Am. J. Hum. Genet. 76:794–803, 2005

Mutations in *ABCA12* Underlie the Severe Congenital Skin Disease Harlequin Ichthyosis

David P. Kelsell,[1] Elizabeth E. Norgett,[1] Harriet Unsworth,[1] Muy-Teck Teh,[1] Thomas Cullup,[2] Charles A. Mein,[2] Patricia J. Dopping-Hepenstal,[5] Beverly A. Dale,[8,9,10] Gianluca Tadini,[13] Philip Fleckman,[10] Karen G. Stephens,[11] Virginia P. Sybert,[10,12] Susan B. Mallory,[14] Bernard V. North,[3] David R. Witt,[15] Eli Sprecher,[16] Aileen E. M. Taylor,[17] Andrew Ilchyshyn,[18] Cameron T. Kennedy,[19] Helen Goodyear,[20] Celia Moss,[21] David Paige,[6] John I. Harper,[7] Bryan D. Young,[4] Irene M. Leigh,[1] Robin A. J. Eady,[5] and Edel A. O'Toole[1]

[1]Centre for Cutaneous Research, Institute of Cell and Molecular Science, [2]Genome Centre, William Harvey Research Institute, [3]Academic Department of Psychiatry, and [4]Molecular Oncology Unit, Institute of Cancer, Barts and the London School of Medicine and Dentistry, Queen Mary, University of London, [5]Genetic Skin Disease Group, Division of Skin Sciences, Guy's, King's, and St. Thomas' School of Medicine, St. Thomas' Hospital, [6]Department of Dermatology, Royal London Hospital, and [7]Department of Paediatric Dermatology, Great Ormond Street Hospital for Children, London; Departments of [8]Oral Biology, [9]Biochemistry, [10]Medicine/Dermatology, and [11]Laboratory Medicine, University of Washington, and [12]Department of Dermatology, Group Health Permanente, Seattle; [13]Institute of Dermatological Sciences, University of Milan, Milan; [14]Division of Dermatology, Department of Internal Medicine, Washington University School of Medicine, St. Louis; [15]Genetics Department, Kaiser Permanente, San Jose, CA; [16]Department of Dermatology and Laboratory of Molecular Dermatology, Rambam Medical Center, Haifa; [17]Department of Dermatology, Royal Victoria Infirmary, Newcastle upon Tyne, United Kingdom; [18]Department of Dermatology, Walsgrave Hospital, Coventry, United Kingdom; [19]Department of Dermatology, Southmead Hospital, Bristol, United Kingdom; and [20]Department of Paediatrics, Birmingham Heartlands Hospital, and [21]Department of Dermatology, Birmingham Children's Hospital, Birmingham, United Kingdom

Harlequin ichthyosis (HI) is the most severe and frequently lethal form of recessive congenital ichthyosis. Although defects in lipid transport, protein phosphatase activity, and differentiation have been described, the genetic basis underlying the clinical and cellular phenotypes of HI has yet to be determined. By use of single-nucleotide–polymorphism chip technology and homozygosity mapping, a common region of homozygosity was observed in five patients with HI in the chromosomal region 2q35. Sequencing of the *ABCA12* gene, which maps within the minimal region defined by homozygosity mapping, revealed disease-associated mutations, including large intragenic deletions and frameshift deletions in 11 of the 12 screened individuals with HI. Since HI epidermis displays abnormal lamellar granule formation, ABCA12 may play a critical role in the formation of lamellar granules and the discharge of lipids into the intercellular spaces, which would explain the epidermal barrier defect seen in this disorder. This finding paves the way for early prenatal diagnosis. In addition, functional studies of ABCA12 will lead to a better understanding of epidermal differentiation and barrier formation.

Introduction

Harlequin ichthyosis (HI [MIM 242500]) is a very severe and usually lethal skin disorder of unknown cause (Hsu et al. 1989; Moreau et al. 1999; Sarkar et al. 2000). HI is also known as "harlequin fetus" because of the tendency for affected babies to be born prematurely. The neonate is covered in a thick "coat of armor" that severely restricts movement (Hsu et al. 1989; Dahlstrom et al. 1995; Sarkar et al. 2000). The skin dries out to form hard diamond-shaped plaques separated by fissures, resembling "armor plating." The normal facial features are severely affected, with distortion of the lips (eclabion), eyelids (ectropion), ears, and nostrils. Historically, an HI-affected neonate would usually die within 2 d of birth because of feeding problems, bacterial infection, and/or respiratory distress. However, a number of patients have survived because of the wider availability of neonatal intensive care and likely benefits from oral retinoids (Hsu et al. 1989; Moreau et al. 1999; Elias et al. 2000; Sarkar et al. 2000). In survivors, the disease comes to resemble severe nonbullous congenital ichthyosiform erythroderma (NBCIE) (Haftek et al. 1996; Choate et al. 1998). The skin barrier remains severely compromised, which leads to increased transepidermal water loss, impaired thermal regulation, and increased risk of secondary infection (Moskowitz et al. 2004).

HI occurs in both sexes and in many ethnic groups. Autosomal recessive (AR) inheritance of HI has been

Received January 4, 2005; accepted for publication February 21, 2005; electronically published March 8, 2005.

Address for correspondence and reprints: Prof. David P. Kelsell, Centre for Cutaneous Research, Institute of Cell and Molecular Science, Barts and the London School of Medicine and Dentistry, 2 Newark Street, London E1 2AT, United Kingdom. E-mail: d.p.kelsell@qmul.ac.uk

0002-9297/2005/7605-0008$15.00

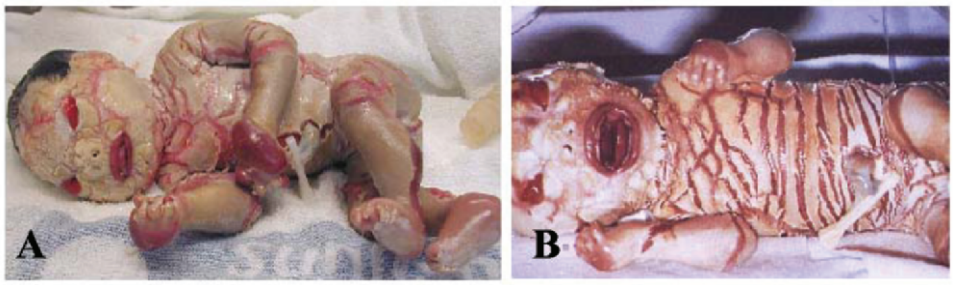


Figure 1 Typical clinical presentation of HI at birth

inferred, but, in a large number of cases, the inheritance pattern cannot be ascertained, and the disorder could be due to a new dominant mutation. It has been subdivided into three types based on the expression of keratin 16 and profilaggrin and the appearance of keratohyalin granules (Dale et al. 1990). Studies have also reported abnormalities in the localization of epidermal lipids and have highlighted the abnormal ultrastructure of epidermal lamellar granules in HI that are associated with the absence of extracellular lamellar structures in all three subtypes (Dale et al. 1990; Akiyama et al. 1996, 1997, 1998). The nuclei of the keratinocytes also show abnormalities, since they appear to become flattened early on in differentiation (in the lower cell layers), but some are retained even as the cornified layer is sloughed off. This parakeratosis along with hyperkeratosis and hypergranulosis (retention of keratohyalin granules in the stratum granulosum) is a characteristic phenotype of the epidermis of neonates with HI (Buxman et al. 1979; Hsu et al. 1989). This may suggest that the process of terminal differentiation is not completed in HI-affected skin. Alterations in calcium-mediated signaling and protein phosphatase activity have also been reported in HI keratinocytes (Kam et al. 1997; Michel et al. 1999). Plausible candidate genes underlying these diverse cellular phenotypes in HI have included *filaggrin, claudins, PP2A,* and *calpain 1* (Kam et al. 1997; Michel et al. 1999).

Mutations in the genes encoding transglutaminase-1 (*TGM1* [MIM 190195]), ichthyin, 4 lipoxygenase 3 (*ALOXE3* [MIM 607206]), and 12(R)-lipoxygenase (*ALOX12B* [MIM 603741]) have all been shown to underlie both NBCIE and lamellar ichthyosis (LI) (Huber et al. 1995; Jobard et al. 2002; Lefevre et al. 2004). Recently, missense mutations in the gene encoding the adenosine triphosphate (ATP)-binding cassette (ABC [GenBank accession number NM_173076]) transporter protein ABCA12 (MIM 607800) have also been shown to cause LI (Lefevre et al. 2003). Unlike the other AR forms of ichthyosis, the genetic basis of HI has not been elucidated.

In the present study, we have used SNP-array technology to localize the HI gene, on the basis of the principle of homozygosity mapping. This identified a major locus for HI on chromosome 2q35 and subsequent disruptive mutations in the *ABCA12* gene in 11 of the 12 analyzed patients with HI. Most of the mutations identified would result in a severely truncated protein. This genetic study immediately establishes the means for early prenatal DNA diagnosis of HI and provides a molecular clue toward understanding the numerous biological abnormalities of epidermal differentiation in HI-affected skin.

Material and Methods

Clinical

Twelve families, from diverse ethnic backgrounds, with individuals affected with HI were included in the present genetic study—three from the United States, seven from Great Britain, and two from Italy. Ethical approval was obtained from the local research ethics committees. The clinical phenotype was verified as HI by at least two clinicians; the typical clinical presentation at birth is shown in figure 1. A summary of the clinical details is shown in table 1. The control population was of mixed ethnicity and consisted of 60 unrelated individuals.

SNP-Array Analysis

A SNP GeneChip Mapping 10K (Affymetrix) array analysis was performed on affected individuals from six families. Two of these families were consanguineous, with AR inheritance of HI. Patient DNA samples were processed in accordance with the standard GeneChip Mapping 10K *Xba* Assay protocol. Briefly, 350 ng of DNA was digested with *Xba*I and ligation to the *Xba*I adaptor prior to PCR amplification by use of AmpliTaq Gold with Buffer II (Applied Biosystems). For each DNA sample, four 100-μl PCRs were set up to obtain sufficient purified PCR product (20 μg), by use of Ultrafree-MC filtration column (Millipore), for subsequent fragmenta-

Table 1

Clinical Details of Patients with HI

Patient	Ethnicity	Gestation (wk)	Birth Weight (g)	Treatment		Outcome	Other Problems
				Retinoid (Dose [mg/kg/d])	Begun at Age (d)		
49	British Bangladeshi	35	2,500	Acitretin (1)	2	SIE, now aged 2.5 years[a]	Pseudomonal SI, FTT, gastrostomy
6	British Pakistani	34	1,750	Etretinate (1.15)	2	SIE, now aged 20 years[b]	Anemia ectropion (surgery)
7	Bangladeshi	38	2,800	Acitretin	NA	SIE, now aged 14 years[c]	FTT, limb deformity
55	British Indian	36	2,600	Acitretin (.75)	4	SIE, now aged 8 years[b]	Neonatal sepsis (SA), hypernatremia, ectropion
40	Italian	40	3,200	Acitretin (1)	3	SIE, now aged 6 years[a]	Neonatal sepsis (CA), ectropion surgery, limb strictures
44	Italian	36	2,000	Acitretin (1)	7	SIE, now aged 6 years[a]	Nystagmus, PDA, neonatal sepsis
63	British Pakistani	36	2,600	Acitretin (.5)	4	SIE, now aged 2 years[b]	Nystagmus, MDD, cataracts, FTT
50	British white	36	2,300	Acitretin (1)	3	SIE, now aged 4 years[b]	Neonatal mild hypothermia
955-01	African-American	36	2,355	Isotretinoin (1)	7	Died at age 6 mo	Septicemia
915-06	Iranian	36	2,980	Isotretinoin (2)	19	Died at age 4 mo	Septicemia (SA)
928-01	Native American	40	NA	Not given		Died at age 1.5 d	Respiratory failure
69	British Pakistani	36	2,820	Not given		Died at age 3 d	Gram-negative sepsis

Note.—CA = *Candida albicans*; FTT = failure to thrive; MDD = motor developmental delay; NA = information not available; PDA = patent ductus arteriosus; SA = *Staphylococcus aureus*; SI = skin infection; SIE = severe ichthyosiform erythroderma.

[a] Remains on retinoids.

[b] Now off retinoids.

[c] Intermittent courses of retinoids.

tion with DNase I. Fragmentation was visualized by 4% agarose-gel electrophoresis to confirm the production of 50–100-bp PCR fragments prior to 3′ labeling with biotin and hybridization to the SNP array. Hybridized arrays were processed with an Affymetrix Fluidics Station 450, and fluorescence signals were detected using the Affymetrix GeneChip Scanner 3000. Raw SNP call data were exported to Microsoft Excel for analysis. The average call rate (expressed as a percentage) ± SEM for the six patients was 97.62% ± 0.41%.

Microsatellite and Linkage Analysis

Data from SNP chips revealed an overlapping region of homozygosity, in five of the six affected individuals, at a position on chromosome 2. Microsatellite markers mapping to this region were used to confirm and refine the minimal region of homozygosity. DNA from patient blood samples was first amplified with the microsatellite markers *D2S325* (chromosome 2q33.3) and *D2S126* (chromosome 2q36.1) from the Applied Biosystems Linkage Mapping Set. Sample DNA was amplified with these primers at 5 μM for 30 cycles at 55°C, by use of Amplitaq Gold PCR Mastermix (Applied Biosystems). *D2S128* (chromosome 2q34) and *D2S1345* (chromosome 2q34) were selected from the University of California–Santa Cruz (UCSC) Genome Bioinformatics database, and PCR was performed using Bioline Biotaq DNA Polymerase, Bioline PCR buffer, 60 nM $MgCl_2$, and 20 pM of each primer, with a final reaction volume of 20 μl. Fluorescent-tagged PCR products were analyzed using the ABI 3700 capillary sequencer. Haplotypes were constructed from the microsatellite data of affected individuals and unaffected siblings. Linkage analysis was performed using the Genehunter program (Kruglyak et al. 1996).

Sequence Analysis of ABCA12

Primer design and PCR conditions were the same as those described elsewhere (Lefevre et al. 2003), with the exception that a 15-μl reaction volume was used. The PCR products covering each of the 53 exons and respective intron boundaries were purified using ExoSAP-IT (Amersham Pharmacia Biotech) and were sequenced on either the forward or reverse strand, by use of ABI BigDye Terminator reagents (Applied Biosystems) and an ABI 3700 sequencer (sequencing reaction conditions: 5 μl of purified product, 1 μl of BigDye Terminator v.1.1, 3 μl of Better Buffer [Microzone], 1 μl water; annealing temperature of 60°C). Sequence analysis was performed using Phred, Phrap, and Consed, and variants were detected using reference sequences taken from the Ensembl Genome Browser (Ewing and Green 1998; Ewing et al. 1998; Gordon et al. 1998). Likely disease-associated mutations were screened in a total of 120 control chromosomes—either by direct sequencing or by RFLP analysis if the mutation altered a restriction site.

Results

Genetic Mapping

From the SNP-array analysis, only one region of the genome, 2q35, was found to have large blocks of homozygosity in common for five of the six affected individuals assessed by means of this methodology (fig. 2). This common interval was calculated to be a genetic distance of ~1.5 Mb. To support linkage of HI to 2q35, microsatellite markers that map to this region were identified using the UCSC Genome Browser. The microsatellite genotype data are consistent with a recessive mode of inheritance of HI and linkage of the disease gene to 2q35 in the largest consanguineous family with HI (fig. 3*A*). Linkage analysis was performed for this family, but the size of the family prohibited the calculation of a statistically significant LOD score (data not shown). This highlights the usefulness of the SNP array for homozygosity mapping in diseases not applicable to classic genetic linkage studies. Affected individuals from families in which the mode of inheritance is unknown were also genotyped with these microsatellite markers. Of the 12 affected individuals analyzed in the present study, 11 were homozygous for one or more of the markers analyzed in the 2q35 region (fig. 3*B*).

Candidate-Gene Analysis

Six genes were identifiable, from the UCSC Genome Browser, that map within the minimal region, including *BARD1,* which interacts with the breast-ovarian cancer–associated protein BRCA1; *ATIC,* in which mutations are the cause of a purine biosynthesis disorder (AICA-ribosuria [MIM 608688]); *Fibronectin 1,* which is involved in cell adhesion and migration; the *FLJ10116* gene, which encodes a protein of unknown biological function; and the *PECR* gene, which encodes a peroxisomal trans 2-enoyl CoA reductase. The gene *ABCA12,* which encodes the ABC transporter protein, also maps within the minimal homozygous region on chromosome 2q35. Missense mutations in this gene have recently been shown to underlie the less severe ichthyotic disorders LI and NBCIE (Lefevre et al. 2003). Since the transport of extracellular epidermal lipids is abnormal in HI (Dale et al. 1990) and a defect in epidermal lipid metabolism has been suggested in the pathogenesis of the disease (Buxman et al. 1979), *ABCA12* could be a plausible candidate gene on the basis of chromosome position and function. The 53 coding exons of the *ABCA12* gene were individually amplified by PCR and were sequenced for 12 patients. Mutations in *ABCA12* were detected in 11

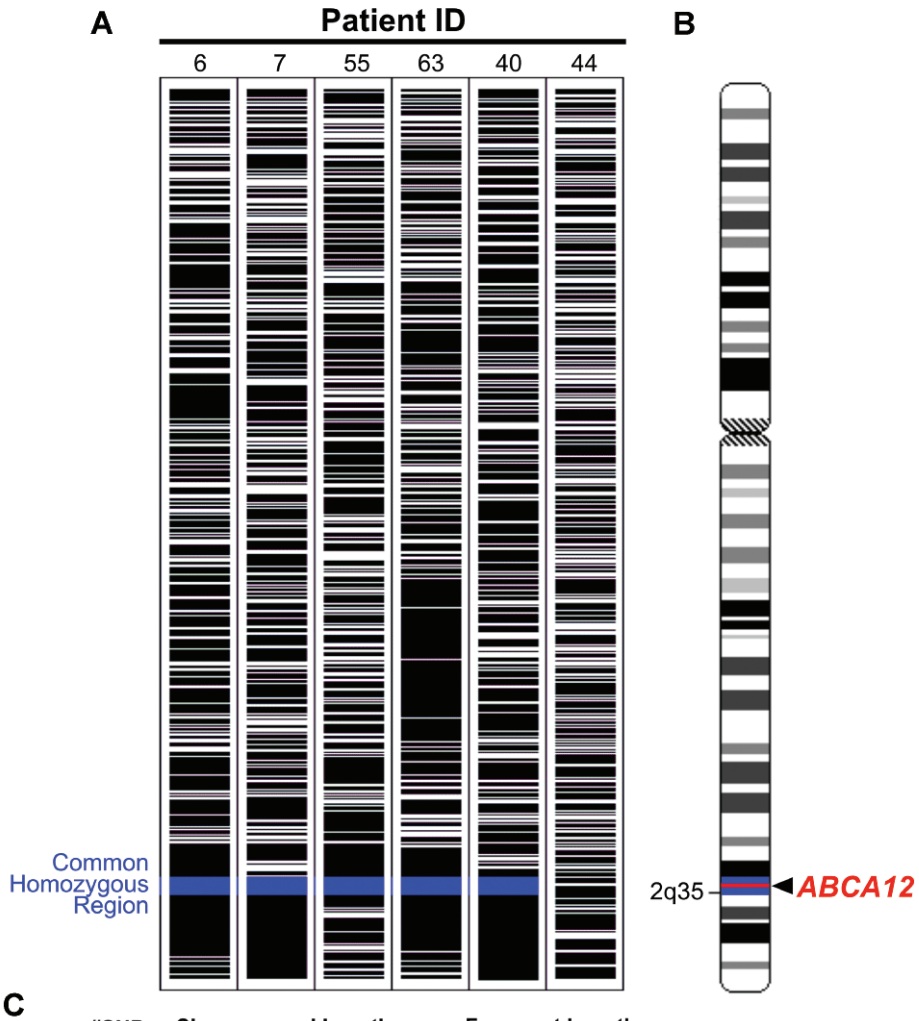
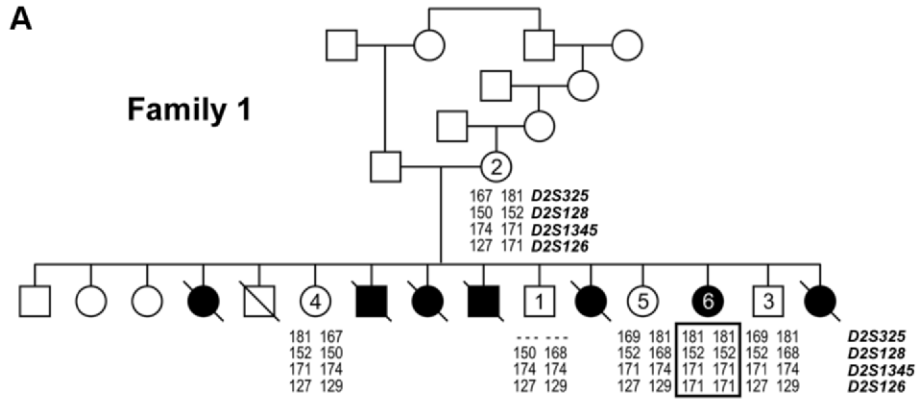


Figure 2 Localization of the HI gene to chromosome 2q35 by use of SNP-array mapping. *A*, Homozygous SNP profiles of chromosome 2 in six patients with HI. Black bars indicate homozygous SNP call data. Blue bars indicate the minimal common region of homozygous SNPs in patients 1–5, a genetic distance of ~1.5 Mb. *B*, Ideogram of chromosome 2 showing the position of *ABCA12* (*red bar*), located within the minimal common region of homozygous SNPs (*blue*) identified in panel A. *C*, The genetic interval of the homozygous SNP "blocks" in each patient with HI, with clear indication that the *ABCA12* gene maps within the common homozygous region for five of the six patients.

of the 12 patients (fig. 4). This indicates that *ABCA12* mutations are a major genetic cause of HI. The majority of the mutations were predicted to result in a truncated protein; indeed, in one patient (40), the last 25 exons were homozygously deleted. Four patients all harbored the same homozygous frameshift mutation and, on the basis of haplotype analysis (see fig. 3*B*) and their shared ethnicity, it is likely to be the same founder mutation in at least three of the four patients. In individual 955–01, a homozygous missense substitution (W198C) in exon 6 was identified in addition to the more disruptive homozygous nonsense substitution in exon 44. The latter sequence change is more likely to be associated with HI. Unlike the other individuals with HI in the mutation study who were homozygous for a specific *ABCA12* mutation, individual 44 was a compound heterozygote for two different *ABCA12* mutations. Both SNP and microsatellite genotypes were heterozygous in the chromo-



B

ABCA12
(chr 2q35)

Affected Patient ID	Marker (chr location)	D2S325 (chr 2q33.3)		D2S128 (chr 2q34)		D2S1345 (chr 2q34)		D2S126 (chr 2q36.1)	
	6	**181**	**181**	**152**	**152**	**171**	**171**	**171**	**171**
	69	---	---	**152**	**152**	**171**	**171**	---	---
	55	**159**	**159**	**152**	**152**	**171**	**171**	---	---
	63	---	---	150	152	171	168	**141**	**141**
	7	**169**	**169**	**160**	**160**	174	177	**141**	**141**
	40	169	173	148	150	---	---	**127**	**127**
	49	**161**	**161**	**160**	**160**	**174**	**174**	**139**	**139**
	50	---	---	154	158	**174**	**174**	---	---
	915-06	**177**	**177**	148	150	**168**	**168**	**126**	**126**
	955-01	**169**	**169**	**143**	**143**	**174**	**174**	**138**	**138**
	928-01	**169**	**169**	152	154	**177**	**177**	**138**	**138**
	44	183	179	156	158	174	177	139	141

Figure 3 Analysis of microsatellite markers flanking the *ABCA12* gene. *A,* Segregation of microsatellite markers flanking *ABCA12* at chromosome 2q35 in a family with HI. Only affected individual 6 is homozygous at this region of chromosome 2. Note that the majority of siblings with HI are deceased. *B,* Haplotype analysis of individuals with HI. Microsatellite markers map around the *ABCA12* gene locus (215, 621, 772–215, 828, 657) at chromosome 2q35. Several patients are homozygous for the markers *D2S325* (208, 096, 142–208, 096, 490), *D2S128* (214, 917, 811–214, 918, 065), *D2S1345* (214, 991, 103–214, 991, 425), and *D2S126* (221, 842, 419–221, 842, 782). The genome positions (Mb) for *ABCA12* and microsatellite markers on chromosome 2 are shown in parentheses.

somal region harboring the *ABCA12* locus in this patient. In only one patient (50) with HI was there no *ABCA12* mutation detected. None of the mutations were detected in the control population.

Discussion

The ABC superfamily is a large family of transmembrane proteins involved in transport of substrates across both the plasma membrane and intracellular membranes. Mutations in ABC genes cause diverse diseases, including cystic fibrosis, X-linked adrenoleukodystrophy, Tangier disease, pseudoxanthoma elasticum, and Dubin-Johnson syndrome (Stefkova et al. 2004). In the present article, we report, we believe for the first time, that mutations in the *ABCA12* gene, a member of the ABCA subfamily, cause HI. Genetic studies of this disorder have been hampered by the fact that families with more than one affected child are rare. Families with only one affected individual are unsuitable for genetic linkage studies be-

A

Patient	Mutation type	Resultant change	Exon	Mutation
49	Exon deletion	Truncated protein	23	Homozygous exon deletion
6	Frameshift	Truncated protein	49	7541delC/7541delC TACAGAACAAATGTTC[C]GTCATCCTCACATCTC
7	Frameshift	Truncated protein	42	6378delGC/6378delGC TCAATTGGTATCATT[GC]GATTTTCAAATTACC
55	Frameshift	Truncated protein	49	7541delC/7541delC TACAGAACAAATGTTC[C]GTCATCCTCACATCTC
40	Exon deletion	Truncated protein	28-53	Homozygous deletion from exon 28 to 53
44	Frameshift/amino acid change	Truncated protein/Reduced protein function	33/47	5229delA AAAGAGTCACAAAAAA[A]TAGTGCTATGAGTC D2363N ATTCCAGAAAAG[G]ATATTAAAGAAACTGTTC
63	Frameshift	Truncated protein	49	7541delC/7541delC TACAGAACAAATGTTC[C]GTCATCCTCACATCTC
50	None found			
955-01	Nonsense substitution	Truncated protein	44	R2203* CATGTTTTTTTCCTTG[C/T]GACTCTTAATCAACGA
915-06	Base change at splice junction	Unknown, possible splice variants	42	TGTATCTCTCCTACTTCTCCTGTTTGG[g/t]taagctgc (lower case are intronic)
928-01	Nonsense substitution	Truncated protein	34	W1742* CCACACCCGCAGGAACT[G/A]GAAAGGTCTCATT
69	Frameshift	Truncated protein	49	7541delC/7541delC TACAGAACAAATGTTC[C]GTCATCCTCACATCTC

B

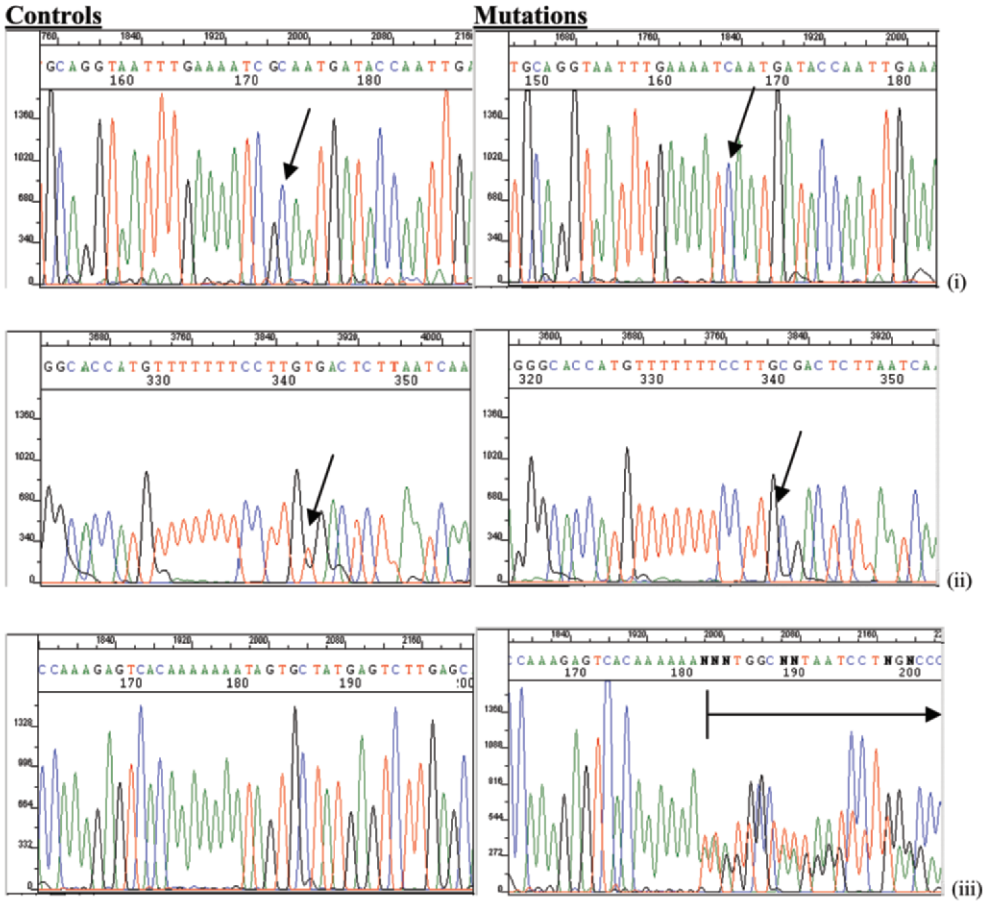


Figure 4 Mutation analysis of the *ABCA12* gene in patients with HI. *A*, Table summarizing mutation analysis. An asterisk (*) indicates a sequence change to a stop codon. *B, i,* Chromatogram showing homozygous GC deletion in exon 42 of patient 7. *ii,* Chromatogram showing homozygous T→C substitution in exon 44 in patient 955-01. *iii,* Chromatogram showing heterozygous A deletion in exon 33 of patient 44.

cause of (1) the difficulties in determining whether the disease has been inherited in an AR mode or is due to a new dominant mutation and (2) the low statistical power for linkage analysis. An additional complicating factor is the possibility of genetic heterogeneity.

The results of the Affymetrix 10K SNP-array genome scan of five unrelated patients with HI showed suggestive linkage to a region on chromosome 2q35 on the basis of having a large homozygous SNP haplotype "block" in common, a genetic distance of ~1.5 Mb. Microsatellite markers from this region were identified from the UCSC Genome Browser, and subsequent genotypes were consistent with a locus for HI on 2q35 on the basis of homozygosity at that locus in 11 of the 12 patients with HI described in this study. The *ABCA12* gene encoding an ABC transporter protein that is postulated to be involved in the transport of epidermal lipids is localized within the chromosomal region encompassed by these microsatellite markers. This was regarded as an excellent candidate gene to underlie the HI phenotype, since abnormal or absent epidermal lipids in HI skin have been described. Mutation analysis revealed that 11 of the 12 analyzed patients with HI had a disease-associated mutation in the *ABCA12* gene. The majority of the mutations were predicted to lead to a severely truncated protein, with the exception of a missense substitution and a splice-site mutation. In contrast, the *ABCA12* mutations in LI/NCBIE were all missense and were restricted to within the ATP-binding domain of the protein. Thus, the mutations in HI are likely to disrupt other aspects of *ABCA12* function and may provide the molecular reason behind the severity of HI compared with the milder skin conditions LI and NCBIE. Further mutation studies are in progress to identify the molecular spectra of mutations in a larger panel of patients with HI, including those subcategorized into the different types of HI (Dale et al. 1990). The data in the present article reveal that *ABCA12* mutations are the major cause of HI.

In normal epidermis, the cytoplasm of cells of the upper spinous and granular cell layers contain small granules, 100–300 nm in size, known as "Odland bodies" (Odland 1960); lamellar granules; lamellar bodies; or membrane-coating granules. These move toward the cell membrane as they enter the granular cell layer. They discharge their lipid components and proteases into the intercellular space and play important roles in barrier function, desquamation, and intercellular cohesion within the stratum corneum (Menon et al. 1992; Elias et al. 2000). Lipid delivery to lamellar bodies has also been shown to be required for subsequent delivery of protein to the organelle (Rassner et al. 1999). The defect in *ABCA12* in HI may explain the observed abnormalities in the lamellar granules, possibly associated with severely compromised skin-barrier function. This is analogous to the situation in lung in which surfactant is stored in lamellar bodies in alveolar type II cells. Mutations in *ABCA3* cause fatal surfactant deficiency in newborns, with markedly abnormal lamellar bodies observed ultrastructurally (Shulenin et al. 2004). The lamellar bodies in epidermis also contain beta-defensin, a microbicidal peptide that is also found in the intercellular space, which suggests that it might be discharged with lipid components of the lamellar body (Oren et al. 2003). The defect in epidermal barrier and the loss of beta-defensin may also result in increased susceptibility to infection. Additionally, mutations in two other genes of the ABCA family cause disorders of lipid transport. The *ABCA1* gene is mutated in Tangier disease and in the less severe, heterozygous, familial hypoalphalipoproteinemia as well as in premature atherosclerosis, the resultant disorder dependent on the site of the mutation (Brooks-Wilson et al. 1999; Rust et al. 1999; Clee et al. 2000). The *ABCA4* gene is mutated in patients with Stargardt disease, in some patients with AR retinitis pigmentosa, and in patients with AR cone-rod dystrophy (Stefkova et al. 2004). Our observation that mutations in the *ABCA12* gene cause HI is analogous to other systemic disorders of lipid transport. In addition, like *ABCA1* and *ABCA4*, the site and type of mutation in the *ABCA12* gene determine the severity of the condition, with the more disruptive genetic lesions, such as deletion or frameshift mutations, causing HI.

Prenatal screening tests for HI historically involved invasive procedures, such as fetoscopically and ultrasonographically guided fetal-skin biopsies that reveal key ultrastructural features, such as multivesicular lamellar granules in affected fetuses at 20–24 wk of gestation (Elias et al. 1980; Suzumori and Kanzaki 1991). Similar morphological changes have been described in amniotic fluid cells at 17 wk of gestation (Akiyama et al. 1994). Other methods that have been used in recent years for the prenatal diagnosis of HI are three-dimensional and four-dimensional real-time sonography (Vohra et al. 2003). A major translational benefit of our present findings is that DNA-based analysis should now be possible, which paves the way for earlier (i.e., first trimester) prenatal testing that is more reliable and conclusive than the other non-DNA–based prenatal screening techniques.

In summary, we have applied the rapid SNP-array genotyping technology to identify, by homozygosity mapping, *ABCA12* mutations as the major underlying genetic cause of the devastating skin disease HI. Previously, this disease had proved difficult to map by classic linkage analysis because of the limited size of pedigrees. The association of *ABCA12* mutations with HI will facilitate prenatal DNA diagnosis of this life-threatening disorder as well as the possibility of preimplantation genetic testing. In addition, results of the present study suggest a major role for ABCA12 in lipid trans-

port, barrier formation, and keratinocyte differentiation. The increased understanding of the biological role of ABCA12 in HI may lead to the development of targeted therapy to lessen the burden of this disease for both surviving children with HI and their parents.

Acknowledgments

The authors acknowledge the patients and their families who have contributed to the study. In addition, we acknowledge the contributions of Drs. Nick Lench and Sarah Hatsell during the early stages of our genetic studies of HI; Tracy Chaplin, who runs the SNP Array facility at Queen Mary; and Drs. Sandra Charles-Holmes (Coventry), Vinnie Biggs (Holyoke, MA), Michael Heffernan (St. Louis), and Richard Mupanemunda (Birmingham), for assistance with the clinical aspects of this study. We also acknowledge the major contribution of Dr. Karen Holbrook (Columbus, OH) to our understanding of the pathogenesis of HI. Funding was from the Wellcome Trust (to R.A.J.E.), a Wellcome Trust Prize Studentship (to E.E.N.), United States Public Health Service grant 2P01 AR 21557 (to P.F.), the Odland Endowed Research Fund (to P.F.), and the Barts and The London Charitable Foundation (to B.D.Y.).

Electronic-Database Information

The accession number and URLs for data presented herein are as follows:

Ensembl Genome Browser, http://www.ensembl.org/
GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for ABC [accession number NM_173076])
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for HI, *TGM1, ALOXE3, ALOX12B,* ABCA12, and AICA-ribosuria)
UCSC Genome Bioinformatics, http://www.genome.ucsc.edu/ (for the Human Genome working draft of chromosome 2)

References

Akiyama M, Dale BA, Smith LT, Shimizu H, Holbrook KA (1998) Regional difference in expression of characteristic abnormality of harlequin ichthyosis in affected fetuses. Prenat Diagn 18:425–436

Akiyama M, Kim DK, Main DM, Otto CE, Holbrook KA (1994) Characteristic morphologic abnormality of harlequin ichthyosis detected in amniotic fluid cells. J Invest Dermatol 102:210–213

Akiyama M, Shimizu H, Yoneda K, Nishikawa T (1997) Collodion baby: ultrastructure and distribution of cornified cell envelope proteins and keratins. Dermatology 195:164–168

Akiyama M, Yoneda K, Kim SY, Koyama H, Shimizu H (1996) Cornified cell envelope proteins and keratins are normally distributed in harlequin ichthyosis. J Cutan Pathol 23:571–575

Brooks-Wilson A, Marcil M, Clee SM, Zhang LH, Roomp K, van Dam M, Yu L, et al (1999) Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. Nat Genet 22:336–345

Buxman MM, Goodkin PE, Fahreenback WH (1979) Harlequin icthyosis with epidermal lipid abnormality. Arch Dermatol 115:189–193

Choate KA, Williams ML, Elias PM, Khavari PA (1998) Transglutaminase 1 expression in a patient with features of harlequin ichthyosis: case report. J Am Acad Dermatol 38:325–329

Clee SM, Kastelein JJ, van Dam M, Marcil M, Roomp K, Zwarts KY, Collins JA, Roelants R, Tamasawa N, Stulc T, Suda T, Ceska R, Boucher B, Rondeau C, DeSouich C, Brooks-Wilson A, Molhuizen HO, Frohlich J, Genest J Jr, Hayden MR (2000) Age and residual cholesterol efflux affect HDL cholesterol levels and coronary artery disease in ABCA1 heterozygotes. J Clin Invest 106:1263–1270

Dahlstrom JE, McDonald T, Maxwell L, Jain S (1995) Harlequin ichthyosis—a case report. Pathology 27:289–292

Dale BA, Holbrook KA, Fleckman P, Kimball JR, Brumbaugh S, Sybert VP (1990) Heterogeneity in harlequin icthyosis, an inborn error of epidermal keratinisation: variable morphology and structural protein expression and a defect in lamellar granules. J Invest Dermatol 94:6–18

Elias PM, Fartasch M, Crumrine D, Behne M, Uchida Y, Holleran WM (2000) Origin of the corneocyte lipid envelope (CLE): observations in harlequin ichthyosis and cultured human keratinocytes. J Invest Dermatol 115:765–769

Elias S, Mazur M, Sabbagha R, Esterly NB, Simpson JL (1980) Prenatal diagnosis of harlequin ichthyosis. Clin Genet 17: 275–280

Ewing B, Green P (1998) Base-calling of automated sequencer traces using Phred. II. Error probabilities. Genome Res 8: 186–194

Ewing B, Hillier L, Wendl MC, Green P (1998) Base-calling of automated sequencer traces using Phred. I. Accuracy assessment. Genome Res 8:175–185

Gordon D, Abajian C, Green P (1998) Consed: a graphical tool for sequence finishing. Genome Res 8:195–202

Haftek M, Cambazard F, Dhouailly D, Reano A, Simon M, Lachaux A, Serre G, Claudy A, Schmitt D (1996) A longitudinal study of a harlequin infant presenting clinically as non-bullous congenital ichthyosiform erythroderma. Br J Dermatol 135:448–453

Hsu WY, Chen JY, Lin WL, Tsay CH (1989) [Harlequin fetus: a case report.] Zhonghua Yi Xue Za Zhi (Taipei) 43:63–66

Huber M, Rettler I, Bernasconi K, Frenk E, Lavrijsen SP, Ponec M, Bon A, Lautenschlager S, Schorderet DF, Hohl D (1995) Mutations of keratinocyte transglutaminase in lamellar ichthyosis. Science 267:525–528

Jobard F, Lefevre C, Karaduman A, Blanchet-Bardon C, Emre S, Weissenbach J, Ozguc M, Lathrop M, Prud'homme JF, Fischer J (2002) 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 11:107–113

Kam E, Nirunsuksiri W, Hager B, Fleckman P, Dale BA (1997) Protein phosphatase activity in human keratinocytes cultured from normal epidermis and epidermis from patients with harlequin ichthyosis. Br J Dermatol 137:874–882

Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES (1996) Parametric and nonparametric linkage analysis: a unified multipoint approach. Am J Hum Genet 58:1347–1363

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 (2003) Mutations in the transporter ABCA12 are associated with lamellar ichthyosis type 2. Hum Mol Genet 12: 2369–2378

Lefevre C, Bouadjar B, Karaduman A, Jobard F, Saker S, Ozguc M, Lathrop M, Prud'homme JF, Fischer J (2004) Mutations in ichthyin a new gene on chromosome 5q33 in a new form of autosomal recessive congenital ichthyosis. Hum Mol Genet 13:2473–2482

Menon GK, Ghadially R, Williams ML, Elias PM (1992) Lamellar bodies as delivery systems of hydrolytic enzymes: implications for normal and abnormal desquamation. Br J Dermatol 126:337–345

Michel M, Fleckman P, Smith LT, Dale BA (1999) The calcium-activated neutral protease calpain I is present in normal foetal skin and is decreased in neonatal harlequin ichthyosis. Br J Dermatol 141:1017–1026

Moreau S, Salame E, Goullet de Rugy M, Delmas P (1999) Harlequin fetus: a case report. Surg Radiol Anat 21:215–216

Moskowitz DG, Fowler AJ, Heyman MB, Cohen SP, Crumrine D, Elias PM, Williams ML (2004) Pathophysiologic basis for growth failure in children with ichthyosis: an evaluation of cutaneous ultrastructure, epidermal permeability barrier function, and energy expenditure. J Pediatr 145:82–92

Odland GF (1960) A submicroscopic granular component in human epidermis. J Invest Dermatol 34:11–15

Oren A, Ganz T, Liu L, Meerloo T (2003) In human epidermis, β-defensin 2 is packaged in lamellar bodies. Exp Mol Pathol 74:180–182

Rassner U, Feingold KR, Crumrine DA, Elias PM (1999) Coordinate assembly of lipids and enzyme proteins into epidermal lamellar bodies. Tissue Cell 31:489–498

Rust S, Rosier M, Funke H, Real J, Amoura Z, Piette JC, Deleuze JF, Brewer HB, Duverger N, Denefle P, Assmann G (1999) Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. Nat Genet 22: 352–355

Sarkar R, Sharma RC, Sethi S, Basu S, Das R, Mendiratta V, Sardana K, Kakar N (2000) Three unusual siblings with harlequin icthyosis in an Indian family. J Dermatol 27:609–611

Shulenin S, Nogee LM, Annilo T, Wert SE, Whitsett JA, Dean M (2004) ABCA3 gene mutations in newborns with fatal surfactant deficiency. N Engl J Med 350:1296–1303

Stefkova J, Poledne R, Hubacek JA (2004) ATP-binding cassette (ABC) transporters in human metabolism and diseases. Physiol Res 53:235–243

Suzumori K, Kanzaki T (1991) Prenatal diagnosis of harlequin ichthyosis by fetal skin biopsy: report of two cases. Prenat Diagn 11:451–457

Vohra N, Rochelson B, Smith-Levitin M (2003) Three-dimensional sonographic findings in congenital (harlequin) ichthyosis. J Ultrasound Med 22:737–739