Novel mutations in the gene encoding ATP-binding cassette 1 in four Tangier disease kindreds

Margaret E. Brousseau,* Ernst J. Schaefer,* Josee Dupuis,† Brenda Eustace,† Paul Van Eerdewegh,†, Allison L. Goldkamp,* Lisa M. Thurston,† Michael G. FitzGerald,§ Diane Yasek-McKenna,†† Gilmore O'Neill,†† Gretchen P. Eberhart,§§ Barbara Weiffenbach,† Jose M. Ordovas,* Mason W. Freeman,§§ Robert H. Brown, Jr.,†† and Jessie Z. Gu[1],†**

Lipid Metabolism Laboratory, JM-USDA Human Nutrition Research Center on Aging at Tufts University and Department of Medicine,* New England Medical Center, Boston, MA 02111; Human Genetics† and Genomics Departments,§ Genome Therapeutics Corporation, Waltham, MA; Department of Psychiatry,** Harvard Medical School, Cambridge, MA; Day Neuromuscular Research Laboratory,†† Massachusetts General Hospital, Charlestown, MA; and Lipid Metabolism Unit,§§ Massachusetts General Hospital, Boston, MA

Abstract Tangier disease (TD) is an autosomal co-dominant disorder in which homozygotes have a marked deficiency of high density lipoprotein (HDL) cholesterol and, in some cases, peripheral neuropathy and premature coronary heart disease (CHD). Homozygotes are further characterized by cholesteryl ester deposition in various tissues throughout the body, most notably in those of the reticuloendothelial system. Several studies have demonstrated that the excess lipid deposition in TD is due to defective apolipoprotein-mediated efflux of cellular cholesterol and phospholipids. Although much progress has been made in our understanding of the metabolic basis of TD, the precise molecular defect had remained elusive until very recently. By positional cloning methods, we: *1*) confirm the assignment of TD to chromosome 9q31, *2*) provide evidence that human ATP-binding cassette-1 (hABC-1) maps to a 250 kb region on 9q31, and *3*) describe novel deletion, insertion, and missense mutations in the gene encoding hABC-1 in four unrelated TD kindreds. These results establish a causal role for mutations in hABC-1 in TD and indicate that this transporter has a critical function in the regulation of intracellular lipid trafficking that dramatically affects plasma HDL cholesterol levels.—Brousseau, M. E., E. J. Schaefer, J. Dupuis, B. Eustace, P. Van Eerdewegh, A. L. Goldkamp, L. M. Thurston, M. G. FitzGerald, D. Yasek-McKenna, G. O'Neill, G. P. Eberhart, B. Weiffenbach, J. M. Ordovas, M. W. Freeman, R. H. Brown, Jr., and J. Z. Gu. **Novel mutations in the gene encoding ATP-binding cassette 1 in four Tangier disease kindreds.** ***J. Lipid Res.*** **2000.** 41: **433–441.**

Supplementary key words ATP-binding cassette • apolipoprotein • cholesterol • high density lipoprotein • Tangier disease

First described by Fredrickson and colleagues in 1961, Tangier disease (TD) is a rare genetic disorder characterized by severe deficiency of high density lipoproteins (HDL) in the plasma (1). A hallmark of this disease is the accumulation of cholesteryl esters (CE) in various tissues of the body, most notably in those of the reticuloendothelial system (RES), leading to hyperplastic orange tonsils, as well as to lymphadenopathy, hepatosplenomegaly, and peripheral neuropathy (2). TD homozygotes typically have plasma HDL cholesterol (HDL-C), apolipoprotein (apo) A-I, and low density lipoprotein cholesterol (LDL-C) levels which are approximately 5%, 1%, and 40% of normal, respectively (3), whereas the HDL-C and apoA-I levels of heterozygotes are 50% of normal (2). Although there is an overall increased incidence of cardiovascular disease in TD homozygotes, it is not as extensive as one might predict based on the strikingly low HDL-C levels (3). This is perhaps due, in part, to the reduction of LDL-C levels observed in these patients.

A number of kinetic studies utilizing radiolabeled HDL and apoA-I have been conducted to determine the metabolic basis for the hypoalphalipoproteinemia in TD (4–6). The collective results of these studies have clearly established that the reduced levels of HDL-C in TD are directly attributable to the hypercatabolism of HDL constituents, rather than to the defective biosynthesis of HDL apolipoproteins. Confirmation of the absence of a biosynthetic defect was provided by the fact that the apoA-I gene sequence is normal in TD patients (7, 8). More recent work has focused on the cellular defect observed in TD patients, namely the inability to translocate intracellular cholesterol to the plasma membrane (9–14). This translocation is stimulated by the binding of HDL and apoA-I to cells (15) and is dependent on protein kinase C (9, 16), implicating impaired signal transduction in the pathogen-

Abbreviations: hABC-1, human ATP-binding cassette 1; apo, apolipoprotein; BAC, bacteria artificial chromosome; C, cholesterol; HDL, high density lipoprotein; RCT, reverse cholesterol transport; TD, Tangier disease.

[1] To whom correspondence should be addressed.

esis of TD. In this regard, Walter et al. (17) have further reported abnormalities in the coordinate activation of phospholipases C and D. It is important to note that the defect in apolipoprotein-mediated cholesterol efflux is not specific to apoA-I binding, as other apolipoproteins containing amphipathic helices such as apoA-II, apoA-IV, apoC-I, apoC-II, and apoC-III show decreased lipid efflux from TD cells as well (18, 19).

While much progress has been made in our understanding of the metabolic basis of TD, the precise molecular defect responsible for TD has remained elusive for a number of years and has been the primary research focus of laboratories throughout the world. Recently, three laboratories reported that mutations in the gene encoding human ATP-binding cassette 1 (hABC-1) are responsible for TD (20–22). This has subsequently been confirmed in two additional reports (23, 24). The search for the TD gene by genetic linkage analysis was complicated by the lack of unique biochemical markers that unambiguously distinguish TD heterozygotes. Although HDL-C levels may be reduced in TD heterozygotes relative to age- and gender-matched controls, there are several different genetic and environmental factors that influence HDL-C levels in the general population (25). In 1982, Suarez, Schonfeld, and Sparkes (26) reported their unsuccessful attempt to link the TD locus to a polymorphic marker that could be used in the diagnosis of heterozygosity. However, using a graphic linkage exclusion method, Rust and colleagues (27) assigned the gene defect underlying TD to an 8 cM region of chromosome 9q31. In this report, we: *1*) confirm the assignment of TD to chromosome 9q31, *2*) provide evidence that hABC-1 maps to a 250 kb region on 9q31, and *3*) report novel deletion, insertion, and missense mutations in the gene encoding hABC-1 in four unrelated TD kindreds.

MATERIALS AND METHODS

Family studies and lipid analyses

The proband of the large kindred (TD16) presented in **Fig. 1**, individual 420, has been described elsewhere in detail (case 52), along with the two siblings of Japanese origin (cases 36 and 37) (3). In this manuscript, these two siblings are identified as TD18-1 and TD18-2. Proband 420 developed neuropathy at age 25, CHD at age 46, and died of this disease at age 58. TD18-1 and TD18-2 do not, as yet, have evidence of neuropathy or CHD. The other two probands are new cases. One patient (TD17) is a 51-year-old male having total cholesterol, HDL-C, and triglyceride levels of 36, 1, and 333 mg/dl, respectively, as well as established CHD requiring bypass at age 46. He has no evidence of neuropathy. The other patient (TD19), a 52-year-old male, presented to the MGH Lipid Clinic in 1996 with mild splenomegaly, a total cholesterol of 72 mg/dl, an HDL-C of 3 mg/dl, and triglycerides of 307 mg/dl, without any symptoms of neuropathy or premature CHD. As shown in **Fig. 2**, TD16, TD17, TD18-1, and TD18-2 were found to have a significant defect in apoA-I-mediated cholesterol efflux from fibroblasts relative to control subjects. A skin biopsy was not available from TD19; however, this individual has a classic TD phenotype. Thirty cc of blood was collected from each of these probands into tubes containing 0.1% EDTA, as well

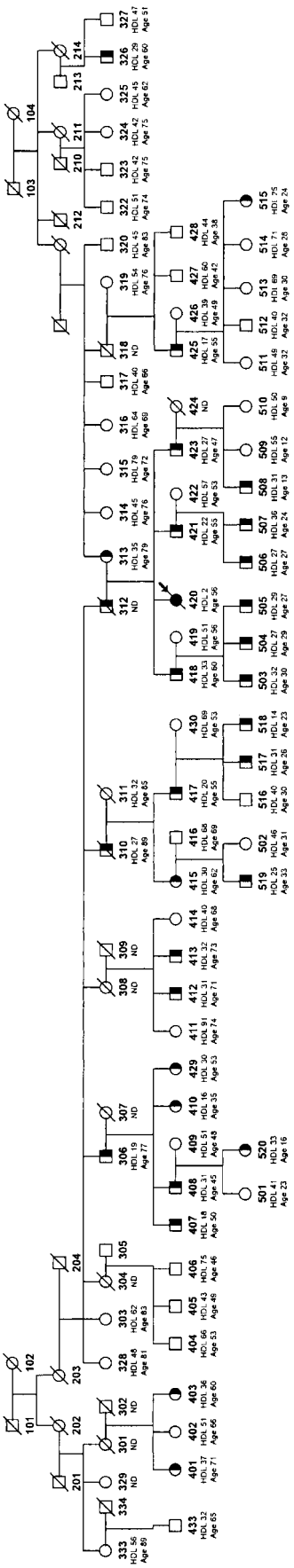

Fig. 1. Pedigree of a large kindred with Tangier disease. Only those who consented to participation in our study are presented in this diagram. The homozygous proband, individual 420, developed neuropathy at age 25, CHD at age 46, and died at age 58. The designation of heterozygosity in this figure was based on haplotyping data. HDL-C (mg/dl) and age (years) are provided for each individual. Individuals 312 and 313, the parents of the proband 420, are obligate heterozygotes.

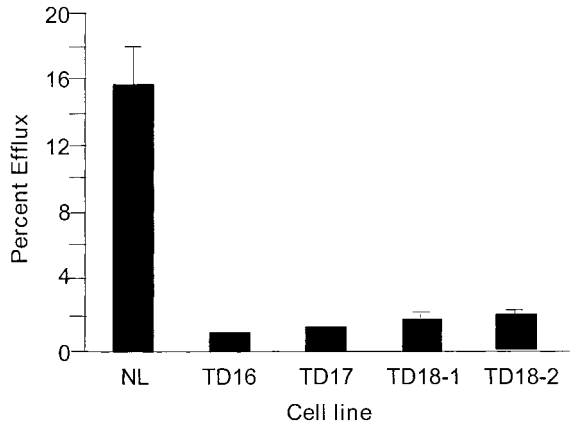

Fig. 2. ApoA-I-mediated cellular cholesterol efflux from the fibroblasts of TD homozygotes (TD16, TD17, TD18-1, TD18-2) and control subjects (n = 5). Subconfluent cells were labeled with [^{3}H]cholesterol, followed by incubation with exogenous unlabeled cholesterol. Efflux was performed in triplicate for each individual using an apoA-I concentration of 5 μg/mL in the medium. Efflux medium was counted, cell lipids were extracted, and extracts were separated by TLC for the isolation of esterified and unesterified cholesterol. Data for all subjects are shown as mean ± SD of medium [^{3}H]cholesterol expressed as a percentage of total (medium plus cell) [^{3}H]cholesterol. The SD values for TD16 and TD17 were less than 0.10%. ApoA-I-mediated cellular cholesterol efflux from the fibroblasts of each TD homozygote was significantly less ($P < 0.001$) than the mean value of control subjects.

as from the extended family members of proband TD16. Plasma HDL-C levels were determined as previously described (28).

Our experimental protocol was approved by the Human Investigation Review Committee of Tufts University and New England Medical Center, and informed consent was obtained from all subjects.

Cellular cholesterol efflux assay

Fibroblast lines were initiated by explant culture from a 3-mm punch biopsy at a 1-mm skin thickness obtained under sterile conditions from the medial aspect of the inner arm and placed in HEPES-buffered saline, pH 7.4. The tissue was transferred to a 100-mm sterile plastic petri dish and diced. Explants were transferred to 25-cm^2 flasks in DMEM media with 15% FBS, penicillin 50 u/ml, and streptomycin 50 ug/ml. Explant culture was performed either in the Cytogenetics Laboratory of the Brigham and Women's Hospital, Boston, MA or in the Cytogenetics Department of the New England Medical Center (NEMC), Boston, MA. Two control primary fibroblast cell lines from the former laboratory, one from the NEMC laboratory, and two from the American Type Culture Collection (Rockville, MD) were used for the comparison studies. All fibroblasts were used between the 5th and 12th passage.

Fibroblasts were grown to 60–80% confluence in DMEM with 10% FBS and labeled by incubating in media with 0.2–0.5 μCi [^{3}H]cholesterol/ml (New England Nuclear, Boston, MA) until confluent, approximately 72 h. Labeled cells were cholesterol enriched by incubation in DMEM with 2 mg/ml of fatty acid-free bovine serum albumin (DMEM/FA-BSA) and 30 μg/ml of nonlipoprotein cholesterol from an ethanol stock solution for 24 h. Cells were incubated for 48 h in DMEM with 1 mg/ml FA-BSA to allow cellular cholesterol pools to equilibrate (29), prior to the initiation of the efflux measurements. Efflux of radiolabeled cholesterol from cells was measured as previously described (30). Briefly, cholesterol-loaded [^{3}H]cholesterol-labeled cells were incubated in DMEM/FA-BSA with the indicated concentration of apoA-I for 16 h at 37°C. After incubation with acceptor, efflux media was removed, centrifuged to remove cells and debris, and an aliquot of media was counted directly in a scintillation counter. Cell lipids were extracted from PBS-washed culture dishes with hexane–isopropanol 3:2(v:v), then evaporated to dryness under nitrogen gas, reconstituted in chloroform, and subjected to thin-layer chromatography (TLC). Cell proteins were dissolved in 0.1 N NaOH and aliquots were quantified by the method of Lowry et al. (31). The amount of cholesterol appearing in the media was expressed as a percentage of the sum of [^{3}H]cholesterol found in the media and in cellular unesterified and esterified cholesterol.

DNA isolation and genome scan

Genomic DNA was isolated by standard methods from whole blood samples using Qiagen columns. Primer pairs for PCR analysis of polymorphisms at approximately 400 loci were used in the genome wide scan. Genotypes were determined using an ABI Prism 377 (Applied Biosystems), and data were analyzed using GeneScan and Genotyper 2.0 software (Perkin Elmer).

Genetic linkage analysis

The pedigree used in the genetic linkage studies is shown in Fig. 1. As a unique biochemical marker for the unambiguous assignment of heterozygosity was not available, low HDL-C was the phenotype used in the linkage analysis. Individuals with HDL-C values below or at the 10th percentile of those for age- and gender-matched controls from the Lipid Research Clinics (32) and Framingham Offspring (33) reference populations were designated affected. Individuals at or below the 10th percentile of only one of the reference populations were classified as phenotype unknown, while all other subjects were assumed to be unaffected. Two-point linkage analysis was performed with the MLINK and ILINK components of the program LINKAGE (34), modeling low HDL-C as a co-dominant trait, with carriers having an 80% chance of being affected. The phenocopy rate was set at 9%, and the proband was assumed to be homozygous at the disease locus. The allele frequencies at each marker were estimated by the USERM13 program (35), and the frequency of the disease allele was assumed to be 0.007. Subsequently, haplotyping analysis was performed using SIMWALK2 software (36).

Southern blotting of BAC filters

The RPCI-11 human male bacteria artificial chromosome (BAC) library from the laboratory of Pieter deJong at the Roswell Park Cancer Institute was used for physical mapping, as well as for preparation of BAC filters (**Fig. 3**). The BACs used ranged from 150 to 250 kb. Glycerol stocks, containing 200 μl LB/12.5 μg/ml chloramphenicol, of confirmed clones were arrayed in a 96-well format, and clones were manually stamped, in duplicate, onto Hybond nylon filters (Amersham, UK) using a 96 pin replicator for a total of 152 BACs per filter. Filters were next placed on LB/chloramphenicol plates and grown overnight at 37°C to enlarge the colonies. Filters were denatured, neutralized, and UV crosslinked, as described in Sambrook, Fritsch, and Maniatis (37), prior to prehybridization with 5 ml of ExpressHyb solution (Clontech) at 68°C for 2 h. Two probes were generated using hABC-1 primer pairs 1 (F, 5′-CCT CAG CAT CTT GTC CAC AG-3′; R, 5′-TGG TGG ACA ATG AAA CCT TC-3′) and 19 (F, 5′-CCA CAT CAA CTT CTG GCT CT-3′; R, 5′-TGG CCT GGC CTC TAT TTA TC-3′) for PCR amplification of normal fibroblast cDNA. Fifty ng of each gel-purified probe was labeled with 50 μCi of α^{32}P-dCTP, using the Prime-It RmT labeling kit (Stratagene), and unincorporated nucleotides were removed with G-50 microspin

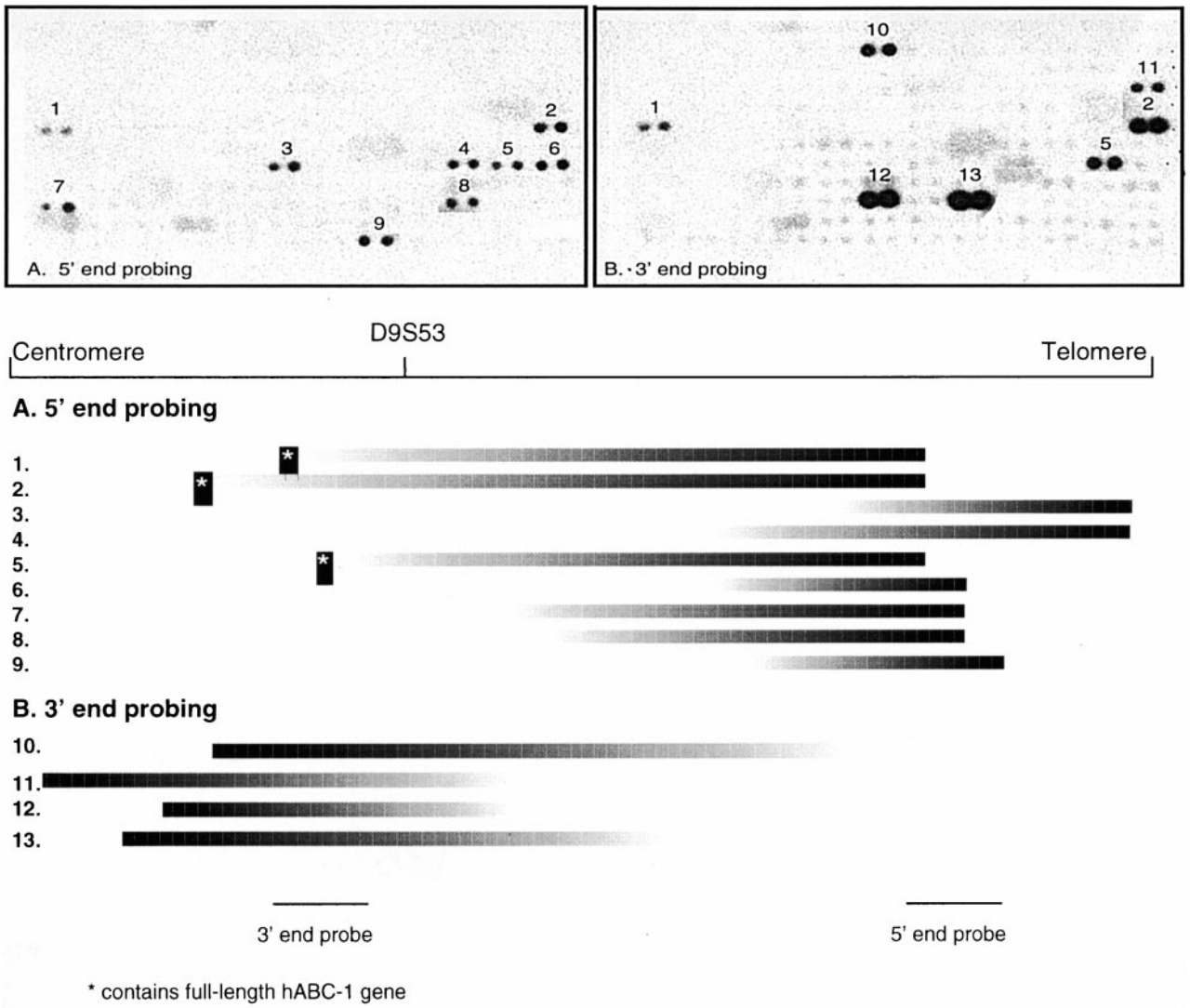

Fig. 3. Hybridization of BAC filters with the 5′-most (panel A) and 3′-most (panel B) sequences of hABC-1 cDNA. As described in Methods, filters containing 152 BAC colonies were hybridized with probes representing either the 5′-most or the 3′-most sequence of the hABC-1 transcript. BACs 1, 2, and 5 contained both the 5′ and 3′ ends of the hABC-1 transcript, suggesting the presence of a full-length genomic clone and localizing hABC-1 to a 250 kb region of 9q31. Each BAC was also positive for D9S53, providing further evidence for the localization of hABC-1 to 9q31.

columns (Amersham Pharmacia Biotech). Filters were hybridized for 2 h at 68°C and were subsequently washed prior to autoradiography at −70°C overnight.

Mutation detection and sequence analysis

A summary of the strategy used for mutation detection is illustrated in **Fig. 4.** Briefly, oligo dT-primed cDNA was generated from total fibroblast RNA using the Superscript One-Step RT-PCR system (Life Technologies). Primary PCR was performed using the Expand PCR Kit (Boehringer Mannheim), resulting in 5′ and 3′ products of 3.7 and 3.3 kb in size, respectively. Next, 19 primer sets were designed for use in secondary PCR, with primer sets 1–10 using the 5′-primary PCR product as template and primer sets 11–19 using the 3′-primary product. PCR products were gel-purified with 96-well plates (VWR Scientific) and were then directly sequenced with Energy Transfer primers (Amersham). The reactions were run on ABI Prism DNA Sequencers (Applied Biosystems), and sequencing data were analyzed with the Sequencher 3.1 (Gene Codes Corporation) and Polyphred (University of Washington) programs.

RESULTS

To identify the genetic locus of TD, two-point linkage analysis was performed using PCR-based polymorphic markers. A region of linkage was observed with microsatellite markers on chromosomal band 9q31, consistent with previous reports (20–22, 27). Also consistent with the report of Rust et al. (27) was the location of the maximum LOD score (5.15) in our study at marker D9S1784, as shown in **Table 1**. Haplotyping analysis in our large TD pedigree narrowed the genetic interval to the region between markers D9S277 and D9S1832, based on two critical crossovers in individuals 316 and 421 (Figure 1). The hap-

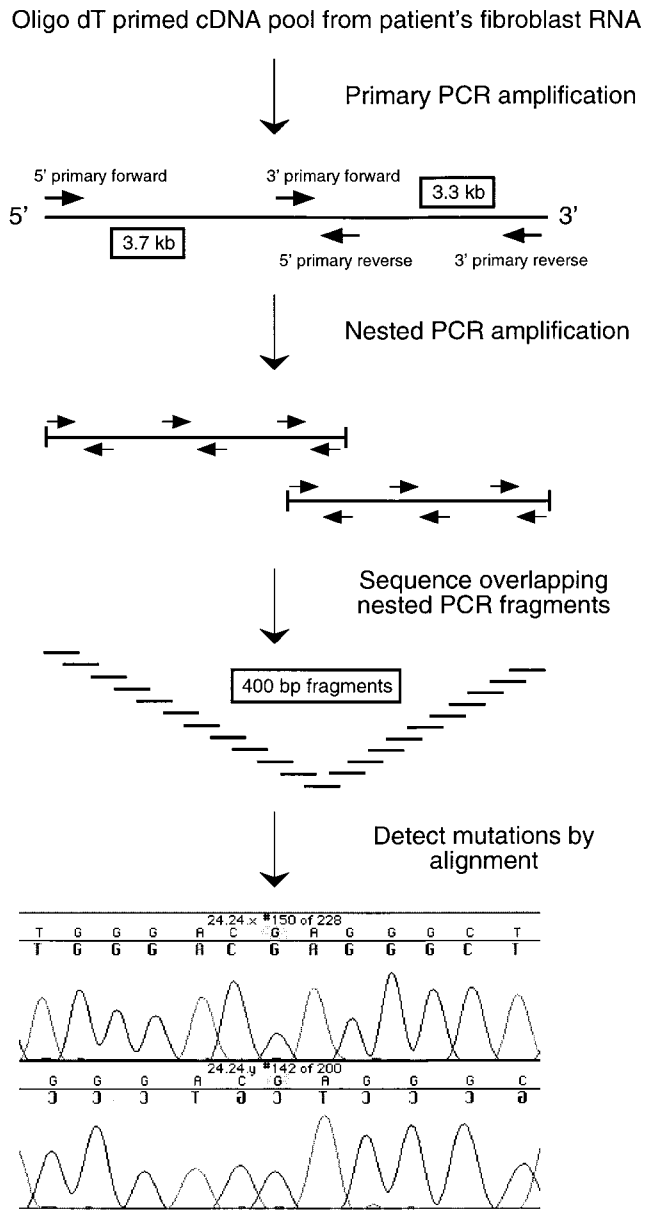

Fig. 4. Summary of the strategy used for mutation detection. Primary PCR amplification was performed on fibroblast-derived cDNA from each patient for the generation of 3.7 kb and 3.3 kb fragments containing the 5′- and 3′-halves of the hABC-1 transcript, respectively, using the following primer pairs: 5′-F(5′-GTC AGC TGT TAC TGG AAG TGG CCT G-3′), 5′-R(5′-CTG TTT CGT CTT GCT GGC AAG GTA C-3′); 3′-F(5′-CCT ATG TGC TGC CAT ATG AAG CTG C-3′), 3′-R(5′- ACT TCT TCC CAC ATC AAC TTC TGG C-3′). The two primary PCR products were used as templates for secondary PCR, resulting in 19 overlapping fragments of about 400 bp in size. The PCR products were purified, sequenced, and aligned to the wild-type hABC-1 sequence for mutation detection.

lotype tracked almost perfectly in this pedigree, with all HDL-deficient individuals having the haplotype. The one exception to this occurred in individual 405, whose HDL-C level was 43 mg/dl, despite having the haplotype. However, this individual's two brothers (404, 406) had very high HDL−C levels relative to those of age-matched males, suggesting that other factors may have influenced HDL-C levels in this nuclear family as well. Homozygosity mapping in additional TD probands further narrowed our centromeric and telomeric boundaries to markers D9S277 and D9S53, respectively, approximately a 1 cM region. The fact that our maximum LOD score occurred at D9S1784 is not inconsistent with our boundaries; rather, it indicates that this marker was most informative in the pedigree presented in Fig. 1.

In order to define this genetic interval, a minimal set of overlapping yeast artificial chromosomes (YACs) and BACs was assembled. Three YACs and 8 BACs independently comprised the physical map for this region (data not shown). Subsequent to physical mapping, we initiated a search for both known and novel candidate genes in this region. One of the candidate genes in the interval defined by Rust et al. (27) was hABC-1, which had previously been mapped to 9q22-q31 (38). Our refined locus, however, placed this gene almost 2 cM outside of the genetic interval of interest, so it was initially not investigated. With the publication by Langmann and colleagues (39) demonstrating that hABC-1 is a sterol responsive gene highly expressed in macrophages, we elected to use its sequence as a probe of our BAC clones. This would allow us to determine whether the locus assignment of hABC-1 was incorrect, or whether a related family member resided within the interval defined by our large pedigree. The results of our work are summarized in Fig. 3. Gel-purified PCR products of primers 1 and 19, which represent the 5′- and 3′-most sequences of hABC-1 cDNA, respectively, were used to screen genomic BACs from the BAC contig map, allowing for the detection, if present, of a full-length genomic clone. As shown in panels A and B, 9 of the 152 BACs screened hybridized to the probe representing the 5′-most sequence of the hABC-1 transcript, whereas 7 BACs hybridized with the probe consisting of the 3′-most sequence of the hABC-1 transcript. Three BACs, designated 1, 2, and 5, contained both the 5′ and 3′ ends of the hABC-1 transcript, suggesting the presence of the full-length genomic clone and localizing hABC-1 to a 250 kb region of 9q31. Moreover, these BACs contained the D9S53 microsatellite marker, confirming the placement of hABC-1 to our defined genetic interval, as well as in that reported by Rust et al. (27).

Sequence analysis of fibroblast cDNA documented the presence of mutations in hABC-1 in four unrelated TD kindreds, as shown in **Table 2**. Proband 420 of kindred TD16 was found to be homozygous for an A→C missense mutation at nucleotide 5338 of the hABC-1 transcript, resulting in the substitution of an uncharged amino acid, asparagine, with a positively charged one, histidine, in the second transmembrane domain. This mutation was also detected in heterozygotes from this pedigree but was not observed in our control population. The Japanese siblings, TD18-1 and TD18-2, were found to be homozygous for a G→A missense mutation at nucleotide position 3805. This mutation causes a change of amino acid from aspartate to leucine at codon 1229, resulting in the substitution of a negatively charged amino acid residue with a nonpolar residue. The most dramatic mutation identified was

TABLE 1. Two-point linkage analysis

Locus[a]	Recombination Fractions (θ)								
	0.00	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40
D9S173	1.52	1.29	1.08	0.89	0.72	0.56	0.40	0.26	0.13
D9S176	1.99	1.76	1.51	1.25	0.99	0.71	0.46	0.25	0.10
D9S1690	3.23	2.88	2.51	2.13	1.73	1.32	0.93	0.56	0.26
D9S271	3.19	3.05	2.75	2.37	1.95	1.51	1.07	0.68	0.35
D9S277	2.42	2.10	1.72	1.33	0.93	0.54	0.19	−0.04	−0.13
D9S938	3.94	3.64	3.24	2.80	2.32	1.82	1.31	0.82	0.40
D9S53	1.37	1.30	1.19	1.06	0.91	0.74	0.56	0.38	0.22
D9S306	1.22	1.19	1.11	1.00	0.86	0.70	0.52	0.35	0.20
D9S1784	5.15	4.59	4.01	3.41	2.80	2.18	1.57	0.99	0.49
D9S1866	1.61	1.50	1.34	1.14	0.92	0.69	0.48	0.29	0.15
D9S172	1.68	1.63	1.50	1.32	1.11	0.88	0.65	0.44	0.26
D9S1832	0.23	2.01	2.08	1.94	1.68	1.37	1.02	0.66	0.34
D9S2026	−1.62	−0.77	−0.41	−0.19	−0.06	0.00	0.03	0.03	0.02

[a] Markers are listed with respect to centromeric→telomeric boundaries.

observed in proband TD17. This individual was discovered to have an 8 bp deletion on one allele of the hABC-1 transcript beginning at nucleotide 755 (TGTTCAGC), causing a premature termination at codon 217 of hABC-1. A premature termination near the C-terminus of the hABC-1transcript was identified on one allele in proband TD19. This was due to a 4 bp insertion (CATT) at nucleotide 6513 in hABC-1, rather than to a deletion. The mutations identified in probands TD17 and TD19 were only confirmed on one allele. As TD17 and TD19 are not from established consanguinous pedigrees, it is likely that they are both compound heterozygotes for TD.

Three independent reports establishing that mutations in the hABC-1 gene cause TD were recently published (20–22), followed by two confirmatory reports (23, 24). The mutations described in these reports are summarized in **Table 3**. Three TD probands were described in more than one report (TD1 = TD8; TD10 = TD15; TD12 = TD7), including the index case of TD from Tangier Island (22, 24). The mutation described in TD1/TD8 causes a frameshift, creating a premature translation stop and, ultimately, a translation product lacking 75% of the amino acid sequence of hABC-1. The proband of our large kindred TD16, individual 420, also had significant premature CHD, despite having only a missense mutation at nt 5338 of the hABC-1 transcript. Two compound heterozygotes with premature CHD have additonally been reported (TD5, TD6): one having a missense mutation at nt 4369 and a G→C mutation in the splice donor site of exon 24 of the hABC-1 transcript (21) and the other two missense mutations (20), influencing the N-terminal NBF and the first intracellular domains of the hABC-1 protein. Proband TD17 in our report, who is very likely a compound heterozygote for TD, has a severe truncation, rather than a point mutation, in the hABC-1 transcript and has extensive premature CHD. Thus, the overall data indicates that a variety of hABC-1 mutations are associated with premature CHD in TD.

TABLE 2. Mutations in the hABC-1 transcript identified in four unrelated TD kindreds

Kindred	Nucleotide Change	Predicted Protein Alteration	Protein Domain(s) Affected
420[a] (TD16)	nt 5338(A→C)	aa 1740 (Asn[b]→His)	2nd transmembrane
TD17[a]	nt 755-762/8 bp del (TGTTCAGC)	frameshift aa 217→stop	all
TD18-1	nt 3805(G→A)	aa 1229(Asp[b]→Leu)	linker
TD18-2	nt 3805(G→A)	aa 1229(Asp[b]→Leu)	linker
TD19	nt 6513/4 bp ins (CATT)	frameshift aa 2155→stop	PDZ-binding (SYV)

TD16, TD18-1, and TD18-2 were homozygous for the reported point mutations, whereas the mutations identified in TD17 and TD19 were only confirmed on one allele. As TD17 and TD19 are not from established consanguinous pedigrees, it is likely that they are both compound heterozygotes for TD; aa, amino acid; del, deletion; ins, insertion; nt, nucleotide.

[a] Premature CHD.

[b] Conserved in human and mouse.

DISCUSSION

Members of the superfamily of ATP-binding cassette transporters couple the hydrolysis of ATP to translocation of solutes, including amino acids, ions, peptides, steroid hormones, and vitamins, across a biological membrane (40, 41). A functional ABC transporter requires the symmetrical association of four protein domains: two nucleotide binding folds (NBFs) with conserved Walker A and B motifs and two highly hydrophobic transmembrane domains, each consisting of six membrane-spanning helices (40, 42, 43). Their activity as transporters is dependent on their interaction with ATP at the NBF (44–46) with further regulation via phosphorylation of serine/threonine residues in the region linking the two symmetric halves in some cases as well (47, 48). ABC transporters are involved in diverse processes, such as signal transduction, protein secretion, and drug and antibiotic resistance. Moreover, most of the mammalian ABC transporters identified thus far are clinically relevant (49). Cystic fibrosis is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-dependent chloride channel (50, 51); mutations in the sulfonylurea receptor (SUR) are associated with hypoglycemia in in-

TABLE 3. Summary of reported mutations in the hABC-1 transcript in Tangier disease kindreds

Kindred	Genotype	Nucleotide Change	Predicted Protein Alteration	Protein Domain(s) Affected
TD1[20]	**homozygote**	**nt 1764 del (G)**	**frameshift (aa 575→stop)**	**all**
TD2	homozygote	3′-genomic del (−1500 nt)	Truncation (−427 aa)	2nd transmembrane; C-terminal NBF
TD3	homozygote	nt 2744 (A→G)	aa 875 (Asn[a]→Ser)	Walker A motif of N-terminal NBF
TD4	compound heterozygote	a. nt 2750 (C→T) b. nt 1136 (T→C)	aa 877 (Ala→Val); aa 339 (Val→Ala)	
TD5	**compound heterozygote**	**a. nt 2750 (C→T); b. nt 1709 (G→C)**	**aa 877 (Ala→Val); aa 530 (Trp→Ser)**	**Walker A motif of N-terminal NBF; 1st intracellular**
TD6[21]	**compound heterozygote**	**a. nt 4369 (T→C) b. exon 24 (G→C)**	**aa 1417 (Cys[a]→Arg)**	**2nd transmembrane; C-terminal NBF**
TD7	homozygote	nt 1730 (A→G)	aa 537 (Gln[a]→Arg)	1st transmembrane
TD8[22]	**homozygote**	**nt 1764 del (G)**	**frameshift (aa 575→stop)**	**all**
TD9	homozygote	nt 1524-37 ins/del (110 bp Alu-sq)	aa 468 (38 aa ins/6 aa del)	N-terminal transmembrane
TD10	homozygote	nt 3105 del (2 bp)	frameshift (aa 1084→stop)	N-terminal NBF
TD11	compound heterozygote	a. nt 1701 (C→G); b. nt 3105 ins	stop codon frameshift (aa 1085→stop)	1st transmembrane; N-terminal NBF
TD12[23]	homozygote	nt 1730 (A→G)	aa 537 (Gln[a]→Arg)	1st transmembrane
TD13	compound heterozygote	a. nt 1700 (C→T) b. not reported	aa 527 (Arg→Trp)	1st transmembrane
TD14	compound heterozygote	a. nt 5062 ins (138 bp) b. nt 5697 ins (14 bp)	frameshift stop codon	N-terminal NBF
TD15[24]	homozygote	nt 3283 del (2 bp)	frameshift (aa 1084→stop)	N-terminal NBF

Boldface, CHD.
[a] Conserved amino acid.

fancy (52); and the human P-glycoprotein confers resistance to chemotherapeutic drugs on tumor cells (53). In this report, we confirm the involvement of the ABC family member hABC-1 in the pathogenesis of TD.

We have identified four novel mutations in hABC-1 in four unrelated TD kindreds. Each of these mutations would be predicted to disrupt hABC-1 function in a different manner based on their locations. The point mutation identified in proband 420 is found in a highly conserved region of the second transmembrane domain of hABC-1 (41), suggesting that it may have functional importance. The charge difference caused by this substitution, uncharged to positive, could induce conformational alterations, ultimately preventing the necessary interaction between the ABC subunit and the transmembrane domain. The missense mutation observed in the Japanese probands (TD18) occurs in a long, charged linker region which is rich in potential phosphorylation sites (38). This region is very similar to that of the regulatory domain of CFTR, where mutations responsible for cystic fibrosis have been localized (54). Frameshift mutations, one due to an 8 bp deletion and the other to a 4 bp insertion, were found to cause premature terminations in hABC-1 in two of our four probands. The deletion results in a severely truncated protein, such that only 217 of the 2201 amino acids of hABC-1 are encoded. Thus, the hABC-1 protein of TD17 consists solely of the first intracellular domain and is devoid of both ATP-binding cassettes. It is interesting to note that this proband has significant atherosclerotic disease, requiring bypass surgery at age 46. Conversely, proband TD19, who has a premature stop codon in the C-terminus of hABC-1, does not have CHD and is the same age as proband TD17. The mutation in TD19 results in the truncation of the final 46 amino acids of hABC-1. This mutation causes a frameshift in the C-terminus of hABC-1, eliminating a sequence (serine–tyrosine–valine or SYV) to which PDZ domain-containing proteins are known to bind. As PDZ domains are thought to play an important role in mediating protein–protein interactions between membrane-associated proteins, this mutation suggests that the hABC-1 transporter requires such a protein interaction to function properly.

Now that the molecular defect in TD has been identified, the next critical step will involve defining those mutations that affect the functionality of hABC-1 to a greater extent than others, improving our understanding of the mechanisms responsible for premature CHD. In this regard, it is clear that the TD probands with CHD described thus far have a variety of mutations in the hABC-1 transcript, ranging from point mutations to severe truncations (20–23). Both of the TD probands with CHD described in the present study had a similar degree of impairment in apoA-I-mediated cholesterol efflux, despite having dramatically different mutations. The proband of TD17 had a severe truncation of the hABC-1 protein, lacking all but the first 217 amino acids of hABC-1, whereas the proband of TD16 had a missense mutation affecting the second transmembrane domain of hABC-1. This suggests that the second transmembrane domain of hABC-1 may be critical to reverse cholesterol transport (RCT) and the progression of atherosclerosis. The compound heterozygote described by Brooks-Wilson and colleagues (21) also had a missense mutation in the C-terminal transmembrane domain of hABC-1. Although the development of premature atherosclerosis in TD patients may be mediated, in part, by the nature of the mutation(s) in hABC-1, the

prevalence of CHD in TD homozygotes is approximately 6-fold higher than normal between the ages of 35 and 65 years (3). This, taken together with the evidence that TD heterozygotes are also at increased risk for premature CHD (55), emphasizes the overall importance of RCT in the process of atherogenesis.

The precise mechanism by which hABC-1 regulates HDL metabolism remains to be elucidated; however, it has been demonstrated that hABC-1 is a sterol-sensitive gene that is inversely regulated by cholesterol import and export (39). As two other ABC family members, multidrug resistant (MDR)-1 and MDR-3 P-glycoproteins, have been shown to translocate lipids across the plasma membrane with broad and limited specificity (56), respectively, it is not unreasonable to propose that hABC-1 may similarly be involved in the transport of cholesterol and/or phospholipids. In support of this hypothesis, it has been shown that cholesterol efflux to HDL is influenced by the cholesterol and sphingolipid content of cell membrane microdomains (39, 57, 58). Thus, a mutant hABC-1 protein would be expected to cause the intracellular accumulation of lipids, precisely what is observed in the macrophages of TD patients in whom cholesterol efflux is clearly defective. The inability of TD homozygotes to efflux cellular cholesterol leads to lipid-poor HDL particles which are rapidly catabolized (4–6), resulting in dramatic reductions of plasma HDL-C levels. Further evidence for the role of hABC-1 in signal transduction and, ultimately, cholesterol efflux comes from the fact that it is up-regulated by cAMP-dependent protein kinases via phosphorylation (59).

In summary, we have localized hABC-1 to a 250 kb region of 9q31, and reported four novel mutations in hABC-1 that are associated with TD in four unrelated kindreds. Although a rare genetic disorder, identification of the precise molecular defect responsible for TD will undoubtedly contribute to our comprehension of HDL metabolism and provide new insight into the role of lipid dysfunction in the genesis of the axonal peripheral neuropathy that often accompanies TD. In the long term, these investigations should lead to the formulation of novel therapies for the treatment of common HDL deficiency states.

The authors are grateful to Patricia Isaia, Jean Zhang, Ziying Liu, Susan Jalbert, and Richard Lirio for their valuable contributions to this work. This research was supported by a contract from the Genome Therapeutics Corporation, Waltham, MA and the Patricia Isaia Research Fund, Tufts University. Dr. Schaefer's laboratory is further supported by the USDA and NIH, and Dr. Freeman's laboratory is funded by NIH awards HL-09319 and HL-45098. Dr. Brown's laboratory is supported by the NINDS, NIA, MDA, ALS Association, and the CB Day Foundation, Inc.

Manuscript received 19 November 1999 and in revised form 7 December 1999.

REFERENCES

1. Fredrickson, D. S., P. H. Altrocchi, L. V. Avioli, D. S. Goodman, and H. C. Goodman. 1961. Tangier disease. *Ann. Intern. Med.* **55:** 1016–1031.
2. Assmann, G., A. von Eckardstein, and H. B. Brewer, Jr. 1995. Familial high density lipoprotein deficiency: Tangier disease. *In* The Metabolic and Molecular Basis of Inherited Disease. C. R. Scriver, A. L. Beaudet, W. S. Sly, D. Valle, editors. McGraw-Hill, New York, NY. 2053–2072.
3. Serfaty-Lacrosniere, C., A. Civeira, P. Lanzberg, P. Isaia, J. Berg, E. D. Janus, M. P. Smith, Jr., P. H. Pritchard, J. Frohlich, R. S. Lees, G. F. Barnard, J. M. Ordovas, and E. J. Schaefer. 1994. Homozygous Tangier disease and cardiovascular disease. *Atherosclerosis.* **107:** 85–98.
4. Schaefer, E. J., C. B. Blum, R. I. Levy, L. L. Jenkins, P. Alaupovic, D. M. Foster, and H. B. Brewer, Jr. 1978. Metabolism of high density lipoproteins apolipoproteins in Tangier Disease. *N. Engl. J. Med.* **299:** 905–910.
5. Schaefer, E. J., D. W. Anderson, L. A. Zech, F. T. Lindren, T. J. Bronzert, E. A. Rubalcaba, and H. B. Brewer, Jr. 1981. Metabolism of high density lipoprotein subfractions and constituents in Tangier Disease following the infusion of high density lipoproteins. *J. Lipid Res.* **22:** 217–228.
6. Bojanovski, D., R. E. Gregg, L. A. Zech, M. S. Meng, C. Bishop, R. Ronan, and H. B. Brewer, Jr. 1987. In vivo metabolism of proapolipoprotein A-I in Tangier disease. *J. Clin. Invest.* **80:** 1742–1747.
7. Law, S. W., and H. B. Brewer, Jr. 1985. Tangier disease: The complete mRNA sequence encoding for preproapoA-I. *J. Biol. Chem.* **260:** 12810-12814.
8. Makrides, S. C., N. Ruiz-Opazo, A. L. Hayden Nussbaum, J. L. Breslow, and V. I. Zannis. 1988. Sequence and expression of Tangier apoA-I gene. *Eur. Biochem.* **173:** 465–471.
9. Schmitz, G., H. Fischer, M. Beuck, K-D. Hoecker, and H. Robenek. 1982. Dysregulation of lipid metabolism in Tangier monocyte-derived macrophages. *Arteriosclerosis.* **10:** 1010–1019.
10. Schmitz, G., G. Assmann, H. Robenek, and B. Brennhausen. 1985. Interaction of Tangier lipoproteins with cholesteryl ester-laden mouse peritoneal macrophages. *Proc. Natl. Acad. Sci. USA.* **82:** 6305–6309.
11. Walter, M., U. Gerdes, U. Seedorf, and G. Assmann. 1994. The high density lipoprotein and apolipoprotein A-I induced mobilization of cellular cholesterol is impaired in fibroblasts from Tangier disease subjects. *Biochem. Biophys. Res. Commun.* **205:** 850–856.
12. Drobnik, W., C. Mollers, T. Resnik, and G. Schmitz. 1995. Activation of phosphatidylinositol-specific phospholipase C in response to HDL_3 and LDL is markedly reduced in cultured fibroblasts from Tangier patients. *Arterioscler. Thromb. Vasc. Biol.* **15:** 1369–1377.
13. Rogler, G., B. Trumbach, B. Klima, K. J. Lackner, and G. Schmitz. 1995. HDL-mediated efflux of intracellular cholesterol is impaired in fibroblasts from Tangier disease patients. *Arterioscler. Thromb. Vasc. Biol.* **15:** 683–690.
14. Francis, G. A., R. H. Knopp, and J. F. Oram. 1995. Defective removal of cellular cholesterol and phospholipids by apolipoprotein A-I in Tangier disease. *J. Clin. Invest.* **96:** 78–87.
15. Oram, J. F., A. J. Mendez, J. P. Slotte, and T. F. Johnson. 1991. High density lipoprotein apolipoproteins mediate removal of sterol from intracellular pools but not from plasma membranes of cholesterol-loaded fibroblasts. *Arterioscler. Thromb.* **11:** 403–414.
16. Mendez, A. J., J. F. Oram, and E. L. Bierman. 1991. Protein kinase C as a mediator of high density lipoprotein receptor-dependent efflux of intracellular cholesterol. *J. Biol. Chem.* **266:** 10104–10111.
17. Walter, M., H. Reinecke, U. Gerdes, N. Jerzy-Roch, G. Hobbel, U. Seedorf, and G. Assmann. 1996. Defective regulation of phosphatidylcholine-specific phospholipases C and D in a kindred with Tangier disease. Evidence for the involvement of phosphatidyl-choline breakdown in HDL-mediated cholesterol efflux mechanisms. *J. Clin. Invest.* **98:** 2315–2323.
18. Hara, H., H. Hara, A. Komaba, and S. Yokoyama. 1992. Alpha-helical requirements for free apolipoproteins to generate HDL and to induce cellular lipid efflux. *Lipids.* **27:** 302–304.
19. Remaley, A. T., U. K. Schumacher, J. A. Stonik, B. D. Farsi, H. Nazih, and H. B. Brewer, Jr. 1997. Decreased reverse cholesterol transport from Tangier disease fibroblasts: acceptor specificity and effect of brefeldin on lipid efflux. *Arterioscler. Thromb. Vasc. Biol.* **17:** 1813–1821.
20. Bodzioch, M., E. Orso, J. Klucken, T. Langmann, A. Bottcher, W. Diederich, W. Drobnik, S. Barlage, C. Buchler, M. Porsch-Ozcurumez, W. E. Kaminski, H. W. Hahmann, K. Oette, G. Rothe, C. Aslanidis, K. J. Lackner, and G. Schmitz. 1999. The gene encoding

ATP-binding cassette transporter 1 is mutated in Tangier disease. *Nat. Genet.* **22:** 347–351.
21. Brooks-Wilson, A., M. Marcil, S. M. Clee, L-H. Zhang, K. Roomp, M. van Dam, L. Yu, C. Brewer, J. A. Colins, H. O. F. Molhuizen, O. Loubser, B. F. F. Ouelette, K. Fichter, K. J. D. Ashbourne-Excoffon, C. W. Sensen, S. Scherer, M. Denis, S. Mott, D. Martindale, J. Frohlich, K. Morgan, B. Koop, S. Pimstone, J. J. P. Kastelein, J. Genest, Jr., and M. R. Hayden. 1999. Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. *Nat. Genet.* **22:** 336–345.
22. Rust, S., M. Rosier, H. Funke, J. Real, Z. Amoura, J-C. Piette, J-F. Deleuze, H. B. Brewer, Jr., N. Duverger, P. Denefle, and G. Assmann. 1999. Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. *Nat. Genet.* **22:** 352–355.
23. Lawn, R. M., D. P. Wade, M. R. Garvin, X. Wang, K. Schwartz, J. G. Porter, J. J. Seilhamer, A. M. Vaughan, and J. F. Oram. 1999. The Tangier disease gene product ABC1 controls the cellular apolipoprotein-mediated lipid removal pathway. *J. Clin. Invest.* **104:** R25–R31.
24. Remaley, A. T., S. Rust, M. Rosier, C. Knapper, L. Naudin, C. Broccardo, K. M. Petersen, C. Koch, I. Arnould, C. Prades, N. Duverger, H. Funke, G. Assmann, M. Dinger, M. Dean, G. Chimini, S. Santamarina-Fojo, D. S. Fredrickson, P. Denefle, and H. B. Brewer, Jr. 1999. Human ATP-binding cassette transporter 1 (ABC1): Genomic organization and identification of the genetic defect in the original Tangier disease kindred. *Proc. Natl. Acad. Sci. USA.* **96:** 12685–12690.
25. Schaefer, E. J., S. Lamon-Fava, J. M. Ordovas, S. D. Cohn, M. M. Schaefer, W. P. Castelli, and P. W. F. Wilson. 1994. Factors associated with low and elevated plasma high density lipoprotein cholesterol and apolipoproteinA-I levels in the Framingham Offspring Study. *J. Lipid Res.* **35:** 871–882.
26. Suarez, B. K., G. Schonfeld, and R. S. Sparkes. 1982. Tangier disease: heterozygote detection and linkage analysis. *Hum. Genet.* **60:** 150–156.
27. Rust, S., M. Walter, H. Funke, A. von Eckardstein, P. Cullen, H. Kroes, R. Hordijk, J. Geisel, J. Kastelein, H. O. F. Molhuizen, M. Schreiner, A. Mischke, H. W. Hahmann, and G. Assmann. 1998. Assignment of Tangier disease to chromosome to 9q31 by a graphical linkage exclusion strategy. *Nat. Genet.* **20:** 96–98.
28. Warnick, G. R., J. Benderson, and J. J. Albers. 1982. Dextran sulfate-Mg2+ precipitation procedure for quantitation of high-density lipoprotein cholesterol. *Clin. Chem.* **28:** 1379–1388.
29. Mendez, A. J., and L. Uint. 1996. Apolipoprotein-mediated cellular cholesterol and phospholipid efflux depend on a functional Golgi apparatus. *J. Lipid Res.* **37:** 2510–2524.
30. Eberhart, G. P., A. J. Mendez, and M. W. Freeman. 1998. Decreased cholesterol efflux from fibroblasts of a patient without Tangier disease, but with markedly reduced high density lipoprotein cholesterol levels. *J. Clin. Endocrinol. Metab.* **83:** 836–846.
31. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
32. Schaefer, E. J. 1984. The clinical, biochemical, and genetic features in familial disorders of high density lipoprotein deficiency. *Arteriosclerosis.* **4:** 303–322.
33. Contois, J., J. R. McNamara, C. Lammi-Keefe, P. W. F. Wilson, T. Massov, and E. J. Schaefer. 1996. Reference intervals for plasma apolipoprotein A-I determined with a standardized commercial immunoturbidimetric assay: results from the Framingham Offspring Study. *Clin. Chem.* **42:** 507–514.
34. Lathrop, G. M., J. M. Lalouel, C. Julier, and J. Ott. 1985. Multilocus linkage analysis in humans: detection of linkage and estimation of recombination. *Am. J. Hum. Genet.* **37:** 482–498.
35. Boehnke, M. 1991. Allele frequency estimation from data on relatives. *Am. J. Hum. Genet.* **48:** 22–25.
36. Sobel, E., and K. Lange. 1996. Descent graphs in pedigree analysis: applications to haplotyping, location scores, and marker-sharing statistics. *Am. J. Hum. Genet.* **58:** 1323–1337.
37. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
38. Luciani, M. F., F. Denizot, S. Savary, M. G. Mattei, and G. Chimini. 1994. Cloning of 2 novel ABC transporters mapping on chromosome 9. *Genomics.* **21:** 150–159.
39. Langmann, T., J. Klucken, M. Reil, G. Liebisch, M-F. Luciani, G. Chimini, W. E. Kaminski, and G. Schmitz. 1999. Molecular cloning of the human ATP-binding cassette transporter 1(hABC1): evidence for sterol-dependent regulation in macrophages. *Biochem. Biophys. Res. Commun.* **257:** 29–33.
40. Higgins, C. F. 1992. ABC transporters: from microorganisms to man. *Annu. Rev. Cell Biol.* **8:** 67–113.
41. Schneider, E., and S. Hunke. 1998. ATP-binding cassette (ABC) transport systems: functional and structural aspects of the ATP-hydrolyzing subunits/domains. *FEMS Microbiol. Rev.* **22:** 1–20.
42. Yoshimura, A., Y. Kuwazuru, T. Sumizawa, M. Ichikawa, S. I. Ikeda, T. Uda, and S. Akiyama. 1989. Cytoplasmic orientation and two-domain structure of the multidrug transporter, P-glycoprotein, demonstrated with sequence-specific antibodies. *J. Biol. Chem.* **264:** 16282–16291.
43. Kerppola, R. E., U. K. Shyamala, P. Klebba, and G. F-L. Ames. 1991. The membrane-bound proteins of periplasmic permeases form a complex. Identification of the histidine permease HisQMP complex. *J. Biol. Chem.* **266:** 9857–9865.
44. Sarkadi, B., E. M. Price, R. C. Boucher, U. A. Germann, and G. A. Scarborough. 1992. Biochemical characterization of the cystic fibrosis transmembrane conductance regulator in normal and cystic fibrosis epithelial cells. *J. Biol. Chem.* **267:** 4854–4858.
45. Shapiro, A. B., and V. Ling. 1994. ATPase activity of purified and reconstituted P-glycoprotein from Chinese hamster ovary cells. *J. Biol. Chem.* **269:** 3745–3754.
46. Ko, Y. H., and P. L. Pedersen. 1995. The first nucleotide binding fold of the cystic fibrosis transmembrane conductance regulator can function as an active ATPase. *J. Biol. Chem.* **270:** 22093–22096.
47. Picciotto, M. R., J. A. Cohn, G. Bertuzzi, P. Greengard, and A. C. Nairn. 1992. Phosphorylation of the cystic fibrosis transmembrane conductance regulator. *J. Biol. Chem.* **267:** 12742–12752.
48. Gill, D. R., S. C. Hyde, C. F. Higgins, M. A. Valverde, G. M. Mintening, and F. V. Sepulveda. 1992. Separation of drug transport and chloride channel functions of the human multidrug resistance P-glycoprotein. *Cell.* **71:** 23–32.
49. Higgins, C. F. 1995. The ABC of channel regulation. *Cell.* **82:** 693–696.
50. Riordan, J. R., J. M. Rommens, B-S. Kerem, N. Alon, R. Rozmahel, Z. Grzelczak, J. Zielenski, S. Lok, N. Plavsic, J. L. Chou, M. L. Drumm, M. C. Iannuzzi, F. S. Collins, and L-C. Tsui. 1989. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science.* **245:** 1066–1073.
51. Hanrahan, J. W., J. A. Tabcharani, F. Becq, C. J. Mathews, O. Augustinas, T. J. Jensen, X-B. Chang, and J. R. Riordan. 1995. Ion Channels and Genetic Diseases. Rockefeller University Press, New York, NY.
52. Thomas, P.M., G. J. Cote, N. Wohlik, B. Haddad, P. M. Mathew, W. Rabl, L. Aguilar-Bryan, R. F. Gagel, and J. Bryan. 1995. Mutations in the sulfonylurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. *Science.* **268:** 426–429.
53. Gottesman, M. M., and I. Pastan. 1993. Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu. Rev. Biochem.* **62:** 385–428.
54. Welsh, M. J., and A. E. Smith. 1993. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. *Cell.* **73:** 1251–1254.
55. Schaefer, E. J., L. A. Zech, D. E. Schwartz, and H. B. Brewer, Jr. 1980. Coronary heart disease prevalence and other clinical features in familial high-density lipoprotein deficiency (Tangier disease). *Ann. Int. Med.* **93:** 261–266.
56. Van Helvoort, A., A. J. Smith, H. Sprong, I. Fritzsche, A. H. Schinkel, P. Borst, and G. Van Meer. 1996. MDR1 P-glycoprotein is a lipid translocase of broad specificity, while MDR3 P-glycoprotein specifically translocates phosphatidylcholine. *Cell.* **87:** 507–517.
57. Harder, T., and K. Simons. 1997. Caveolae, DIGs, and the dynamics of sphingolipid-cholesterol microdomains. *Curr. Opin. Cell. Biol.* **9:** 534–542.
58. Nion, S., O. Briand, S. Lestavel, G. Torpier, F. Nazih, C. Delbart, J. C. Fruchart, and H. Clavey. 1997. High-density-lipoprotein subfraction 3 interaction with glycosylphos-phatidylinositol-anchored proteins. *Biochem. J.* **328:** 415–423.
59. Becq, F., Y. Hamon, A. Bajetto, M. Gola, B. Verrier, and G. Chimini. 1997. ABC1, an ATP binding cassette transporter required for phagocytosis of apoptotic cells, generates a regulated anion flux after expression in *Xenopus laevis* oocytes. *J. Biol. Chem.* **272:** 2695–2699.

ERRATA

In the article "Novel mutations in the gene encoding ATP-binding cassette 1 in four Tangier disease kindreds" by Margaret E. Brousseau et al. published in the March 2000 issue of the *Journal of Lipid Research* (Volume **41**, pages 433–441), the authors incorrectly reported the amino acid substitution in the TD18 kindred. The corrections shown below appear in boldface.

The G→A substitution, as shown in Table 2 on page 438, results in the replacement of an aspartic acid (Asp) residue with **asparagine (Asn)**, not leucine (Leu). Therefore **(Asp→Asn)** should appear instead of (Asp→Leu). Additionally, in the Results section on page 437, four lines from the bottom in the right column, the sentence should read "This mutation causes a change of amino acid from aspartate to **asparagine** at codon 1229, resulting in the substitution of a negatively charged amino acid residue with **an uncharged** residue."

In a letter to the Editor, the authors state "Because this mutation involves a conserved amino acid (Asp) and results in the replacement of a negatively charged amino acid with an uncharged residue in the highly charged linker region, an important regulatory domain of ABCA1, this error does not change our conclusions about this mutation in any way. Moreover, we clearly identified this mutation on both alleles in each TD-18 subject, indicating homozygosity for this missense mutation."

The *Journal of Lipid Research* and the authors apologize for this error.