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Visions & Reflections

Tangier disease: still more questions than answers

J.-R. Nofer [a, b, *] and A. T. Remaley [c]

[a] Institut für Klinische Chemie und Laboratoriumsmedizin, Westfälische Wilhelms-Universität, Albert Schweitzer Str. 33, 48129 Münster (Germany), Fax: + 492518347225, e-mail: nofer@uni-muenster.de
[b] Institut für Arteriosklerosеforschung an der Universität Münster, Münster (Germany)
[c] Department of Laboratory Medicine, Warren Grant Magnuson Clinical Center, National Institutes of Health, Bethesda, Maryland (USA)

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Abstract. High-density lipoproteins (HDLs) play a central role in transporting cholesterol from peripheral tissues to the liver for elimination from the body. Impairment of HDL-mediated cholesterol transport favors cholesterol deposition in the arterial wall and promotes development of arteriosclerosis. Tangier disease is a severe HDL deficiency syndrome characterized by the accumulation of cholesterol in tissue macrophages and prevalent atherosclerosis. A three-decade search for a culprit in Tangier disease led to the identification of mutations in a cell membrane protein called ABCA1, which mediates the secretion of excess cholesterol from cells into the HDL metabolic pathway. Because of its ability to deplete cells of cholesterol and to raise plasma HDL levels, ABCA1 has become a promising therapeutic target for preventing cardiovascular disease.

Key words. Tangier disease; high-density lipoprotein (HDL); arteriosclerosis; reverse cholesterol transport; cholesterol efflux; ATP-binding cassette transporter 1 (ABCA1).

Tangier disease – a malady born on an island

In the history of American literature, the name Tangier is usually associated with the eccentric writer William Borroughs, who conceived some of his novels in this Moroccan town on the Straight of Gibraltar. The eponym Tangier disease (TD), however, was coined by Dr Donald S. Fredrickson to commemorate Tangier Island, a small isle located in Chesapeake Bay, approximately 20 miles west of the eastern shore of Virginia, where the first case of the disease was reported [1]. Tangier Island was discovered by the English explorer Captain John Smith, and owes its name to the poetic imagination of the sailor, who was reminded by its sandy shores of the white dunes of the port of Tangier. After its discovery, the island was without a single lipidosis, in fact without a single inhabitant for the next 78 years, until 1686 when John Crockett together with a few comrades founded a settlement there. Except for a calamitous outbreak of cholera in 1886, nothing particular happened for the next 300 years, and the inhabitants made a modest living from the fishery. Tangier Island was well insulated from the mainland both economically and socially, so when Dr Fredrickson traveled there in the early 1960s, he found only one road, one car and about 900 residents, many of them speaking a unique local dialect and bearing the surname Crockett. Geographic isolation is well known to favor the development of communities with a much greater incidence of rare diseases, particularly autosomal recessive disorders, and Dr Fredrickson was indeed in search of further cases of a strange malady, which was first reported in a 5-year-old male patient from Tangier Island. In this patient, unusual tonsils – large, yellowish gray, and with lobulated areas – were noticed by a physician, during a tonsillectomy,

* Corresponding author.

which was the most common surgical procedure performed on children at the time. Histopathological examinations revealed many foam cells along the surface of the tonsils, in the septa and extending into the lymph follicles. The boy was referred to the nearby National Institutes of Health, in Bethesda, Maryland, with the tentative diagnosis of Hand-Schüller-Christian disease or Niemann-Pick disease. It soon became clear that neither diagnosis was correct. No evidence of granuloma formation or reticulohistiocytic hyperplasia typical for Hand-Schüller-Christian disease could be found, and the foamy cells were accumulating cholesteryl esters rather than sphingolipids, the characteristic lipid that accumulates in Niemann-Pick disease. Another striking feature of the patient, which especially intrigued Dr. Fredrickson, was the almost complete absence of high-density lipoproteins (HDLs) in his plasma. A similar phenotype encompassing abnormal-appearing tonsils and extremely low HDL was also found by Dr. Fredrickson in another patient, a young girl, during his explorations on Tangier Island. Given the genetic insularity of the islanders, it is perhaps not surprising that this girl happened to be the younger sister of the original patient. These two children became the index cases for TD, a genetic disorder characterized by an abnormal plasma lipoprotein profile and a variety of seemingly disparate clinical findings, such as enlarged tonsils, splenomegaly, peripheral neuropathy and atherosclerosis (fig. 1).

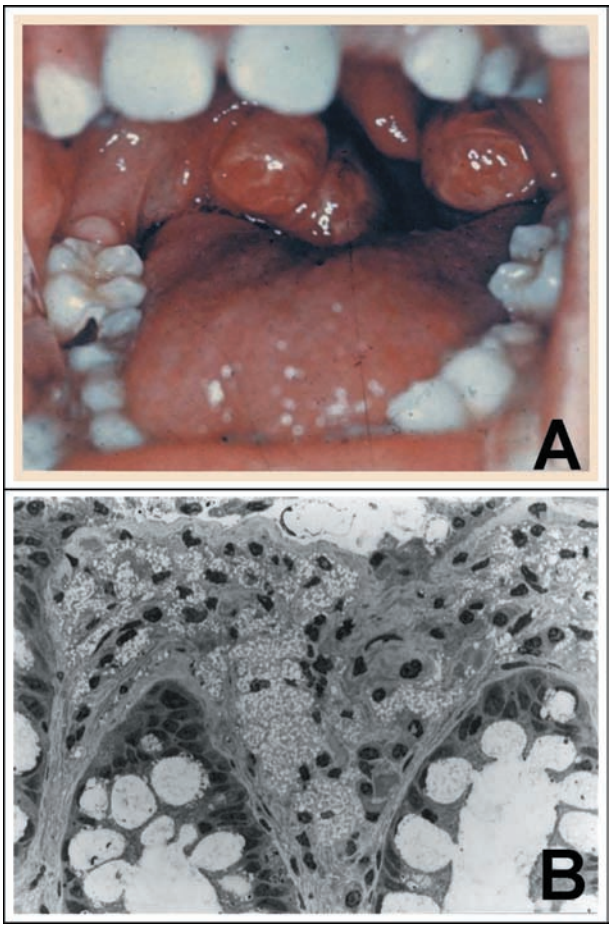

Figure 1. Pathological changes in Tangier disease. (*A*) Macroscopic findings in an original infant patient with Tangier disease. Note the yellow/orange appearance of enormously enlarged tonsils. (*B*) Microscopic demonstration of a lymphatic tissue from a patient with Tangier disease. Note numerous lipid droplets in macrophages and other cells arising due to accumulation of cholesteryl esters and other lipids. Both illustrations from Fredrickson [1].

A bumpy road to the discovery of the Tangier gene

Little was known about lipoprotein physiology and its links to the pathogenesis of atherosclerosis when Tangier disease was first identified. This, however, was soon to change. In the 1970s, in a series of ground-breaking experiments, Joseph L. Goldstein and Michael S. Brown discovered a receptor for low-density lipoproteins (LDL). Furthermore, they demonstrated that the LDL receptor was defective in familial hypercholesterolemia, an inborn error of metabolism characterized by several-fold increased plasma cholesterol and excess deposition of cholesterol in peripheral tissues, particularly in the vessel wall of arteries [2]. The association of familial hypercholesterolemia with premature coronary heart disease (CHD), which in a homozygotic state is expressed even in children, pointed to the pivotal role of LDL in the pathogenesis of atherosclerosis. This notion was enormously strengthened by epidemiological data from the Framingham Heart Study, which showed that hypercholesterolemia is associated with increased levels of plasma LDL and that LDL-cholesterol is a major coronary risk factor in the general population [3]. Pharmacological reduction of LDL-cholesterol became a natural target of therapeutic intervention in CHD, and this eventually led to the development of the statin drugs, which have become the mainstay for the treatment of CHD.

Whereas major efforts in the field of lipoprotein research were concentrated on LDL, HDL remained somewhat neglected, and little effort was devoted toward understanding its physiology or defects in metabolism, such as TD. By the end of the decade, however, the analysis of Framingham data also revealed that plasma HDL-cholesterol is inversely related to CHD risk [4]. This observation corroborated well with the hypothesis of John Glomset that HDL acts as a shuttle in a process termed reverse cholesterol transport, in which excess cholesterol is removed from peripheral tissues, such as the arterial wall, and transported to the liver for excretion [5]. With in vitro experiments, Goldstein and Brown demonstrated that incubation of macrophages with HDL decreases their cholesterol content and thereby showed that HDL acts an initiator of cholesterol efflux, which is the first step of the

reverse cholesterol transport [6]. In subsequent years, many of the details of cholesterol efflux from cells were described by George Rothblat and Michael Phillips [7], but the exact molecular mechanisms for cholesterol efflux were still not fully understood. It slowly became evident, however, that HDL, because of its ability to protect against atherosclerosis, would be a good target for therapeutic intervention in CHD. The hope was that deciphering the defect underlying Tangier disease would provide a valuable clue for understanding how the HDL concentration in plasma is normally regulated, thus providing a potential strategy for developing drugs to increase HDL. For the next 20 years, TD became a Holy Grail of lipoprotein research, and the race to discover its cause began.

If K. R. Popper needed an example to illustrate his theory of scientific knowledge development by empirical falsification of theoretical assumptions, he could not find a better one than TD. The history of TD research is full of seemingly plausible hypotheses for the genetic defect, which soon proved to be false or, at the very least, not causally related to the disease. However, the determination and undiminished enthusiasm of a relatively small number of dedicated investigators did eventually lead to solving the TD puzzle.

Early on, Gerd Schmitz and colleagues suggested that the underlying defect in TD might be faulty conversion of pro-apolipoprotein (pro-apo) A-I to mature apo A-I, either due to a defect in the converting enzyme activity or to a specific structural defect in Tangier apo A-I [8]. Despite intense research, however, neither apo A-I defects nor deficiency of the converting-enzyme activity could be found in Tangier patients [9, 10]. A seminal observation by John F. Oram revealed the presence of HDL-binding sites in fibroblasts, analogous to the situation with the LDL receptor. Interestingly, the density of the HDL-binding sites was found to be reciprocally related to the cellular level of cholesterol [11, 12]. An obvious implication of this discovery was the hypothesis that TD is caused by a deficiency or by structural defects in a putative HDL receptor. Not surprisingly, discoveries of new HDL-binding receptors began to shoot up like mushrooms. More than 40 different binding proteins were reported, including such exotic candidates as glycoprotein IIb/IIIa or cytoskeletal band III protein (chloride/bicarbonate exchanger) [13, 14]. Of four HDL receptors (HBP/vigilin, HB1 and 2 and SR-B1), which were cloned, sequenced and actually proven to act as HDL-binding partners, none turned out to be missing or dysfunctional in TD [15–17]. A failure to link the defect in TD to an HDL receptor shifted the focus of research toward HDL-induced signal transduction. Again, a plethora of peculiarities were described in TD cells, such as decreased or increased activation of large or small G proteins, phosphoinositide- and phosphatidylcholine-specific phospholipases C and D, kinases, such as protein kinase C, and even defective calcium fluxes [18–20]. Once again, none of these apparent defects proved to be causal in TD. Another observation that the interconversion between various subfractions of HDL, such as the pre-β and α fraction, was impaired in Tangier patients suggested that this disease was caused by a deficiency of a hypothetical plasmatic factor [21]. In the early 1990s, pre-β-HDL and α-HDL were demonstrated to differ by their content of phosphatidylinositol and, as could be expected, plasma and fibroblasts of TD patients were soon reported to be disturbed in their capacity to transfer this phospholipid onto HDL particles [22, 23]. In this deluge of experimental findings, some of which perhaps reflected minor phenotypic features of TD, whereas others were plainly incorrect, one observation proved to be entirely true and crucial to our understanding of TD. Within a short time period, four different groups reported that Tangier cells were specifically impaired in their ability to efflux cholesterol and phospholipid not to HDL but to lipid-free apo A-I or lipid-poor apo A-I, such as pre-β-HDL [24–27]. Lipid efflux to lipid-free apo A-I had been previously described by Hara and Yokoyama [28], but its physiologic relevance was not clear until it was shown to be defective in TD cells. This key observation demonstrated that the generation of large mature HDL particles from apo A-I is impaired in TD, and thereby also explains the hypercatabolism of HDL in TD patients, which is the cause of their hypoalphalipoproteinemia [9, 29].

For the first time, a true TD phenotype could now be defined at the cellular level; yet, another 4 years would pass until the cause of TD could be pinpointed at the genome level. The localization, in 1998, of the genetic defect in TD to chromosome 9q31, by a genome-wide linkage exclusion strategy complemented by classical lod score calculations by Rust and colleagues, was a major breakthrough [30]. The number of candidate genes in TD was thus dramatically narrowed, and the final identification of the Tangier gene was clearly only a matter of time. In August 1999, in a single issue of *Nature Genetics*, three groups independently reported defects in the ATP-binding cassette (ABC) transporter 1 (ABCA1) in TD [31–33]. The ABCA1 transporter had been previously cloned by the laboratory of Giovanna Chimini [34], but it had no known function, although an early clue was the observation that the ABCA1 gene was up-regulated in cholesterol-loaded cells [35]. Shortly after the initial reports of the TD mutation, an ABCA1 defect was also demonstrated in the original TD kindred [36], thus completing the first chapter of TD research.

ABCA1 – what is it and how does it work?

The identification of the underlying molecular defect in TD as mutations in ABCA1 was a breakthrough in our

understanding of this enigmatic disease. A question, was immediately raised, however, as to how the various apparently disparate phenotypic manifestations of TD, both at the clinical and cellular level, could be explained by an impaired function of this protein. ABCA1 belongs to a large family of evolutionarily conserved transmembrane proteins that transport a wide variety of substrates, including ions, drugs, peptides and lipids across the plasma membrane [37, 38]. Mutations in other ABC family members cause a variety of genetic disorders, such as cystis fibrosis, intrahepatic cholestasis, macular degeneration, peroxisomal dysfunction and immunodeficiency syndromes. Specifically, ABCA1 is a member of the ABCA subfamily of ABC transporters, many of which are involved in lipid transport. In addition, the ABCA subfamily only occurs in multicellular organisms. This is consistent with the notion that in the course of evolution, the ABCA1 transporter specifically developed to modulate extracellular lipoprotein metabolism [39].

Bearing in mind that the impaired efflux of cholesterol to apo A-I is a major phenotypic feature of cells from Tangier patients, it was straightforward to assume that ABCA1 acts as a cholesterol transporter. In analogy to other ABC transporters, several models were put forward to explain how ABCA1 facilitates the movement of cholesterol across the plasma membrane [40]. For example, a V-shaped structure was proposed, where the bundles of transmembrane helices touch each other either at the outer or the inner side of the membrane. The transport of a cholesterol molecule would occur in a tilting mechanism, in which the substrate binds to the V-shaped structure, when it is open to the cytoplasmic site, and is released, when it is open to the outside. An alternative mechanism was also suggested, in which the transmembrane helices of ABCA1 spin round their long axes parallel to the membrane, so that substrate bound on the membrane side of the helical ring would be transferred to the inside cavity and released. Unfortunately, none of these models has yet been substantiated by any empirical data. On the contrary, evidence was obtained, using a photoactivable cholesterol analog, that ABCA1 is not a cholesterol-binding protein [41]. Furthermore, by depleting cell cholesterol or by using pharmacological inhibitors, specific cholesterol and phospholipid efflux could be effectively dissociated [42, 43]. A two-step mechanism was then proposed, in which ABCA1 transports only phospholipid across the plasma membrane to form apo A-I-phospholipid complexes. As mentioned above, such lipid-poor apo A-I complexes (HDL precursor particles) are effective acceptors of cholesterol. The identity of the putative phospholipid substrates of ABCA1, however, has not been unequivocally established to date. Mature HDL particles are rich in phosphatidylcholine (PC) and sphingomyelin (SM); therefore, these two phospholipids would be natural candidates for ABCA1 substrates. PC and SM, however, are typically located in separate domains in the plasma membrane, and, therefore, ABCA1 is unlikely to facilitate translocation of these phospholipids with equal efficacy. There is some circumstantial evidence that PC- rather than SM-enriched domains serve as a primary source for phospholipid efflux by ABCA1, but, regrettably, no formal studies of ABCA1-mediated phospholipid translocation, using fluorescent or spin-labeled analogs have been conducted to date [43–45]. In addition to neutral lipids, negatively charged phospholipids, such as phosphatidylserine (PS), were also postulated to be translocated across the plasma membrane by ABCA1 [46, 47]. The increased presence of PS in the exofacial leaflet of the plasma membrane has been suggested to influence the arrangements of lipids in a way favoring apo A-I tethering and the formation of apo A-I-phospholipid complexes. However, observations that phospholipids effluxed from cells by ABCA1 are not enriched in PS and that annexin V, a protein which avidly complexes PS, neither impairs cholesterol efflux nor cell-binding activity of apo A-I, argues against the notion that cell surface PS is sufficient to mediate apo A-I-dependent lipid efflux [48]. Phosphadidylinositols (PIs) represent another important class of negatively charged phospholipids. The involvement of ABCA1 in transmembranous PI transport could explain disturbed transfer of this phospholipid onto HDL particles, and the defective interconversion between pre-β-HDL and α-HDL previously reported in plasma from Tangier patients (see above). Unfortunately, no information concerning the substrate specificity of ABCA1 toward PI is currently available.

If cholesterol and phospholipid translocation across the plasma membrane are not a primary function of ABCA1, what other mechanisms could possibly account for ABCA1-mediated lipid efflux? Perhaps intuitively easier to comprehend is a model in which ABCA1 serves as a docking site for an apolipoprotein acceptor, to which the lipids from the exofacial leaflet of the plasma membrane could be directly transferred. Compelling evidence has accumulated recently showing that ABCA1 actually acts as a binding partner for apo A-I. Several studies have demonstrated that the number of apo A-I membrane-binding sites correlates closely with the ABCA1 expression level and that pharmacological ABCA1 inactivation is accompanied by reduced apo A-I binding at the cell surface [49–51]. Membrane topological models of ABCA1 predict the presence of two exocytoplasmic domains, which could act as apo A-I-docking sites [52–54]. The direct interaction of apo A-I with ABCA1 was most strongly suggested by cross-linking studies. Here, free apo A-I could be co-precipitated with ABCA1 but not with SR-BI [41]. Cross-linking of apo A-I to ABCA1 was saturable, occurred with high affinity and was transient, as apo A-I rapidly dissociated from the complex and was released to the medium. Several mutations in the exo- but

not in the endocytoplasmic domains of ABCA1 were also shown to prevent apo A-I cross-linking. In addition, apo A-I variants and even peptides derived from apo A-I were shown to bind to ABCA1 with different affinity and their binding properties correlated with their ability to promote cholesterol efflux [55–57].

The ABCA1 transporter, however, has some unusual characteristics for a typical receptor, which suggest that the interaction of apo A-I with the cell may also involve a membrane lipid interaction. The ABCA1 transporter is not specific for apo A-I, but instead promotes cell binding and lipid efflux by other apolipoproteins [27, 58]. Apparently there is not a strict requirement for any particular primary amino acid sequence for a protein to be a ligand for the ABCA1 transporter, but simply the presence of an amphipathic helix is sufficient [59]. Furthermore, synthetic amphipathic peptides made with either all-L or -D amino acids bind equally well to ABCA1-expressing cells and efflux lipid to the same degree, which suggests that there is also not a stereoselective requirement for at least peptide binding by the ABCA1 transporter [59]. Although the data from the synthetic peptides do not preclude the possibility of regions of apo A-I that may specifically bind to the ABCA1 transporter, they do suggest that the binding to membrane lipids, perhaps adjacent to the transporter, by the amphipathic helices of apolipoproteins may also be involved in ABCAI ligand binding and the lipid efflux process.

The logical extension of the finding that apo A-I binds to ABCA1 would be demonstration that this transporter acts as an apo A-I receptor mediating outside-in signaling. Actually, some observations appear to support this conjecture. For example, apo A-I-induced generation of cAMP, as well as activation of protein tyrosine kinase JAK-2, were shown to be enhanced in ABCA1-overexpressing cells and abolished in Tangier cells [60, 61]. In addition, several intermediary proteins involved in intracellular signaling, such as β2-syntrophin or Cdc42, which is an upstream activator of PC-specific phospholipases C and D, were shown to directly interact with cytoplasmatic domains of ABCA1 (fig. 2) [62–64]. It seems, therefore, that, as was presumed almost two decades ago, the protein defect in TD is indeed an apo A-I receptor and that it is sometimes worthwhile to adhere to original intuitions, even if they appear to be contradicted by subsequent empirical data.

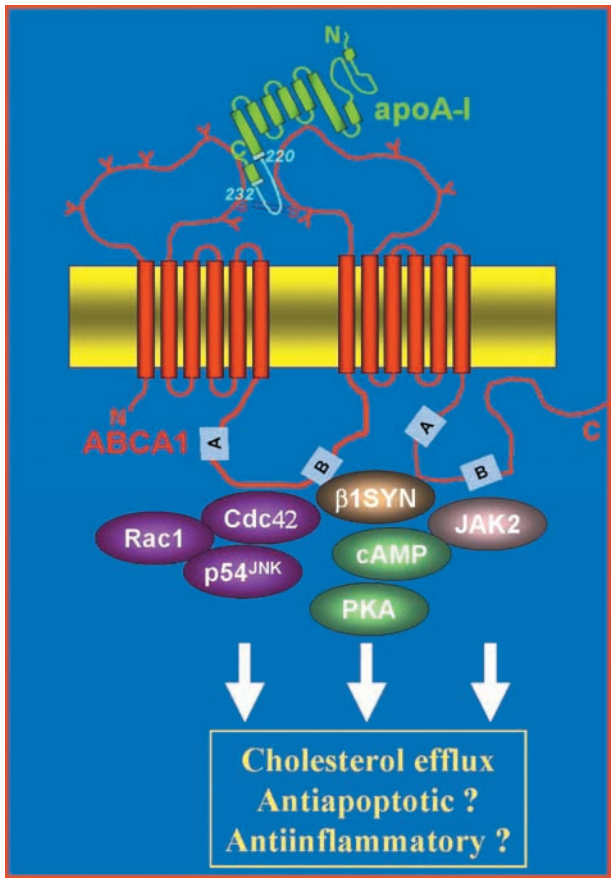

Figure 2. Current concepts of ABCA1 function. In addition to its function as a cholesterol and/or phospholipid transporter, ABCA1 serves as a binding partner for apo A-I. This interaction not only promotes phospholipid and cholesterol efflux from cells, but in addition results in activation of several intermediary proteins involved in intracellular signal transduction such as the small G proteins Cdc42 and Rac1, protein kinases PKA, JAK2 and p54^{JNK}, as well as adapter proteins α- and β1-syntrophin. Intracellular signals generated by apo A-I/ABCA1 interaction facilitate intracellular and/or trans-membranous cholesterol transport and thereby apo A-I lipidation. In addition to lipid removal, apo A-I-induced outside-in signaling conceivably fulfills other anti-atherogenic functions such as inhibition of apoptosis or inflammation. The exact role played by various elements of the apo A-I-inducible signaling cascade in regulation of anti-atherogenic processes distinct from cholesterol efflux has not yet been elucidated.

Do defects in ABCA1 explain the Tangier phenotype?

How do the findings about ABCA1 function explain the phenotypic manifestations of TD? As reviewed above, impaired cholesterol efflux to apo A-I is a major phenotypic feature of Tangier cells. Several studies have convincingly demonstrated the involvement of ABCA1 in cholesterol efflux. For example, treatment of macrophages with cell-permeable cAMP analogs caused parallel increases in apo A-I-mediated cholesterol efflux, ABCA1 mRNA and protein levels and incorporation of ABCA1 into the plasma membrane [49]. Similarly, apo A-I-induced efflux of cholesterol and phospholipid was much enhanced after overexpression of intact but not truncated ABCA1 [50]. Conversely, inhibition of ABCA1-induced cholesterol efflux could be achieved by overexpression of dominant-negative ABCA1 mutants or in the presence of glibenclamide, an effective inhibitor of several ABC transporters [41]. A close correlation be-

tween ABCA1 mRNA expression levels and cholesterol efflux has also been observed in several cell lines [65]. The interaction of apo A-I with ABCA1 was also demonstrated to be required for generation of lipid-poor HDL precursors. In this process, apo A-I is released from the complex with ABCA1 in the form of phospholipid-containing, discoidal particles with pre-β-HDL and α-HDL [66]. Formation of HDL precursor particles was enhanced in cells overexpressing ABCA1 and, depending on experimental conditions, was substantially reduced or completely abolished in the presence of ABCA1 inhibitors or in ABCA1-deficient cells [64–69]. Furthermore, ABCA1 variants defective in apo A-I binding and/or promoting cholesterol efflux failed to support formation of HDL precursors.

The contribution of ABCA1 to the formation of HDL particles was further examined in vivo in transgenic animals. When human ABCA1 was overexpressed under the control of the apo E promoter, which targets expression of the transgene to liver and macrophages, transgenic mice exhibited increased plasma levels of HDL and apo A-I [70, 71]. Pre-β-HDL levels were also elevated in these animals. Similar observations were made in mice after selective overexpression of ABCA1 in the liver [72, 73]. Conversely, liver- and intestine-specific ABCA1 knockout mice presented with HDL-cholesterol concentrations that were about 20% and 80% those of wild-type littermates, respectively [74, 75]. These observations support an unequivocal and essential role for hepatic and intestinal ABCA1 in HDL production. The finding that plasma HDL and apo A-I levels were dramatically reduced in the absence of hepatic ABCA1 but in the presence of functional ABCA1 in extrahepatic tissues also suggests that lipidation of the nascent apo A-I molecule occurs during or soon after secretion of the particle into the circulation. Assembly of lipid-free apo A-I with phospholipids in the liver, and to a lesser extent in the intestine, would provide a source of lipid poor HDL precursor particles which, following to maturation to spherical HDL in plasma by lecithin-cholesterol acyltransferase and phospholipid transfer protein, would be available to initiate reverse cholesterol transport by means of ABCA1-dependent or -independent mechanisms, such as by other ABC transporters (see below), SR-B1 [7] or by nonspecific aqueous diffusion [7], and subsequently to direct the flux of cholesterol to the liver upon HDL particle catabolism by this organ. The realization that liver and not peripheral ABCA1 is a single most important regulator of nascent HDL production leads to a profound revision of our views on the role played by this transporter in reverse cholesterol transport.

In marked contrast to liver-specific ABCA1 expression, transplantation of ABCA1-expressing macrophages into ABCA1-deficient animals had virtually no effect on plasma HDL and apo A-I levels [76, 77]. Macrophages, therefore, are apparently not major contributors to plasma HDL levels [76, 77]. What then is the primary function of ABCA1 in these cells? First, although macrophages may not participate significantly in the generation of plasma HDL and overall reverse cholesterol transport, cholesterol efflux from these cells by ABCA1 is still, nevertheless, important in blocking the progression of atherosclerosis, by preventing the transformation of macrophages into foam cells. Macrophages, unlike many other cells, are prone to the accumulation of excess cholesterol, because they express scavenger receptors and because of lipid uptake from the phagocytosis of cell debris. Macrophages, therefore, probably rely on both ABCA1-dependent and -independent mechanisms for cholesterol efflux. Another intriguing possibility for the expression of ABCA1 by macrophages is that ABCA1 may also modulate their immune and inflammatory responses, which are the principal functions of macrophages. Some observations indeed suggest that anti-atherogenic mechanisms of ABCA1 may not be solely confined to initiation of cholesterol efflux. For example, ABCA1-deficient macrophages exhibit increased recruitment into the arterial wall of atherosclerosis-prone animals and enhanced responsiveness to chemotactic factors, such as macrophage chemoattractant protein-1 (MCP-1) [78].

As cholesterol efflux is a primary function of ABCA1, defects in this process would be predicted to result in the accumulation of cholesteryl esters in macrophages and other cells in the arterial wall, which should result in significant acceleration of atherosclerosis. However, the risk for early atherosclerosis in TD, although enhanced, is not as dramatic as might be expected. As a comparison, adults with heterozygous familial hypercholesterolemia, who have markedly elevated plasma levels of LDL-C (mean ~300 mg/dl), have a three- to fourfold-higher risk of premature coronary artery disease (<60 years old) than do those with TD, who have virtually no plasma HDL-C. To what extent does ABCA1 prevent deposition of cholesterol in peripheral tissues and thereby protect against development of atherosclerosis? Data from studies with ABCA1 knockout and transgenic animals allow only a partial answer to these questions. Increased lipid deposition in various tissues has been noted in the ABCA1$^{-/-}$ mouse [79]. In ABCA1-deficient, hypercholesterolemic animals (ABCA1$^{-/-}$/apo E$^{-/-}$ or ABCA1$^{-/-}$/LDL-R$^{-/-}$ double-knockout mice), where the imbalance between centripetal and basipetal cholesterol flux is expected to be particularly aggravated, the accumulation of foam cells in peripheral tissue was especially pronounced and led to the development of xanthomatosis typical for patients with genetic disorders of lipoprotein metabolism [76]. However, no accelerated development of atherosclerotic lesions could be observed in ABCA1 knockout mice with an absence of other defects. Several explanations can potentially account for this unexpected finding. The re-

duced cholesterol efflux resulting from ABCA1 deficiency could be balanced by decreased cholesterol influx caused by a less atherogenic profile (lower total and LDL-cholesterol, lower triglycerides) in ABCA1 knockouts. Consistent with this hypothesis, the selective inactivation of ABCA1 in macrophages significantly increased atherosclerosis development in the absence of any of the beneficial changes in plasma lipid profiles, which normally occurs in complete ABCA1 knockout animals. Alternatively, impairment of cholesterol efflux in macrophages from mice lacking ABCA1 could be counter balanced by activation of some cholesterol efflux 'back-up' mechanisms. For example, other ABC transporters could compensate for ABCA1 dysfunction. Of interest in this regard is that at least two other ABC transporters were reported to be involved in mediating cholesterol efflux. ABCA7, a ubiquitously expressed close relative of ABCA1, was shown to promote efflux of phospholipid and, to a lesser extent, cholesterol to apo A-I, when overexpressed in a fibroblast cell line [80, 81]. However, this transporter does not appear to contribute to cholesterol efflux from macrophages, where it is primarily located in the cell interior [82]. Accordingly, apo A-I-induced phospholipid and cholesterol effluxes were normal in macrophages obtained from ABCA7$^{-/-}$ mice, which were characterized by normal or only slightly decreased serum HDL-cholesterol levels [83]. In addition to ABCA7, ABCG1 and ABCG4 are also able to initiate cholesterol efflux from cells [84]. In contrast to ABCA1 and 7, these two transporters utilize mature HDL particles (HDL3 and 2) rather than apo A-I as cholesterol acceptors. Accordingly, these transporters would not be expected to provide a major contribution to the generation of poorly lipidated nascent HDL particles, the generation of which is generally attributed to ABCA1. As with ABCA1 and ABCA7, ABCG1 and ABCG4 are all able to efflux cholesterol from cells and thereby initiate reverse cholesterol transport; therefore, under certain circumstances they can conceivably compensate for defective ABCA1 function. Possibly, only when these compensating mechanisms do not function properly would atherosclerosis develop in Tangier patients. Unfortunately, the crosses between ABCA7-deficient and atherosclerosis-prone animals have not yet been reported and, thus, the actual contribution of ABCA7 to the development of atherosclerosis remains enigmatic. Likewise, no information is available concerning the in vivo role played by ABCG1 and ABCG4 in animal models of atherosclerosis.

Experiments with ABCA1 transgenic animals have produced controversial results. Initially, two different transgenic mouse lines containing the human ABCA1 gene were reported to exhibit no changes in plasma lipids or in HDL-cholesterol levels [70, 85]. However, ABCA1 expression was relatively low in these animals and, therefore, could fail to affect the plasma lipid phenotype. Later studies with a transgenic mouse strongly expressing ABCA1 showed an anti-atherogenic lipid profile with elevated levels of plasma cholesterol, HDL-cholesterol and apo A-I. In addition, significantly less aortic atherosclerosis has been reported [86]. The latter observations give strong support to the contention that ABCA1, whatever its mode of action, exerts a strong anti-atherogenic effect and provides an explanation for the accelerated development of atherosclerosis in at least some Tangier patients.

Quite understandably, most of the research on TD in recent years was focused on deciphering links between ABCA1 and HDL metabolism and atheroclerosis. The role played by ABCA1 in the development of other phenotypic features of TD was investigated less intensely. The yellow or orange discoloration of the tonsils, adenoids, and colonic reticuloendothelial cells, which is a hallmark of TD, may be caused by the accumulation in these cells of yellow/orange lipophilic compounds, such as vitamin E, retinyl esters and carotenoids. Recent results suggest that ABCA1 indeed mediates export of cellular α-tocopherol to HDL and lipid-poor apolipoproteins [87]. Peripheral neuropathy, which manifests either as syringomyelia-like disease, with progressive loss of sensory and motor function in the upper body or as peripheral neuropathy with loss of sensory function, is another feature of TD, where little progress has been made. The major pathological finding on peripheral nerve biopsy in TD is lipid accumulation in Schwann cells. Regrettably, not a single study has yet been devoted to investigation of the role of ABCA1 in oligodendrocyte physiology and the mechanism underlying development of lipid-engorged Schwann cells in TD. Interestingly, ABCA1 was shown to be abundantly expressed in pyramidal neurons and disturbed function of ABCA1 has been reported in conjunction with some neurodegenerative diseases, such as Alzheimer disease [88]. ABCA2, another close cousin of ABCA1, is primarily expressed in neural tissues and associations between ABCA2 and Alzheimer disease have also been postulated [89, 90]. It would be tempting to speculate that ABCA1 and 2 act complementary to each other and that neurological syndromes develop only in those patients with TD, in which ABCA2 cannot fully substitute for ABCA1.

Future therapies for the treatment of cardiovascular disease?

The promise that the discovery of the genetic cause of TD would lead to new therapies for the treatment of CHD has not yet been fulfilled, but recent developments suggest that such treatments may be on the horizon. The expression of ABCA1 by cells is exquisitely regulated by their cellular content. Increased cholesterol accumulation by cells leads to several homeostatic processes [91], one

of which is the induction of the ABCA1 transporter gene [35], which enables cells to efflux excess cholesterol. This regulation is largely controlled by the LXR elements in the promoter of ABCA1 [92]. LXR is a transcription factor that binds oxysterols, which are produced by cholesterol-replete cells. Several experimental therapeutic agents are potent LXR agonists and are now being actively investigated [93]. LXR agonists, however, regulate many genes, many of which are potentially beneficial for the treatment of atherosclerosis [94], but other gene changes, such as those that lead to hypertriglyceridemia [95] and hepatic steatosis [96], may be detrimental. Recently, selective LXR agonists that may more specifically induce the ABCA1 transporter and avoid some of the negative gene changes have been described [97].

Another exciting finding was the recent result of a phase I clinical trial that showed that intravenous infusion of a variant of apo A-I called apo A-I Milano could rapidly decrease the size of atherosclerotic plaques [98]. Although there had been many previous experimental studies in various animal models of atherosclerosis that showed the benefit of HDL administration [99, 100], the rapidity and the effectiveness of HDL treatment in humans was largely unexpected. Apparently, in addition to ABCA1, the level of apo A-I that is available to extract lipid from ABCA1-expressing cells is also a rate-limiting factor in reverse cholesterol transport. As already discussed, HDL appears to also have other beneficial properties [101], and its effect on reducing atherosclerotic plaques may also involve other mechanism, such as reducing inflammation [102, 103]. Peptide mimetics of apo A-I have also been shown to be effective in various animal models of atherosclerosis and some of them are orally available [104]. They are also now being investigated as possible therapeutic agents [105].

TD research in perspective

It has been a long journey since the discovery of TD on an obscure island by Dr. Fredrickson over 40 years ago, but much has been learned along the way about TD disease and HDL metabolism. The history of TD research is an excellent example of how the study of a rare and seemingly unimportant disease can lead to new insights into normal physiology that may have great implications for the treatment of common disorders. There is, however, still much to be learned about TD and the ABCA1 transporter, and future research in this area is likely to uncover even more secrets about HDL and its connection to cardiovascular disease.

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