

# Human ATP-binding cassette transporter 1 (ABC1): Genomic organization and identification of the genetic defect in the original Tangier disease kindred

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**Tangier disease is characterized by low serum high density lipoproteins and a biochemical defect in the cellular efflux of lipids to high density lipoproteins. *ABC1*, a member of the ATP-binding cassette family, recently has been identified as the defective gene in Tangier disease. We report here the organization of the human *ABC1* gene and the identification of a mutation in the *ABC1* gene from the original Tangier disease kindred. The organization of the human *ABC1* gene is similar to that of the mouse *ABC1* gene and other related *ABC* genes. The *ABC1* gene contains 49 exons that range in size from 33 to 249 bp and is over 70 kb in length. Sequence analysis of the *ABC1* gene revealed that the proband for Tangier disease was homozygous for a deletion of nucleotides 3283 and 3284 (TC) in exon 22. The deletion results in a frameshift mutation and a premature stop codon starting at nucleotide 3375. The product is predicted to encode a nonfunctional protein of 1,084 aa, which is approximately half the size of the full-length *ABC1* protein. The loss of a *MnI* restriction site, which results from the deletion, was used to establish the genotype of the rest of the kindred. In summary, we report on the genomic organization of the human *ABC1* gene and identify a frameshift mutation in the *ABC1* gene of the index case of Tangier disease. These results will be useful in the future characterization of the structure and function of the *ABC1* gene and the analysis of additional *ABC1* mutations in patients with Tangier disease.**

high density lipoproteins | cholesterol | atherosclerosis

**T**angier disease is an autosomal codominant disease characterized by the accumulation of cholesteryl ester in various tissues, most notably the tonsils, liver, spleen, and intestinal mucosa (1, 2). Macrophages, present as foam cells in affected tissue, are the principal cell type that accumulates excess tissue cholesterol (2, 3). The genetic defect in Tangier disease has been proposed to be associated with decreased reverse cholesterol transport (4–8), the pathway by which excess cellular cholesterol is transported back to the liver for excretion. In particular, the efflux of cholesterol to high density lipoprotein (HDL), which is the first step in the reverse cholesterol transport pathway, has been reported to be defective in Tangier disease (4–8).

According to current concepts (4, 9–11), two separate pathways are involved in the efflux of cholesterol from cells to HDL: the aqueous diffusion pathway and the apolipoprotein-mediated pathway. In the aqueous diffusion pathway, cholesterol spontaneously desorbs from the cell membrane and associates with lipoprotein acceptors, such as HDL, in the extracellular space (4, 9). For many cell types this appears to be the principal pathway through which cholesterol efflux occurs (11). Alternatively, in cells such as macrophages (12–14) and fibroblasts (4, 8), cholesterol and phospholipid efflux also can be mediated by lipid-poor or lipid-free apolipoproteins, such as apoA-I, apoA-II, and

apoE. Apolipoprotein-mediated efflux is an energy-dependent process that requires the direct interaction of the apolipoprotein with the cell surface, the lipidation of the apolipoprotein, and the subsequent dissociation of the lipid-apolipoprotein complex from the cell (4, 8, 10, 15). Cholesterol loading of cells induces the apolipoprotein-mediated cholesterol efflux pathway (4, 13, 16). Fibroblasts from patients with Tangier disease have been shown to be normal with regard to aqueous diffusion of cholesterol but defective in apolipoprotein-mediated efflux of both cholesterol and phospholipid (4, 8). Lipid efflux from Tangier disease fibroblasts to HDL is also partially reduced (4, 8). In addition to removing cholesterol by aqueous diffusion, a component of lipid efflux by HDL is believed to occur by apolipoproteins that dissociate from HDL and remove lipid from the cell by the apolipoprotein-mediated pathway (4, 8, 10).

Initial studies to elucidate the genetic defect in Tangier disease, using positional cloning, mapped the defective gene to a region on chromosome 9 (17). In a recent report, we (18) and others (48, 49) have identified that mutations in the *ABC1* gene are the molecular defect in Tangier disease.

The *ABC1* protein is a member of the large ATP-binding cassette family of proteins that transport a wide assortment of molecules, such as ions, sugars, vitamins, lipids, and proteins across the plasma membrane (19–22). Mutations in specific *ABC* proteins have been described as causes of several human diseases, including cystic fibrosis (23), adrenoleukodystrophy (24), Zellweger disease (25), and macular degeneration (26). The *ABC1* gene has been cloned from mice (27) and humans (28) and has been proposed to play a role in macrophage function (27–29). Like other *ABC* proteins, the *ABC1* protein contains two ATP-binding domains and 12 transmembrane domains, which are believed to form a channel-like structure and are essential for the transport function of *ABC* proteins (20, 23).

In the present study, we determined the genomic organization of human and mouse *ABC1* and identified the genetic defect in the original kindred from Tangier Island (1). The two hallmarks of the disease, enlarged lipid laden tonsils and low serum HDL, are based on the initial description of this kindred (1). Subsequent studies of this kindred also have provided key insights into the metabolic and the biochemical abnormalities in Tangier

Abbreviations: HDL, high density lipoprotein; EMEM, Eagle's modified minimum essential medium; apoA, apolipoprotein A.

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disease. Metabolic kinetic studies established that there is no defect in the biosynthesis of apoA-I in Tangier disease, but rather the low plasma HDL levels are the result of its hypercatabolism (31). More recently, studies of cultured cells from this (4) and other Tangier disease kindreds (5–8) have been pivotal in confirming the importance of the apolipoprotein-mediated pathway in cholesterol and phospholipid cellular efflux in the reverse cholesterol transport pathway. Structural analysis of the *ABCI* gene in this study revealed a mutation in the original kindred that results in the production of a nonfunctional truncated *ABCI* protein, thus confirming mutations in the *ABCI* gene as the cause of Tangier disease.

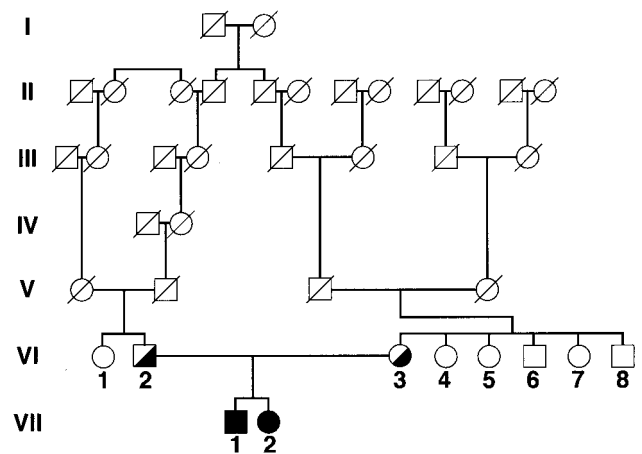
## Materials and Methods

**Cell Culture.** Primary human skin fibroblasts were obtained by explant cultures of skin biopsies taken from the upper arm. Patients from which Tangier cell lines were obtained have been described (1). Normal human skin fibroblasts were from the American Type Culture Collection. Fibroblasts were routinely grown in Eagle's modified minimum essential medium (EMEM) (GIBCO/BRL), supplemented with 10% FCS, 2 mM glutamine, 100 units/ml penicillin, and 100  $\mu$ g/ml streptomycin (EMEM10).

**Cholesterol Efflux Assay.** As described (4, 32, 33), fibroblasts grown on 24-well plates were incubated with EMEM10 containing 1  $\mu$ Ci/ml of 1,2-<sup>3</sup>H-cholesterol (50 Ci/mmol) (DuPont) for 48 hr. After washing the cells, fibroblasts were cholesterol-loaded by a 24-hr incubation with 50  $\mu$ g/ml of nonlipoprotein cholesterol in EMEM, containing 2 mg/ml of BSA (fraction V) (EMEM/BSA). Efflux medium was prepared by adding the indicated acceptor to EMEM/BSA and then was added to washed cells for a 20-hr incubation period. After the incubation, medium was collected, centrifuged (10,000  $\times$  *g* for 5 min), and counted for radioactivity by liquid scintillation counting. Residual radioactivity in the cell fraction was determined after overnight extraction with isopropanol. Percentage efflux is radioactive counts in the efflux medium divided by the sum of the radioactive counts in the medium plus cell fraction. Results from the blank (EMEM/BSA media only) were subtracted from the radioactive counts obtained in the presence of an acceptor in EMEM/BSA.

**Phospholipid Efflux Assay.** As described (4), choline-containing phospholipids were labeled by incubating confluent cholesterol-loaded fibroblasts grown on 24-well plates with EMEM10 medium containing 5  $\mu$ Ci/ml methyl-<sup>3</sup>Hcholine chloride (84 Ci/mmol, Amersham Pharmacia) for 24 hr. Cells then were washed three times with EMEM/BSA, followed by a 1-hr preincubation in EMEM/BSA, before incubation with the indicated acceptor in EMEM/BSA. After a 20-hr efflux period, medium was collected and centrifuged (10,000  $\times$  *g*; 5 min). Samples of supernatants were extracted with chloroform/methanol (2:1), and radioactivity in the organic phase was quantified by liquid scintillation counting. Radioactivity remaining in the cell fraction was determined after overnight extraction with isopropanol, followed by a 1-hr extraction with hexane/isopropanol (3:2). Percentage efflux was calculated as for cholesterol efflux.

**Lipoprotein and Apolipoprotein Isolation and Analysis.** HDL (*d* = 1.063–1.021 g/ml) was isolated from human plasma by density gradient ultracentrifugation. Apolipoproteins purified from human plasma by column chromatography (34) were determined to be over 99% pure, as assessed by SDS/PAGE and amino-terminal sequence analysis. Plasma lipoprotein analysis was performed on a Hitachi 911 analyzer (Roche Diagnostics), using Roche reagents.



**Fig. 1.** Pedigree of Tangier disease kindred. Roman numerals indicate the number of the generation. Subjects in generations VI and VII are identified with arabic numbers. Solid symbols indicate homozygotes and half-filled symbols indicate obligate heterozygotes.

**DNA Sequence Analysis.** DNA was extracted from leukocytes from whole blood, using a Promega DNA extraction kit (Promega). Total RNA was extracted from cultured fibroblast with a Qiagen RNA extraction kit (Qiagen, Chatsworth, CA). Reverse transcription-PCR (RT-PCR) was performed by using Superscript One-Step system, as described by the manufacturer (Life Technologies, Gaithersburg, MD). *ABCI* cDNA for sequencing was obtained by RT-PCR amplification of four overlapping DNA fragments, using the following primers pairs: (i) 75S (5'-CTA CCC ACC CTA TGA ACA AC-3') and 2561R (5'-CTC TTC TCA TCA CTT TCC TC-3'), (ii) 1991S (5'-GCT GGT TCA TTA GTA GCC TCA-3') and 3731R (5'-CCA TCT GAG GTC TCA GCA TCC-3'), (iii) 3651S (5'-TGG CAT CTC AGA GAC GAC CCT-3') and 4999R (5'-GGT GTT TTG CTT TGC TGA CCC-3'), and (iv) 4741S (5'-GCA ATC AGC TCT TTC CTG AAT-3') and 6751R (5'-AGC CAC GCC GTA TGA ACA GGA-3').

Synthetic oligonucleotide primers were synthesized, using phosphoramidite chemistry on a DNA synthesizer (PE Applied Biosystems, Foster City, CA). Sequencing was performed by ABI Prism Big dye cycle sequencing and analyzed on an ABI 310 DNA sequencer.

Identification of exon/intron boundaries was performed by alignment of genomic DNA sequence with the human *ABCI* cDNA (28). Genomic DNA sequence was obtained either by direct sequencing of bacterial artificial chromosome (BAC) clones or by PCR amplification of genomic DNA. BAC clones were identified by screening for the *ABCI* gene from either Genome Systems human release II library (Genome Systems, St. Louis) or from Research Genetics (Huntsville, AL) library CITB-HSP-D. Primers for genomic sequencing were based on the exon/intron boundaries from the mouse *ABCI* gene (GenBank accession no. g495256). Mouse genomic clones were isolated for DNA sequence analysis by screening a phage genomic library from 129SV mouse strain (Stratagene).

## Results

**Characterization of Tangier Disease Kindred.** The pedigree of the original Tangier disease kindred (1) shows consanguinity at generations V and VI (Fig. 1). The proband (subject VII-1) has one sibling (subject VII-2), who also is affected with Tangier disease. The parents (subjects VI-2 and VI-3) are obligate heterozygotes for Tangier disease. A recent analysis of the plasma lipoprotein profile of the kindred is shown in Table 1.

**Table 1. Lipoprotein analysis of kindred**

Subject	Total cholesterol	HDL-C	LDL-C, mg/dl	TG	ApoA-I	ApoA-II
VI-1	228	64	125	193	161	46
VI-2	127	17	61	158	85	26
VI-3	188	23	136	784	118	39
VI-4	232	16	136	552	147	40
VI-5	200	35	91	371	145	39
VI-6	169	27	114	142	99	25
VII-1	35	8	28	352	3	2
VII-2	86	7	33	471	35	2

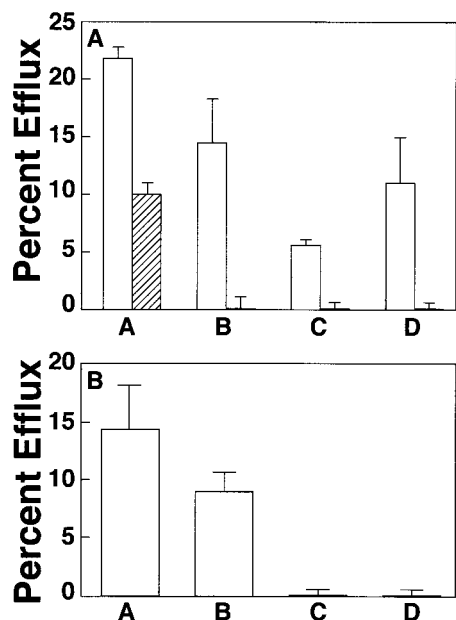
Subject number corresponds to number shown in pedigree in Fig. 1. LDL, low density lipoprotein; TG, triglyceride.

Characteristic of Tangier disease, the homozygotes (subjects VII-1 and VII-2) had a marked reduction of HDL-C, as well as for apoA-I and apoA-II. The obligate heterozygote parents (subjects VI-2 and VI-3) had approximately a 50% reduction in HDL-C. No samples from subjects VI-7 and VI-8 were available for analysis. Based on lipoprotein analysis and clinical history, there do not appear to be any other homozygotes for Tangier disease in the current living members of the kindred. The heterozygous state of the rest of the family could not be established with certainty based on the plasma lipoprotein analysis (Table 1).

A lipid efflux study was performed on skin fibroblasts from the Tangier disease proband (subject VII-1). As shown in Fig. 2A, fibroblasts from the proband had a lower than normal level of

HDL-mediated cholesterol efflux (column A). In contrast, apolipoprotein-mediated cholesterol efflux by either apoA-I (column B) or apoA-II (column C) was nearly absent compared with normal fibroblasts. In addition, phospholipid efflux to apoA-I (column D) also was significantly reduced from the Tangier disease cells. These results are consistent with previous studies (4, 8) that showed a marked reduction of apolipoprotein-mediated lipid efflux from Tangier disease cells, but only a partial reduction in HDL-mediated efflux.

The results of a cholesterol efflux study using apoA-I as acceptor are shown for the affected sibling and one of the parents. Compared with normal cells (Fig. 2B, column A), cells from the parent, (column B: subject VI-3) had only a partial reduction of apolipoprotein efflux. In contrast, the proband (column C; subject VII-1) and the affected sibling (column D; subject VII-2) both had almost a complete absence of apolipoprotein-mediated efflux from cells. These results are consistent with the assigned genotypes for Tangier disease shown in the pedigree (Fig. 1).



**Fig. 2.** Lipid efflux study of kindred. (A) Lipid efflux from normal control fibroblast cells (open columns) and proband fibroblast (subject VII-1) (lined columns) was measured, using cholesterol-labeled cells (columns A-C) or phospholipid-labeled cells (column D), with either 100  $\mu$ g/ml HDL (column A), 10  $\mu$ g/ml apoA-I (columns B and D), or 10  $\mu$ g/ml apoA-II (column C) as the acceptor. Results for control cells are the mean percent efflux  $\pm$  SD of triplicate determinations. (B) Lipid efflux of kindred. Normal cells (column A), and cells from subjects VI-3 (column B), VII-1 (column C), and VII-2 (column D) were radiolabeled with cholesterol and incubated with 10  $\mu$ g/ml apoA-I. Efflux from control cells is the mean percent efflux  $\pm$  SD from three normal cell lines. Results for the subjects from the kindred are the mean percent efflux  $\pm$  SD of triplicate determinations.

**Organization of the ABC1 Gene.** The organization of the human *ABC1* gene was investigated by alignment of the genomic sequence with the previously published cDNA sequence for *ABC1* (28). Genomic DNA sequence was obtained either by direct sequencing of bacterial artificial chromosome (BAC) clones spanning the *ABC1* gene or by sequence analysis of PCR-amplified fragments of genomic DNA. A total of 49 splice junctions were observed in the human *ABC1* gene (Table 2). The location of the splice sites and the overall structure were very similar for the human and mouse *ABC1* genes. The sequences of all identified donor and acceptor splice sites are consistent with consensus sequences for splice junctions and contain the invariant AG (5' splice) and GT (3' splice) sites. The exons, ranging in size from 33 to 249 bp, are relatively small compared with other genes but similar to those in other *ABC* genes (19–22). The coding region resides in exons 3–49. Based on the insert sizes of the BACs used for the analysis, the *ABC1* gene is estimated to span over 70 kb.

**DNA Sequence Analysis of ABC1 Gene in Tangier Disease.** Sequence analysis was performed on DNA from the proband for the *ABC1* gene. Four overlapping PCR fragments were produced by reverse transcription-PCR, as described in *Materials and Methods*, and both strands were sequenced. A 2-bp TC deletion at nucleotides 3283 and 3284 was identified in the cDNA from the proband (Fig. 3). The deletion causes a frameshift, starting at amino acid 1055 and results in a premature stop codon after amino acid 1084. The truncated protein encoded by the mutated *ABC1* gene would be predicted to be nonfunctional, because it contains only approximately half of the amino acids as the intact protein. The truncation would occur between the predicted sixth



**Table 2. Intron-exon boundaries of the ABC1 gene**

Exon num.	Exon size, bp	5' splice site	3' splice site	Position, bp	Exon num.	Exon size, bp	5' splice site	3' splice site	Position, bp5	
1	h			6	26	h	114	gccttcagATGGTACC	AGACCCAGgtctgtta	3841
	m		GACAAACgtaagctg	149		m	114	ccatcttagATGGTACT	AGACCCAGgtctgcac	3984
2	h	94	ccactcagTGTCAGCT	100	27	h	149	tcttcagAATCCAGA	TTGCTCAGgtgagacg	3990
	m	94	ctgttcagTGTCAGCT	243		m	149	attacaagAATCCAGG	TTGCTCAGgtgaggggt	4133
3	h	142	ttoacacagGCCATTTT	242	28	h	125	gggtaaagATTGTCTT	TTTGTACAGtatagttt	4115
	m	142	ttcaatagGCCACTTT	385		m	125	gtttatagATTGTCTT	TTTGTACAGtatagttt	4258
4	h	119	ctttacagTIGGCCIC	361	29	h	99	accocacagCAATGATG	CCAATCCCgtgagtgctc	4214
	m	119	ctttcagCGTGTCTC	504		m	99	atctctagTAATGATG	CCAATCCCgtgagtgctc	4357
5	h	122	ctcaatagACTTGAAG	483	30	h	190	ctttcagAGACACGC	CTCCACAgtgagtgca	4404
	m	122	tcoaatagATTTGAAG	626		m	190	ttttacagAGATACCC	CTCCACAgtgagtgcc	4547
6	h	177	gcttcacagGATATTTT	660	31	h	95	gatgcagAGAAAACA	GCCAAAAGgtgacttt	4499
	m	177	octtcacagGATATTTT	803		m	95	gatgcagAGAAAACA	GCAAGAGgttatogtc	4642
7	h	93	cattacagAGAACACT	753	32	h	33	ctctgcagCTTAAAGA	GAGTTTACgttaagttg	4532
	m	93	ctctctagACAAAAC	896		m	33	ctctgcagCTTAAAGA	GAGTTTACgttaagttg	4675
8	h	241	gacgcagCIGTTCAG	994	33	h	106	tcttttagGATGGGG	TGGCCAAGgttaataa	4638
	m	241	gacaccagCIGTTCAG	1137		m	106	tcttttagGATGGGG	TGACCAAGgttacagca	4781
9	h	140	cttcacagTCCCTTAC	1134	34	h	75	gtttacagGACAGTTC	ATGTCAGgttaaacccg	4713
	m	140	ctttctagTCCCTTAT	1277		m	75	gtttacagGACAGTTC	CTGTATGGgttaacacc	4856
10	h	117	gactccagGTGAACAA	1251	35	h	170	tctttcagGTTGGTIT	GTTGGTCCgttaagttg	4883
	m	117	gactgttagGTGAACAA	1394		m	170	tacttcagGTTGGTIT	GTTGGTCTgttaagttg	5026
11	h	198	aatggcagATGCTGIT	1449	36	h	178	ctctccagGATGACCA	GGGATATgttaaggac	5061
	m	198	aatggcagACGCTGIT	1592		m	178	ccttctagGATGACCA	GGGATATgttgaggac	5204
12	h	206	tctatcagTIGTICAA	1655	37	h	116	acttttagTGCAAITA	CTGTATGGgttaagttc	5177
	m	206	tctgtcagTIGTICAA	1798		m	116	tcttttagTGCAAITA	CTGTATGGgttaagttc	5320
13	h	177	ggttttagGTACTGGG	1832	38	h	145	ctcaacagTIGTICAA	CGGCAATgtgagttca	5322
	m	177	gatttcagGTACTGGG	1975		m	145	ctcaacagTIGTICAA	CAAACAATgttaagttg	5465
14	h	223	gcctccagCTTCTGTC	2055	39	h	124	tttttttagAAGCTGAA	AAGGTTTgtgagttga	5446
	m	223	gcctccagCTTCTGTC	2198		m	124	atctgcagAAGCTCAA	GAGGTTTgtgagttga	5589
15	h	222	cttccacagTTAGGAAA	2277	40	h	130	tctactagGGGAGAAT	AGSCCCAGgtgagctt	5576
	m	222	ctctccagTTAGGAAA	2420		m	130	tctcacagGGGAGAAC	AGSCCCAGgtgagctt	5719
16	h	205	tctccacagAGCCCTGT	2482	41	h	121	cccccaagACCTGTAA	TGACGAAGgtgagaga	5697
	m	205	tctctcagAGCCCTGT	2625		m	121	ctccccagACCTGTAA	TGACCAAGgtgggacg	5840
17	h	114	gctttcagGCCAGTAC	2596	42	h	63	tggttcagATATATAG	CTGGTACgttaaacgac	5760
	m	114	gctttcagGACAGTAT	2739		m	63	gggttcagATCTATAG	CCGGAGAGgttaaatgc	5903
18	h	172	tgctcacagTCTGCATG	2768	43	h	107	cttttcagTCTTTTGG	AGAAATAGgtgagaaa	5867
	m	172	tgctcacagTCTGCATG	2911		m	107	cttttcagTCTTTTGG	AAAACAGgttgaggcaa	6010
19	h	132	gtcctcagGTCATATC	2900	44	h	142	cttttcagTATCTTAT	TTGGCAAGgtactagtt	6009
	m	132	gtcctcagGTCATATC	3043		m	142	ccttacagCATCTTAT	TTGGCAAGgtactagtt	6152
20	h	143	gtcccacagGCTGACTG	3043	45	h	135	atcactagGTTGGTGA	TGTTTCTgttataact	6144
	m	143	tccccacagGCTGACTG	3186		m	135	ctctctagGTTGGTGA	TGTTTCTgtgagttgt	6287
21	h	138	ttttgttagGTGGATG	3181	46	h	104	aattacagGATGAACC	TCTCATAGgtccgtag	6248
	m	138	tcttttagGTGGGATG	3324		m	104	aattatagGATGAACC	TCTCATAGgtctgtac	6391
22	h	221	octggcagGCCGACC	3402	47	h	93	taccgcagTATGGAAG	AAAAATAGgtataaaa	6341
	m	221	octggcagGCCGACC	3545		m	93	ccccacagTATGGAAG	AAAAACAGgttaacggg	6484
23	h	73	gccatcagGAGGACAG	3475	48	h	244	gctttcagGTTTGGAG	TTGACCAAgtaagctt	6585
	m	73	gtcatcagGAGGACAG	3618		m	244	gctttcagGTTTGGAG	TTGACCAAgtaagctt	6728
24	h	203	ccctgcagATGCTCTT	3678	49	h		tttaacagGATTTTGT		
	m	203	ccctgcagATGCTCTT	3821		m		tttgccagGATTTTGT		
25	h	49	ttttccagATATTCCT	3727						
	m	49	ttttccagATATTCCT	3870						

h, human; m, mouse.

and seventh transmembrane domains in the linker region of the ABC1 protein (Fig. 4). No other mutations were discovered in the ABC1 cDNA sequence of the proband.

The 2-bp deletion shown in Fig. 3 results in a loss of a *Mnl1* restriction site. The genotypes of the rest of the kindred, in regard to the mutation discovered in the proband, were evaluated by restriction fragment polymorphism (Fig. 5). A 172-bp fragment was amplified by PCR from genomic DNA of the indicated members of the kindred, digested with *Mnl1*, and analyzed by electrophoresis. The position of the 172-bp fragment

amplified by PCR from control DNA and not digested with *Mnl1* is shown in Fig. 5, lane A. *Mnl1* cleavage of the control DNA fragment in Fig. 5, lane A resulted in two bands of approximately 111 and 61 bp (Fig. 5, lane B), as expected. The PCR-amplified band from the proband (subject VII-1, Fig. 5, lane C) was not cut by *Mnl1*, as predicted by the DNA sequence analysis (Fig. 3). Complete absence of the 111- and 61-bp fragments in Fig. 5, lane C indicates that the proband is homozygous for the TC deletion shown in Fig. 5. The affected sibling (subject VII-2, Fig. 5, lane D) had a pattern similar to that of the proband, thus showing that

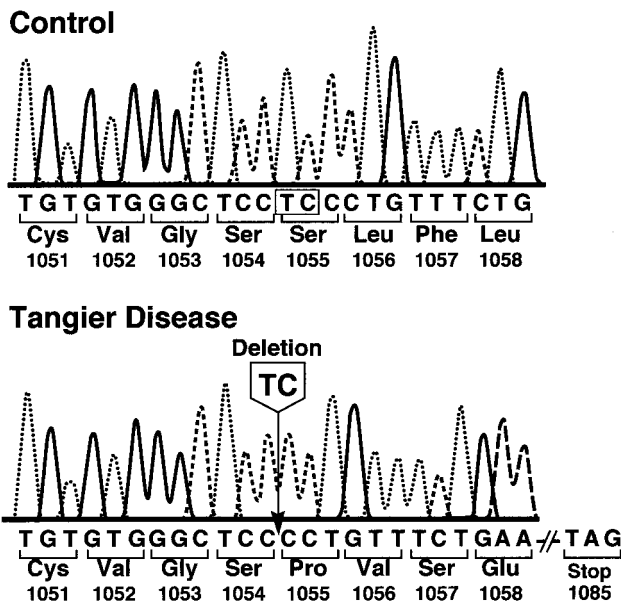


Fig. 3. DNA sequence analysis of *ABC1* gene in proband. DNA sequences of control subject and proband (subject VII-1) are shown for part of exon 22 of the *ABC1* gene. Arrow indicates the site of the TC deletion.

she is also homozygous for the same mutation. The obligate heterozygote parents (Fig. 5, lanes E and F) had DNA fragment patterns expected for a heterozygote. They had one mutant allele, which was resistant to the *Mnl1* digestion and migrated as a 172-bp band. The other apparently normal allele yielded bands of approximately 111 and 61 bp after digestion with *Mnl1*. The DNA fragment pattern in Fig. 5, lane G from subject VI-6 is also consistent with heterozygosity for the mutation. The lipoprotein analysis of subject VI-6 is similar to those of the obligate heterozygote parents (Table 1). The other members of the kindred (Fig. 5, lanes H-J) had normal patterns of DNA fragments and apparently do not have the mutation. The presence of the 2-bp deletion was confirmed in all subjects identified to be heterozygotes or homozygotes by DNA sequence analysis.

### Discussion

In this report, we describe the organization of the human *ABC1* gene and identify an *ABC1* mutation in the index case for Tangier disease. The organization of the *ABC1* gene is similar to that of other *ABC* genes (19–22) and contained 49 exons that

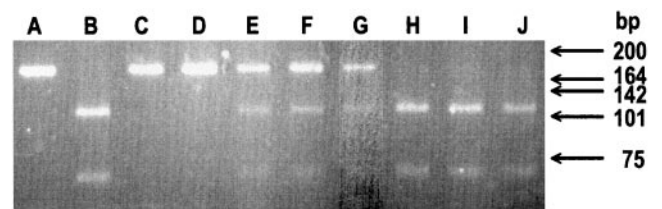


Fig. 5. Restriction digest polymorphism analysis of Tangier disease kindred. A 172-bp fragment was amplified from DNA extracted from peripheral blood leukocytes by PCR, using a sense primer starting at nucleotide 3181 (5'-GGC-CGACCATTATTCCTCT3') and a reverse primer starting at nucleotide 3353 (5'-GATTCCACATCCTTCTTGACC3'). PCR fragments were digested with *Mnl1* and analyzed on 3% Nusieve/0.5% Me Seakem gel. Letters above each lane correspond to the following samples: undigested control DNA (A), *Mnl1*-digested DNA from control (B), subject VII-1 (C), subject VII-2 (D), subject VI-3 (E), subject VI-2 (F), subject VI-6 (G), subject VI-4 (H), subject VI-5 (I), and subject VI-1 (J).

spanned greater than 70 kbp in length (Table 2). Because of the frequency of mutations in splice site junctions (35), particularly in a gene like *ABC1* with a relatively large number of splice sites, the description of the *ABC1* genomic organization will be useful in the future identification of *ABC1* mutations in Tangier disease patients. The mutation described in the proband of the kindred is a 2-bp deletion that results in a frameshift (Fig. 3) and a truncated protein that is predicted to be nonfunctional, because it would be only approximately half the size of the intact protein. The genotype analysis of the rest of the kindred is consistent with this mutation being the cause of Tangier disease (Fig. 5). The identification of an *ABC1* mutation in the original Tangier disease kindred thus establishes that defects in the *ABC1* gene are a cause for Tangier disease. Additional studies, however, will be necessary to determine whether there are other genetic defects that can lead to the Tangier disease phenotype.

Although mutations in the *ABC1* gene now have been identified as a cause for Tangier disease, the exact physiologic role of the ABC1 protein and how a defect in the protein can lead to a loss of apolipoprotein-mediated efflux is still not known. Prior studies of ABC proteins, however, suggest several possibilities. The ABC1 protein along with ABC2, ABC3, and ABCR are members of the ABCA subfamily (36–39) that, unlike other ABC proteins, are not present in prokaryotes or in yeast, which suggests a specialized role for these proteins in multicellular organisms (20, 36, 40, 41). All members of the ABCA subfamily also contain a relatively large hydrophobic region between the sixth and seventh transmembrane domains (Fig. 4). The exact orientation of the hydrophobic region in relation to the plasma

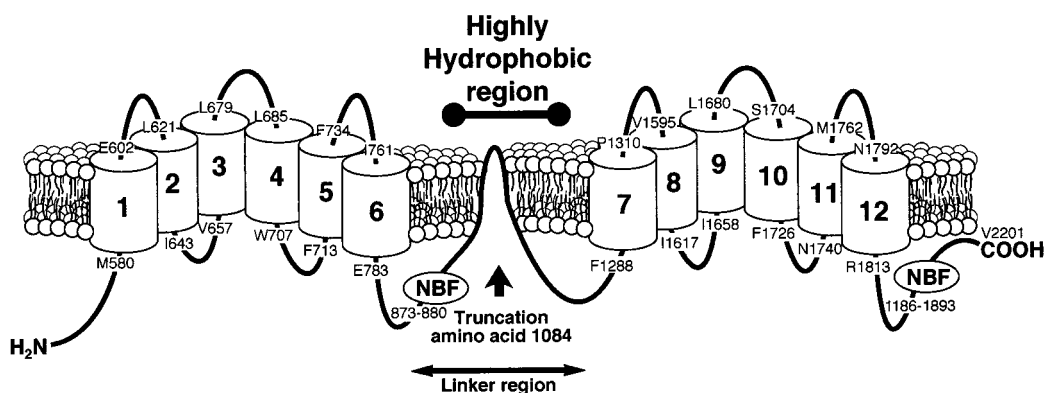


Fig. 4. Diagram of the secondary structure of the ABC1 transporter. Arrow indicates the site of the TC deletion and the approximate location of the carboxyl terminus of the truncated protein. NBF indicates the positions of the nucleotide-binding folds.

membrane is not known, but it has been suggested that it may facilitate the transport of lipid across the membrane bilayer (36, 38, 39). The ligands for transport by the ABC1 subfamily are not known except for ABCR, which transports retinoids that are conjugated to phosphatidylethanolamine in the retinal rod photoreceptor cells (39). In addition to the ABCR, several other ABC proteins also transport lipids (42–44). For example, disruption of the *mnr2* gene in mice leads to defective secretion of phospholipid and cholesterol into bile (45).

If ABC1 transports lipid, a defect in ABC1 function could directly lead to a disruption of lipid efflux from cells. In one simple model, apoA-I and other apolipoproteins could interact directly with the ABC1 protein. ApoA-I then could undergo lipidation with free cholesterol and/or phospholipid transported by the ABC1 protein, and the lipidation of apoA-I would lead to the formation and dissociation of nascent HDL from the cell. A functional defect in ABC1 therefore would lead to low plasma HDL, because of the rapid catabolism of lipid poor apoA-I (4, 8, 31).

Several other aspects of ABC1 expression are consistent with a direct role for ABC1 in lipid efflux from cells. Macrophages abundantly express the ABC1 protein (27, 28, 30) and are the predominant cell type that is affected by Tangier disease (2). Unlike many other cells, macrophages can efflux lipid by the apolipoprotein-mediated pathway (13, 16, 46). Macrophages may be particularly prone to lipid accumulation in the absence of the apolipoprotein-mediated pathway, because of their scavenger role in the clearance of cellular debris, such as lipid-rich membranes and oxidized lipids. The induction of ABC1 expression in macrophages after cholesterol loading (28) is also consistent with a requirement for cells to be cholesterol-loaded before they are able to undergo apolipoprotein-mediated efflux (4, 8).

Previous studies also have identified several other potential roles for ABC1 that may relate indirectly to lipid efflux. Both ABC1 and *Ced7*, a similar protein expressed in *Caenorhabditis elegans*, have been proposed to be important in the phagocytosis of apoptotic cells (29, 30). The mechanism by which this occurs is not known but has been proposed to involve a function of ABC1 in the transport of adhesion proteins to the cell surface

(29, 30). Alternatively, the translocation of lipids by ABC1 may modify the plasma membrane of macrophages and facilitate the process of phagocytosis. Like other ABC proteins, ABC1 also has been proposed to play a part in the transport and secretion of leaderless proteins, such as IL-1 $\beta$  (47). Leaderless proteins lack a signal peptide and are secreted from the cell through a pathway that bypasses the classical endoplasmic reticulum-Golgi pathway. The ABC1 protein therefore might mediate lipid efflux from cells indirectly by its role in the transport of a cell surface protein that is essential for lipid efflux.

Now that the genetic defect of Tangier disease has been identified, it will be useful to carefully examine the causes for the phenotypic heterogeneity of the disease in regard to the development of premature cardiovascular disease. Minimal atherosclerosis is present in the original kindred with Tangier disease (1), but premature cardiovascular disease has been identified in another Tangier disease kindred that also has a *ABC1* gene mutation (17). Additional studies will be required to elucidate the genetic, as well as the environmental factors, that may modulate the phenotypic expression of Tangier disease.

In the first description (1) of Tangier disease in 1961, one of the authors (D.S.F) wrote the following: "One of the remarkable opportunities that patients with rare genetically determined diseases offer is an occasional view of normal processes obtainable in no other way. Possibly Tangier disease will provide us with just such a view into the now-clouded aspects of fat transport and metabolism." It took nearly 40 years to unravel the biochemical and genetic defect in Tangier disease but now this prediction has come true. The identification of *ABC1* mutations as the defect in Tangier disease has provided a key insight into the normal process of reverse cholesterol transport. Future studies aimed at understanding the function of ABC1 in lipid efflux represent a promising new direction for atherosclerosis research.

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