thematic review

ABCA1: the gatekeeper for eliminating excess tissue cholesterol

John F. Oram[1,*] and Richard M. Lawn[†]

Department of Medicine,* Box 356426, 1959 NE Pacific Pl., University of Washington, Seattle, WA 98195-6426; and CV Therapeutics, Inc.,[†] Palo Alto, CA 94304

Abstract **It is widely believed that HDL functions to transport cholesterol from peripheral cells to the liver by reverse cholesterol transport, a pathway that may protect against atherosclerosis by clearing excess cholesterol from arterial cells. A cellular ATP-binding cassette transporter (ABC) called ABCA1 mediates the first step of reverse cholesterol transport: the transfer of cellular cholesterol and phospholipids to lipid-poor apolipoproteins. Mutations in ABCA1 cause Tangier disease (TD), a severe HDL deficiency syndrome characterized by accumulation of cholesterol in tissue macrophages and prevalent atherosclerosis. Studies of TD heterozygotes revealed that ABCA1 activity is a major determinant of plasma HDL levels and susceptibility to CVD. Drugs that induce ABCA1 in mice increase clearance of cholesterol from tissues and inhibit intestinal absorption of dietary cholesterol. Multiple factors related to lipid metabolism and other processes modulate expression and tissue distribution of ABCA1. Therefore, as the primary gatekeeper for eliminating tissue cholesterol, ABCA1 has a major impact on cellular and whole body cholesterol metabolism and is likely to play an important role in protecting against cardiovascular disease.**—Oram, J. F., and R. M. Lawn. **ABCA1: the gatekeeper for eliminating excess tissue cholesterol.** ***J. Lipid Res.*** **2001.** 42: **1173–1179.**

Supplementary key words HDL • reverse cholesterol transport • cellular cholesterol trafficking • cholesterol efflux • phospholipid efflux • cardiovascular disease • Tangier disease • LXR • ABCA1 gene regulation

Numerous population studies have shown an inverse correlation between plasma HDL levels and risk for cardiovascular disease (CVD), implying that factors associated with HDL protect against atherosclerosis. Some of these factors appear to have antioxidant and anti-inflammatory effects (1), which may ablate processes that initiate atherogenesis. The most widely accepted view, however, is that HDL is atheroprotective because of its role in reverse cholesterol transport (2).

REVERSE CHOLESTEROL TRANSPORT AND ABCA1

Reverse cholesterol transport is a metabolic pathway whereby excess cholesterol in peripheral tissues is transported to the liver for elimination from the body (3). The model in **Fig. 1** illustrates some of the processes involved in this pathway [reviewed in (4)]. The liver produces lipid-poor apolipoprotein (apo)A-I that circulates to peripheral cells and picks up cholesterol and phospholipids. A fraction of this apoA-I may also interact with hepatocytes and acquire lipids before exiting the portal circulation. By a complex series of steps involving acquisition of more lipids and proteins and esterification of cholesterol, this partially lipidated apoA-I matures into spherical particles that represent the bulk of HDL. These particles are processed and remodeled by the combined actions of cholesteryl ester transfer protein, phospholipid transfer protein, scavenger receptor B1, and hepatic lipase, which transfer HDL cholesteryl esters to other lipoproteins and cells and regenerate lipid-poor apoA-I.

The gatekeeper of this reverse cholesterol transport pathway is an ATP-binding cassette (ABC) transporter called ABCA1 (5–11), a 2,261-amino acid integral membrane protein. ABC transporters are a superfamily of proteins that use ATP as a source of energy to transport substrates between different cellular compartments and from the cell (12, 13). ABC transporters are defined by the presence of nucleotide-binding domains containing two conserved peptide motifs known as Walker A and Walker B that are present in many proteins that utilize ATP (14). ABC transporters also have a unique amino acid signature between the two Walker motifs, which defines the family. ABC transporters are integrated into the membrane by domains containing six transmembrane helices. The minimum requirement for an active ABC transporter is two nucleotide-binding and two 6-helix transmembrane domains. The Human Gene Nomenclature Committee recently divided the ABC transporters into eight alphabetized (A–H) subfamilies based on their amino acid

Abbreviations: ABCA1, ATP-binding cassette transporter A1; apo, apolipoprotein; CVD, cardiovascular disease; TD, Tangier disease; LXR, liver X receptor; RXR, retinoid X receptor; PPAR, peroxisome proliferator-activated receptor.

[1] To whom correspondence should be addressed.
e-mail: joram@u.washington.edu

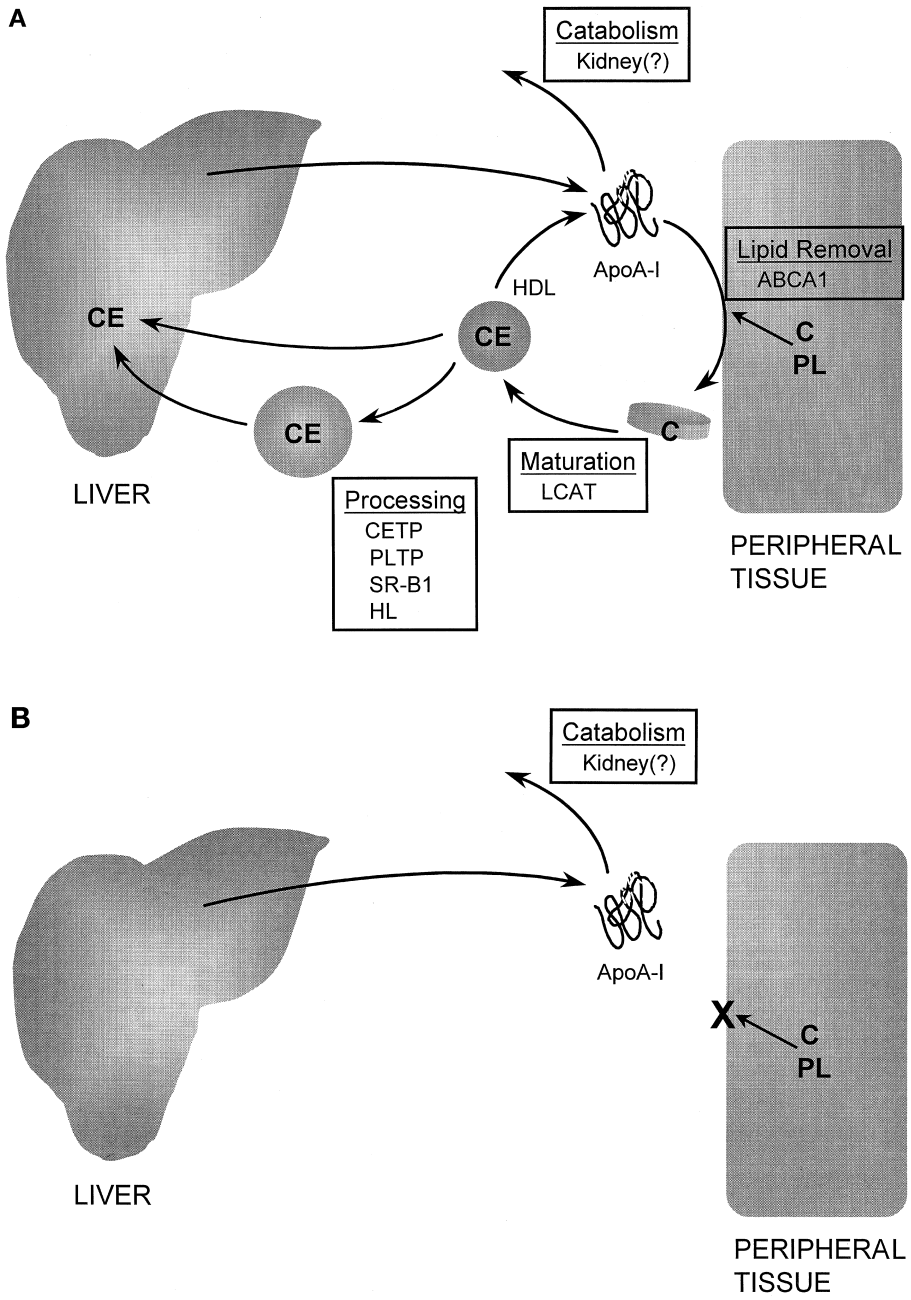

Fig. 1. A: Model for the major steps in reverse cholesterol transport. B: Consequences of a nonfunctional ABCA1, as in Tangier disease. C, Free cholesterol; CE, cholesteryl esters; HL, hepatic lipase; LCAT, lysolecithin-cholesterol acyltransferase; PL, phospholipid; PLTP, phospholipid transfer protein; SR-B1, scavenger receptor B1.

sequences. In the case of the ABCAs, a single polypeptide chain encodes for the two transmembrane and the two nucleotide-binding domains (**Fig. 2**). The ABCAs, unlike other ABC transporters, also have a regulatory domain between the two halves of the protein that contains a highly hydrophobic segment (15).

ABCA1 transports cellular cholesterol and phospholipids (mostly phosphatidylcholine) to cell surface-bound apolipoproteins (4, 16–18). This protein therefore represents the first and rate-controlling step in the reverse cholesterol transport pathway. Knowledge of how ABCA1 functions is still in its early stage of development. Although the structure of ABCA1 is unknown, electron microscopic analysis has suggested a structural model for a closely related ABC transporter called P-glycoprotein (19). This model predicts that the two membrane-spanning domains form a large aqueous chamber in the plasma membrane that opens through pores on the cell surface and within the membrane lipid phase (**Fig. 3**). Because the chamber does not open to the cytosol, P-glycoprotein is believed to act as a "floppase" by translocating lipophilic compounds from the inner aspect of the plasma membrane

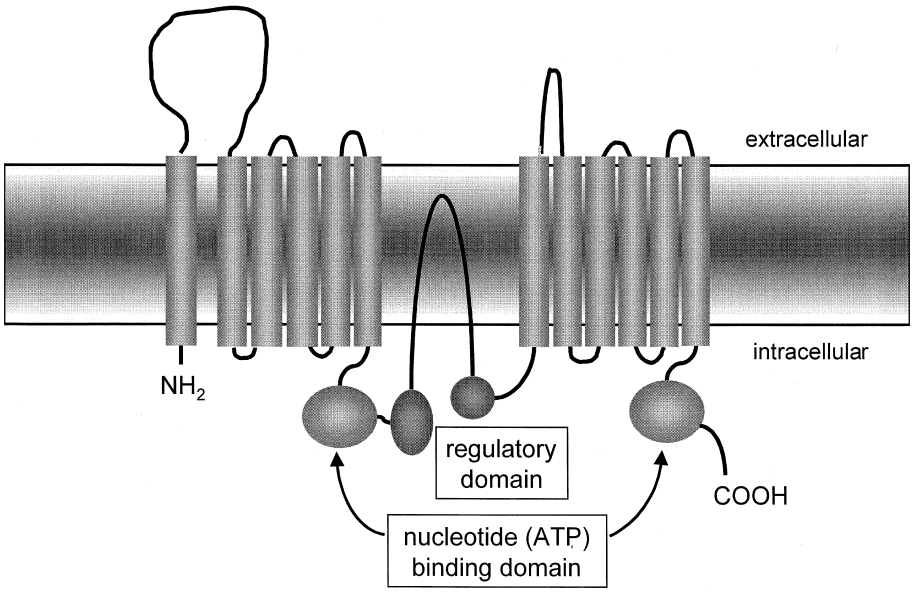

Fig. 2. The predicted structure of ABCA1. The membrane topography model is revised from ref. (71).

into the aqueous environment of the ABC transporter chamber. Both the cell-surface and inner membrane pores are estimated to be relatively large (2–3 nm), allowing for simultaneous translocation of multiple molecules. This may explain why the ABCA1 pathway generates diffuse and irregular structures that protrude from the plasma membrane and interact with apolipoproteins (20). The ABC transporter MDR3 P-glycoprotein, which has a similar lipid transport function as ABCA1, also forms vesicles that protrude from the plasma membrane (21).

Because cholesterol is an integral and necessary membrane component, it is likely that ABCA1 targets specific pools of excess cellular cholesterol for secretion. Its is still unclear what mechanisms are involved in this process, but studies have shown that the interaction of apolipoproteins with cholesterol-loaded cells stimulates translocation of free cholesterol away from intracellular esterifying enzymes to sites accessible to apolipoproteins (22–27) that presumably contain ABCA1. The properties of these ABCA1 membrane domains are unknown, but they differ from cholesterol- and sphingolipid-rich rafts that contain caveolae (28). As one possible mechanism, the interaction of apolipoproteins with ABCA1 or a partner protein stimulates translocation of intracellular cholesterol and phospholipids from the Golgi to plasma-membrane ABCA1 by a signal-responsive vesicular transport pathway (Fig. 3). As another possibility, ABCA1-containing vesicles travel to intracellular lipid deposits, ABCA1 pumps lipids into the

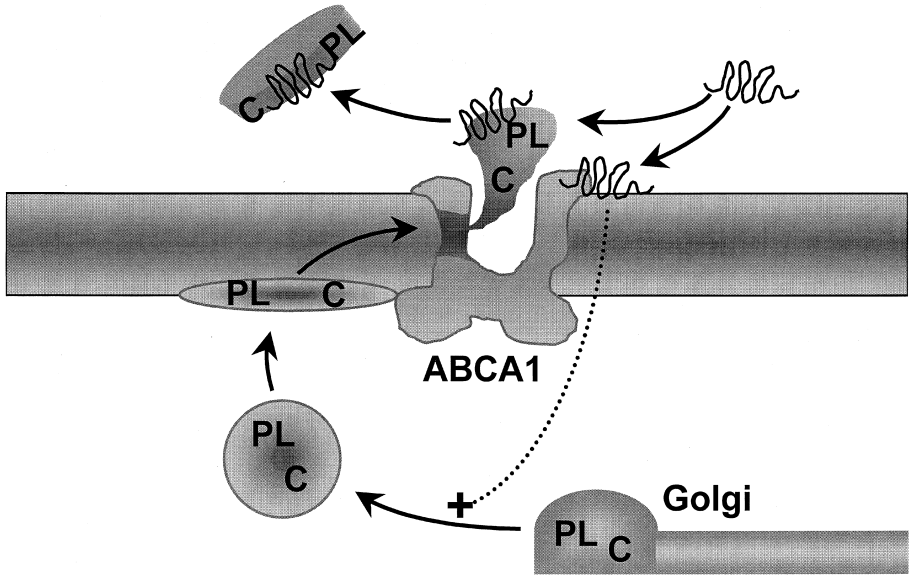

Fig. 3. Model for the cellular ABCA1 lipid secretory pathway.

vesicle lumen, and the vesicles transport their lipid cargo back to the plasma membrane (10, 29). Either mechanism would provide a rapid and effective means of ridding cells of excess cholesterol.

DEFECTIVE ABCA1

Mutations in ABCA1 cause a severe HDL deficiency syndrome called Tangier disease (TD) (5–8, 11, 30–32). Lipid-free apolipoproteins are unable to remove cholesterol and phospholipids from fibroblasts isolated from TD patients (33, 34), consistent with a defective ABCA1. Presenting features of TD include large yellow-orange tonsils, neuropathies, splenomegaly, hepatomegaly, ocular abnormalities, hypocholesterolemia, and CVD (35). Homozygotes have a virtual absence of plasma HDL and apoA-I, low plasma LDL levels (~40% normal), and hypertriglyceridemia (>300 mg/dl). They are characterized by an accumulation of cholesteryl esters in reticuloendothelial cells of tissues including tonsils, thymus, lymph node, bone marrow, spleen, liver, gall bladder, and intestinal mucosa. Many patients also have lipid deposits in neuronal Schwann cells, smooth muscle cells, and fibroblasts.

Targeted disruption of the *Abca1* gene in mice produces a phenotype similar to that of human TD (36–38). The absence of ABCA1 in mice leads to accumulation of sterols in some tissues, but no one has yet determined whether this also enhances atherogenesis. Interestingly, inactivation of the *Abca1* gene increases absorption of dietary cholesterol, suggesting that ABCA1 may facilitate resecretion of cholesterol into the intestinal lumen. Thus, ABCA1 may also suppress the flux of dietary cholesterol into the body, providing another protective mechanism against excess cholesterol.

The Wisconsin Hypoalpha Mutant (WHAM) chicken is a naturally occurring animal model of TD (11, 39). ABCA1 in these chickens has a missense mutation near the N-terminus that produces a defective protein. Similar to human TD patients and ABCA1 knockout mice, the WHAM chicken hypercatabolizes apoA-I and accumulates cholesteryl esters in tissues. The most severe lipid accumulation occurs in hepatic parenchymal and intestinal epithelial cells.

ABCA1, HDL, AND CVD

Studies of TD patients and animal models indicate that loss-of-function mutations in ABCA1 have a major impact on lipoprotein metabolism. A failure to lipidate apolipoproteins by the ABCA1 pathway leads to a rapid catabolism of lipid-poor apoA-I (Fig. 1B) (40, 41) and accumulation of sterols in tissue macrophages, intestinal cells, and hepatocytes (11, 35, 42). This implies that ABCA1 activity is a major determinant of plasma HDL levels. Studies of TD heterozygotes further support this conclusion. As a group, heterozygotes have approximately half-normal plasma HDL levels and cholesterol efflux activity when measured in their cultured fibroblasts (43–45). Moreover, the relative activity of this efflux pathway significantly correlates with both the concentration and size of plasma HDL particles (44, 45). One study (45) showed that compared with unaffected family members, ABCA1 heterozygotes and homozygotes have more prevalent and severe atherosclerosis and an earlier onset of disease. These findings implicate ABCA1 activity as a major factor contributing to the inverse relationship between HDL levels and CVD observed in the general population.

REGULATION AND TISSUE EXPRESSION OF ABCA1

As befitting a protein that is a key modulator of cellular sterol homeostasis, the regulation of the ABCA1 gene is highly tuned to respond to appropriate stimuli. The apoA-I-mediated cholesterol efflux from cultured macrophages and fibroblasts increases upon cholesterol loading and inhibition of cell proliferation (4, 46–48), and ABCA1 gene transcription responds in a similar manner (8, 49). Hence, the elucidation of control elements of the ABCA1 gene has become a topic of basic and applied biomedical interest.

The human ABCA1 gene comprises 50 exons spanning 150 kb (50). Primer extension and rapid amplification of cDNA ends (5′ RACE) cloning identified two mRNA start sites within 100 nucleotides in RNA derived from human monocytes, fibroblasts, and placenta, defining two 5′ noncoding exons of the human ABCA1 gene (50–53). Isolation of genomic clones surrounding these start sites revealed TATAA boxes and other putative control elements. Deletion, mutagenesis, and transfection of luciferase reporter constructs revealed that a region of ~1,500 bp immediately upstream of the transcription start site was sufficient to allow enhancement of ABCA1 transcription in response to macrophage exposure to acetylated LDL (52, 53). A major finding was the presence of a direct repeat response element (DR-4) for the nuclear hormone receptor liver X receptor (LXR). This sequence binds the heterodimer receptor pair, LXR and retinoid X receptor (RXR), and mutation of this site abolishes sterol-mediated activation of the promoter (52–54). LXR and RXR bind and are activated by oxysterols and 9-cis-retinoic acid, respectively (**Fig. 4**) (55). To induce gene expression, these ligands can activate transcription either separately or together. Treatment of cells either with a hydroxycholesterol or 9-cis-retinoic acid induces ABCA1, but their combined treatment has marked synergistic effects (52, 53). Because treatment of cells with oxysterol-free cholesterol induces ABCA1, cells must contain mechanisms for generating oxysterols from cholesterol, an area of cell biology still poorly understood.

In vivo studies in mice have confirmed that LXR is an important regulator of ABCA1 gene expression (56). This has ignited the search for LXR agonists as possible therapeutic agents to increase transcription of ABCA1 and the beneficial flux of cholesterol from peripheral tissues. Another consequence of the presence of an LXR response element in the ABCA1 gene is the demonstration that the

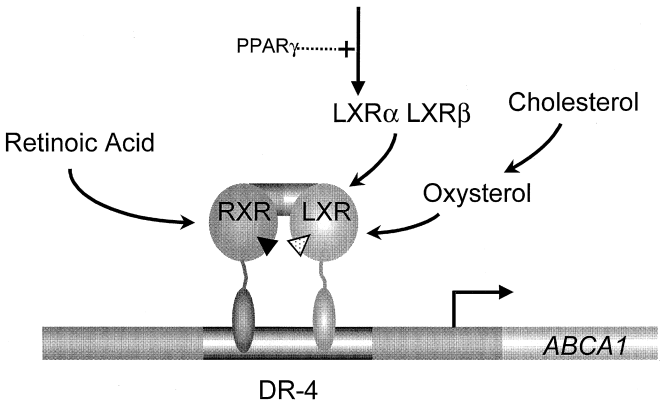

Fig. 4. Regulation of ABCA1 gene transcription by oxysterols, retinoic acid, and PPAR activators. DR-4, direct repeat response element.

induction of cholesterol efflux from macrophages by peroxisome proliferator-activated receptor (PPAR) activators is likely explained by the enhanced transcription of LXR-α caused by activation of PPAR-γ (Fig. 4) (57, 58). The proposal that ABCA1 reduces intestinal cholesterol uptake has increased the prospects that such ligands would have additional beneficial effects on cholesterol metabolism by reducing absorption of dietary sterols. However, the recent discovery that LXR induces lipogenic genes in the liver and raises plasma triglycerides has dampened the promise of such drugs (59, 60).

It is likely that multiple factors related to lipid metabolism and other cell-specific functions coordinate ABCA1 expression. For example, the zinc finger protein 202, which regulates expression of various genes involved in lipid metabolism and is linked to hypoalphalipoproteinemia, represses ABCA1 expression (61). Moreover, analogs of cAMP stimulate ABCA1 transcription in some cell lines, particularly macrophages, even when depleted of cholesterol (8, 48, 62–66). Interferon-γ can repress ABCA1 expression in macrophages (67), suggesting that inflammatory cytokines may have local influences on the activity of the ABCA1 lipid secretory pathway.

Although ABCA1 is highly expressed in macrophages where it plays an obvious role in cholesterol secretion, ABCA1 mRNA is widely distributed among multiple tissues including placenta, liver, brain, kidney, and intestine (49). In situ hybridization studies showed that although in many tissues, ABCA1 expression occurs in resident macrophages, it may also play a role in cells lining vessels or collecting ducts in several tissues (68). The observed 62% increase in dietary cholesterol absorption in ABCA1 knockout mice fed a high fat diet uncovered an unexpected role in modulating uptake of cholesterol from the gut (37). In addition, treatment of mice with selective LXR agonists significantly reduced the amount of intestinal cholesterol absorption while increasing ABCA1 mRNA in the small intestine (56). However, the interpretation of such pharmacological experiments has been complicated by the discovery of other LXR target genes in the intestine that are linked to cholesterol absorption and that, incidentally, are also members of the ABC gene family (69).

Control elements of ABCA1 transcription operative in these different tissues are still incompletely understood. One intriguing development is the discovery that hepatocarcinoma Hep G-2 cells produce an alternately spliced ABCA1 mRNA that utilizes a novel first exon (52, 70). Even though ABCA1 may transport lipids from hepatocytes to circulating apolipoproteins, it may also function to lipidate newly synthesized apolipoproteins prior to secretion. Such divergent functions might require unique regulatory sequences located adjacent to alternative 5′ ends of the transcribed region.

CONCLUSION

Recent studies have established ABCA1 as the gatekeeper for modulating flux of tissue cholesterol into the reverse cholesterol transport pathway. Because it promotes cholesterol secretion from macrophages and may inhibit absorption of dietary cholesterol, ABCA1 has become an attractive target for drug development aimed at preventing atherosclerosis. It is now apparent that multiple factors related to lipid metabolism and, perhaps, other cell-specific processes coordinate ABCA1 expression. Continuing studies of the ABCA1 gene must proceed to fully understand its regulation and its varied impact on physiological functions.

The authors' work was supported by National Institutes of Health grants HL18645 (J.O.), HL55362 (J.O.), and DK02456 (J.O.) and by CV Therapeutics (D.L. and J.O.).

REFERENCES

1. Navab, M., J. A. Berliner, A. D. Watson, S. Y. Hama, M. C. Territo, A. J. Lusis, D. M. Shih, B. J. Van Lenten, J. S. Frank, L. L. Demer, P. A. Edwards, and A. M. Fogelman. 1996. The Yin and Yang of oxidation in the development of the fatty streak. A review based on the 1994 George Lyman Duff Memorial Lecture. *Arterioscler. Thromb. Vasc. Biol.* **16:** 831–842.
2. Miller, G. J., and N. E. Miller. 1975. Plasma-high-density-lipoprotein concentration and development of ischaemic heart-disease. *Lancet.* **1:** 16–19.
3. Glomset, J. A. 1968. The plasma lecithins:cholesterol acyltransferase reaction. *J. Lipid Res.* **9:** 155–167.
4. Oram, J. F., and S. Yokoyama. 1996. Apolipoprotein-mediated removal of cellular cholesterol and phospholipids. *J. Lipid Res.* **37:** 2473–2491.
5. Brooks-Wilson, A., M. Marcil, S. M. Clee, L. H. Zhang, K. Roomp, M. van Dam, L. Yu, C. Brewer, J. A. Collins, H. O. Molhuizen, O. Loubser, B. F. Ouelette, K. Fichter, K. J. Ashbourne-Excoffon, C. W. Sensen, S. Scherer, S. Mott, M. Denis, D. Martindale, J. Frohlich, K. Morgan, B. Koop, S. Pimstone, J. J. Kastelein, and M. R. Hayden. 1999. Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency [see comments]. *Nat. Genet.* **22:** 336–345.
6. 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 [see comments]. *Nat. Genet.* **22:** 347–351.
7. Rust, S., M. Rosier, H. Funke, J. Real, Z. Amoura, J. C. Piette, J. F. Deleuze, H. B. Brewer, N. Duverger, P. Denefle, and G. Assmann. 1999. Tangier disease is caused by mutations in the gene encoding

ATP-binding cassette transporter 1 [see comments]. *Nat. Genet.* **22:** 352–355.
8. 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 [see comments]. *J. Clin. Invest.* **104:** R25–R31.
9. Oram, J. F., and A. M. Vaughan. 2000. ABCA1-mediated transport of cellular cholesterol and phospholipids to HDL apolipoproteins. *Curr. Opin. Lipidol.* **11:** 253–260.
10. Santamarina-Fojo, S., A. Remaley, E. Neufeld, and H. B. Brewer. 2001. Regulation of intracellular trafficking of ABCA1. *J. Lipid Res.* In press.
11. Hayden, M., J. Kastelein, and A. Attie. 2001. Insights derived from mutations in ABCA1 in humans and animal models. *J. Lipid Res.* In press.
12. Klein, I., B. Sarkadi, and A. Varadi. 1999. An inventory of the human ABC proteins. *Biochim. Biophys. Acta.* **1461:** 237–262.
13. Dean, M., H. Yannick, and G. Chimini. 2001. The human ATP-binding cassette (ABC) transporter superfamily. *J. Lipid Res.* **42:** 1007–1017.
14. Walker, J. E., M. Saraste, M. J. Runswick, and N. J. Gay. 1982. Distantly related sequences in the α and β subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. *EMBO J.* **8:** 945–951.
15. Luciani, M. F., F. Denizot, S. Savary, M. G. Mattei, and G. Chimini. 1994. Cloning of two novel ABC transporters mapping on human chromosome 9. *Genomics.* **21:** 150–159.
16. Yokoyama, S. 1998. Apolipoprotein-mediated cellular cholesterol efflux [published erratum appears in *Biochim. Biophys. Acta.* 1998. Jul 31; **1393(1):** 222] *Biochim. Biophys. Acta.* **1392:** 1–15.
17. Rothblat, G. H., M. de la Llera-Moya, V. Atger, G. Kellner-Weibel, D. L. Williams, and M. C. Phillips. 1999. Cell cholesterol efflux: integration of old and new observations provides new insights. *J. Lipid Res.* **40:** 781–796.
18. Oram, J. F. 2000. Tangier disease and ABCA1. *Biochim. Biophys. Acta.* **1529:** 321–330.
19. Rosenberg, M. F., R. Callaghan, R. C. Ford, and C. F. Higgins. 1997. Structure of the multidrug resistance P-glycoprotein to 2.5 nm resolution determined by electron microscopy and image analysis. *J. Biol. Chem.* **272:** 10685–10694.
20. Lin, G., and J. F. Oram. 2000. Apolipoprotein binding to protruding membrane domains during removal of excess cellular cholesterol. *Atherosclerosis.* **149:** 359–370.
21. Crawford, A. R., A. J. Smith, V. C. Hatch, R. P. Oude Elferink, P. Borst, and J. M. Crawford. 1997. Hepatic secretion of phospholipid vesicles in the mouse critically depends on mdr2 or MDR3 P-glycoprotein expression. Visualization by electron microscopy. *J. Clin. Invest.* **100:** 2562–2567.
22. Slotte, J. P., J. F. Oram, and E. L. Bierman. 1987. Binding of high density lipoproteins to cell receptors promotes translocation of cholesterol from intracellular membranes to the cell surface. *J. Biol. Chem.* **262:** 12904–12907.
23. Aviram, M., E. L. Bierman, and J. F. Oram. 1989. High density lipoprotein stimulates sterol translocation between intracellular and plasma membrane pools in human monocyte-derived macrophages. *J. Lipid Res.* **30:** 65–76.
24. 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.
25. 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.
26. 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.
27. 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.
28. Mendez, A. J., G. Lin, D. P. Wade, R. M. Lawn, and J. F. Oram. 2000. Membrane lipid domains distinct from cholesterol/sphingomyelin-rich rafts are involved in the ABCA1-mediated lipid secretory pathway. *J. Biol. Chem.* **276:** 3158–3166.
29. Takahashi, Y., and J. D. Smith. 1999. Cholesterol efflux to apolipoprotein A-I involves endocytosis and resecretion in a calcium-dependent pathway [see comments]. *Proc. Natl. Acad. Sci. USA.* **96:** 11358–11363.
30. Hayden, M. R., S. M. Clee, A. Brooks-Wilson, J. Genest, Jr., A. Attie, and J. J. Kastelein. 2000. Cholesterol efflux regulatory protein, Tangier disease and familial high-density lipoprotein deficiency. *Curr. Opin. Lipidol.* **11:** 117–122.
31. 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. 2000. Novel mutations in the gene encoding ATP-binding cassette 1 in four tangier disease kindreds. *J. Lipid Res.* **41:** 433–441.
32. Marcil, M., A. Brooks-Wilson, S. M. Clee, K. Roomp, L. H. Zhang, L. Yu, J. A. Collins, M. van Dam, H. O. Molhuizen, O. Loubster, B. F. Ouellette, C. W. Sensen, K. Fichter, S. Mott, M. Denis, B. Boucher, S. Pimstone, J. Genest, Jr., J. J. Kastelein, and M. R. Hayden. 1999. Mutations in the ABC1 gene in familial HDL deficiency with defective cholesterol efflux. *Lancet.* **354:** 1341–1346.
33. 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.
34. 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.
35. Assmann, G., A. von Eckardstein, and H. B. Brewer. 1995. Familial high density lipoprotein deficiency: Tangier disease. *In* The Metabolic and Molecular Bases of Inherited Disease. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill, New York. 2053–2072.
36. Orso, E., C. Broccardo, W. E. Kaminski, A. Bottcher, G. Liebisch, W. Drobnik, A. Gotz, O. Chambenoit, W. Diederich, T. Langmann, T. Spruss, M. F. Luciani, G. Rothe, K. J. Lackner, G. Chimini, and G. Schmitz. 2000. Transport of lipids from golgi to plasma membrane is defective in tangier disease patients and Abc1-deficient mice. *Nat. Genet.* **24:** 192–196.
37. McNeish, J., R. J. Aiello, D. Guyot, T. Turi, C. Gabel, C. Aldinger, K. L. Hoppe, M. L. Roach, L. J. Royer, J. de Wet, C. Broccardo, G. Chimini, and O. L. Francone. 2000. High density lipoprotein deficiency and foam cell accumulation in mice with targeted disruption of ATP-binding cassette transporter-1. *Proc. Natl. Acad. Sci. USA.* **97:** 4245–4250.
38. Christiansen-Weber, T. A., J. R. Voland, Y. Wu, K. Ngo, B. L. Roland, S. Nguyen, P. A. Peterson, and W. P. Fung-Leung. 2000. Functional loss of ABCA1 in mice causes severe placental malformation, aberrant lipid distribution, and kidney glomerulonephritis as well as high-density lipoprotein cholesterol deficiency. *Am. J. Pathol.* **157:** 1017–1029.
39. Attie, A. D., A. Brooks-Wilson, M. E. Gray-Keller, L-H. Zhang, A. Tebon, J. Mulligan, J. J. Bitgood, M. E. Cook, J. J. Kastelein, and M. R. Hayden. 2000. Massive cholesterol ester accumulation in hepatocytes and intestinal epithelial cells caused by ABC1 mutation in WHAM chickens. *Circulation.* **102:** II-312.
40. 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 lipoprotein apolipoproteins in Tangier disease. *N. Engl. J. Med.* **299:** 905–910.
41. Schaefer, E. J., D. W. Anderson, L. A. Zech, F. T. Lindgren, T. B. 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.
42. 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. Intern. Med.* **93:** 261–266.
43. Marcil, M., L. Yu, L. Krimbou, B. Boucher, J. F. Oram, J. S. Cohn, and J. Genest, Jr. 1999. Cellular cholesterol transport and efflux in fibroblasts are abnormal in subjects with familial HDL deficiency. *Arterioscler. Thromb. Vasc. Biol.* **19:** 159–169.
44. Brousseau, M. E., G. P. Eberhart, J. Dupuis, B. F. Asztalos, A. L. Goldkamp, E. J. Schaefer, and M. W. Freeman. 2000. Cellular cholesterol efflux in heterozygotes for tangier disease is markedly reduced and correlates with high density lipoprotein cholesterol concentration and particle size. *J. Lipid Res.* **41:** 1125–1135.
45. Clee, S. M., J. J. Kastelein, M. van Dam, M. Marcil, K. Roomp, K. Y.

Zwarts, J. A. Collins, R. Roelants, N. Tamasawa, T. Stulc, T. Suda, R. Ceska, B. Boucher, C. Rondeau, C. DeSouich, A. Brooks-Wilson, H. O. Molhuizen, J. Frohlich, J. Genest, Jr., and M. R. Hayden. 2000. Age and residual cholesterol efflux affect HDL cholesterol levels and coronary artery disease in ABCA1 heterozygotes. *J. Clin. Invest.* **106:** 1263–1270.

46. Mendez, A. J., G. M. Anantharamaiah, J. P. Segrest, and J. F. Oram. 1994. Synthetic amphipathic helical peptides that mimic apolipoprotein A-I in clearing cellular cholesterol. *J. Clin. Invest.* **94:** 1698–1705.
47. Mendez, A. J. 1997. Cholesterol efflux mediated by apolipoproteins is an active cellular process distinct from efflux mediated by passive diffusion. *J. Lipid Res.* **38:** 1807–1821.
48. Oram, J. F., A. J. Mendez, J. Lymp, T. J. Kavanagh, and C. L. Halbert. 1999. Reduction in apolipoprotein-mediated removal of cellular lipids by immortalization of human fibroblasts and its reversion by cAMP: lack of effect with Tangier disease cells. *J. Lipid Res.* **40:** 1769–1781.
49. 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.
50. Santamarina-Fojo, S., K. Peterson, C. Knapper, Y. Qiu, L. Freeman, J. F. Cheng, J. Osorio, A. Remaley, X. P. Yang, C. Haudenschild, C. Prades, G. Chimini, E. Blackmon, T. Francois, N. Duverger, E. M. Rubin, M. Rosier, P. Denefle, D. S. Fredrickson, and H. B. Brewer, Jr. 2000. Complete genomic sequence of the human ABCA1 gene: analysis of the human and mouse ATP-binding cassette A promoter. *Proc. Natl. Acad. Sci. USA.* **97:** 7987–7992.
51. Pullinger, C. R., H. Hakamata, P. N. Duchateau, C. Eng, B. E. Aouizerat, M. H. Cho, C. J. Fielding, and J. P. Kane. 2000. Analysis of hABC1 gene 5′ end: additional peptide sequence, promoter region, and four polymorphisms. *Biochem. Biophys. Res. Commun.* **271:** 451–455.
52. Costet, P., Y. Luo, N. Wang, and A. R. Tall. 2000. Sterol-dependent transactivation of the ABC1 promoter by the liver X receptor/retinoid X receptor. *J. Biol. Chem.* **275:** 28240–28245.
53. Schwartz, K., R. M. Lawn, and D. P. Wade. 2000. ABC1 gene expression and apoA-I-mediated cholesterol efflux are regulated by LXR. *Biochem. Biophys. Res. Commun.* **274:** 794–802.
54. Venkateswaran, A., B. A. Laffitte, S. B. Joseph, P. A. Mak, D. C. Wilpitz, P. A. Edwards, and P. Tontonoz. 2000. Control of cellular cholesterol efflux by the nuclear oxysterol receptor LXRalpha [in process citation]. *Proc. Natl. Acad. Sci. USA.* **97:** 12097–12102.
55. Repa, J. J., and D. J. Mangelsdorf. 1999. Nuclear receptor regulation of cholesterol and bile acid metabolism. *Curr. Opin. Biotechnol.* **10:** 557–563.
56. Repa, J. J., S. D. Turley, J. A. Lobaccaro, J. Medina, L. Li, K. Lustig, B. Shan, R. A. Heyman, J. M. Dietschy, and D. J. Mangelsdorf. 2000. Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers [see comments]. *Science.* **289:** 1524–1529.
57. Chinetti, G., S. Lestavel, V. Bocher, A. T. Remaley, B. Neve, I. P. Torra, E. Teissier, A. Minnich, M. Jaye, N. Duverger, H. B. Brewer, J. C. Fruchart, V. Clavey, and B. Staels. 2001. PPAR-alpha and PPAR-gamma activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway. *Nat. Med.* **7:** 53–58.
58. Chawla, A., W. A. Boisvert, C. H. Lee, B. A. Laffitte, Y. Barak, S. B. Joseph, D. Liao, L. Nagy, P. A. Edwards, L. K. Curtiss, R. M. Evans, and P. Tontonoz. 2001. A PPAR gamma-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. *Mol. Cell.* **7:** 161–171.
59. Repa, J. J., G. Liang, J. Ou, Y. Bashmakov, J. M. Lobaccaro, I. Shimomura, B. Shan, M. S. Brown, J. L. Goldstein, and D. J. Mangelsdorf. 2000. Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRalpha and LXRbeta [in process citation]. *Genes Dev.* **14:** 2819–2830.
60. Schultz, J. R., H. Tu, A. Luk, J. J. Repa, J. C. Medina, L. Li, S. Schwendner, S. Wang, M. Thoolen, D. J. Mangelsdorf, K. D. Lustig, and B. Shan. 2000. Role of LXRs in control of lipogenesis [in process citation]. *Genes Dev.* **14:** 2831–2838.
61. Porsch-Ozcurumez, M., T. Langmann, S. Heimerl, H. Borsukova, W. E. Kaminski, W. Drobnik, C. Honer, C. Schumacher, and G. Schmitz. 2001. The zinc finger protein 202 (znf202) is a transcriptional repressor of atp binding cassette transporter a1 (abca1) and abcg1 gene expression and a modulator of cellular lipid efflux. *J. Biol. Chem.* **276:** 12427–12433.
62. Smith, J. D., M. Miyata, M. Ginsberg, C. Grigaux, E. Shmookler, and A. S. Plump. 1996. Cyclic AMP induces apolipoprotein E binding activity and promotes cholesterol efflux from a macrophage cell line to apolipoprotein acceptors. *J. Biol. Chem.* **271:** 30647–30655.
63. Sakr, S. W., D. L. Williams, G. W. Stoudt, M. C. Phillips, and G. H. Rothblat. 1999. Induction of cellular cholesterol efflux to lipid-free apolipoprotein A- I by cAMP. *Biochim. Biophys. Acta.* **1438:** 85–98.
64. Bortnick, A. E., G. H. Rothblat, G. Stoudt, K. L. Hoppe, L. J. Royer, J. McNeish, and O. L. Francone. 2000. The correlation of ATP-binding cassette 1 mRNA levels with cholesterol efflux from various cell lines. *J. Biol. Chem.* **275:** 28634–28640.
65. Oram, J. F., R. M. Lawn, M. R. Garvin, and D. P. Wade. 2000. ABCA1 is the cAMP-inducible apolipoprotein receptor that mediates cholesterol secretion from macrophages [in process citation]. *J. Biol. Chem.* **275:** 34508–34511.
66. Takahashi, Y., M. Miyata, P. Zheng, T. Imazato, A. Horwitz, and J. D. Smith. 2000. Identification of cAMP analogue inducible genes in RAW264 macrophages. *Biochim. Biophys. Acta.* **1492:** 385–394.
67. Panousis, C. G., and S. H. Zuckerman. 2000. Interferon-gamma induces downregulation of Tangier disease gene (ATP-binding-cassette transporter 1) in macrophage-derived foam cells. *Arterioscler. Thromb. Vasc. Biol.* **20:** 1565–1571.
68. Lawn, R. M., D. P. Wade, T. L. Couse, and J. N. Wilcox. 2001. Localization of human atp-binding cassette transporter 1 (abc1) in normal and atherosclerotic tissues. *Arterioscler. Thromb. Vasc. Biol.* **21:** 378–385.
69. Berge, K. E., H. Tian, G. A. Graf, L. Yu, N. V. Grishin, J. Schultz, P. Kwiterovich, B. Shan, R. Barnes, and H. H. Hobbs. 2000. Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters [in process citation]. *Science.* **290:** 1771–1775.
70. Cavelier, L. B., Y. Qiu, J. K. Bielicki, V. Afzal, J. F. Cheng, and E. M. Rubin. 2001. Regulation and activity of the human ABCA1 gene in transgenic mice. *J. Biol. Chem.* **276:** 18046–18051.
71. Fitzgerald, M. L., A. J. Mendez, K. J. Moore, L. P. Andersson, H. A. Panjeton, and M. W. Freeman. 2001. ABCA1 contains an N-terminal signal-anchor sequence that translocates the proteins first hydrophilic domain to the exoplasmic space. *J. Biol. Chem.* **276:** 15137–15145.