© 2002 Oxford University Press *Human Molecular Genetics*, 2002, Vol. 11, No. 1 **107–113**

ARTICLE

Lipoxygenase-3 (*ALOXE3*) and 12(*R*)-lipoxygenase (*ALOX12B*) are mutated in non-bullous congenital ichthyosiform erythroderma (NCIE) linked to chromosome 17p13.1

Florence Jobard, Caroline Lefèvre, Aysen Karaduman[1], Claudine Blanchet-Bardon[2], Serap Emre[3], Jean Weissenbach[4], Meral Özgüc[3], Mark Lathrop, Jean-François Prud'homme[5] and Judith Fischer*

Centre National de Génotypage, 91057 Evry, France, [1]Department of Dermatology, Hacettepe University, Ankara, Turkey, [2]Department of Dermatology, Saint-Louis Hospital, 75010 Paris, France, [3]Department of Medical Biology and Tübytak DNA/Cell Bank, Hacettepe University, Ankara, Turkey, [4]Genoscope and CNRS UMR 8030, 91057 Evry, France and [5]Généthon, 91002 Evry, France

Received October 29, 2001; Revised and Accepted November 7, 2001

We report the identification of mutations in lipoxygenase-3 (*ALOXE3*) and 12(*R*)-lipoxygenase (*ALOX12B*) genes in non-bullous congenital ichthyosiform erythroderma (NCIE) linked to chromosome 17. Linkage disequilibrium analysis of six families affected by NCIE permitted us to reduce a recently reported interval of 8.4 cM on chromosome 17p13.1 to a 600 kb region around the marker D17S1796, which contains LOX genes. LOX products have long been implicated in skin disorders. Two point mutations and one deletion were found in *ALOXE3* and three point mutations were found in *ALOX12B* in these consanguineous families from the Mediterranean basin. *ALOXE3* and *ALOX12B* are two genes which are physically linked and functionally related. They are separated by 38 kb, have one more exon than the other LOX genes and are mainly expressed in epithelial cells including keratinocytes. Although the main substrate(s) of the two enzymes is (are) still unknown, the products of ALOX12B obtained in experimental systems have been demonstrated to be of *R*-chirality. It seems likely that the product of one of these enzymes may be the substrate of the other, and that they belong to the same metabolic pathway.

INTRODUCTION

Autosomal recessive congenital ichthyosis (ARCI) comprises a clinically and genetically heterogeneous group of disorders of keratinization characterized by skin desquamation over the whole body, often associated with erythema (1,2). Two non-syndromic forms have been clinically defined: lamellar ichthyosis (LI) and non-bullous congenital ichthyosiform erythroderma (NCIE). To date, five genes for ARCI have been localized on chromosomes 2q33–q35 (LI2, MIM 601277), 14q11.2 (LI1, MIM 242300; NCIE1, MIM 242100), 17p13.1, 19p13.1–p13.2 (NNCI, MIM 604781) and 19p12–q12 (LI3, MIM 604777) (3,4–6). One of them, the transglutaminase 1 gene (*TGM1*, MIM 190195) on chromosome 14, was identified in 1995 (7,8). Mutations in *CGI-58* gene at 3p21 (NCIE2, MIM 604780) were recently found to underlie Chanarin–Dorfman syndrome (CDS, MIM 275630) (9).

The localization on chromosome 17p was described in two families of German and Turkish origin, in which the six affected subjects presented a mild form of NCIE with small, light brown, adherent scales and no palmoplantar keratoderma (3). The 8.4 cM interval was defined by loss of homozygosity between markers D17S938 and D17S1879. We tested 56 consanguineous ARCI families, in which all known loci except 17p13.1 had been excluded. The patients in six of these families were found to have a homozygous haplotype in this interval, which we saturated with additional markers from public databases. Linkage disequilibrium analysis permitted us to reduce the interval to ~600 kb between markers D17S1796 and D17S1812. There were seven genes in the region including two genes from the lipoxygenase (LOX) family, which are known to be expressed in the epidermis and which we therefore considered to be good candidate genes. Mutations were found in these genes in all six families: three families presented mutations in

*To whom correspondence should be addressed. Tel: +33 1 60 87 83 57; Fax: +33 1 60 87 83 83; Email: fischer@cng.fr

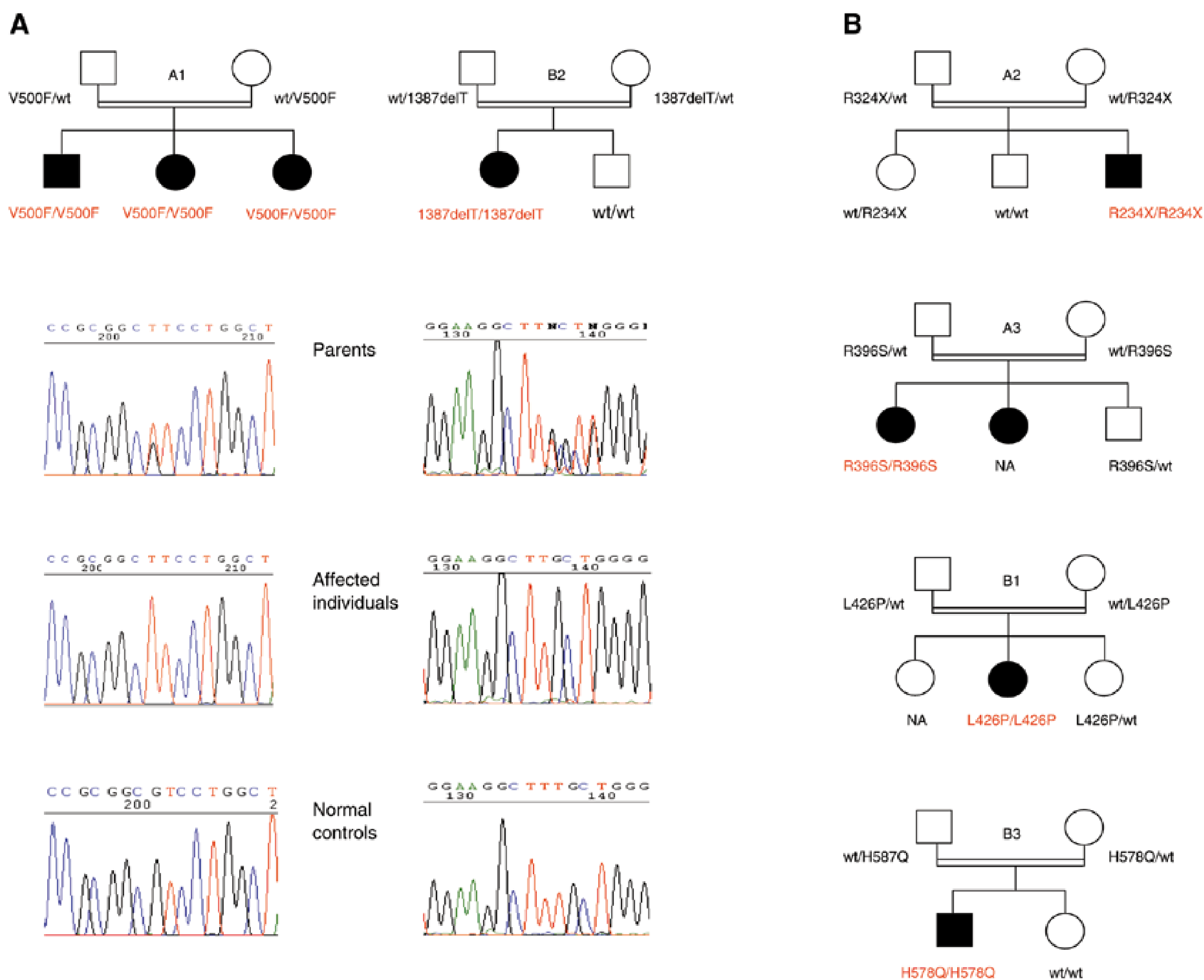

Figure 1. (**A**) Pedigrees and sequence mutations in the ALOXE3 gene in family A1 and in ALOX12B in family B2. (**B**) Pedigrees and mutations in the other four families. NA, not available; wt, wild-type.

ALOXE3 and three in ALOX12B. Mutations in either one of them lead apparently to the same phenotype, indicating the possibility of their participation in a common metabolic pathway.

RESULTS

Patients

Eight patients and 18 non-affected subjects from six families (Fig. 1A and B) were analyzed. All the families originate from the Mediterranean basin: three from Turkey and two from North Africa which are known to be consanguineous from first cousin marriages, and one from France, for which a consanguinity of the third degree was reported. All the parents were available for the study. All the affected subjects presented the clinical features of NCIE and were born as collodion babies. Patients from two of the Turkish families, B2 and B3, are shown in Figures 2 and 3. The 3-year-old boy (family B3) presented a mild ichthyosiform erythroderma with fine, white desquamation over the whole body and a palmoplantar keratoderma. The 12-year-old girl (family B2) presented an ichthyosiform erythroderma with fine, white scaling on the face, the neck, the trunk and the flexures, but darker, larger, polygonal scales on the buttocks and the extension sites of the arms and legs. She had an ectropion, an eclabion and severe palmoplantar keratoderma. A mild diffuse alopecia and thin nails were also noted.

Linkage, haplotype and disequilibrium analyses

The original cosegregating region of 8.4 cM on 17p13.1 was defined by loss of homozygosity in a German family between the loci D17S938 and D17S1879 (3). Previous studies in our large collection of 190 ARCI families, in which 106 are known to be consanguineous, had shown linkage in 50 consanguineous and 10 non-consanguineous kindreds to one of the known ARCI loci (2q33–q35, 14q11.2, 19p13.2–p13.1, 19p12–q12) or to the Chanarin–Dorfman locus at 3p21. Linkage to a given locus was defined by all or some of the following criteria: the presence of a homozygous haplotype in patients around the

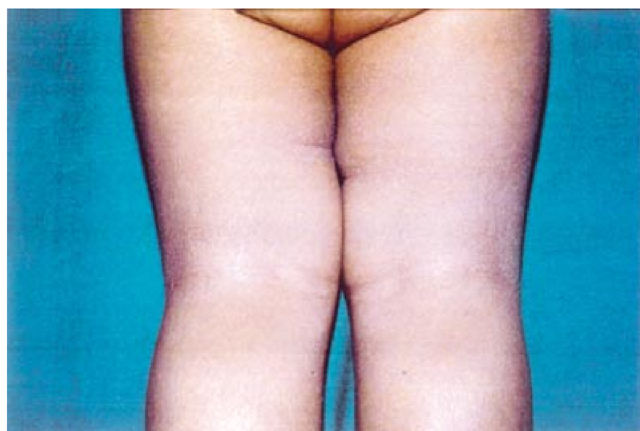

Figure 2. Patient from family B3 at age 3 years. He presents a mild ichthyosiform erythroderma with fine, white scales over the whole body.

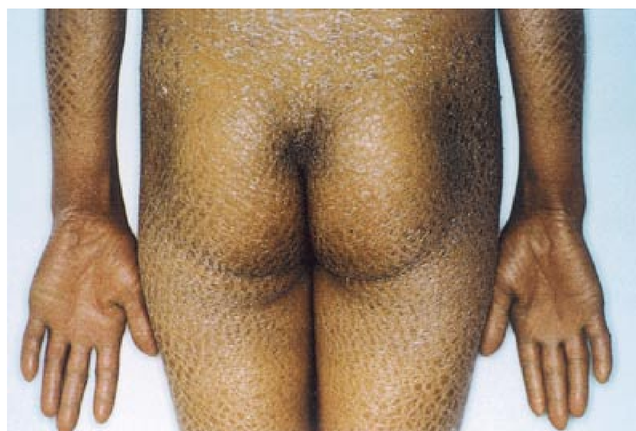

Figure 3. Twelve-year-old girl (family B2) with brownish, large scales on the buttocks and the extension sites of the arms and legs, including the flexures. Note also the palmoplantar keratoderma.

locus and the exclusion of all other known loci, LOD score above the expected LOD score and the identification of mutations in the known genes.

The 56 remaining consanguineous families were genotyped in the 17p interval with six microsatellites, spaced 1.5 cM apart (10). Six kindreds from the Mediterranean basin demonstrated linkage to this region. In these six families we performed further genotyping with a total of 26 markers (spaced at ~0.5 cM) from public databases. All the patients showed homozygous alleles in the interval between the loci D17S960 and D17S1858. The haplotypes for these markers are presented in Figure 4. A common haplotype was observed in three families of Turkish and North African origin for markers D17S1796, D17S1812 and D17S1805, and five of the families shared a common allele for marker D17S1796.

The maximum pairwise LOD score at $\Theta = 0.00$ for the marker D17S1844 was 6.63 and the multipoint LOD score at the same locus was 10.04. Linkage disequilibrium analysis showed p_{excess} values of 0.8, 0.6 and 0.5 for markers D17S1796, D17S1812 and D17S1805, respectively ($\chi^2 = 0.03, 0.02, 0.02$). The size of the interval was thus reduced to ~600 kb between the markers D17S1796 and D17S1812, although this figure is only a rough estimate because the region is incompletely sequenced and contains several gaps (Human Genome Project Working Draft: http://genome.ucsc.edu). This interval contains two LOX genes from the LOX family, *ALOXE3* and *ALOX12B*, which were considered to be promising candidate genes for NCIE.

ALOXE3 and *ALOX12B* gene structure and mutation analysis

ALOXE3 and *ALOX12B* belong to the same subfamily of LOX genes and are physically linked and functionally related; they have the same gene structure with 15 exons instead of 14 as in the other LOX genes and have an insertion of a 31–41 proline-rich amino acid stretch close to the N-terminus (11–14). They are separated by a 38 kb intergenic interval and have the same orientation. They display a high level of sequence similarity (54% identity at the protein level). They are mainly expressed in epithelial cells, including keratinocytes (14,15).

Mutation analysis of the 15 coding exons and exon–intron boundaries of *ALOXE3* and *ALOX12B* genes revealed five different point mutations and one deletion (Figs 1 and 4); all mutations were homozygous in the six consanguineous families. In *ALOXE3*, three point mutations were found: C700T, C1186A and G1498T, which change arginine to a stop codon (R234X), arginine to serine (R396S), and valine to phenylalanine (V500F), respectively. In *ALOX12B*, we identified two other point mutations at nucleotide positions T1277C and C1734A, which change leucine at amino acid position 426 to proline (L426P), and histidine to glutamine at amino acid position 578 (H578Q). In a third family, a small deletion of one T nucleotide at position 1387 was identified leading to a frame-shift and a premature stop codon at amino acid position 466. None of these sequence variations was found in 120 unaffected individuals from France, North Africa and Turkey (240 chromosomes).

DISCUSSION

The identification of mutations in two LOX genes in eight patients affected by NCIE confirms the long suspected role of LOX products in the maintenance of the cutaneous permeability barrier and more generally the biological action of essential fatty acids (16–18). NCIE is one of the main clinical forms of ichthyosis characterized by disruption of the permeability barrier of the skin, inducing transepidermal water loss (TEWL) (19) and accelerated mitotic rates in the epidermidis (20). The permeability barrier, located in the stratum corneum, is mediated by lipid-enriched membrane layers, composed primarily of ceramides, cholesterol and free fatty acids.

Numerous studies of essential fatty acid metabolism and enzymes including LOXs in skin have been published, notably in psoriasis, atopic dermatitis and other dermatoses including ichthyoses (21–23). Since 1929, it has been known that an essential fatty acid (EFA) deficiency induces a scaly skin condition, with a concomitant increase in TEWL (24,25), which can be reversed by adequate feeding or topical application of EFA to the skin (26,27). The role of LOX products of EFA metabolism in skin hydration was suspected when it was shown that the curative effect of EFA was not inhibited by

Gene	*ALOXE3*						*ALOX12B*					
Family	A2		A1		A3		B1		B2		B3	
Origin	FR		TR		NA		NA		TR		TR	
Marker												
Tel												
D17S1845	4	1	2	3	5	1	1	1	1	1	1	1
D17S1828	3	8	2	3	5	3	6	5	2	6	3	3
D17S1876	8	2	1	4	7	2	8	8	2	5	8	8
D17S1810	6	4	3	4	8	5	7	7	6	5	8	8
D17S1854	1	2	2	2	2	1	2	2	1	2	2	2
D17S1832	7	8	7	7	6	5	8	9	6	5	5	5
D17S938	7	8	0	0	6	6	10	8	2	2	8	8
D17S796	1	6	1	4	7	7	7	1	4	5	1	1
D17S1881	6	9	7	3	6	6	3	4	3	3	3	3
D17S960	2	2	4	4	2	2	4	2	2	2	4	4
D17S1353	2	2	1	1	5	5	6	6	12	12	7	7
D17S1796	3	3	3	3	3	3	3	3	3	3	1	1
D17S1812	3	3	4	4	4	4	4	4	4	4	3	3
D17S1805	2	2	1	1	2	2	2	2	2	2	4	4
D17S1844	2	2	7	7	9	9	6	6	3	3	7	7
D17S786	5	5	0	0	3	3	4	4	5	5	3	3
D17S1858	7	7	6	6	6	6	4	3	6	6	4	4
D17S1791	11	11	13	13	9	9	3	12	4	4	8	8
D17S952	3	3	4	4	2	2	4	1	3	3	1	1
D17S945	6	5	5	5	7	7	6	9	4	4	9	9
D17S1879	2	2	1	1	1	2	2	9	4	4	3	3
D17S804	3	5	9	9	6	6	6	5	5	5	6	6
D17S1852	8	12	8	8	4	9	10	13	7	7	12	12
D17S954	6	6	5	5	4	4	6	2	2	2	4	4
D17S1875	2	6	3	3	4	4	1	2	3	3	3	3
D17S799	6	2	0	0	6	6	2	8	7	7	2	2
Cen												
Mutation	C700T		G1498T		C1186A		T1277C		1387delT		C1734A	
Effect	R234X		V500F		R396S		L426P		stop aa 466		H578Q	

Figure 4. Patient haplotypes and corresponding mutations. Common haplotypes in families from France (FR, A2), Turkey (TR, A1, B2, B3) and North Africa (NA, A3, B1). Loss of homozygosity is indicated by arrowheads. The three markers in disequilibrium are in bold type and the shared haplotypes are shaded.

indomethacin, a powerful cyclooxygenase (COX) inhibitor, and that feeding rats eicosatetraynoic acid, a potent COX and LOX inhibitor, gave rise to an extremely dry and scaly skin (28). Moreover, topical application of LOX products from human platelets and rat epidermal homogenate to EFA-deficient rats resulted in nearly complete resolution of their scaly dermatitis (29). More recently it was shown that TEWL is increased in platelet-type 12-LOX deficient mice but there were no changes in the basal mitotic activity of epidermal cells (30).

LOXs are a class of structurally and functionally related non-heme iron-containing dioxygenases that catalyze the oxygenation of free and esterified polyunsaturated fatty acids to produce the corresponding hydroperoxy derivatives. LOX enzymes also use alternative substrates such as phospholipids and even, as recently demonstrated in plants, triglycerides (31). Categorized in mammals according to the positional specificity of the oxygen insertion using arachidonic acid as a substrate, several families of LOXs are known. The major products of EFA metabolism in normal human skin and keratinocytes have been demonstrated to be 12- and 15-hydroperoxy eicosatetraenoic acids from arachidonic acid and 13-hydroxy octadecadienoic acid from linoleic acid (reviewed in 32). The products of enzymatic activity of ALOX12B of which 98% were of R-chirality have been difficult to obtain, since there was a 10- to 20-fold lower activity than with the reticulocyte type of 15-LOX used as a positive control (11,12,33). For ALOXE3, no enzymatic activity was found with arachidonic and linoleic acid, as well as with methyl and cholesteryl arachidonate as substrates (13). Although the role of 5-LOX in the biosynthesis of leukotrienes is generally accepted (34,35), and 15-LOX has been implicated in cell maturation and differentiation (36), knowledge of the biological roles of the other LOX isoforms is limited (37).

Originally found in different lineages of bone marrow-derived blood cells (34,35), other mammalian LOXs have been shown to have their expression restricted mainly to epithelial cells, including the skin. A 5-LOX, an 8-LOX in mice, four 12-LOXs, two 15-LOXs and ALOXE3 or e-LOX-3, its murine homolog, are known to be present in mammalian skin and hair follicles (11,12,38). Moreover, evidence suggests distinct cell-specific expression patterns for the different LOXs within the epidermis. e-LOX-3 and ALOX12B are predominantly expressed in the suprabasal cell layers of neonatal and adult mouse epidermis and also in footsole, trachea, lung, tongue, forestomach, brain and testis (15). It was suggested that the late expression of both e-LOX-3 and ALOX12B in late granular and squamous keratinocytes supports a critical function for

Table 1. Primer sequences for *ALOXE3* and *ALOX12B* genes

Name	Forward sequences	Reverse sequences	PCR conditions
ALOXE3			
Exon amplification			
1	CATCCTCAGCCCTACCCAG	GATCTCAAGAGTCCAGGAGGAA	I
2	ATTCATACAAAAGCCCGGTATG	CCCAGAGTTTCTGACCTCAATC	II
3	CACGTGTTTCACAGGGATGT	TCCAAATAAGAGTCCTGATCCAA	II
4a/4b	CATCCTTAAATGCTACCCTATGTGA	CATGTAGAGAGGGAAGACTGTGG	III
5	GTCAGTTCCTCCAGGCAGAG	CTGTTTTTGTCCCCTCCTCA	III
6	TGATTCCTACCTCATAGAGTTTTTG	CAGGCCATTTCCATACCCT	II
7/8/9	CCATTAATTCAGGCACCAAGA	CAGTGATTGTACAGGTGTTCTCACA	IV
10	TGCTTGGTGAACCGAAGAAG	CTAGGGTGTGAAAACACCTGAAAT	II
11	GAGTGAGTGGGACTTAAGGGAA	TAACTGATGGGAGTAGGGTGTG	II
12	CGGTGTTCCCTACTTCCTCA	CCACTCCAACCCCAAGTCTA	II
13	GATCCCCTCATTTCCCAACT	TAAATCCCGTCGATCAGTCC	II
14	AGCCACCTTGACTGGATTTG	CTGAGAACAGGGAGGATGGA	II
Internal sequencing			
4a_int		CTTGAATGAAACTGCATTGTCATAG	
4b_int	GACTTGGAAGGCAGGAAAGAGGGA		
7_int		CTGGCGGTTAGCACCTGAGC	
8_int	GCTCAGGTGCTAACCGCCAG		
ALOX12B			
Exon amplification			
1	CAGCTTTTCCAGAATTTGGCT	CAGCTCTCCAGCCTCTCCT	II
2	ATGAGCAGGGCCTAGGTGAG	CTCTGGCTTGATGGGAGC	II
3/4a/4b	GGCACAGAAGCTGAACTCC	CTGGGGCTGTCTTGGAGG	I
5	CAGAGACCTAAGTGGATGGGG	GGAGTCGGGGAGGAAAAC	I
6/7	CATAGTCTTCTTCTGTCCCATGC	CAATCTCTCAAGGATAACGAGAGAC	I
8/9	CTTAGGAGTGTTTTCACCTTCTCATACT	CTTTCTAGACAGGAGAACCAACTTCAT	II
10	GAGCAGGATTGTTCCTGGAG	CTAGAAGCTCCCCACACCCT	II
11	CAAGCCATCCTCTCCACTTC	AGACTGCAATTCCGGATCAC	II
12	TTTGCAAAACGGTGAGAGC	GAGGCTGGACCAGGGATTAT	II
13/14	GGGAGTCCATCTCAGCGAC	GAGAACGGAATCGCGGTG	I
Internal sequencing			
4b_int	GCCTGAGTAAGGACCCGAAGC		
3/4a_int		GCAGGGCAACTGGGATCCAG	
6_int		GGCTTGCCTGGGACTGGC	
7_int	GCCAGTCCCAGGCAAGCC		
8_int		CTCTGACTGCTCAGTGGGCC	
9_int	GGCCCACTGAGCAGTCAGAG		
13_int		TCAGTGGCCTCTCACTCCCTG	
14_int	CAGGGAGTGAGAGGCCACTGA		

PCR conditions: I: T_m = 60°C, Advantage-GC genomic PCR kit; II: 60°C, AmpliTaq (Applied Biosystems, Roche); III: 56°C, AmpliTaq; IV: 60°C, LA PCR kit (Takara).

these isoenzymes in advanced stages of terminal differentiation such as the establishment of the epidermal lipid barrier.

The clinical characteristics of the two patients shown in Figures 2 and 3 are quite different even though they carry mutations in the same gene (*ALOX12B*). However, these patients have different ages (3 and 12 years), and the severity of the lesions may increase with age. The patient in family B2 resembles two patients from one family from the United Arab Emirates previously described by Krebsova *et al.* (3) who presented a NCIE which also increased in severity with age and was more marked on the extensor surfaces of the limbs and on the upper back. However, the disease in this family was said to be unlinked to chromosome 17. In three of our consanguineous families, we found a relatively small homozygous haplotype with a size between 1.5 and 4 Mb; linkage to this locus could be easily missed unless closely spaced markers are used. Surprisingly these families which show diverse mutations in either *ALOXE3* or *ALOX12B* share a common haplotype. Accurate genotype–phenotype correlations will require analysis of more affected individuals.

Four of the mutations in LOX genes have deleterious consequences on enzyme function: a nonsense mutation leading to a stop codon in *ALOXE3* (R234X), a deletion (1387delT) leading to a premature stop codon in *ALOX12B*, a missense mutation in *ALOX12B* (H578Q) that changes a histidine which participates in the binding of an iron atom (12,39) and another missense mutation, also in *ALOX12B* (L426P). This latter mutation is localized inside the consensus pattern of the second iron-binding region and includes two histidine residues essential for the activity of LOXs (39). In the alignment of *ALOXE3* with rabbit 15-LOX, which is the only mammalian LOX for which the crystalline structure is known (40), the two other missense mutations, R396S and V500F, correspond to amino acids 348 and 452 in the rabbit enzyme. By site-directed mutagenesis, a neighboring phenylalanine at position 353 has been demonstrated to be essential for the positional specificity of mammalian 15-LOXs (41).

The implication of two LOX enzymes in NCIE should contribute to a better understanding of the metabolic pathway which leads to the formation of the permeability barrier in the skin.

MATERIALS AND METHODS

Subjects and specimens

Signed informed consent was obtained from each patient and family member. Medical records including dermatologic examination and pedigree information were collected. DNA was extracted from whole blood using standard procedures and lymphoblastoid cell lines were established for most of the patients and their parents.

Genetic analysis

Genotyping with fluorescent markers was carried out as described previously by Fischer *et al.* (6). Haplotypes were constructed assuming the most parsimonious linkage phase. Linkage programs were used on the assumption of autosomal recessive inheritance, full penetrance and a disease frequency of 1/300 000 in the general population. Pairwise LOD scores were calculated with the MLINK program of the LINKAGE 5.1 package (42) incorporating consanguineous loops into the pedigree files. Multipoint analysis was performed with the Allegro 1.1 program (43). For linkage disequilibrium analysis (44), the excess of the disease-associated alleles was calculated using the p_{excess} equation: $p_{excess} = (p_{affected} - p_{normal}) / (1 - p_{normal})$, in which $p_{affected}$ and p_{normal} denote the frequency of the disease-associated allele on disease-bearing chromosomes [11] and normal chromosomes [12]. Chromosomes shared by two sibs or homozygous were counted only once. For χ^2 estimation, we used the combined-allele method (45). The significance level ($\alpha = 0.05$) was corrected by using Bonferroni's procedure (46) as follows: $\alpha' = 1 - (1 - \alpha^{1/L})$, with L being the number of individual tests (eight in our study).

Mutation screening

Mutation analysis was performed in affected patients, both parents and siblings in the six families. We designed intronic oligonucleotide primers for both genes flanking the coding exons and internal primers for sequencing (Table 1), using the Primer3 program (http://intranet.cng.fr/primer3/primer3_www.cgi) for the genomic sequences AJ305020, AJ305021, AJ305023 for ALOXE3 and AJ305026, AJ305027 for *ALOX12B*. We used the original nomenclature including exons 4a and 4b in both genes. Four different sets of PCR conditions were employed as indicated in Table 1. The PCR reaction was performed in a 45 µl volume containing 50 ng of genomic DNA (in 10 µl) using standard procedures. After an initial denaturation step at 96°C for 5 min, *Taq* polymerase was added at 94°C (hot start) and 35 cycles of amplification were performed consisting of 30 s at 94°C, 40 s at the optimal annealing temperature (between 56 and 60°C) followed by a 10 min terminal elongation step. One microliter of purified PCR products was added to 1 µl of sense or antisense primer (10 µM) and 3 µl of BigDye terminator mix (PE Applied Biosystems). The linear amplification consisted of an initial denaturation step at 96°C, 25 cycles of 10 s of denaturation at 96°C, a 5 s annealing step (56–60°C) and a 4 min extension step at 60°C. The reaction products were purified and sequenced on an Applied Biosystems Sequencer 3700. Both strands from all subjects and controls were sequenced for the entire coding region and the intron–exon boundaries.

GenBank accession numbers

Homo sapiens ALOXE3 protein mRNA, complete cds (AF151816); genomic sequences AJ305020, AJ305021 and AJ305023. *H.sapiens* ALOX12B protein mRNA, complete cds (AF059250); genomic sequences AJ305026 and AJ305027.

ACKNOWLEDGEMENTS

We wish to thank the members of the families for their participation in this study. We would like to acknowledge the continuous technical support of the Généthon DNA bank and its team. We are especially grateful to Susan Cure for help in writing the manuscript. This study was supported by the Centre National de Génotypage (CNG), Association Française contre les Myopathies (AFM) and Généthon.

REFERENCES

1. Williams,M.L. and Elias,P.M. (1985) Heterogeneity in autosomal recessive ichthyosis. Clinical and biochemical differentiation of lamellar ichthyosis and nonbullous congenital ichthyosiform erythroderma. *Arch. Dermatol.*, **121**, 477–488.
2. Traupe,H. (1989) *The Ichthyoses: A Guide to Clinical Diagnosis, Genetic Counseling, and Therapy.* Springer, Heidelberg, Germany.
3. Krebsova,A., Kuster,W., Lestringant,G.G., Schulze,B., Hinz,B., Frossard,P.M., Reis,A. and Hennies,H.C. (2001) Identification, by homozygosity mapping, of a novel locus for autosomal recessive congenital ichthyosis on chromosome 17p, and evidence for further genetic heterogeneity. *Am. J. Hum. Genet.*, **69**, 216–222.
4. Parmentier,L., Clepet,C., Boughdene-Stambouli,O., Lakhdar,H., Blanchet-Bardon,C., Dubertret,L., Wunderle,E., Pulcini,F., Fizames,C. and Weissenbach, J. (1999) Lamellar ichthyosis: further narrowing, physical and expression mapping of the chromosome 2 candidate locus. *Eur. J. Hum. Genet.*, **1**, 77–87.
5. Virolainen,E., Wessman,M., Hovatta,I., Niemi,K.M., Ignatius,J., Kere,J., Peltonen,L. and Palotie,A. (2000) Assignment of a novel locus for autosomal recessive congenital ichthyosis to chromosome 19p13.1–p13.2. *Am. J. Hum. Genet.*, **66**, 1132–1137.
6. Fischer,J., Faure,A., Bouadjar,B., Blanchet-Bardon,C., Karaduman,A., Thomas,I., Emre,S., Cure,S., Özgüc,M., Weissenbach,J. and Prud'homme,J.F. (2000) Two new loci for autosomal recessive ichthyosis on chromosomes 3p21 and 19p12–q12 and evidence for further genetic heterogeneity. *Am. J. Hum. Genet.*, **66**, 904–913.
7. Huber,M., Rettler,I., Bernasconi,K., Frenk,E., Lavrijsen,S.P., Ponec,M., Bon,A., Lautenschlager,S., Schorderet,D.F. and Hohl,D. (1995) Mutations of keratinocyte transglutaminase in lamellar ichthyosis. *Science*, **267**, 525–528.
8. Russell,L.J., DiGiovanna,J.J., Rogers,G.R., Steinert,P.M., Hashem,N., Compton,J.G. and Bale,S.J. (1995) Mutations in the gene for transglutaminase 1 in autosomal recessive lamellar ichthyosis. *Nat. Genet.*, **9**, 279–283.
9. Lefèvre,C., Jobard,F., Caux,F., Bouadjar,B., Karaduman,A., Heilig,R., Lakhdar,H., Wollenberg,A., Verret,J.L., Weissenbach,J. *et al.* (2001) Mutations in CGI-58, a new protein of the esterase/lipase/thioesterase family, in Chanarin–Dorfman syndrome. *Am. J. Hum. Genet.*, **69**, 1002–1012.
10. Dib,C., Faure,S., Fizames,C., Samson,D., Drouot,N., Vignal,A., Millasseau,P., Marc,S., Hazan,J., Seboun,E. *et al.* (1996) A comprehensive genetic map of the human genome based on 5264 microsatellites. *Nature*, **380**, 152–154.
11. Boeglin,W.E., Kim,R.B. and Brash,A.R. (1998) A 12R-lipoxygenase in human skin: mechanistic evidence, molecular cloning, and expression. *Proc. Natl Acad. Sci. USA*, **95**, 6744–6749.
12. Sun,D., McDonnell,M., Chen,X.S., Lakkis,M.M., Li,H., Isaacs,S.N., Elsea,S.H., Patel,P.I. and Funk,C.D. (1998) Human 12(R)-lipoxygenase and the mouse ortholog. Molecular cloning, expression, and gene chromosomal assignment. *J. Biol. Chem.*, **273**, 33540–33547.
13. Kinzig,A., Heidt,M., Furstenberger,G., Marks,F. and Krieg,P. (1999) cDNA cloning, genomic structure, and chromosomal localization of a novel murine epidermis-type lipoxygenase. *Genomics*, **58**, 158–164.
14. Krieg,P., Marks,F. and Furstenberger,G. (2001) A gene cluster encoding human epidermis-type lipoxygenases at chromosome 17p13.1: cloning, physical mapping, and expression. *Genomics*, **73**, 323–330.
15. Heidt,M., Furstenberger,G., Vogel,S., Marks,F. and Krieg,P. (2000) Diversity of mouse lipoxygenases: identification of a subfamily of epidermal isozymes exhibiting a differentiation-dependent mRNA expression pattern. *Lipids*, **35**, 701–707.
16. Nugteren,D.H., Christ-Hazelhof,E., van der Beek,A. and Houtsmuller,U.M. (1985) Metabolism of linoleic acid and other essential fatty acids in the epidermis of the rat. *Biochim. Biophys. Acta*, **834**, 429–436.
17. Nugteren,D.H. and Kivits,G.A. (1987) Conversion of linoleic acid and arachidonic acid by skin epidermal lipoxygenases. *Biochim. Biophys. Acta*, **921**, 135–141.
18. Mao-Qiang,M., Elias,P.M. and Feingold,K.R. (1993) Fatty acids are required for epidermal permeability barrier function. *J. Clin. Invest.*, **92**, 791–798.
19. Lavrijsen,A.P., Bouwstra,J.A., Gooris,G.S., Weerheim,A., Bodde,H.E. and Ponec,M. (1995) Reduced skin barrier function parallels abnormal stratum corneum lipid organization in patients with lamellar ichthyosis. *J. Invest. Dermatol.*, **105**, 619–624.
20. Ghadially,R., Williams,M.L., Hou,S.Y. and Elias,P.M. (1992) Membrane structural abnormalities in the stratum corneum of the autosomal recessive ichthyoses. *J. Invest. Dermatol.*, **99**, 755–763.
21. Baer,A.N., Klaus,M.V. and Green,F.A. (1995) Epidermal fatty acid oxygenases are activated in non-psoriatic dermatoses. *J. Invest. Dermatol.*, **104**, 251–255.
22. Iversen,L. and Kragballe,K. (2000) Arachidonic acid metabolism in skin health and disease. *Prostaglandins Other Lipid Mediat.*, **63**, 25–42.
23. Fogh,K. and Kragballe,K. (2000) Eicosanoids in inflammatory skin diseases. *Prostaglandins Other Lipid Mediat.*, **63**, 43–54.
24. Melnick,B. (1989) Epidermal lipids and the biochemistry of keratinization. In Traupe,H. (ed.), *The Ichthyoses: A Guide to Clinical Diagnosis, Genetic Counseling, and Therapy*. Springer, Heidelberg, pp. 14–42.
25. Proksch,E., Holleran,W.M., Menon,G.K., Elias,P.M. and Feingold,K.R. (1993) Barrier function regulates epidermal lipid and DNA synthesis. *Br. J. Dermatol.*, **128**, 473–482.
26. Prottey,C., Hartop,P.J. and Press,M. (1975) Correction of the cutaneous manifestations of essential fatty acid deficiency in man by application of sunflower-seed oil to the skin. *J. Invest. Dermatol.*, **64**, 228–234.
27. Ziboh,V.A., Miller,C.C. and Cho,Y. (2000) Significance of lipoxygenase-derived monohydroxy fatty acids in cutaneous biology. *Prostaglandins Other Lipid Mediat.*, **63**, 3–13.
28. Tobias,L.D. and Hamilton,J.G. (1979) The effect of 5, 8, 11, 14-eicosatetraynoic acid on lipid metabolism. *Lipids*, **14**, 181–193.
29. Elliott,W.J., Morrison,A.R., Sprecher,H.W. and Needleman,P. (1985) The metabolic transformations of columbinic acid and the effect of topical application of the major metabolites on rat skin. *J. Biol. Chem.*, **260**, 987–992.
30. Johnson,E.N., Nanney,L.B., Virmani,J., Lawson,J.A. and Funk,C.D. (1999) Basal transepidermal water loss is increased in platelet-type 12-lipoxygenase deficient mice. *J. Invest. Dermatol.*, **112**, 861–865.
31. Fuller,M.A., Weichert,H., Fischer,A.M., Feussner,I. and Grimes,H.D. (2001) Activity of soybean lipoxygenase isoforms against esterified fatty acid indicates functional specificity. *Arch. Biochem. Biophys.*, **388**, 146–154.
32. Lomnitski,L., Sklan,D. and Grossman,S. (1995) Lipoxygenase activity in rat dermis and epidermis: partial purification and characterization. *Biochim. Biophys. Acta*, **1255**, 351–359.
33. Siebert,M., Krieg,P., Lehmann,W.D., Marks,F. and Furstenberger,G. (2001) Enzymic characterization of epidermis-derived 12-lipoxygenase isoenzymes. *Biochem. J.*, **355**, 97–104.
34. Brash,A.R. (1999) Lipoxygenases: occurrence, functions, catalysis, and acquisition of substrate. *J. Biol. Chem.*, **274**, 23679–23682.
35. Spokas,E.G., Rokach,J. and Wong,P.Y. (1999) Leukotrienes, lipoxins, and hydroxyeicosatetraenoic acids. *Methods Mol. Biol.*, **120**, 213–247.
36. van Leyen,K., Duvoisin,R.M., Engelhardt,H. and Wiedmann,M. (1998) A function for lipoxygenase in programmed organelle degradation. *Nature*, **395**, 392–395.
37. Kuhn,H. and Thiele,B.J. (1999) The diversity of the lipoxygenase family. Many sequence data but little information on biological significance. *FEBS Lett.*, **449**, 7–11.
38. Funk,C.D., Keeney,D.S., Oliw,E.H., Boeglin,W.E. and Brash,A.R. (1996) Functional expression and cellular localization of a mouse epidermal lipoxygenase. *J. Biol. Chem.*, **271**, 23338–23344.
39. Hammarberg,T., Zhang,Y.Y., Lind,B., Radmark,O. and Samuelsson,B. (1995) Mutations at the C-terminal isoleucine and other potential iron ligands of 5-lipoxygenase. *Eur. J. Biochem.*, **230**, 401–407.
40. Gillmor,S.A., Villasenor,A., Fletterick,R., Sigal,E. and Browner,M.F. (1997) The structure of mammalian 15-lipoxygenase reveals similarity to the lipases and the determinants of substrate specificity. [Published erratum appears in *Nat. Struct. Biol.* (1998), **3**, 242.] *Nat. Struct. Biol.*, **12**, 1003–1009.
41. Borngraber,S., Kuban,R.J., Anton,M. and Kuhn,H. (1996) Phenylalanine 353 is a primary determinant for the positional specificity of mammalian 15-lipoxygenases. *J. Mol. Biol.*, **264**, 1145–1153.
42. Lathrop,G.M., Lalouel,J.M., Julier,C. and Ott,J. (1985) Multilocus linkage analysis in humans: detection and estimation of recombination. *Am. J. Hum. Genet.*, **37**, 482–498.
43. Gudbjartsson,D.F., Jonasson,K., Frigge,M.L. and Kong,A. (2000) Allegro, a new computer program for multipoint linkage analysis. *Nat. Genet.*, **25**, 12–13.
44. Hästbacka,J., de la Chapelle,A., Kaitila,I., Sistonen,P., Weaver,A. and Lander,E. (1992) Linkage disequilibrium mapping in isolated founder populations: diastrophic dysplasia in Finland. *Nat. Genet.*, **2**, 204–211.
45. Aksentijevich,I., Pras,E., Gruberg,L., Shen,Y., Holman,K., Helling,S., Prosen,L., Sutherland,G.R., Richards,R.I., Dean,M. *et al.* (1993) Familial Mediterranean fever (FMF) in Moroccan Jews: demonstration of a founder effect by extended haplotype analysis. *Am. J. Hum. Genet.*, **53**, 644–651.
46. Weir,B.S. (1990) *Genetic Data Analysis*. Sinauer, Sunderland, MA.