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An ATP-binding cassette gene (ABCG5) from the ABCG (White) gene subfamily maps to human chromosome 2p21 in the region of the Sitosterolemia locus

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Abstract. We characterized a new human ATP-binding cassette (ABC) transporter gene that is highly expressed in the liver. The gene, ABCG5, contains 13 exons and encodes a 651 amino acid protein. The predicted protein is closely related to the Drosophila white gene and a human gene, ABCG1, which is induced by cholesterol. This subfamily of genes all have a single ATP-binding domain at the N-terminus and a single C-terminal set of transmembrane segments. ABCG5 maps to human chromosome 2p21, between the markers D2S117 and D2S119. The abundant expression of this gene in the liver suggests that the protein product has an important role in transport of specific molecule(s) into or out of this tissue.

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ABC proteins bind and hydrolyze ATP providing energy for the transport of an array of substrates (Dean and Allikmets, 1995). Several ABC genes are exclusively expressed in the liver and are involved in the energy-dependent transport of bile salts and bile acids from the liver into the bile (Muller and Jansen, 1998). For example the ABCC2 (MRP2) protein is involved in the elimination of amphipathic organic anions, ABCB4 (MDR3) mediates the secretion of phosphatidylcholine, and ABCB11 (SPGP/BSEP) transports taurocholate and other bile salts from the liver into the bile (Gerloff et al., 1998; Strautnieks et al., 1998). The ABCA1 (ABC1) gene was recently shown to be mutated in Tangier disease, a disorder involving abnormal accumulation of cholesterol in macrophages and very low high-density lipoproteins (HDL) in the serum (Bodzioch et al., 1999; Brooks-Wilson et al., 1999; Remaley et al., 1999; Rust et al., 1999). Current models of ABCA1 function suggest that the gene is involved in the transfer of cholesterol from the plasma membrane to apolipoprein A molecules outside of the cell, to initiate HDL formation

Eukaryotic ABC transporters are divided into seven distinct subfamilies based on phylogenetic analysis of the sequence of ATP-binding domains as well as the structure and arrangement of their domains (Allikmets et al., 1996; Decottignies and Goffeau, 1997; Michaelis and Berkower, 1995). ABC proteins are either full size, as are ABCB11, ABCB1, ABCB4, and ABCC2, with approximately 12 transmembrane domains and two ATP binding sites on each molecule; or they are half size with six transmembrane domains and one ATP binding site, such as ABCG1. The half-transporters are presumably required to dimerize to form a functional transporter. While the best studied half-transporters form heterodimers, homodimerization is certainly possible.

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Table 1. ABCG5 splice junction sequences

Exon	Size (bp)	Splice acceptor	Splice donor	Intron (kb)
1	**5'**		ACAGCGTCAG**gt**aaggcagagccctt	0.6
2	122	ggggtttccttttaa**ag**CCACCGCGTG	GGAAGCTCAG**gt**aagcttgggaagga	<6
3	137	tgttgtcgccccgc**ag**GCTCCGGGAA	CGTCCTGCAG**gt**gggcgcgtccccca	2
4	99	cccgagtctcctgc**ag**AGCGACACCC	CCAGAAGAAG**gt**gggtgcagcccccc	3
5	133	tttgtgtctcctgc**ag**GTGGAGGCCG	CAGGATCCTA**gt**aagtggcacccaga	1.4
6	140	tctttgctgctggc**ag**AGGTCATGCT	GCTTTTTCAG**gt**aagaggttcaactc	1.5
7	130	tctgttgtctggtc**ag**CTCTTTGACA	GACTTCTATA**gt**aagtttttctttca	0.45
8	214	tgggaaaaactttt**ag**TGGACCTGAC	TTCTCCTGAG**gt**aagaggctcacaaa	0.1
9	206	ggttgtttgttttc**ag**GAGAGTGACA	GTGAATCTGT**gt**aagtgcccacgtgc	1
10	139	tgccttccatcccc**ag**TTCCCGTGCT	TGTGCTACTG**gt**gaggggttgttcag	2.5
11	186	gcttatgcttttct**ag**GACGCTGGGC	GATTCCTCAG**gt**aagatatcataatt	>5
12	113	ttttctttttcttaa**ag**AAACATACCC	TTCACTTGTG**gt**aagtattctatttg	1.3
13	**3'**	atcttttccttgac**ag**GCAGCTCAAA		

Most recently, the ABCG1 gene was characterized as a putative cholesterol and phospholipid transport regulator in macrophages (Klucken et al., 2000). ABCG1 expression is significantly up-regulated by low density lipoproteins (LDLs) and down-regulated by HDLs, suggesting its direct involvement in macrophage lipid export process (Klucken et al., 2000). Whether ABCG1 functions as a homodimer or heterodimer is not known. Heterodomerization of the Drosophila white protein with brown and scarlet suggests that ABCG1, the human ortholog of white, could form functional complexes (dimers) with other human half-transporters. Here, we describe a novel human ABC half-transporter, ABCG5, a very closely related ABC transporter from the same subfamily.

Materials and methods

RNA Expression Analysis

Labeling of cDNAs and of individual probes was accomplished using the Rediprime II random prime labeling system according to the manufacturer's instructions (Amersham, Arlington Heights, Il). Probes were hybridized to multiple tissue Northern blots from Clontech (Palo Alto, CA) according to the manufacturers protocol. A quantitative real-time PCR analysis assay was developed for ABCG5 and several other ABC genes using the Cyber-green expression system (Perkin-Elmer, Foster City, CA).

cDNA, genomic cloning and exon/intron structure

Primers were designed from the sequence of the EST clones and used for the amplification of White3 gene fragments from a fetal liver cDNA library (Clontech). Primers White3RACE3c (5′-AGTCGGTCTGCCACATGGCTCAGACTC) and WhiteRACE4 (5′-CGCAGCGCCCGGCCGTTCACATACACC) were used for 5′ RACE reactions using Marathon-Ready cDNA (Clontech). PCR products were cloned into the pCR2.1-TOPO vector (Invitrogen). Primers for amplification of genomic fragments were designed from White3 cDNA sequence. Platinum Taq DNA Polymerase High Fidelity (GibcoBRL) was used for Long Range PCR. The positions of the introns were determined by comparison between genomic and cDNA sequences. Primers for amplification of individual exons were designed from adjacent intron sequence 30–50 bp from the splice site. Amplification of exons was performed with AmpliTaq Gold Polymerase (Perkin Elmer) according to protocol. Sequencing was performed with DNA Sequencing Kit (Applied Biosystems), sequencing reactions were resolved on an ABI 373A automated sequencer. The nucleotide sequence data reported in this manuscript have been deposited at the NCBI/Genbank Data Library under accession number AF312715.

Results

Searches of the dbEST database (www.ncbi.nih.gov/dbEST) with the BLAST program led to the identification of several overlapping mouse and human sequences that shared high homology to White/ABCG subfamily genes but that appeared to encode a unique gene. Cloning and sequencing identified a cDNA with a single open reading frame encoding 651 amino acids, designated ABCG5. This was the longest clone obtained by 5′ RACE analysis and the predicted initiation codon matches the Kozak consensus sequence. Amplification across each of the introns was used to determine that there are 13 exons. The exon size, boundary and splice acceptor and donor sequences and approximate intron sizes are given in Table 1.

Figure 1 displays an alignment of the ABCG5 protein with the amino acid sequence of the other ABCG subfamily genes. While all human ABCG genes are half transporters, yeast contain ABCG-type genes that are both half (ADP1) and full transporters (YOL075, PDR5, bfr1). Considerable identity is seen in the ATP-binding domain and substantial homology throughout the entire coding region. ABCG5 is most closely related to the C-terminal half of the yeast YOL075 gene with 30% amino acid identity overall and 38% in the NBF and 26% in the TM region. The alignment was used to generate a phylogenetic tree of the genes confirming that ABCG5 and YOL075 are closely related (Fig. 2a). Expression of ABCG5 in normal human tissues was examined by RNA blot hybridization and revealed a 3.5-kb transcript predominantly in the liver (Fig. 2b). Real-time PCR assays gave equivalent results (data not shown).

Using radiation hybrid analysis the ABCG5 gene was mapped to chromosome 2p21→p13 between markers D2S117 and D2S119 consistent with data from an ABCG5 EST (T99836). The mouse *Abcg5* gene was also mapped by radiation hybrids to chromosome 17, 53–55 cM from the centromere (data not shown). The gene for sitosterolemia, a disorder involving abnormal sterol absorption, and defective secretion, also maps to this region. To test for an involvement of ABCG5 in sitosterolemia all of the coding exons of the gene were sequenced in six unrelated affected individuals. The only variants detected were the Q609E polymorphism and an arginine to cystine change in codon 50 (R50C). The R50C variant was seen in the father of an affected individual and alters a residue that is con-

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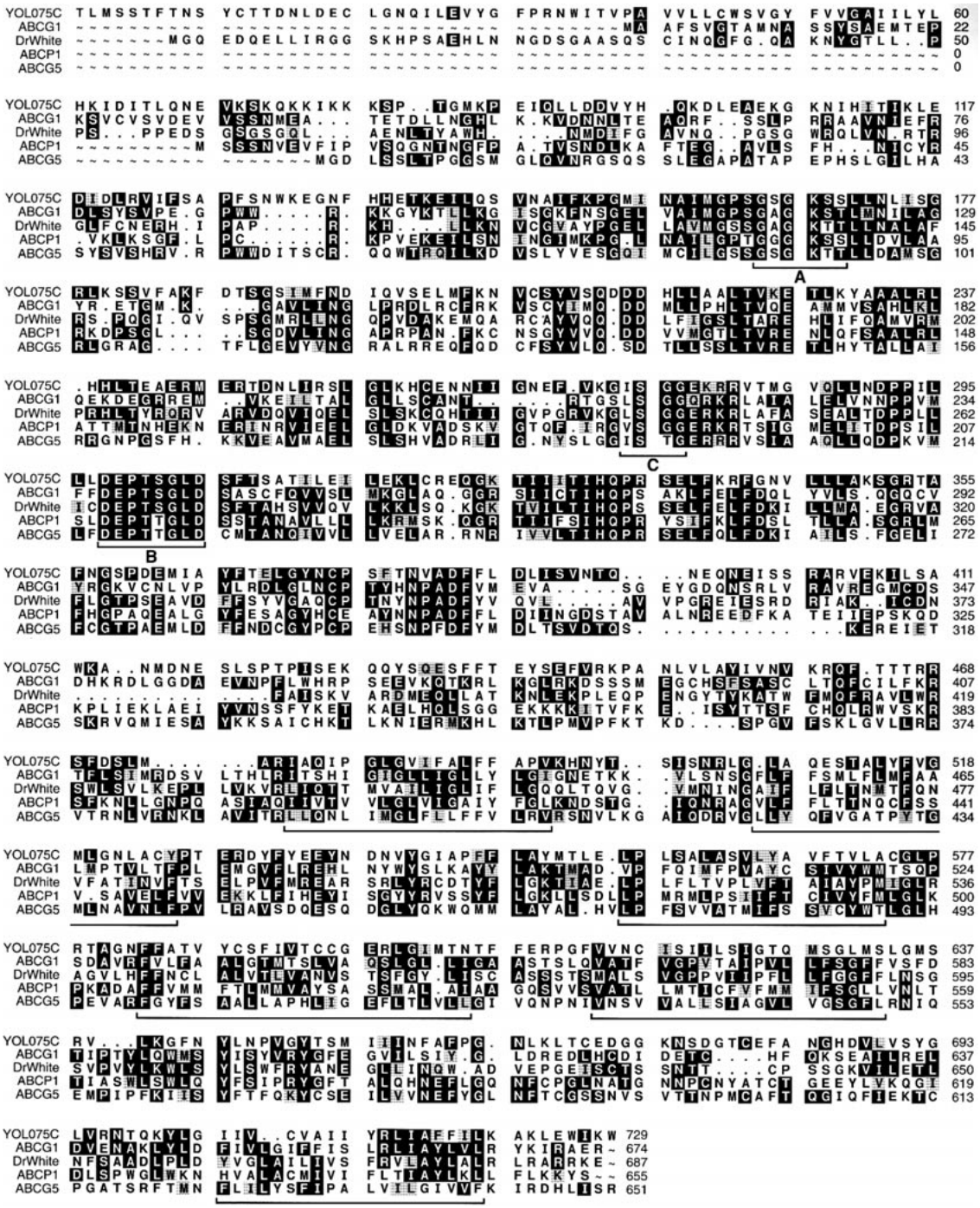

Fig. 1. Alignment of ABCG5 with White subfamily genes. The predicted amino acid sequence of ABCG5 is shown along with the human ABCG2 (ABCP), Drosophila white (DrWhite), human ABCG1, and the C-terminal half of the yeast YOL075 genes. Identical residues are shaded in black and similar residues in gray. The Walker A, B and Signature motifs are underlined (A, B, and C) as well as the predicted transmembrane segments.

served in the murine *Abcg5* gene. However this variant did not segregate with disease in this family. This data is not sufficient to either confirm or exclude ABCG5 as the gene responsible for sitosterolemia, and further studies with a larger number of pedigrees will be required.

Discussion

We have identified a new ABC gene that encodes a half-transporter highly expressed in the liver. The gene is a member of the ABCG/White subfamily that currently includes two

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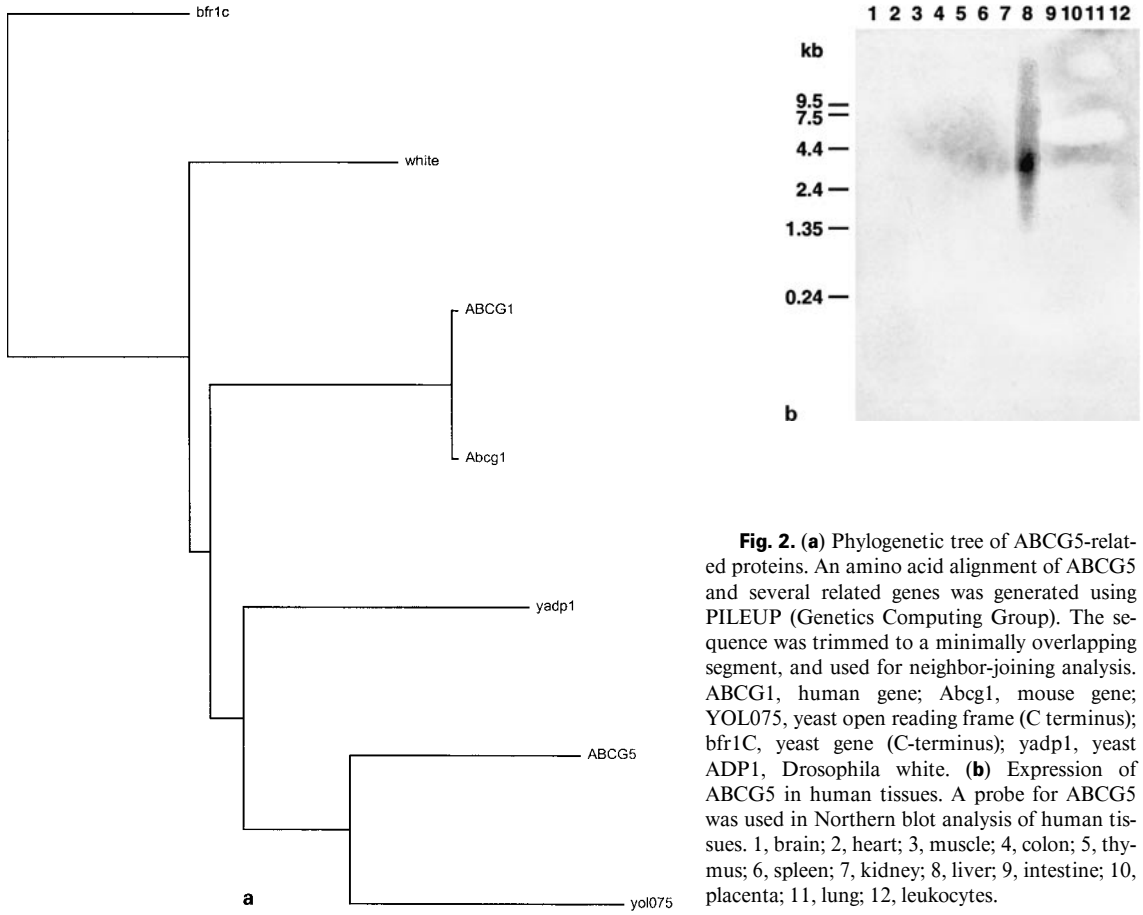

Fig. 2. (**a**) Phylogenetic tree of ABCG5-related proteins. An amino acid alignment of ABCG5 and several related genes was generated using PILEUP (Genetics Computing Group). The sequence was trimmed to a minimally overlapping segment, and used for neighbor-joining analysis. ABCG1, human gene; Abcg1, mouse gene; YOL075, yeast open reading frame (C terminus); bfr1C, yeast gene (C-terminus); yadp1, yeast ADP1, Drosophila white. (**b**) Expression of ABCG5 in human tissues. A probe for ABCG5 was used in Northern blot analysis of human tissues. 1, brain; 2, heart; 3, muscle; 4, colon; 5, thymus; 6, spleen; 7, kidney; 8, liver; 9, intestine; 10, placenta; 11, lung; 12, leukocytes.

other proteins, ABCG1 (white1) and ABCG2 (MXR/ABCP/BCRP). The mouse ortholog of ABCG5 shares a high degree of amino acid identity with the human protein (data not shown). ABCG5 could function as a homodimer, or as a heterodimer with the other known ABCG subfamily members. A possible candidate for a heterodimerization partner is ABCG1 that is also expressed in the liver and is involved in cholesterol and phospholipid transport (Klucken et al., 2000). At the same time, it is feasible that ABCG5 functions as a homodimer, since another ABCG subfamily member, ABCG2 (ABCP), can confer a drug resistance phenotype to cells upon transfection, suggesting that it functions as a homodimer (Rabindran et al., 2000).

The ABCG5 gene maps into the genetic interval that has been defined for sitosterolemia (Patel et al., 1998). A principal phenotype of sitosterolemia is the lack of transport of plant and fish sterols from the liver into the bile (Salen et al., 1997). This leads to an accumulation of these sterols with resultant xanthomas and, in some cases, arthritis. Given that several other ABC genes play crucial roles in the transport of substances into the bile, it is reasonable to speculate that ABCG5 could also be involved in excretion of compounds from the liver. There is precedence for ABC genes playing a role in sterol transport from the finding that ABCA1 is involved in cholesterol transport from cells onto HDL. Another member of the ABCG subfamily, ABCG1, is induced by cholesterol loading suggesting that it also plays a role in cholesterol transport, presumably as a regulator (Klucken et al., 2000). It will be important to determine the cell type(s) in the liver where ABCG5 is expressed and to study the regulation of the gene in response to sterol loading of these cells.

Note added in proof

Following acceptance of this manuscript, Berge et al. (2000) and Lee et al. (2001) reported mutations in ABCG5 in some sitosterolemia patients and the identification of an adjacent gene, ABCG8, that is also mutated in this disorder. We identified that an Amish American sitosterolemia patient is homozygous for the G574R variant in ABCG8, in agreement with the report of Lee et al. (2001).

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References

Allikmets R, Gerrard B, Hutchinson A, Dean M: Characterization of the human ABC superfamily: isolation and mapping of 21 new genes using the expressed sequence tags database. Hum molec Genet 5:1649–1655 (1996).

Berge KE, Tian H, Graf GA, Yu L, Grishin NV, Schultz J, Kwiterovich P, Shan B, Barnes R, Hobbs HH: Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. Science 290:1771–1775 (2000).

Bodzioch M, Orso E, Klucken J, Langmann T, Bottcher A, Diederich W, Drobnik W, Barlage S, Buchler C, Porsch-Ozcurumez M, Kaminski WE, Hahmann HW, Oette K, Rothe G, Aslanidis C, Lackner KJ, Schmitz G: The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. Nature Genet 22:347–351 (1999).

Brooks-Wilson A, Marcil M, Clee SM, Zhang LH, Roomp K, van Dam M, Yu L, Brewer C, Collins JA, Molhuizen HO, Loubser O, Ouelette BF, Fichter K, Ashbourne-Excoffon KJ, Sensen CW, Scherer S, Mott S, Denis M, Martindale D, Frohlich J, Morgan K, Koop B, Pimstone S, Kastelein JJ, Hayden MR, et al: Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. Nature Genet 22:336–345 (1999).

Dean M, Allikmets R: Evolution of ATP-binding cassette transporter genes. Curr Opin Genet Dev 5:79–785 (1995).

Decottignies A, Goffeau A: Complete inventory of the yeast ABC proteins. Nature Genet 15:137–145 (1997).

Gerloff T, Stieger B, Hagenbuch B, Madon J, Landmann L, Roth J, Hofmann AF, Meier PJ: The sister of P-glycoprotein represents the canalicular bile salt export pump of mammalian liver. J biol Chem 273:10046–10050 (1998).

Klucken JCB, Ors E, Kaminski WE, Porsch-Ozcurumez M, Liebisch G, Kapinsky M, Diederich W, Drobnik W, Dean M, Allikmets R, Schmitz G: ABCG1 (ABC8) the human homolog of the Drosophila white gene is a regulator of macrophage cholesterol and phospholipid transport. Proc natl Acad Sci, USA 97:817–822 (2000).

Lee MH, Lu K, Hazard S, Yu H, Shulenin S, Hidaka H, Kojima H, Allikmets R, Sakuma N, Pegoraro R, Srivastava AK, Salen G, Dean M, Patel SB: Identification of a gene, ABCG5, important in the regulation of dietary cholesterol absorption. Nature Genet 27:79–83 (2001).

Michaelis S, Berkower C: Sequence comparison of yeast ATP binding cassette (ABC) proteins. Cold Spring Harbor Symposium (Cold Spring Harbor Laboratory Press, (Cold Spring Harbor 1995).

Muller M, Jansen PL: The secretory function of the liver: new aspects of hepatobiliary transport. J Hepatol 28:344–354 (1998).

Patel SB, Salen G, Hidaka H, Kwiterovich PO, Stalenhoef AF, Miettinen TA, Grundy SM, Lee MH, Rubenstein JS, Polymeropoulos MH, Brownstein MJ: Mapping a gene involved in regulating dietary cholesterol absorption. The sitosterolemia locus is found at chromosome 2p21, J Clin Invest 102: 1041–1044 (1998).

Rabindran SK, Ross DD, Doyle LA, Yang W, Greenberger LM: Fumitremorgin C reverses multidrug resistance in cells transfected with the breast cancer resistance protein. Cancer Res 60:47–50 (2000).

Remaley AT, Rust S, Rosier M, Knapper C, Naudin L, Broccardo C, Peterson KM, Koch C, Arnould I, Prades C, Duverger N, Funke H, Assman G, Dinger M, Dean M, Chimini G, Santamarina-Fojo S, Fredrickson DS, Denefle P, Brewer HB Jr: 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 (1999).

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

Salen G, Shefer S, Nguyen L, Ness GC, Tint GS, Batta AK: Sitosterolemia. Subcell Biochem 28:453–476 (1997).

Strautnieks S, Bull LN, Knisely AS, Kocoshis SA, Dahl N, Arnell H, Sokal E, Dahan K, Childs S, Ling V, Tanner MS, Kagalwalla AF, Nemeth A, Pawlowska J, Baker A, Mieli-Vergani G, Freimer NB, Gardiner RM, Thompson RJ: A gene encoding a liver-specific ABC transporter is mutated in progressive familial intrahepatic cholestasis. Nature Genet 20:233–238 (1998).

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