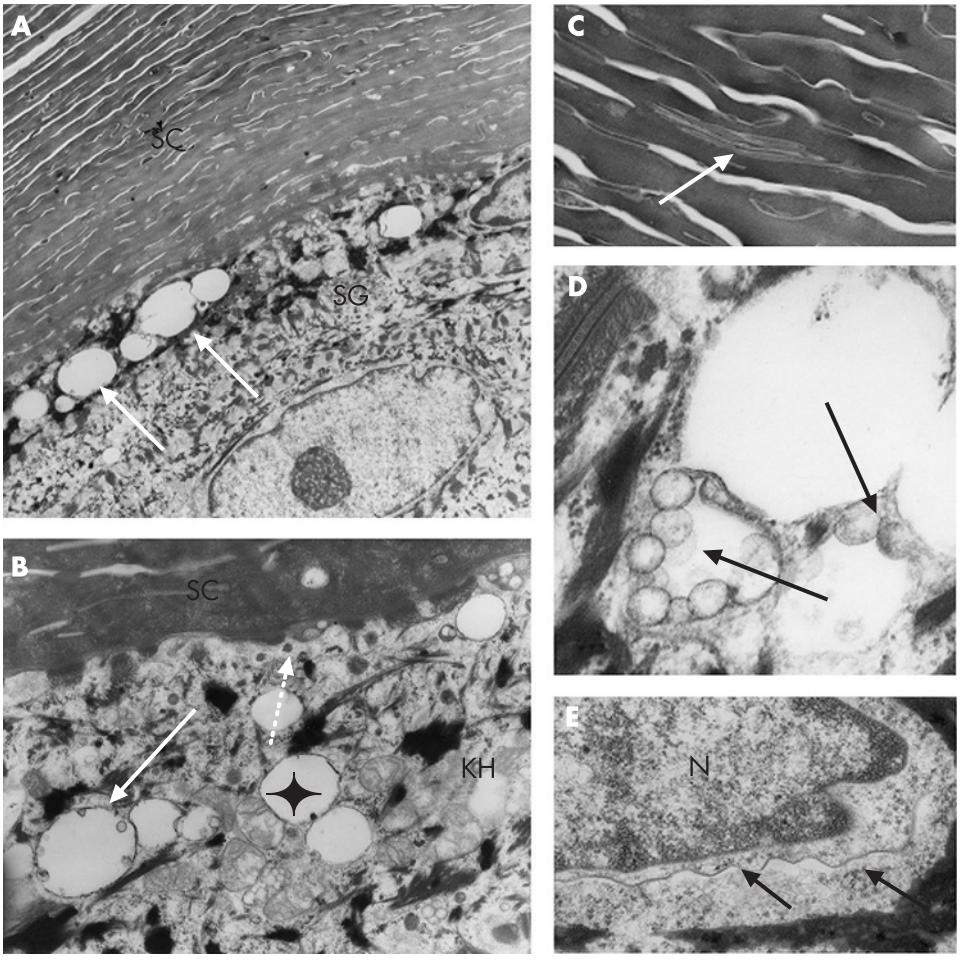

Figure 2 EM of epidermis of ARCI patient classified as type III. (A) Hyperkeratotic horny layer (SC) and granular layer (SG) with vacuoles (arrows); magnification ×5500. (B) Vacuolar structures within the granular layer, consisting of larger vesicles, partly empty (asterisk), partly containing some marginal small vesicles (filled arrow). There are also morphologically normal lamellar bodies (dotted arrow). SC, stratum corneum; KH, keratohyalin; magnification ×17 000. (C) Small stack of elongated membranes (arrow) within horny scales; magnification ×25 000. (D) Higher magnification of vesicular complexes (arrows), putatively defective lamellar bodies, within the granular layer. D, desmosome; magnification ×50 000. (E) Perinuclear elongated membraneous structure (arrows). N, nucleus; magnification ×20 000.

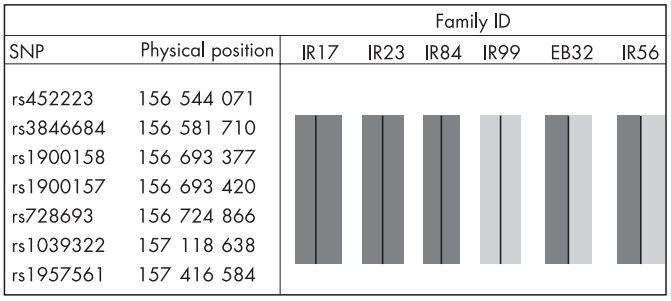

Figure 3 SNP array results from the chromosome 5q33 region in 6 patients with ichthyosis type III from families IR17, IR23, IR84, IR99, EB32 and IR56. The two haplotypes are illustrated as either dark or light grey bars. Probands of families IR17, IR23, IR84 are homozygous for one haplotype, whereas proband of family IR99 is homozygous for a different haplotype. Affected members of EB32 and IR56 are compound heterozygous for the two haplotypes.

the c.527C→A mutation and the proband of IR99 was homozygous for the c.688G→A mutation. The affected members of families EB32 and IR56 were compound heterozygous for the two mutations c.527C→A and c.688G→A, consistent with the SNP array results. The p.A176D c.527C→A missense mutation was the most prevalent mutation, found on 37 of 54 (69%) alleles associated with ARCI EM type III. The p.G230R c.688G→A transition was less prevalent, found on nine (17%) alleles. In addition, two new splice-site mutations were identified in the *ichthyin* gene. One patient was found to be compound heterozygous for an AG→AA transition in the acceptor splice site of exon 3 and another patient was compound heterozygous for a GT→AT transition in the donor splice site of exon 5.

The exon 5 splice-site mutation was further analysed on patient-derived *ichthyin* cDNA. DNA sequencing showed that the mutant mRNA retained 68 nucleotides of intronic sequence by the activation of a cryptic splice site, which resulted in a premature stop codon (fig 4). None of the four alterations was found on 200 chromosomes from a Scandinavian control population. In the control group of 18 *TGM1*-negative patients with ARCI who had EM results inconsistent with ARCI EM type III, we identified one who was homozygous for the *ichthyin* missense mutation p.A176D c.527C→A.

In total, we identified *ichthyin* gene mutations on both alleles in 23 of the 27(85%) patients diagnosed as having ARCI EM type III (15 of 18 families). Two affected siblings were found to have a single heterozyous p.G230R, c.688G→A missense mutation (table 1).

Expression analysis

The expression of *ichthyin* in keratinocytes from six patients with ARCI EM type III with *ichthyin* gene mutations was analysed by quantitative PCR. Five patients were homozygous for the missense mutation c.527C→A and one was compound heterozygous for c.527C→A and the donor splice-site mutation in exon 5. We found no significant differences in *ichthyin* expression levels between affected and healthy people. The *ichthyin* mRNA levels showed a large variation within both groups (data not shown).

DISCUSSION

In this study, we identified a strong correlation between patients with ARCI with specific ultrastructural abnormalities of the epidermis and mutations in the *ichthyin* gene. The EM morphology is characterised by abnormal lamellar bodies and perinuclear elongated membranes in stratum granulosum, and is classified as EM type III. Homozygosity mapping using high-density SNP arrays revealed a candidate region spanning the *ichthyin* gene, which was found to be mutated in 25 of 27 (93%) patients.

Six different mutations have previously been described in the *ichthyin* gene.[7] In our cohort of patients from 18 families, we identified four *ichthyin* gene mutations, of which three are novel. One is a missense mutation (c.688G→A) that results in an amino acid substitution from glycine to arginine (nonpolar to charged polar). This missense mutation (p.G230R) affects an evolutionarily conserved residue according to the ConSeq database (conseq.bioinfo.tau.ac.il/). Two novel mutations affect splice sites, of which one is a donor splice-site mutation in intron 5 and another an acceptor splice-site mutation in intron 2. Quantitative reverse transcriptase PCR was used to study the levels of mutant *ichthyin* mRNA in five patients homozygous for the c.527C→A missense mutation and in one patient compound heterozygous for the c.527C→A missense mutation and the donor splice-site mutation in exon 5. No differences in the levels of *ichthyin* cDNA were found when comparing healthy and affected people. This suggests that these mutations do not alter the stability of the transcript, and further studies are required to clarify the effect of the mutations at a molecular level. In two siblings, diagnosed with ARCI EM type III, we identified only one mutant allele in the *ichthyin* gene. This is possibly due to an as yet unidentified mutation on the other allele. In 2 of the 27 individuals diagnosed with EM type III, we did not find any mutation in the *ichthyin* gene, which may be explained by yet unidentified mutations in *ichthyin* or by genetic heterogeneity in ARCI EM type III. Of the 18 control patients with ARCI who had EM pictures that did not fulfill ARCI EM type III criteria, 1 carries a mutant *ichthyin*. This indicates that

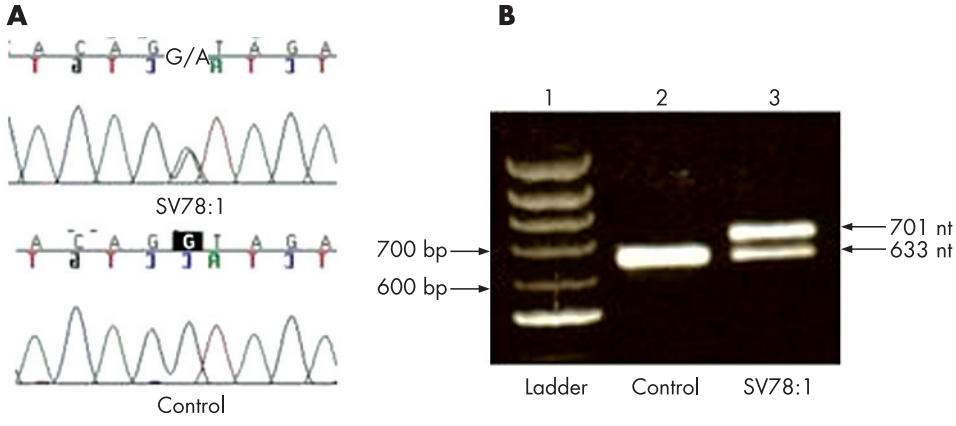

Figure 4 (A) DNA sequence of patient SV78:1 (top) showing a splice-site mutation in the donor splice-site of *ichthyin* exon 5 on one allele and a control sequence (bottom). (B) Reverse transcriptase PCR of *ichthyin* from keratinocyte-derived mRNA of patient SV78:1 (lane 3) and a healthy control (lane 2). A normal 633 nucleotide amplicon is generated by a primer pair in exon 1 and exon 6. The cDNA derived from SV78:1 is heterozygous for a transcript that has retained 68 nucleotides of intron 5.

Table 1 Distribution of *ichthyin* mutations in patients with ARCI with EM type III

Ichthyin mutations			
Allele 1	**Allele 2**	**Families (n)**	**Patients (n)**
527C→A	527C→A	9	15
688G→A	688G→A	1	1
527C→A	688G→A	3	5
688G→A	Unknown	1	2
527C→A	AG→AA exon 3	1	1
527C→A	GT→AT exon 5	1	1
Unknown	Unknown	2	2
Total		18	27

Four different mutations were identified in 16 families.
Both alleles are affected in 23 probands and one allele is affected in two siblings.
In two individuals, no mutation was identified.
The c.527C→A missense mutation was the most common mutation, found on 37 of 54 alleles.

Ichthyin deficiency does not necessarily result in structural changes detected as EM type III.

Several genes are implicated in ARCI, and the clinical presentation varies even within families.[1 2 21] Previous studies report that a large proportion of patients with the clinical presentation of LI or NCIE carry mutations in the *TGM1* gene.[3 4 20 21 25] The phenotypes of LI and NCIE are, however, non-specific, and both clinical forms have been found associated with mutations in all known ARCI genes.[4–8 26] Two ultrastructural phenotypes in ARCI have to some extent been associated with different genotypes: EM type II is associated with mutations in the *TGM1* gene and the EM type IV morphology is associated with an as yet unidentified ARCI gene mapped to chromosome 9q34.[14 20–22 24] Previous attempts have also been made to correlate the ultrastructural changes in epidermis with the ARCI phenotype, and associations have been reported between NCIE and EM type I, between LI and EM type II, and between IPS and EM type IV.[18 20–22]

There is a high level of expression of the *ichthyin* gene in keratinocytes, but its role in the skin remains unknown.[7] Other mutant genes identified in ARCI have been suggested to be involved in lipid pathways essential for the permeability barrier of the skin.[3–6 8] Examples are the genes *ALOXE3* and *ALOX12B*, which encode the enzymes epidermal lipoxygenase 3 (eLOX3) and 12R-LOX, both of which are believed to metabolise arachidonic acid in the skin. People with mutations in these genes have a defect in the epidermal lipid barrier.[11] It has been hypothesised that *ichthyin* plays a role as a receptor in the same metabolic process.[7] The ultrastructural characteristics of ARCI EM type III, with abnormal lamellar bodies aggregated in the granular cells and elongated membrane structures within the same cells,[16] suggest that Ichthyin is involved in the formation or the transport of lamellar bodies. The lamellar bodies are beaded tubular structures continuous with the *trans*-Golgi network and contain phospholipids, glycoproteins and acid phosphates that are released into the intercellular space.[9] These substances are essential for the lipid barrier of the skin, and defective Ichthyin could thus interfere with their normal transport to the intercellular space. A possible function for Ichthyin in the lamellar body membrane may be either as a receptor for 12R-LOX and eLOX3 enzymatic products and/or for the fusion of lamellar bodies with the plasma membrane. Abnormal lamellar bodies in stratum granulosum of the epidermis are found in other disorders such as CEDNIK (*c*erebral *d*ysgenesis, *n*europathy, *i*chthyosis and *k*eratoderma; OMIM 609528), harlequin ichthyosis (HI; OMIM 242500) and neutral lipid storage disease (OMIM 275630), all of which show symptoms of ichthyosis.[27–29] In HI, the lamellar bodies appear to be deficient in lipid content. One mutant gene has been identified in HI, *ABCA12*, and it has been proposed that this is involved in lipid transport within the epidermis.[30] The synthesis and transport of lipids mediated by ABCA12 seem to be essential, and it is possible that a dysfunction of Ichthyin results in a similar but milder defect in epidermis.

From our findings, we conclude that mutations in the *ichthyin* gene are associated with ARCI type III as defined by EM. The patients in our study have large variation in severity of clinical symptoms but with similar ultrastructural abnormalities in epidermis. The ultrastructural characteristics of ARCI EM type III are consistent with a defect in the stratum granulosum. Further studies of these abnormalities may shed light on the normal cellular processes mediated by Ichthyin as well as the general mechanisms of terminal differentiation and keratinisation in epidermis. We suggest that a combination of ultrastructural and genetic analysis may provide a tool for an accurate diagnosis and clinical delineation for some patients in this heterogeneous group of disorders.

Electronic database information

- Mutation p.A176D, c.527C→A: NCBI GenBank accession number EF599763.
- Mutation p.G230R, c.688G→A: NCBI GenBank accession number EF599764.
- Mutation AG→AA acceptor splice-site exon 3: NCBI GenBank accession number EF599765.
- Mutation GT→AT donor splice-site exon 5: NCBI GenBank accession number EF599766.

ACKNOWLEDGEMENTS

We thank the patients and their families for their contribution to this study. We thank Malin Melin for technical assistance, Inger Pihl-Lundin for her assistance with cell culturing and professor Kirsti-Maria Niemi and professor Per Westermark for reviewing the EM pictures of the Swedish patients. This work was supported by grants from the Swedish Research Council, the Torsten and Ragnar Söderbergs Fund, Uppsala University and University Hospital, Astra Zeneca and the Network for Ichthyoses and Related Keratinisation disorders, German Ministry of Education and Research.

Authors' affiliations

J Dahlqvist*, J Klar*, M Hellström Pigg, N Dahl, Department of Genetics and Pathology, Uppsala University, The Rudbeck Laboratory, Uppsala, Sweden
I Hausser, I Anton-Lamprecht, Department of Dermatology, University of Heidelberg, Heidelberg, Germany
T Gedde-Dahl Jr, Department of Dermatology, Rikshospitalet University Hospital and Institute of Forensic Medicine, University of Oslo, Oslo, Norway
A Gånemo, A Vahlquist, Department of Medical Science, Uppsala University Hospital, Uppsala, Sweden

Competing interests: None declared.

*The first two authors have contributed equally to this work.

T Gedde-Dahl died February 2006.

Informed consent was obtained from the patient or parent/guardian for publication of figure 1.

REFERENCES

1 **Richard G**. Molecular genetics of the ichthyoses. *Am J Med Genet C Semin Med Genet* 2004;**131C**:32–44.

2 **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**:477–88.
3 **Huber M**, Rettler I, Bernasconi K, Frenk E, Lavrijsen SP, Ponec M, Bon A, Lautenschlager S, Schorderet DF, Hohl D. Mutations of keratinocyte transglutaminase in lamellar ichthyosis. *Science* 1995;**267**:525–8.
4 **Russell LJ**, DiGiovanna JJ, Rogers GR, Steinert PM, Hashem N, Compton JG, Bale SJ. Mutations in the gene for transglutaminase 1 in autosomal recessive lamellar ichthyosis. *Nat Genet* 1995;**9**:279–83.
5 **Jobard F**, Lefevre C, Karaduman A, Blanchet-Bardon C, Emre S, Weissenbach J, Ozguc M, Lathrop M, Prud'homme JF, Fischer J. 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**:107–13.
6 **Lefevre C**, Audebert S, Jobard F, Bouadjar B, Lakhdar H, Boughdene-Stambouli O, Blanchet-Bardon C, Heilig R, Foglio M, Weissenbach J, Lathrop M, Prud'homme JF, Fischer J. Mutations in the transporter ABCA12 are associated with lamellar ichthyosis type 2. *Hum Mol Genet* 2003;**12**:2369–78.
7 **Lefevre C**, Bouadjar B, Karaduman A, Jobard F, Saker S, Ozguc M, Lathrop M, Prud'homme JF, Fischer J. Mutations in ichthyin a new gene on chromosome 5q33 in a new form of autosomal recessive congenital ichthyosis. *Hum Mol Genet* 2004;**13**:2473–82.
8 **Lefevre C**, Bouadjar B, Ferrand V, Tadini G, Megarbane A, Lathrop M, Prud'homme JF, Fischer J. Mutations in a new cytochrome P450 gene in lamellar ichthyosis type 3. *Hum Mol Genet* 2006;**15**:767–76.
9 **Madison KC**. Barrier function of the skin: ''la raison d'etre'' of the epidermis. *J Invest Dermatol* 2003;**121**:231–41.
10 **Elias PM**, Schmuth M, Uchida Y, Rice RH, Behne M, Crumrine D, Feingold KR, Holleran WM, Pharm D. Basis for the permeability barrier abnormality in lamellar ichthyosis. *Exp Dermatol* 2002;**11**:248–56.
11 **Eckl KM**, Krieg P, Kuster W, Traupe H, Andre F, Wittstruck N, Furstenberger G, Hennies HC. Mutation spectrum and functional analysis of epidermis-type lipoxygenases in patients with autosomal recessive congenital ichthyosis. *Hum Mutat* 2002;**26**:351–61.
12 **Oji V**, Traupe H. Ichthyoses: differential diagnosis and molecular genetics. *Eur J Dermatol* 2006;**16**:349–59.
13 **Hennies HC**, Kuster W, Wiebe V, Krebsova A, Reis A. Genotype/phenotype correlation in autosomal recessive lamellar ichthyosis. *Am J Hum Genet* 1998;**62**:1052–61.
14 **Ganemo A**, Pigg M, Virtanen M, Kukk T, Raudsepp H, Rossman-Ringdahl I, Westermark P, Niemi KM, Dahl N, Vahlquist A. Autosomal recessive congenital ichthyosis in Sweden and Estonia: clinical, genetic and ultrastructural findings in eighty-three patients. *Acta Derm Venereol* 2003;**83**:24–30.
15 **Lesueur F**, Bouadjar B, Lefevre C, Jobard F, Audebert S, Lakhdar H, Martin L, Tadini G, Karaduman A, Emre S, Saker S, Lathrop M, Fischer J. Novel mutations in ALOX12B in patients with autosomal recessive congenital ichthyosis and evidence for genetic heterogeneity on chromosome 17p13. *J Invest Dermatol* 2007;**127**:829–34.
16 **Arnold ML**, Anton-Lamprecht I, Melz-Rothfuss B, Hartschuh W. Ichthyosis congenita type III. Clinical and ultrastructural characteristics and distinction within the heterogeneous ichthyosis congenita group. *Arch Dermatol Res* 1988;**280**:268–78.
17 **Anton-Lamprecht I**. The skin. In: Papadimitrou J, Spagnolo D, eds. *Diagnostic ultrastructure of non-neoplastic diseases*. Edinburgh: Churchill and Livingstone, 1992:459–550.
18 **Niemi KM**, Kanerva L, Kuokkanen K. Recessive ichthyosis congenita type II. *Arch Dermatol Res* 1991;**283**:211–18.
19 **Niemi KM**, Kanerva L, Wahlgren CF, Ignatius J. Clinical, light and electron microscopic features of recessive ichthyosis congenita type III. *Arch Dermatol Res* 1992;**284**:259–65.
20 **Pigg M**, Gedde-Dahl T Jr, 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**:589–96.
21 **Laiho E**, Niemi KM, Ignatius J, Kere J, Palotie A, Saarialho-Kere U. Clinical and morphological correlations for transglutaminase 1 gene mutations in autosomal recessive congenital ichthyosis. *Eur J Hum Genet* 1999;**7**:625–32.
22 **Klar J**, Gedde-Dahl T Jr, Larsson M, Pigg M, Carlsson B, Tentler D, Vahlquist A, Dahl N. Assignment of the locus for ichthyosis prematurity syndrome to chromosome 9q33.3–34.13. *J Med Genet* 2004;**41**:208–12.
23 **Woods CG**, Valente EM, Bond J, Roberts E. A new method for autozygosity mapping using single nucleotide polymorphisms (SNPs) and EXCLUDEAR. *J Med Genet* 2004;**41**:e101.
24 **Melin M**, Klar J Jr, Gedde-Dahl T, Fredriksson R, Hausser I, Brandrup F, Bygum A, Vahlquist A, Hellstrom Pigg M, Dahl N. A founder mutation for ichthyosis prematurity syndrome restricted to 76 kb by haplotype association. *J Hum Genet* 2006;**51**:864–71.
25 **Choate KA**, Williams ML, Khavari PA. Abnormal transglutaminase 1 expression pattern in a subset of patients with erythrodermic autosomal recessive ichthyosis. *J Invest Dermatol* 1998;**110**:8–12.
26 **Akiyama M**, Sawamura D, Shimisu H. The clinical spectrum of nonbullous congenital ichthyosiform erythroderma and lamellar ichthyosis. *Clin Exp Dermatol* 2003;**28**:235–40.
27 **Sprecher E**, Ishida-Yamamoto A, Mizrahi-Koren M, Rapaport D, Goldsher D, Indelman M, Topaz O, Chefetz I, Keren H, O'Brien TJ, Bercovich D, Shalev S, Geiger D, Bergman R, Horowitz M, Mandel H. A mutation in SNAP29, coding for a SNARE protein involved in intracellular trafficking, causes a novel neurocutaneous syndrome characterized by cerebral dysgenesis, neuropathy, ichthyosis, and palmoplantar keratoderma. *Am J Hum Genet* 2005;**77**:242–51.
28 **Akiyama M**, Sawamura D, Nomura Y, Sugawara M, Shimizu H. Truncation of CGI-58 protein causes malformation of lamellar granules resulting in ichthyosis in Dorfman-Chanarin syndrome. *J Invest Dermatol* 2003;**121**:1029–34.
29 **Akiyama M**, Dale BA, Smith LT, Shimizu H, Holbrook KA. Regional difference in expression of characteristic abnormality of harlequin ichthyosis in affected fetuses. *Prenat Diagn* 1998;**18**:425–36.
30 **Kelsell DP**, Norgett EE, Unsworth H, Teh MT, Cullup T, Mein CA, Dopping-Hepenstal PJ, Dale BA, Tadini G, Fleckman P, Stephens KG, Sybert VP, Mallory SB, North BV, Witt DR, Sprecher E, Taylor AE, Ilchyshyn A, Kennedy CT, Goodyear H, Moss C, Paige D, Harper JI, Young BD, Leigh IM, Eady RA, O'Toole EA. Mutations in ABCA12 underlie the severe congenital skin disease harlequin ichthyosis. *Am J Hum Genet* 2005;**76**:794–803.

International Forum on Quality and Safety in Health Care

23–25 April 2008
Le Palais des Congrès de Paris

Call for Abstracts: Submission deadline 3 October 2007

Why submit an abstract?
- Your achievement showcased to key international opinion leaders
- Communicate your organisation's work
- Enhance your organisation's profile
- Network with others in your area/field
- Share your success, learn from your failures

For more details and to submit your abstract online visit internationalforum.bmj.com