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Mutations in the transporter ABCA12 are associated with lamellar ichthyosis type 2

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Lamellar ichthyosis type 2 (LI2) is a rare autosomal recessive skin disorder for which a gene has been localized on chromosome 2q33–35. We report the identification of five missense mutations in the *ABCA12* gene in nine families from Africa affected by LI2. The mutations were homozygous in eight consanguineous families and heterozygous in one non-consanguineous family. Four of these mutations are localized in the first ATP-binding domain (nucleotide-binding fold), which is highly conserved in all ABC proteins. The ABCA12 protein belongs to a superfamily of membrane proteins that translocate a variety of substrates across extra- and intracellular membranes. ABCA transporters have been implicated in several autosomal recessive disorders, notably of lipid metabolism. By analogy with ABCA3, a lamellar body membrane protein in lung alveolar type II cells, ABCA12 could function in cellular lipid trafficking in keratinocytes.

INTRODUCTION

Autosomal recessive congenital ichthyoses (ARCI) comprise 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). Both forms have an estimated incidence of between one in 300 000–500 000 live-born babies. Clinically, LI can be distinguished from NCIE by the characteristics of the scales, which are large, adherent, dark and pigmented, and by the absence of erythema. In NCIE, scales are fine, white and on an erythematous background, with the exception of the extension sites on the limbs, where the scales are larger and grayish (3). However, overlapping phenotypes exist and a clear differentiation between the groups is often not evident. Histologic characteristics of LI are orthohyperkeratosis and mild focal parakeratosis; the stratum corneum is at least twice as thick as in NCIE patients (3,4), and there is a normal or increased granular layer. Biochemically, the terminal differentiation of the epidermis is disturbed in LI, with a reduced barrier function, defects in the stratum corneum lipid composition (4,5), a rise in ceramides (sphingolipids) and in free sterols in severe cases of LI (1). LI has been considered to be a retention ichthyosis, in contrast to NCIE or psoriasis, which are hyperproliferative diseases (5,6).

Besides clinical heterogeneity, both LI and NCIE have been demonstrated to be genetically heterogeneous. For LI, four genes have been localized to date: LI1 (MIM 242300) on chromosome 14q11; LI2 (MIM 601277) on chromosome 2q33–35; LI3 (MIM 604777) on chromosome 19p12–q12; and LI5 (MIM 606545), also known as NCIE1 (MIM 242100), on chromosome 17p13 (7–10). Three genes for two of these loci have been identified to date: transglutaminase 1 (*TGM1*) for LI1 (11,12), and two lipoxygenases (*ALOXE3* and *ALOX12B*) for LI5/NCIE1 (13).

The low incidence of LI or NCIE and the large clinical and genetic heterogeneity of these disorders have hampered the attempts to better refine the classification of these disorders using clinical (2,6), biochemical (5,6,14) and ultrastructural

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characteristics (15), and research has failed to yield a consistent and replicable classification scheme. We analyzed nine families from Africa with LI linked to the LI2 locus, of which six had been previously reported (8,16). We saturated a 7 cM interval between the markers D2S157 and D2S1371 with 25 microsatellite markers. Based on recombination events and haplotype analysis, we reduced the linkage interval and sequenced candidate genes. Three genes were excluded before we identified five different missense mutations in four exons (28, 30, 31 and 32) of the *ABCA12* gene, four of which were situated in a conserved ATP binding domain of the protein.

RESULTS

Clinical features and patient origins

A total of nine families were analyzed, including 15 patients (seven females, eight males) and 41 non-affected family members for which DNA was available (Fig. 1). Eight of these families were known to be consanguineous from first cousin marriages and for one family (G) no consanguinity was known (Table 1). All the families were from Africa, four from Algeria, four from Morocco and one from Mali. Six of these families (A, C–G) have been previously reported (8,16). In three other families (H, I, J) from our collection of families affected by ichthyosis (9,13), the disorder was found to be linked to the LI2 locus. The clinical features of patients from families A–G (8,16) were similar to those patients of the additional families, H–J. The diagnostic criteria for lamellar ichthyosis were fulfilled by all patients (2); they were all born as collodion babies and presented a generalized lamellar ichthyosis with large adherent dark pigmented scales, with ectropion and palmoplantar keratoderma, except one female patient from family J, who presented a milder form of ichthyosis with smaller and whitish scales. She also had leukonychia, clubbing of nails, and deformation of the second phalange of hands and feet. None of the patients presented erythema or extracutaneous symptoms, which are associated with ichthyosis in some syndromes.

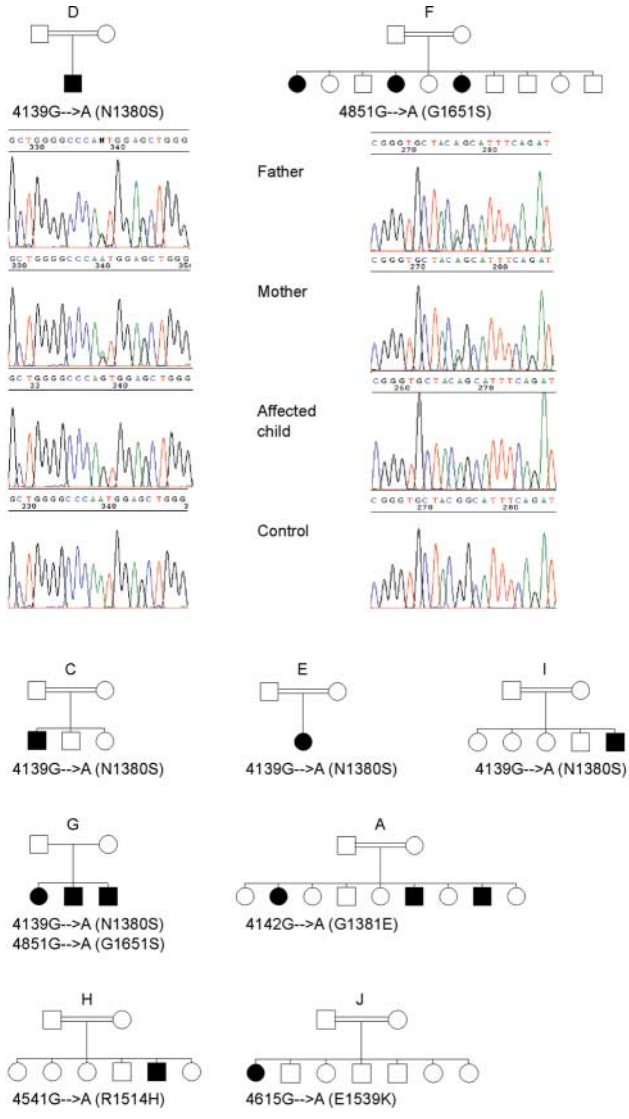

Figure 1. Sequences from patients and parents from family D (4139G→A) and F (4851G→A) and normal controls are shown. Pedigrees of the other affected families are also depicted. Affected individuals are indicated by blackened symbols.

Linkage, linkage disequilibrium and haplotype analysis

Linkage of the LI2 locus to chromosome 2q33–34 has been previously reported in seven families (8,16); we used six of them (A, C–G) for further genotyping to narrow the interval and for mutation analysis. To identify additional families linked to the LI2 locus, we genotyped 112 consanguineous ichthyosis families with a microsatellite panel we developed. This panel consists of 30 microsatellite markers covering the six known loci for recessive ichthyoses on chromosomes 2q33–35, 3p21 (MIM 275630/604780), 14q11, 17p13, 19p12–q12, and 19p13.2–p13.1 (MIM 604781) (7–10,17). Three families (H–J) were found with linkage to 2q33–35, in which the other five loci could be excluded. The nine families (A, C–J) were genotyped with a total of 25 microsatellite markers in a 7 cM interval between markers D2S157 and D2S1371. The distribution of markers in the interval was unequal with a large gap of 1.32 Mb between D2S1345 and D2S2361 as only few markers were available in this interval. A co-segregating region of 5.27 Mb was defined by recombination events (loss of homozygosity) observed in the patient from family E for the centromeric marker AFM212ze9 (D2S157), and in the patients from families H and I for the telomeric marker AFMa052tc1 (D2S2361). All the patients were homozygous for the microsatellite markers inside this interval, with the exception of the three affected children from family G, which was non-consanguineous. In the middle of the homozygous haplotype, the marker CNG_K2_08 was heterozygous in two affected children from families E and I, most likely as a consequence of a mutation in one of the ancestors, as we previously observed in haplotypes from a family carrying a mutation in the *kindlerin* gene (18). The maximum pairwise LOD score at $\Theta = 0.00$ for D2S143 (AFM191xb8) was 12.32.

The genotypes are presented in Figure 2. One haplotype (yellow) was shared by four families (C, E, G, I) and a

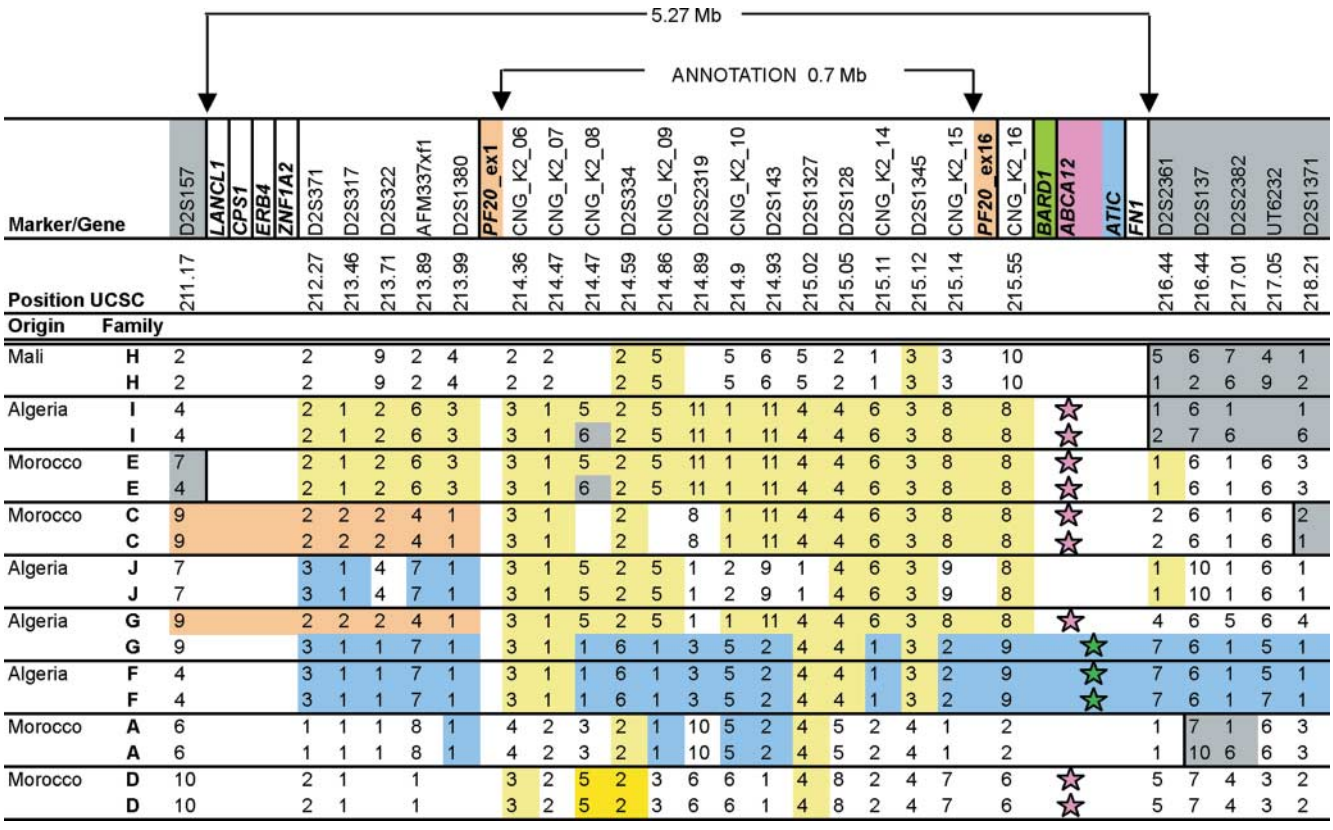

Figure 2. Construction of haplotypes. Genotypes of one patient per family for 25 microsatellite markers from a 7 cM region on chromosome 2q33–35 are shown. The most centromeric marker is on the left. Common haplotypes are in the same color; colored stars indicate the common mutations. Position UCSC indicates the Mb position from the Golden Path database (http://genome.ucsc.edu). Genes are in bold.

Table 1. Origin of families and mutations

Family	Number of patients	Degree of consanguinity	Origin	Mutation	Effect	Exon number
A	3	1st	Morocco	4142G→A	G1381E	28
C	1	1st	Morocco	4139A→G	N1380S	28
D	1	1st	Morocco	4139A→G	N1380S	28
E	1	1st	Morocco	4139A→G	N1380S	28
F	3	1st	Algeria	4951G→A	G1651S	32
G	3	No	Algeria	4139A→G	N1380S	28
				4851G→A	G1651S	32
H	1	1st	Mali	4541G→A	R1514H	30
I	1	1st	Algeria	4139A→G	N1380S	28
J	1	1st	Algeria	4615G→A	E1539K	31

second haplotype (blue) was common in two families (F, G) which permitted us to refine the interval to 1.55 Mb between markers AFM275vh5 (D2S2319) at the centromeric border and AFMa052tc1 (D2S2361) at the telomeric border.

Exclusion of candidate genes and identification of mutations in the *ABCA12* gene

Eight genes in the initial 5.27 Mb interval were known and one gene which now corresponds to PF20 has been annotated. Three candidate genes were sequenced in LI2 patients in which no mutations were found: *PF20* which codes for a WD repeat protein; *ATIC* (5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase, and *BARD1* (BRCA-associated RING domain 1). In the fourth candidate gene, *ABCA12*, we identified five missense mutations.

Structure of the *ABCA12* gene and its cDNA

The GenBank mRNA sequence of the *ABCA12* gene consisted of 7187 bp (AF418105), coding for a protein of 2347 amino acids (AAN40735) comprising 47 exons. This sequence was first extended by 160 bp using 5′-RACE. This extension showed a strong homology with two cDNAs from 10 day

neonate mouse skin (AK028781, AK029031). Since the mouse cDNAs were 827 bp longer at the 5′ end than the human homolog from GenBank, we compared the mouse cDNA with the sequence from human genomic contig NT_005403 using the BLAST program (www.ncbi.nlm.nih.gov/blast/). We found six additional sequences with homologies varying between 83 and 93%, corresponding to the first five exons of the mouse *abca12* gene. Our new ABCA12 mRNA sequence was checked by overlapping RT–PCR, and all the products were sequenced. This mRNA sequence corresponds to the recently described patent sequence 1 (AX587156), which was however reported as genomic DNA (19). We found that the complete *ABCA12* gene consists of 53 exons with a total length of the coding sequence of 7785 bp, which is translated into a protein of 2595 amino acids, with a complete 5′-UTR of 220 bp, and a long 3′-UTR (1092 bp). The coding portion begins with an ATG codon at nucleotide residue 221 in exon 1. This methionine is preceded by an in-frame stop codon 6 bp upstream in the 5′-UTR. These data are in accordance with the recent description of *ABCA12*, which was published after completion of our own work (19).

Mutation analysis in the *ABCA12* gene

Mutation analysis of the 53 exons and of the exon–intron boundaries of the *ABCA12* gene revealed five different mutations in the nine families we studied (Table 1). As expected, the mutations were homozygous in the eight consanguineous families, and heterozygous in the non-consanguineous family (G). All of the mutations were missense mutations and were found in the region of the protein encoded by exons 28–32: 4139A→G (N1380S) and 4142G→A (G1381E) in exon 28, 4541G→A (R1514H) in exon 30, 4615G→A (E1539K) in exon 31 and 4951G→A (G1651S) in exon 32. The 4139A→G mutation was found in five families, four of which shared a common haplotype, suggesting a founder effect: two of these families are from Morocco (C and E) and two from Algeria (I and G). The Moroccan family D carried the same mutation, but displayed a different haplotype. Two other families from Algeria presented the G1651S mutation (including the family G of the compound heterozygote) and a common haplotype (Fig. 2). These mutations were not found in 100 normal chromosomes from the same North African population.

Expression analysis

The RAPID-SCAN^{TM} gene expression panel was analyzed with ABCA12-specific RT–PCR1 primer pairs for a 673 bp fragment. The ABCA12 transcript was found to be expressed in seven of the 24 tissues tested; it was highly expressed in placenta, testis and lung, and was present at a lower level in brain, stomach, prostate, fetal brain and fetal liver. No expression was detectable in the other tested tissues including skin. However, a strong expression of ABCA12 transcripts was observed in cultured keratinocytes from normal skin biopsies, and a less strong expression in lymphocytes. No expression was found in cultured fibroblasts from skin biopsies (Fig. 3).

Sequence analysis of the ABCA12 protein and identification of conserved residues

The sequence of 2595 amino acids corresponds to a protein with a calculated molecular weight of 293.4 kDa. It was submitted to the SMART protein analyzing program (http://smart.embl-heidelberg.de), protein analyzing tools from the Biology workbench (http://biowb.sdsc.edu/) and homology searches through protein databases (www.expasy.org). Several functional domains were identified: a signal sequence of 45 amino acids (SignalP) deleting a first transmembrane domain, two AAA domains (ATPases associated with a variety of cellular activities) with an ATP nucleotide binding site between residues 1370–1554 and 2282–2467 (pfam00005, smart0382), two transmembrane domains between residues 1063–1271 and 1987–2293, each consisting of six hydrophobic membrane-spanning helices, and a JmjN motif found in the jumonji family of transcription factors (residues 743–780, IPR003349, smart00545). Four of the mutations (N1380S, G1381E, R1514H, E1539K) were situated in the first highly conserved ATP binding domain of the protein (Fig. 4).

DISCUSSION

The identification of mutations in *ABCA12* in patients with LI2 adds a fourth gene to those already implicated in the clinically and genetically heterogeneous group of autosomal recessive LI disorders, and another mechanism to their pathogenesis.

The first gene identified for a form of lamellar ichthyosis (LI1) in 1995 was transglutaminase 1 (*TGM1*) on chromosome 14 (11,12). It encodes an enzyme that catalyzes the formation of isodipeptide bonds between proteins of the cornified envelope of the skin such as loricrin and involucrin during terminal stages of epidermal differentiation. Mutations of TGM1 cause the most frequent form of recessive LI as shown by the numerous case reports, and represent around 30–40% of recessive LI in the largest series (20–22) and around one third in our own analysis (35 of 112 consanguineous families). Patients have a predominant clinical presentation of LI, sometimes with mild erythroderma, but unlike that of NCIE.

A second gene family coding for the lipoxygenases *ALOXE3* and *ALOX12B* was identified in LI5/NCIE1 linked to chromosome 17 in 2002 (13). These enzymes catalyze the oxygenation of free and esterified polyunsaturated fatty acids to produce the corresponding hydro derivatives. The main substrate(s) of these two enzymes is (are) still unknown. It has been proposed that the product of one of these enzymes may be the substrate of the other, and that the two enzymes are components of the same metabolic pathway, because they are implicated in the same disease (13). This form of LI is rare, since only eight families have been described to date (10,13); the clinical presentation seems to be similar to NCIE in young patients and more like LI in adults.

Few patients affected by LI2 have been described since the gene localization on chromosome 2 in 1996 (8), and since no other group has confirmed this locus in another population outside North Africa, it seems that this form of ichthyosis is rare. The LI2 patients present a relatively homogenous phenotype, with large dark pigmented scales, which are characteristic of LI (2). One female patient showed a serious

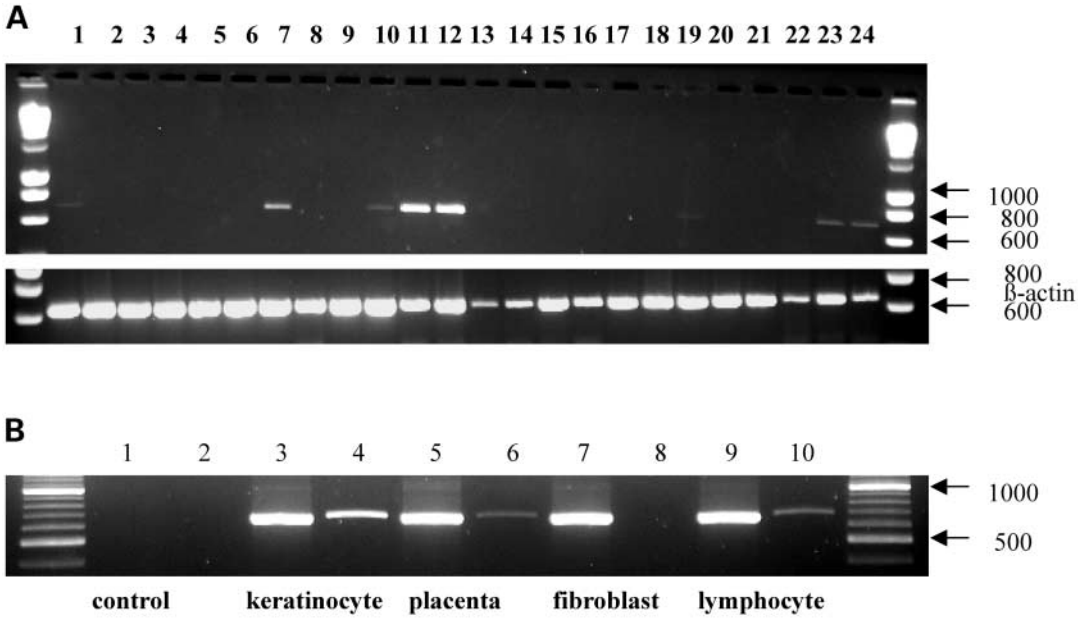

Figure 3. (**A**) Expression of ABCA12 transcripts by RT–PCR in 24 different tissues from the Rapid Scan Expression panel (OriGene Technologies). Lanes: 1, brain; 2, heart; 3, kidney; 4, spleen; 5, liver; 6, colon; 7, lung; 8, small intestine; 9, muscle; 10, stomach; 11, testis; 12, placenta; 13, salivary gland; 14, thyroid; 15, adrenal gland; 16, pancreas; 17, ovary; 18, uterus; 19, prostate; 20, skin; 21, peripheral blood lymphocytes; 22, bone marrow; 23, fetal brain; 24, fetal liver. Strong expression was found in testis (lane 11), and placenta (lane 12); lower expression was detectable in lung (lane 7), and very low expression was found in brain (lane 1), stomach (lane 10), prostate (lane 19), fetal brain (lane 23) and fetal liver (lane 24). No expression was detectable in the other tissues including skin. The molecular marker for quantification and fragment size determination (lane A and B) is Quanti-Ladder^{TM} DNA. The corresponding β-actin expression is shown below. (**B**) Expression analysis of the ABCA12 mRNA by RT–PCR. RT–PCR of ABCA12 in negative controls (lanes 1 and 2), cultured keratinocytes (lanes 3 and 4), placenta (lanes 5 and 6), cultured fibroblasts (lanes 7 and 8) and cultured lymphoblasts (lanes 9 and 10) using RT10 primers which amplify a transcript of 673 bp (lanes 4, 6, 8 and 10) using β-actin as a positive control (661 bp) (lanes 3, 5, 7 and 9); left and right lanes—molecular marker (XIV, Roche Molecular Biochemicals). A stronger expression of ABCA12 was observed in keratinocytes than in placenta or lymphocytes, and no expression was observed in cultured fibroblasts from skin biopsies.

involvement of the nails, which was not observed in the other patients, and she also exhibited a milder form of ichthyosis, with small and whitish scales.

The mutated gene in LI2, *ABCA12*, belongs to the large superfamily of the ATP-binding cassette (ABC) transporter genes, which bind ATP for the transport of various molecules across the cell membrane (23–25). The functional protein contains two transmembrane (TM) domains typically containing six membrane-spanning α-helices, and two ATP-binding domains or nucleotide-binding folds (NBF). The 48 currently known ABC genes are classified into seven subfamilies, based on sequence homology and organization of the NBFs, which contain three characteristic highly conserved motifs: Walker A, Walker B and signature C (23–25). The NBFs are located in the cytoplasm and transfer the energy to transport the substrate across the cell membrane. ABC genes are widely dispersed in eukaryotic genomes and highly conserved between species (26). The ABCA subfamily, of which the *ABCA12* gene is a member, comprises 12 full transporters and one pseudogene (ABCA11). Five genes of this subfamily (ABCA5, 6, 8, 9, 10) are clustered on chromosome 17q24, and the seven others are dispersed on six different chromosomes (ABCA1, 2, 3, 4, 7, 12, 13). As expected, *ABCA12* is highly expressed in keratinocytes, although no expression was detected in skin from a commercial expression panel.

Two genes of the ABCA subfamily have been implicated in the development of genetic diseases affecting cellular lipid transport. The ABCA1 protein is mutated in recessive disorders of cholesterol and phospholipid transport: Tangier disease (MIM 205400), familial hypoalphalipoproteinemia (MIM 604091) and premature atherosclerosis, depending on the site of the mutations in the protein (27–29). The ABCA4 protein is mutated in Stargardt disease (MIM 248200), as well as in some forms of autosomal recessive retinitis pigmentosa (MIM 601718), and in the majority of cases of autosomal recessive cone–rod dystrophy (MIM 604116), depending on the mutation site or the combination of mutations (30–32). Heterozygous mutations in ABCA4 have also been implicated in some cases of macular degeneration (MIM 153800) (31,32). A defective transport of retinal within the rod outer segment of the retina has been described.

All of the five mutations in ABCA12 in the nine families were missense mutations localized in the region of the protein coded by exons 28–32, four of which are in a conserved functional domain. In Stargardt disease, 80% of the mutations are missense, many of which occur in conserved domains of ABCA4 (33); in Tangier disease, 60% of the mutations are missense, also in conserved domains of ABCA1 (34). Since mutations in each of these two proteins are associated with different genetic disorders, the same hypothesis could apply to ABCA12 as recently suggested by investigators who noticed that several different genetic

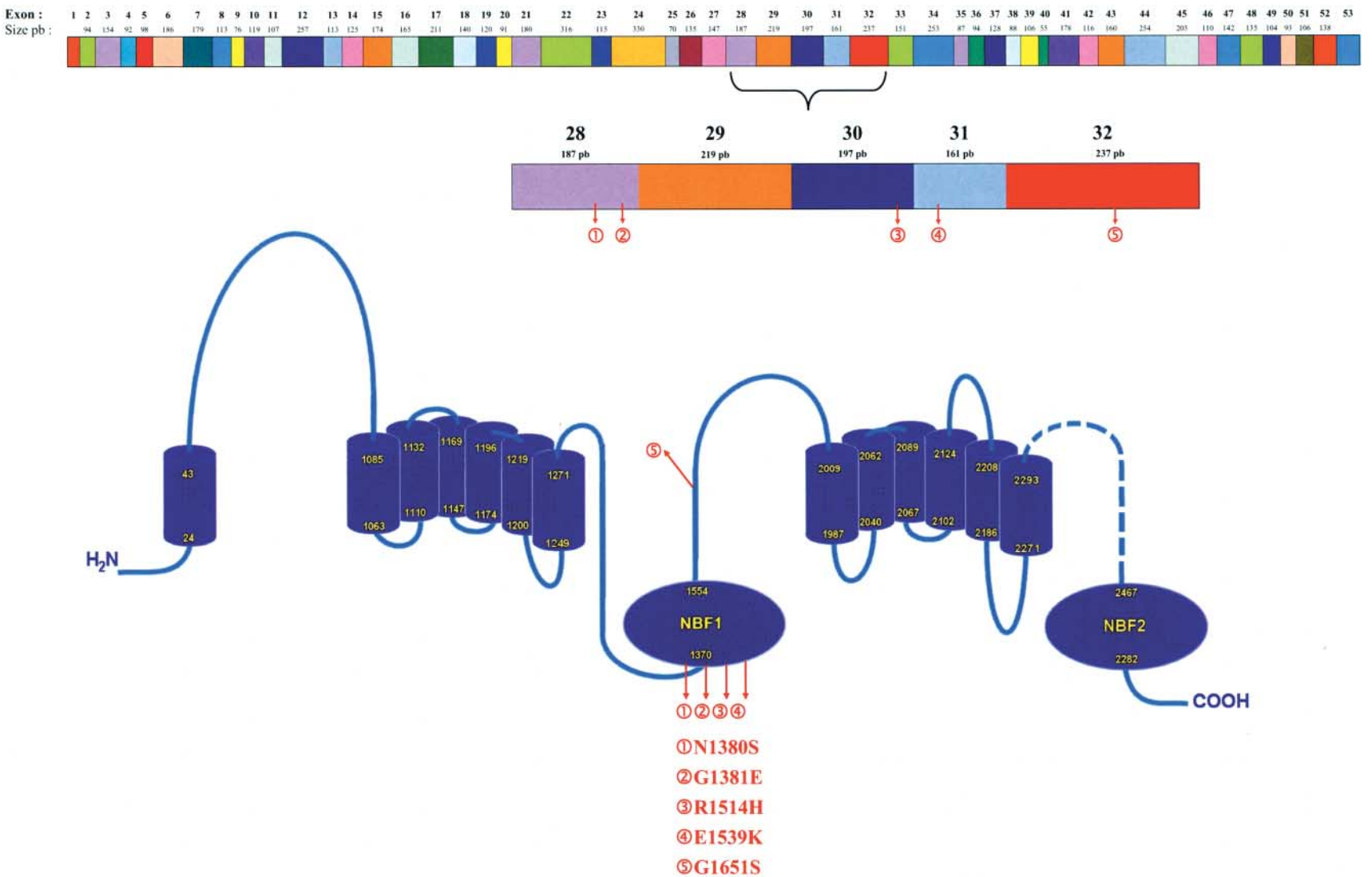

Figure 4. Diagram of ABCA12 cDNA and schematic representation of the predicted structure of the ABCA12 protein showing transmembrane domains, nucleotide-binding folds (NBF) and location of mutations.

disorders map to the ABCA12 locus, including LI2, nonnuclear polymorphic congenital cataract (MIM 601286) and insulin-dependent diabetes mellitus (MIM 601318) (19,35).

The exact nature of the transport mechanism effected by ABC proteins is still partially unknown (36–38). By analogy with ABCA3, which has been found to be a lamellar body membrane protein in human lung alveolar type II cells, ABCA12 could have a similar role in keratinocytes (39). The isolation of ABCA12 in LI2 offers opportunities to better understand the dermo-epidermal barrier and the function of lipids in the skin, to define the different types of ichthyoses on a genetic basis, and to study the phenotype–genotype correlations in these disorders.

MATERIALS AND METHODS

Subjects and samples

Four dermatologists (B.B., C.B.-B., H.L. and O.B.-S.) recorded the clinical data and pedigree information of the families. Blood samples were collected from each participating family member after obtaining written informed consent. DNA extraction from peripheral blood leukocytes and establishment of cell lines were performed at the DNA bank of Genethon using standard procedures.

Genetic analysis

Genotyping with polymorphic fluorescent microsatellite markers was carried out as described previously using ABI 377 sequencers (40). Haplotypes were constructed assuming the most parsimonious linkage phase. Linkage programs were used based on the assumption of autosomal recessive inheritance, full penetrance and a disease frequency of 1/500 000 in the general population. Pairwise LOD scores were calculated with the MLINK program of the LINKAGE 5.1 package (41) incorporating consanguineous loops into the pedigree files. In order to analyse a potential linkage disequilibrium in the 1.19 Mb region, we developed new microsatellites from six BAC sequences (AC027009, AC064841, AC009964, AC008169, AC008279 and AC016708). They were checked for dinucleotide motifs (AC, GT) with at least five repeat motifs and primers (one fluorescently labeled) were designed for a maximal length of the PCR product of around 400 bp (Table 2).

Mutation screening

Mutation analysis was performed in affected patients and in both parents in the nine families, and in supplementary non-affected sibs in cases of missing parents. We designed intronic oligonucleotide primers flanking the exons and internal primers from the chromosome 2 genomic contig NT_005403 for sequencing the *ABCA12* gene (Table 2) using the Primer3 program (http://intranet.cng.fr/primer3/primer3_www.cgi). Sets of PCR conditions were used as indicated in Table 2. The touch-down PCR reaction was performed in a 45 µl volume containing 50 ng of genomic DNA (in 5 µl) using standard procedures. After an initial denaturation step at 95°C for 5 min, *Taq* polymerase was added at 94°C (hot start) and six cycles of amplification were performed consisting of 40 s at 94°C, 30 s 68°C and a 30 s elongation step at 72°C, followed by 30 cycles of 40 s at 94°C, 30 s at optimal annealing temperature, 30 s at 72°C and one 5 min terminal elongation step. One to two microliters of purified PCR products were added to 0.5 µl of sense or antisense primer (20 µM) and 1 µl of BigDye terminator mix (Applied Biosystems) in a 15 µl volume. The linear amplification consisted of an initial 5 min denaturation step at 96°C, 25 cycles of 10 s of denaturation at 96°C and a 4 min annealing/extension step at 56–60°C. The reaction products were purified and sequenced on an Applied Biosystems Sequencer 3700. The forward or reverse strands from all patients and controls were sequenced for the entire coding region and the exon/intron boundaries.

Establishment of lymphoblastoid cell lines, keratinocyte and fibroblast cell culture, and RNA extraction

Lymphoblastoid cell lines were established for normal controls, using standard procedures. Total RNA from lymphocytes was obtained with the RNA-PLUS (Quantum-Appligene) kit following the manufacturer's instructions. Human keratinocytes and fibroblasts were obtained from a skin biopsy removed during routine plastic surgery of a normal individual. The skin sample was processed for primary keratinocyte culture and cells were grown according to the standard procedure described by Invitrogen Life Technologies using products from the same company in serum-free keratinocyte medium supplemented with bovine pituitary extract (25 µg/ml) and recombinant epidermal growth factor (0.1 ng/ml). For primary fibroblast culture we used DMEM (Dulbecco's modified Eagle's medium) with 10% fetal calf serum and 2% L-glutamine. Cultures were grown for two passages and harvested when they reached 90% confluence. Total RNA was isolated using the QIAamp RNA Mini Protocol for isolation of total RNA from cultured cells (Qiagen) following the manufacturer's instructions. The mRNA was isolated following the Oligotex direct mRNA protocol as provided by the manufacturer (Qiagen).

5′-RACE (rapid amplification of cDNA ends)

5′-RACE was performed with Marathon-Ready cDNA from placenta and melanoma (Clontech) using Advantage 2 Polymerase Mix (Clontech) following the manufacturer's instructions. A first PCR was run with AP1 primer and the gene-specific antisense primer of the cDNA (5′-RACE_1; Table 2), with an initial denaturation step at 94°C for 1 min, 30 cycles at 94°C for 30 s and 68°C for 4 min. PCR products were loaded on a 2% agarose gel. Ten microliters of the purified 5′-RACE PCR product were used for a second PCR with the same primers and the same conditions, but with a shorter elongation step of 1 min instead of 4 min. The purified products of the second PCR (from placenta) were sequenced with an internal primer 5′-RACE_int as described above.

RT–PCR and Rapid-Scan™ gene expression panel

RT–PCR was performed using the RT–PCR kit (Life Technologies) with oligo dT primers to generate the first strand of cDNA. Amplification of cDNA from keratinocytes, fibroblasts, placenta and lymphocytes were performed with

Table 2. Primer sequences for *ABCA12* exon amplification, RT–PCR, internal sequencing, 5′-RACE and surrounding markers

Name	Forward sequences	Reverse sequences	PCR conditions	Length (bp) of amplicon
Exon amplification				
1	CAGCCCTCAAGGAAGCTATG	TTGGGGGCATGTTAAGACTC	I	389
2	CAGTTGGTCAAATATTCAGCTTG	CTTTCTGACTAACTTGAAAATCTAGGG	I	392
3	CATAACACCCTCCAAGACTGC	CTAAGCTTGCATGGCTTCCT	I	425
4	CATTGGTGTGTGGTTAGGTGA	CCATACTCAGGCAACCATGA	I	407
5	GGAATTTGCCACTTTTCCAG	GGTGCTCCAAACATCTTGCT	I	362
6	CACATTCATGCATTCGCTCT	CCAGTCAGCATTTCCCAGTC	I	465
7	TGGAGTTACAGGGAAAAGATCC	TCCATAACCTTGATGACCACAG	I	594
8	TTTGTTGAGATTTTGTGCATGA	TCCCTTTCTCCACACTTTGATT	I	459
9	CAAATATTTCCAACAAAAGAGCA	TGCAAGCTCAGAACTGAAAAAG	I	487
10	CCGGCCAACTGCATAGTATT	CAAATCACCAGACTGAAACTGA	I	395
11	CTGGTTTCCTAAATGACCTTGC	GTCGTAAGTAGGGCCATTATAAATCT	I	349
12/13	CCAAATGCCAACTAACCACATA	GAGTTTGGTTGGGAACTTCAAA	I	696
14	AGACATCTGCCCCTGAGAAG	TGAGGCCTCCAGGTTTATCA	I	395
15	GTTTGATTCTTGAGGACCTTTTG	CCAAAAGCCGAATAAATGGA	II	410
16	TCTTAGGTAAACGCCCAGGAAT	TGGCAAAAAGTGTTGCTAGGTA	I	493
17	AAAAGGAATTATGGAAACTGTAAGGA	TCTTGACATGTGGTAAGTGCTG	I	483
18	CTGTCAATTTGCCATGTGGA	GCACATATCCAGGGCAGAAG	III	386
19	GCTGTCATTTGAAACTTTTGACC	CCCTGTCTCAGACCTTCTCTCT	I	439
20	CCAACCCCCAATCTTCTCTT	CCAGGGTCTCATTTTTCTTTG	I	404
21	CGACCCATTTTCTTTGGTGT	TGGTTGCCTACATGAAAGGTT	I	502
22	TCTGAGGTTGACACCTCCAAT	TGGTGAATGTTGTTCCTTTCA	I	594
23	TGGGGTAATACCGATTATTTTCA	TCAGCTGCTTTTGTGATTTG	I	412
24	GGCATATTTCACATAGCAAGCA	TTTCAGTCTGAGAATCCAAAAACA	I	584
25	GCATGGTTGGTAAGGGACTG	CTGTGACACACAGCACAGTTG	I	288
26	CAACAGCATTCAGCTATGCAAG	GAAGTCAACCATAAAAAGCATGTATCTG	I	326
27	TGGAAACTGAGACCACCTTTT	GAGTCCAAAGACGCATGTGTAG	I	443
28	GAACCCTGGTTCTCCACTCTTA	CTTCCTCCATCTGGGAAATGTA	III	517
29	GTTTGCCCTTGACTGAGAAGA	CTGGCCGGTAAGGATAATGA	I	519
30	TGAATCCTCAAGAGTTTATTGTACC	CTCAAACTCCCAGGCTCAAG	I	495
31	CTGGCCCTGAATTTTTCTTG	CCAGGATTTCGATGCTCACT	I	492
32	GCTCATCACCTCACCCTCTG	CTGTCTAGCTGGGGCAACAT	I	504
33	CCCAGCTAGACAGCACGTATC	GGAGGCTTAAATTTCCTTAGTGTT	I	461
34	CAATGGAAGGTCCAAGGCTA	CTCATGGCCTTCATTCAGGT	I	506
35	CACGGCAAGACTCTGTCTCA	CAGCTTTCTTCCAGGCAAAT	I	326
36	TGAAAAATCAGTAAATTGTTCTGTGA	TGAGCTGCCCAGATCCATA	I	370
37	TCTTGGTGTAGGTGAGATGACTTC	AAGGCTGTTTAAATAAAACTGAGAA	IV	441
38	CAGAAACAAAAAGTTGAGCTCCT	CAGAATTGGAACCACTGTGC	I	330
39	GAGAACAGTGCATAATCTTCCAA	CTGCCACCTGTGAAGAAACA	I	349
40	AAAAAGGTCCCCCAAATAAAAT	CCAGCCCTTCTAGATTGACATT	I	302
41	CCTTATTTGTGTCACAGCCAAC	CACTGGATGAAGATAAGCCTGA	I	462
42	GAATGTTAGAAGCAATGGGAACA	CATGTCAAATGAAACCCCAAG	I	387
43	TGCCTCAGCCTCCTAAAGTG	GATGAGGCCCAAAAAGAATTT	I	409
44	CAAATTCAGCCTATATGGGAAA	GCCAAACATTTCCATATTACCAA	IV	510
45	GTGACATGAAAACCCATCAAAA	CCAGGCTGGTCTCAAACTCT	I	458
46	CCAGGGAGAAGAGGGAGAGA	CATTTCCACCCACCTTAATAGC	I	335
47	GTCACTGACCACCATGACCA	CCACTGCCAGAAGGAAAATG	V	395
48	GCTTTAAGGGTTTTGGCACA	GCTTTGCACAATAGCTAGCACA	I	326
49	TTGAACTTTTTGACAGCAGCA	CTTTTCCCACCTGTCATCCT	I	334
50	TAGCCTGGGCAACAGAGTG	GCTTCCAAAGATTAGCTTGTCC	I	390
51	CCAGTTTGCAACATGTCCTG	GTAGAGACGGGGTTTCACCA	I	400
52	CTGTAGCATCATTTTCAGTGGA	TGCTAAGTTTTTCAGGTGCAAG	I	398
53	CGCTTGCACTAGGAGAGAGG	GCAACAACACTCACTGACCTT	I	406
RT–PCR				
RT_2	CCAGAGGTTCACCAGAAAATCTA	AATAGTTCAACAGCATCGAGTCC		1498
RT_3	AAGCCAGTAGAAAAGATGATGGA	GCAATAACCGAGAAGCTGTAGTC		1499
RT_4	TCCATCACACAACCAGATCTATG	CAACTCCAGTAGATGGTTCATCC		1499
RT_5	ACGACGTCTTGTTCAGTTACCTC	ACCATACCTTGGCAAGTGTTCTA		1509
RT_6	TCTCTTTATGGTACCTCCGAACA	GTATCCCAATGCTGATGTTGTTT		1498
RT_7	ATGGCCTTCATCACTTACGTCT	CATCTGGTCATCTTGTGAGTCAA		1491
RT_8	CAGGGAAAAATCCAGCAGAA	GGAACCATTGCGAAGAAAAG		1420
RT_9	TTCCCAGTCCAAGTTCTGATTT	GCATGGCTTCTATGATCGGTAT		1200
RT_10	AGAACAAATGTTCCGTCATCCT	AGCAACAACACTCACTGACCTT		673
Internal sequencing of RT–PCR products				
RT_1_int	CCGATCATAGAAGCCATGCTG			
RT_4_int	CATTGCAGCTCTGATCGGAAG			
RT_6_int	CTGCCCGACATGGCATCAT			
RT_7_int	GTGCGGGCTGAGAGATTAAG			

Table 2. (*Continued*)

Name	Forward sequences	Reverse sequences	PCR-conditions	Length (bp) of amplicon
RT_7_int2	CTCCTTAGGAGACTTCACCTG			
RT_3_int	CCGATGTAACTCTGAGACAGC			
RT_3_int2	CCATCCGGATGAGTCTCAAGA			
RT_4_int3	CCTTCCTATTGGAAGGAGCGA			
RT_5_int2	GGAGCACCTTCTCCTATATGG			
RT_5_int3	GAAGCCTACCTCAAGGAGGAT			
RT_5_int4	GATGGCTTTGGACTGTTGCTG			
RT_8_int2	GTCAGAATGCTGTCTTTCTTCTC			
5′-RACE amplification				
5′-RACE1		CTGGTGAACCTCTGGCCAAACTGTCAGTCAC		
5′RACE internal sequencing				
5′-RACE_int		CATGCCAAGTAAGGAATATAAGG		
Genotyping of newly developed microsatellites				*Size range*
CNG_K2_06	CCATTCTCTCTCCTCTATTGC	CAGAATGGAGAAGCCTTAAA		197–211
CNG_K2_07	GCCAAGTCTCCTTTGTTAAA	TGAAACTGCATAGCAAAAAG		178–182
CNG_K2_08	GTGTCCTAACATGCACACAG	TTTGTTCTTTGTCCTGATCC		330–363
CNG_K2_09	CCCATATTTAATGCTTCTTAGC	CATCCAAAACATATGCAAAA		184–204
CNG_K2_10	CATGCCTGAAACAAAATTAAG	AACATCTGTTTGCCAGAATC		184–210
CNG_K2_14	GATTGGTGCATTCATTAGGT	TTGTACATCTGTTGATTGTGG		255–275
CNG_K2_15	GGACTGCTCTACCTATGGAGT	TAGCACTAGGAAACCCTTCA		162–178
CNG_K2_16	CAAATGCTCATCAGTCAATG	CTCCAATGTCATCCAGGTC		155–179

PCR conditions: I, as described in Material and Methods; II, TM 56°C; III, TM 58°C using Hot Master *Taq* (Eppendorf); IV, 100 ng of DNA; V, TM 59°C using Advantage GC genomic Polymerase Mix (Clontech).

primer pairs (Table 2) covering the entire coding region, the 3′-UTR and the 5′-UTR region.

The Rapid-Scan gene expression panel including 24 human tissues was tested following the manufacturer's instructions (OriGene Technologies) using the ABCA12 mRNA specific primer pair RT10. The expression of RT10 on cDNAs from keratinocytes, fibroblasts, placenta and lymphocytes was also tested (Fig. 3).

GenBank accession numbers

ABCA12—NM_015657 (NCBI RefSeq); chromosome 2 contig NM_005403; sequence 1 from patent WO02064827, AX587156; *ATIC*—NM_004044; *BARD1*—U76638; *PF20*: NM_024532. Online Mendelian Inheritance in Man (www.ncbi.nlm.nih.gov/Omim/searchomim.html); Chanarin–Dorfman syndrome (CDS, MIM 275630); non-bullous ichthyosiform erythroderma (NCIE1, MIM 242100); non-bullous ichthyosiform erythroderma (NCIE2, MIM 604780); lamellar ichthyosis (LI; MIM 242300); lamellar ichthyosis, 1 (LI1; MIM 604777); lamellar ichthyosis, 2 (LI2; MIM 601277); lamellar ichthyosis, 3 (LI3; MIM 604777); lamellar ichthyosis, 5 (LI5; MIM 606545); ichthyosis nonlamellar and nonerythrodermic congenital (NNCI; MIM 604781); transglutaminase 1 (TGM1; MIM 190195); arachidonate lipoxygenase 3 (ALOXE3; MIM 607206); arachidonate 12-lipoxygenase, R type (ALOX12B; MIM 603741); comparative gene identification 58 (CGI58; MIM 604780); Tangier disease (TGD; MIM 205400); hypoalphalipoproteinemia, primary (FHA; MIM 604091); ATP-binding cassette, subfamily A, member 1 (ABCA1; MIM 600046); Stargardt disease (STGD1; MIM 248200); retinitis pigmentosa 19 (RP19; MIM 601718); cone–rod dystrophy 3 (CORD3; MIM 604116); macular degeneration age-related 2 (ARMD2; MIM 153800); ATP-binding cassette, subfamily A, member 4 (ABCA4; MIM 601691); cataract, nonnuclear polymorphic congenital, autosomal dominant (CCP; MIM 601286); diabetes mellitus, insulin-dependent, 13 (IDDM13; MIM 601318).

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