Mechanisms and genetic determinants regulating sterol absorption, circulating LDL levels, and sterol elimination: implications for classification and disease risk

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Abstract This review integrates historical biochemical and modern genetic findings that underpin our understanding of the low-density lipoprotein (LDL) dyslipidemias that bear on human disease. These range from life-threatening conditions of infancy through severe coronary heart disease of young adulthood, to indolent disorders of middle- and old-age. We particularly focus on the biological aspects of those gene mutations and variants that impact on sterol absorption and hepatobiliary excretion via specific membrane transporter systems (NPC1L1, ABCG5/8); the incorporation of dietary sterols (MTP) and de novo synthesized lipids (HMGCR, TRIB1) into apoB-containing lipoproteins (APOB) and their release into the circulation (ANGPTL3, SARA2, SORT1); and receptor-mediated uptake of LDL and of intestinal and hepatic-derived lipoprotein remnants (LDLR, APOB, APOE, LDLRAP1, PCSK9, IDOL). The insights gained from integrating the wealth of genetic data with biological processes have important implications for the classification of clinical and presymptomatic diagnoses of traditional LDL dyslipidemias, sitosterolemia, and newly emerging phenotypes, as well as their management through both nutritional and pharmaceutical means.

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INTRODUCTION

This review covers the dietary and biochemical origins and fates of key classes of sterol molecules in humans, namely, cholesterol and the relatively under-recognized and often unappreciated noncholesterol sterols and stanols; the intra- and intercellular systems that govern their transport; and the contribution of innate genetic programs to the biochemically observed levels of plasma LDL-cholesterol (LDL-C). The reasons for these foci are both biological and medical. The former is the burgeoning knowledge of the normal physiological roles that cholesterol performs within cell membranes in supporting receptor-mediated signaling activities (1–4), the movement of diverse molecules through different membrane-bound compartments (5–8), and multiple other cell functions (9–11), including myelination (12). The latter,

Supplementary key words intestinal sterol absorption and efflux • cellular cholesterol synthesis • lipoprotein assembly • gallstones • LDL uptake

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the view that the pathological consequences of deranged LDL levels can arise from the type, properties, and amounts of individual lipid classes conveyed by LDL particles (21–24).

NEW INSIGHTS INTO STEROL ABSORPTION AND HEPATOBILIARY SECRETION

Humans exhibit net selective absorption of dietary cholesterol over its biochemical look-alikes, the noncholesterol sterols (e.g., sitosterol) and stanols (e.g., 5α-sitostanol), for example, ~50% of dietary cholesterol versus <5% of noncholesterol sterols and 5α-stanols (20, 25, 26). In recent years, two fundamental discoveries have provided an unexpected explanation for this phenomenon, thereby considerably expanding our knowledge of how the membrane transport systems employed by enterocytes and, it turns out, the hepatobiliary system bring about net selective absorption of cholesterol (Figs. 1, 2). The new players on the block to be considered are Niemann-Pick C1 like-1 protein (NPC1L1), the major, and relatively unselective inward transporter of all three sterol lipid classes and vitamin E (27), and ABCG5 and ABCG8, the medical rationale is itself duplex: to raise awareness of several newly recognized, genetically determined lipid metabolic phenotypes/disorders and how they are identified in the clinical setting; and, in the light of these and other better-known genetic disorders, to argue the case for replacing the traditional, but now 45-year-old, Fredrickson and Lees, essentially phenotypic, classification of hyperlipidemia (13) with one rising from biological and genetic foundations. Toward this end, we provide a commentary for the biological bases of the effects of gene variants on LDL, sterol disorders, and subclinical phenotypes, other than those of primary cholesterol biosynthesis (14) and the egress of cholesterol (and other lipids) from late endosomes and lysosomes to other cellular compartments (15–17).

On the basis of the results from recent genome-wide association studies (GWAS), we primarily focus on sterol absorption and hepatobiliary cholesterol efflux (Figs. 1, 2); the incorporation of cholesterol and other lipid moieties, including noncholesterol sterols (18–22) into apolipoprotein (apo)B-containing lipoproteins for their transport to distant sites; and receptor-mediated uptake of apoB-containing lipoproteins, especially LDL. Implicit in this approach is the view that the pathological consequences of deranged LDL levels can arise from the type, properties, and amounts of individual lipid classes conveyed by LDL particles (21–24).

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Biological and genetic classification of LDL dyslipidemias

Residual low level of cholesterol absorption could not be blocked by ezetimibe. In humans, NPC1L1 is not only localized to the brush border of enterocytes (33, 34) where it acts as a unidirectional transporter of cholesterol and noncholesterol sterols into these absorptive cells (35), but it also resides on the apical (biliary canalicular) membranes of hepatocytes where it retrieves biliary cholesterol for its transport back into these cells (18, 35), presumably to inhibit excessive biliary cholesterol loss (Fig. 1).

In humans, the effects of ezetimibe on sterol metabolism and LDL-C levels are well documented (36–40). For example, when von Bergman and colleagues (36–39) examined its effects (10 mg/d, two-week treatment period, two-week washout period) on several metabolic indices in a placebo-controlled, cross-over study involving 18 men (mean age, 25.8 years; BMI, 25.5 kg/m²) with mild to moderate hypercholesterolemia (LDL-C 130-180 mg/dl), they found that ezetimibe decreased fractional cholesterol absorption rates on average by >50% (ezetimibe 22.7 ± 25.8%; placebo 49.8 ± 13.8%); plasma total- and LDL-cholesterol...
levels by 15.1 and 20.4%, respectively; and campesterol and sitosterol by 48% and 41%, respectively. Accompanying these decreases were significant rises in de novo cholesterol synthesis (89%, primarily hepatic) and fecal excretion of neutral sterols (72%). Moreover, in a similarly designed study involving strict vegetarians whose dietary cholesterol intake was low (29.4 ± 16.8 and 31.4 ± 14.4 mg/day during the placebo and ezetimibe phases, respectively), ezetimibe decreased mean fractional cholesterol absorption by 58% (placebo 48.2 ± 8.2%; ezetimibe 20.2 ± 6.2%, \( P < 0.001 \)). This was paired with increased fecal excretion of neutral (81%, \( P < 0.001 \)) and acidic (35%, \( P = 0.052 \)) sterols, plus de novo cholesterol synthesis (72%, \( P < 0.001 \)) (41). By contrast, total plasma cholesterol and LDL-C levels were decreased (9.9 and 17.3%, both \( P < 0.001 \)), suggesting the cholesterol-lowering effect of ezetimibe, at least in vegetarians, is mediated largely through inhibiting endogenous (biliary) cholesterol absorption (Fig. 1).

The most direct evidence that NPC1L1 is a relatively nonselective transporter of sterols derives from studies performed in mice (42, 43). Thus, in NPC1L1−/− mice cholesterol and sitosterol uptake into the proximal third of the small intestine were reduced to similar degrees: 64 and 52%, respectively, compared with control animals (42). This was accompanied by a ~4-fold increase in intestinal cholesterol synthesis and elevated intestinal HMG-CoA reductase mRNA levels but no change in ABCG5 and ABCG8 mRNA (Fig. 1). In ABCG5/8-deficient mice, NPC1L1 deficiency largely prevented the accumulation of phytosterols that normally occurs in these animals (43), as well as in ABCG5−/− (29) and ABCG8−/− (50) only mice.

**NPC1L1 genetic determinants affect circulating sterol and LDL-C levels**

Consistent with the high heritability figures for the otherwise very variable population values of cholesterol (20, 44), campesterol, and sitosterol absorption rates (45, 46), genetic studies have unearthed robust associations between NPC1L1 variants, surrogate markers of sterol absorption efficiency and LDL-C levels (47, 48) (Table 1).

**Rare NPC1L1 variants.** Cohen et al. (47), sequenced the coding regions of NPC1L1 in 256 individuals from the Dallas Heart Study (population-based sample of Dallas County residents, comprising 1,043 whites, 1,832 African Americans, and 601 Hispanics) who had the highest (n = 128) and lowest (n = 128) campesterol:lathosterol (Ca:L) ratios. The rationale was that this ratio serves as a good marker for rates of intestinal cholesterol absorption (20, 49). Thirteen nonsynonymous (NS) coding sequence variants were identified in both the “high” and “low” cholesterol-absorber groups compared with five and 19 such variants present only in the “high” and “low” cholesterol-absorbing groups, respectively. The cumulative frequency of the 19 NS alleles (plus one nonsense mutation) in the “low” cholesterol-absorbing group was higher than in the “high” cholesterol-absorbing group (26/256 alleles compared with 5/256 alleles, \( P < 0.001 \)). In the African-American contingent, the 20 variants reached an appreciable frequency: 6.2% compared with 1.8% and 1.7% in the whites and Hispanics, respectively. Moreover, in this subpopulation, this group of alleles was associated with a lower mean plasma Ca:L ratio (1.7 ± 1.2 versus 2.2 ± 2.4, \( P = 0.005 \)) and LDL-C values (96 ± 36 mg/dl versus 105 ± 37 mg/dl, \( P = 0.005 \)). Consistent with estimated rates of cholesterol absorption, stable isotope methodology confirmed that two NS variants (no others tested) were indeed associated with lower net cholesterol absorption in two pedigrees, one with the I647N variant (n = 37 family members) and the other with the R695C allele (n = 16) (50).

Crucially, functional data now corroborate the genetic evidence that the 20 rare NPC1L1 alleles found solely in the “low” cholesterol absorption group impair NPC1L1-mediated cholesterol uptake (50, 51). Implicated mechanisms include defective glycosylation (e.g., N132S), aberrant folding (e.g., S620C), enhanced degradation through the endoplasmic reticulum (ER)-associated protein degradation pathway (e.g., S881L), impaired transport to the plasma membrane (e.g., A395V), decreased cholesterol-binding (e.g., L110F), as well as perturbed recycling of NPC1L1 to the plasma membrane in response to low cellular/ER-cholesterol concentrations (Fig. 2). As such, the experimental approach of resequencing genes in individuals at the extreme of the population distribution to identify putative causal variants has amply borne fruit. However, whether NPC1L1 null alleles exist in “low” cholesterol absorbers or, conversely, gain-of-function variants increase cholesterol/non-cholesterol sterol uptake remains to be established. Additionally, further investigations are required to explain the rather surprising in vitro finding, given the in vivo data (42, 43), that only cholesterol (ergosterol, β-sitosterol, campesterol, stigmasterol, and brassicasterol) tested had a significant capacity to internalize NPC1L1 via clathrin/AP2-mediated endocytosis (52).

**Common NPC1L1 variants.** Table 1 summarizes the associations between relatively common NPC1L1 variants and LDL-C levels (48, 53, 54). In the PROSPER cohort, homozygotes with the rare allele at the c.-18C>A locus had significantly higher (albeit modest) LDL-C levels than cohort members who had two copies of the common allele, while the heterozygote individuals had intermediate levels (48). Supporting this association, CHD death and nonfatal myocardial infarction (MI) during the 3.2 year follow-up period were more common in the rare allele carriers. However, whether this association is attributable to the causative NPC1L1 allele(s) merely enhancing cholesterol uptake remains an open question. LDL particles are major carriers of phytosterols (21), lathosterol (21), sphingomyelin, and ceramide (22), none of which was analyzed in the patient cohort.

Table 1 also shows the ezetimibe (10 mg/day for 6 weeks) add-on to statin (any dose, any brand) for effectiveness trial (EASE) found association between the rare allele at the c.-18C>A locus and LDL-C lowering response (53),...
<table>
<thead>
<tr>
<th>Gene</th>
<th>Sample (Reference)</th>
<th>Type/d Variation (Proxy)</th>
<th>Position (Effect)</th>
<th>Allele (MAF)</th>
<th>Effect on LDL-C</th>
<th>Other Associated Traits/Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPC1L1</td>
<td>5,804 members of PROSPER cohort (mean age 75 ± 3 year) with or at risk of vascular disease (48)</td>
<td>rs41279653 -18A&gt;C</td>
<td>C(0.15)</td>
<td>C/C +4.2/1.6% (men/women) versus A/A, (combined P = 0.02).</td>
<td>Increased risk of CHD death/nonfatal MI for C/C; HR 1.67 [95% CI 1.10-2.54], (P = 0.04).</td>
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<td></td>
<td>1) rs17655652 -133A&gt;G</td>
<td>G(0.33)</td>
<td>G/G +4.4% versus A/A, (P = 0.023).</td>
<td>Women only; men, NS.</td>
<td>Increased risk of CHD death/nonfatal MI for G/G; HR 1.50 [95% CI 1.09-2.06], (P = 0.04).</td>
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<td></td>
<td>1) rs2072183 (rs41279653) -816C&gt;G(L272L)</td>
<td>G(0.20)</td>
<td>G/G +3.8/2.9% (men/women) versus C/C, (combined P = 0.02).</td>
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<td>1,003 statin-treated patients of Caucasian origin (EASE cohort) (53)</td>
<td>rs2072183 (rs41279653)</td>
<td>c.816C&gt;G(L272L)</td>
<td>G(0.20)</td>
<td>Greater reduction in LDL-C with ezetimibe treatment: C/C, 18.9/23.3%; C/G+G/G, 23.3/33.7% (P &lt; 0.06/0.07).</td>
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<td>65 heterozygous ADH-1 patients/50 statin-resistant primary hypercholesterolemia patients (54)</td>
<td>rs2072183 (rs41279653)</td>
<td>c.816C&gt;G(L272L)</td>
<td>A(0.16)</td>
<td>Greater reduction in LDL-C with ezetimibe treatment: C/C, 18.9/23.3%; C/G+G/G, 23.3/33.7% (P &lt; 0.06/0.07).</td>
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<td></td>
<td>GLGC (32)</td>
<td>rs2072183 (rs41279653)</td>
<td>c.816C&gt;G(L272L)</td>
<td>A(0.16)</td>
<td>Greater reduction in LDL-C with ezetimibe treatment: C/C, 18.9/23.3%; C/G+G/G, 23.3/33.7% (P &lt; 0.06/0.07).</td>
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<td></td>
<td>ABCG8</td>
<td>rs11887534</td>
<td>c.55G&gt;C (D19H)</td>
<td>C</td>
<td>Not determined</td>
<td>Lower Ca:cholesterol ratio (~cholesterol absorption) in carriers (n = 85) versus noncarriers (n = 591), (WMD ~0.50 µg/mg, [95% CI ~0.80 to ~0.20 µg/mg], P = 0.001). Higher Lox cholesterol ratio (~cholesterol synthesis) in carriers (n = 79) versus noncarriers (n = 541), (WMD ~0.26 µg/mg, [95% CI 0.10 to 0.41 µg/mg], P = 0.001).</td>
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<td>96 German gallstone disease (GD) cases and 205 controls/Replication in 1105 cases and 873 controls (98)</td>
<td>rs11887534</td>
<td>c.55G&gt;C (D19H)</td>
<td>C(0.08/0.04)</td>
<td>Not determined</td>
<td>Associated with GD (P = 2x10^-6). Carrier OR combined samples, 2.2 [95% CI 1.8-2.6], P = 1.1x10^-13. Increased biliary cholesterol in patients (P &lt; 0.001).</td>
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<td>226 Indian GD patients plus 222 controls (102)</td>
<td>rs11887534</td>
<td>c.55G&gt;C (D19H)</td>
<td>C(0.08/0.04)</td>
<td>Not determined</td>
<td>Associated with GD (P = 0.017). OR = 2.274 [95% CI 1.17-4.41]. Increased biliary cholesterol in patients (P &lt; 0.001).</td>
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<td>Gene</td>
<td>Sample Reference</td>
<td>Tiled Variant (Proxy)</td>
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<td>Allele/MAP</td>
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<tr>
<td>NPC1L1</td>
<td>GLGC (32)</td>
<td>1) rs11887534 c.55G&gt;C (D19H) C(0.06)</td>
<td>G/C</td>
<td>Total cholesterol 1.6% (G/C) and 2.4% (C/C) versus noncarriers (P&lt;0.001). 11% increase plasma LDL-C levels by increasing the alleles in LD represent gain-of-function variants that unmask a previously neutral or neutral variant.</td>
<td>HR, 1.9 [95% CI 1.7-2.1] for G/C, 3.3 [95% CI 2.3-4.6] for C/C.</td>
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<td>2) rs4299376 c.166-718G&gt;T G(0.30)</td>
<td>Z = 14.476, P = 2×10^{-10}</td>
<td>Other Associated Traits/Comments</td>
<td>Total cholesterol +3.01 mg/dl per copy (Z = 7.037, P = 2×10^{-8})</td>
<td>Other Associated Traits/Comments</td>
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Table 1 highlights another noteworthy result to emerge from the PROSPER and EASE studies: the "promoter" variant c.-133A>G. In PROSPER, women, but not men, with the A allele had higher LDL-C levels than those who did not. Additionally, following randomization to the pravastatin arm of the study (40 mg/day), women homozygous for the A allele displayed greater reduction in LDL-C than those with the G allele. Similarly, in EASE, the mean percentage drop in LDL-C levels following addition of ezetimibe to the patients’ statin medications was highest in Caucasians with the AA genotype. Because the LD between the alleles at this locus and the g.-18C>A is relatively low, two independent haplotypic arrangements at the NPC1L1 locus appear to contribute to LDL-C levels.

The associations between common NPC1L1 variants and LDL-C levels prompt three important questions, all of which have yet to be addressed. First, are the alleles themselves neutral and simply in LD with functional variants that alter the primary sequence of NPC1L1, thereby promoting, for example, its recycling to the plasma membrane for further rounds of cholesterol/sterol uptake (Fig. 2)? Second, does decreased allelic expression of NPC1L1 translate into decreased functional protein and a consequent decrease in cellular and plasma sterol levels? Third, could the rare alleles at the c.-133A>G or c.-18C>A loci or the alleles in LD represent gain-of-function variants that increase plasma LDL-C levels by increasing NPC1L1 transcript numbers and the amount of NPC1L1 available for transport to the plasma membrane? Regarding NPC1L1 expression, Davis et al. (42) showed that the proximal small intestine of the NPC1L1^{-/-} mice fed standard chow diet contained ~80% of wild-type levels of NPC1L1 mRNA, suggesting that haploinsufficiency in enterocytes induces a compensatory rise in transcription from the intact allele. Nonetheless, the mice had lower plasma sitosterol (37% reduction, P = 0.05) and campesterol (50%, P = 0.05) concentrations, presumably attributable to reduced NPC1L1 abundance and, in turn, NPC1L1-mediated sterol uptake, although neither of these parameters was measured. Moreover, in marked contrast to wild-type mice, NPC1L1^{-/-} animals were resistant to a high-cholesterol diet (1% cholesterol, 0.5% sodium cholate for seven days); thus, their plasma cholesterol levels remained virtually the same as when they consumed the standard chow diet. Moreover, they accumulated less cholesterol in both their bile (7.36 ± 0.58 μmol/ml versus 11.1 ± 1.01 μmol/ml, P < 0.05) and liver (79% lower, P < 0.01) than the control mice.
receiving the same cholesterol-enriched diet. Although these results support the premise that human NPC1L1 variants could modulate LDL-C levels by directly affecting NPC1L1 transcript numbers, their translatability, or both, other data reveal a disconnect between determinants of NPC1L1 gene expression (55, 56) and NPC1L1 protein levels (57). Thus, in a crossover study involving 22 men with modestly raised LDL-C (i.e., >50th percentile value for their age (38.1 ± 9.8 years), it was found that atorvastatin (12 weeks, 40 mg/day or placebo) increased intestinal NPC1L1 expression by 18.7% (P = 0.03), as judged by RNA concentrations in duodenal biopsy samples. However, the atorvastatin-induced increases in NPC1L1 transcript numbers were not correlated with changes in NPC1L1 protein values (average increase 33.5% [not significant]).

Notwithstanding, plasma levels of sitosterol and campesterol, two surrogate markers of intestinal cholesterol absorption, were markedly increased (69.7% and 64.7%, respectively; both P < 0.0001), as were intestinal RNA levels of HMGCR (59%), LDL receptor (LDLR) (52%, Fig. 4) and proprotein convertase subtilisin/kexin type 9 (PCSK9) (187%, Fig. 4). As such, it would be interesting to establish the nature of the molecular events mediating the atorvastatin-induced increases in intestinal NPC1L1 and HMGCR expression, as well as how these relate to cholesterol/sterol concentrations in human enterocytes and the cellular distribution of the NPC1L1 protein (Fig. 2).

Efflux functions of ABCG5/8

The ABCG5/8 locus became the focus of attention when two groups (58, 59) showed that mutations in either ABCG5 or ABCG8 cause the rare recessive disorder sitosterolemia (SSL) [OMIM #210250]. Subsequently, it was shown that ABCG5 and ABCG8 were required to form a heterodimer in the endoplasmic reticulum (ER) to reach the surfaces of cultured cells and mouse hepatocytes (60). Moreover, ABCG5 and its obligate partner ABCG8 require bile salt micelles to mediate cholesterol efflux from dog gall-bladder epithelial cells (61). The study of ABCG5(−/−) (29) and ABCG8(−/−) (30) only mice further confirmed that null alleles of either gene are sufficient to cause sitosterolemia.

Understanding the efflux functions of the ABCG5/8 heterodimer requires consideration of its cellular expression pattern and the biochemical milieu to which such cells are exposed (Fig. 1). Northern blotting showed that gene expression is largely confined to human small intestine and liver (58) and that immunocytochemistry resolves the specific cellular and subcellular distribution of the corresponding protein within these organs. ABCG5 and ABCG8 are restricted to small intestinal enterocytes, hepatocytes, and biliary epithelium (two organs but three distinct cell-types) in both mouse (60) and human (62). More specifically, mouse small intestinal enterocytes express ABCG5 (ABCG8 not studied) on their absorptive brush border, as well as in a cytoplasmic punctuate fashion (60). Human enterocytes exhibit the same apical staining for ABCG5 and ABCG8, although the former has an additional diffuse cytoplasmic component (62).

In mouse hepatobiliary tissues, data on cellular expression patterns of ABCG5 and ABCG8 protein remain incomplete. By immunofluorescence microscopy, native ABCG5 protein epitopes are certainly expressed on the hepatocyte apical (i.e., biliary canaliculi) membranes. However, the argument for ABCG8 hepatocytic colocalization is based upon visualization of specific epitopes in a transgenic strain expressing 14 copies of human ABCG5 and ABCG8. Issues of biliary ductal expression were not addressed (60). In man, immunocytochemical data on hepatobiliary cellular expression patterns of the two transporters are more comprehensive and comprehensible, largely tallying with mouse findings. Thus, both proteins could be identified in hepatocytes framing biliary canaliculi, with ABCG5 dominating and displaying a more restricted apical distribution, whereas ABCG8 was expressed on the plasma membrane generally and probably intracellular membrane systems as well. Bile ductular and gall bladder epithelia both immunocytochemically expressed ABCG5 and ABCG8 (62). Cell culture and derivative cDNA studies provide further evidence of distinct biliary epithelial and hepatocytic expression (60, 63).

Kinetic studies performed on sitosterolemia patients and their parents (25, 26, 64, 65) and, more recently, mice (31, 66) have helped adduce the sterol efflux functions of ABCG5/8. For instance, in an early study, Salen and colleagues (26) measured weekly, over a 10-week period, sterol absorption and turnover rates in a 28-year-old female sitosterolemic patient and her obligate heterozygote parents, all of whom received control diets containing ~500 mg/day cholesterol and 100 mg/day sitosterol. Both the patient and her parents absorbed more cholesterol (patient, mother, father, and controls, respectively, 62 ± 7.6%, 59 ± 6.7%, 84 ± 9%, and 48 ± 4%) and sitosterol (34 ± 6%, 15 ± 10%, 17 ± 8%, and 5 ± 4%) than control participants, but despite these increases, only the proband displayed notably increased plasma cholesterol (233 ± 12, 210 ± 26, 194 ± 14, and 185 ± 6 mg/dl), sitosterol (21.0 ± 2.0, 0.95 ± 0.17, 0.36 ± 0.09, and 0.22 ± 0.20 mg/dl) and apoB (131, 77, 72, and 69 mg/dl) levels. Thus, while both parents displayed near-normal capacities to eliminate sterols, especially noncholesterol sterols, from their bodies, their sitosterolemic daughter did not.

Investigations on wild-type (66), ABCG5(−/−) (29), ABCG8(−/−) (30), and ABCG5/8-deficient mice (31) have produced data largely concordant with human findings. Thus, Igel et al. (66) examined sterol metabolism in wild-type mice that had received an intragastric dose of plant oil, containing rough equal quantities of three deuterated sterols and their corresponding 5α-stanoles. They found the mice rapidly (~15 min) internalized all of the lipids with similar efficiencies, as judged by the sterol/stanol:cholesterol ratios in their upper intestinal enterocytes (i.e., campesterol:cholesterol 0.91 ± 0.05, sitosterol:cholesterol 0.75 ± 0.11, cholesterol:cholesterol 0.70 ± 0.08, campestanol:cholesterol 0.52 ± 0.08, and sitostanol:cholesterol 0.41 ± 0.08). However, thereafter, marked differences occurred. First, in the elimination of sterols and stanoles from enterocytes (sitostanol >
In marked contrast to wild-type mice, ABCG5/8-deficient animals retain noncholesterol sterols in both their plasma and liver (i.e., sitosterol, 85 and 116×; campesterol, 14 and 16×; and cholestanol, 2 and 1.5× relative to wild-types animals). Cholesterol levels, in comparison to the human sitosterolemia (59, 67–71, 73, 76, 79, 81–84). In most kindred, the mutations definitively reside on both copies of either one gene or the other, but not on one chromosome of each. A few of the mutations are over-represented in certain ethnic groups (e.g., ABCG5, R389H in Japanese, Chinese (71, 85), ABCG8, W361X in Europeans (45, 59, 81), implying founder effects, but otherwise, each mutation is confined to one or two kindred. Many of the mutations are predicted to encode truncated ABCG5 or 8 polypeptides, resulting in the loss of their single nucleotide binding domain, putative transmembrane domain (six α-helices), glycosylated extracellular loop (∼70 amino acids), or short, highly conserved cytosolic C-terminal tail. Some (mutations) are missense, including five affecting the ATP binding cassette motif of either ABCG5 or ABCG8 and, thus, the presumed coupling of ATP hydrolysis to sterol transport (61, 86, 87). Others appear to impair the formation of stable G5G8 heterodimers and their subsequent trafficking out of the ER (88). A few may affect the ABCG5/8 sterol binding sites, which have yet to be mapped.

ABCG5/8 genetics

Below, we summarize the clinical presentation of sitosterolemia, the spectrum of reported ABCG5/8 mutations, and the phenotype of the carrier state (Table 2), as well as the associations among relatively common ABCG5/8 variants, plasma sterol profiles, and risk of gallstone disease and coronary heart disease (CHD; Table 1).

**Sitosterolemia mutations.** Clinically, sitosterolemia (also known as phytosterolemia) manifests either in children as tender and tenuous xanthomas (67–71) or in young adults with severe CHD attributable to massive accumulation of sterols and stanols in monocyte-derived macrophages (25, 68, 72–75). Other clinical manifestations include arthralgia and intermittent arthritis ascribed to sitosterol deposits (45, 70); liver disease (45); and hematological abnormalities (45, 69, 76, 77), including abnormally shaped, fragile erythrocytes and large platelets. In one patient, adrenal insufficiency was present (76).

Biochemically, sitosterolemia is characterized by raised (~30×) plasma levels of plant (e.g., sitosterol, the main

dietary sterol, campesterol, and stigmasterol), their 5α-saturated derivatives (e.g., 5α-cholestanol and 5α

-sitostanol) and shell-fish sterols (22-dehydrocholesterol, brassicasterol, C-26 sterol, and 24-methylene cholesterol) (25, 72, 78). Plasma cholesterol levels may also be severely elevated, especially in children (58, 59, 71, 79). At the tissue level (except brain), sterols and stanols accumulate in a range of cell types in approximately the same ratio as present in blood (72, 80).

So far, ~40 different ABCG5 or G8 mutations have been described in 65+ sitosterolemia families (59, 67–71, 73, 76, 79, 81–84). In most kindred, the mutations definitively reside on both copies of either one gene or the other, but not on one chromosome of each. A few of the mutations are over-represented in certain ethnic groups (e.g., ABCG5, R389H in Japanese, Chinese (71, 85), ABCG8, W361X in Europeans (45, 59, 81), implying founder effects, but otherwise, each mutation is confined to one or two kindred. Many of the mutations are predicted to encode truncated ABCG5 or 8 polypeptides, resulting in the loss of their single nucleotide binding domain, putative transmembrane domain (six α-helices), glycosylated extracellular loop (~70 amino acids), or short, highly conserved cytosolic C-terminal tail. Some (mutations) are missense, including five affecting the ATP binding cassette motif of either ABCG5 or ABCG8 and, thus, the presumed coupling of ATP hydrolysis to sterol transport (61, 86, 87). Others appear to impair the formation of stable G5G8 heterodimers and their subsequent trafficking out of the ER (88). A few may affect the ABCG5/8 sterol binding sites, which have yet to be mapped.

**Table 2.** ABCG8 haploinsufficiency and ABCG5 missense mutation increase net sterol absorption in a Micronesian population

<table>
<thead>
<tr>
<th></th>
<th>Noncarriers</th>
<th>ABCG8 p.Q24HfsX8 Carriers</th>
<th>ABCG5 p.D450H Carriers</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency (%)</td>
<td>~</td>
<td>11.1</td>
<td>1.8</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Campesterol:cholesterol (~cholesterol absorption)</td>
<td>1.33 ± 0.52</td>
<td>2.00 ± 0.87</td>
<td>2.78 ± 1.01</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Lathosterol:cholesterol (~cholesterol synthesis)</td>
<td>1.52 ± 0.67</td>
<td>1.33 ± 0.55</td>
<td>0.84 ± 0.38</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>165.48 ± 34.35</td>
<td>170.29 ± 34.48</td>
<td>167.55 ± 34.92</td>
<td>0.022*</td>
</tr>
</tbody>
</table>

Data (e.g., plasma campesterol:cholesterol ratio) are from up to 2,819 individuals (83, 89).

*P = difference between all genotype groups.

*P = difference between ABCG5 p.Q24HfsX8 carriers and noncarriers only.
this variant has greater impact on plasma noncholesterol sterol levels than the more common and truncating (i.e., null) mutant ABG5 allele (Table 2). Furthermore, inferred endogenous cholesterol synthesis rates are more markedly decreased in ABG5 missense mutation carriers than in ABG5 null carriers, which may explain why mean plasma cholesterol values in islanders with the ABG5 missense allele are midway between those islanders with no ABG5/8 mutation and the ABG5 null allele carriers (Table 2).

Historical but more direct evidence of reduced cholesterol synthesis in sitosterolemia patients (as well as heterozygote carriers) derives from the era preceding genetic diagnosis (64, 90–93). Nguyen and colleagues (93), for example, showed that hepatocytes from two affected sisters (ages 27 and 29 years) contained 24% less cholesterol than control subjects, as well as barely detectable levels of HMGCR mRNA. HMGCR protein (0.18 ± 0.04 versus 1.43 ± 0.41 units/mg cell protein) and activity (15.0 ± 2.0 versus 98.1 ± 28.8 pmol/mg protein/min) were also markedly reduced. It seems, however, that certain cell types may compensate for any potential shortfall in hepatic-derived cholesterol by increasing uptake of their exogenous supplies, as both peripheral blood mononuclear cells and hepatocytes themselves exhibited substantial increases in LDL-C high-affinity (receptor-mediated) binding (253 versus 95.1 ± 8.2 ng/mg). Moreover in the follow-up study (91) involving an additional sitosterolemic sister, neither colesterol ( bile acid sequestrant) nor ileal bypass surgery (aimed at lowering sterol absorption and stimulating bile acid synthesis) increased de novo cholesterol synthesis, as measured in mononuclear leukocytes. Rather, receptor-mediated uptake of LDL was increased.

Opposing effects of ABG8 variants on CHD and gallstone disease risk. Prior to GLGC (32), three small studies had indicated that the D19H ABG8 variant (or allele in strong LD (e.g., rs41360247), represents a gain-of-function (94–96) as 19H carriers had plasma sterol profiles suggestive of increased sterol efflux plus a compensatory rise in de novo cholesterol synthesis (Table 1). Accordingly, in the GLGC cohort, 19H allele ownership translates into only a modest reduction in LDL-C levels (Table 1). In another GWAS (97), an allele in strong LD (r^2 = 0.93) with the 19H ABG8 allele (not genotyped) displayed association with markedly lower plasma noncholesterol sterol levels and, more importantly, decreased CHD risk (Table 1).

The strong association of ABG8 19H variant (or allele in LD) with gallstone disease was originally detected in German patients suffering from all types of stones and even more convincingly for stones in which cholesterol was the major component (Table 1) (98). Since then, associations between 19H ownership and gallstone disease have been reported in Chileans (98), Romanians (99), Swedes (100), Taiwanese (101), Indians (102), and the Danish general population (103). Tantalizingly, in Indian patients, 19H ownership was associated with higher cholesterol levels in bile (102). Sadly, in this study, noncholesterol sterols were not measured.

Genetic data for two further ABG8 alleles (rs4299376, rs4245791) also point to the clinical importance of the ABG8/8 transporter in regulating long-term, whole-body sterol homeostasis (Table 1). In the GLGC cohort, the rare allele at rs4299376 (in strong LD with the rare allele at rs4245791) was associated with increased LDL-C and 40% lower levels of hepatic ABG8 mRNA (P = 0.009). Similarly, in the case-control study of Teupser et al. (97), the proxy for this allele displayed association with increased plasma phytosterol and cholesterol, and increased CHD risk.

In summary, the convergence of biological and genetic data indicate that the rare allele at ABG5/8 single-nucleotide polymorphism (SNP) site rs41360247 (or allele[s] in LD; e.g., ABG8 19H), increases sterol efflux, thereby lowering plasma phytosterols and LDL-C levels, and CHD risk (but increased risk of gallstone disease). Conversely, the rare allele at SNP site rs4245791 (or allele in LD) is associated with increased plasma phytosterol concentrations, modestly raised LDL-C, and increased CHD risk.

Downstream CYP7AI variant associated with increased LDL-C levels

Variant rs2081687, located ~14 kb 3′ of CYP7AI, is the first to be robustly associated with LDL-C levels in the population at large (Table 1). Even if this phenotype arises from a so-far undiscovered variant(s) in strong LD with rs2081687, rather than the variant itself, the genetic architecture of this genomic region suggests the causative lesion will reside outside the structural gene, its complex 5′ promoter elements, and 3′ untranslated region (UTR) (104, 105). Hence, an unsuspected, distant 3′ sequence may regulate CYP7AI expression and, in turn, cholesterol conversion to bile acids (Fig. 1).

HMGCR variant associated with LDL-C levels

It seems to have been known for a considerable time, albeit anecdotally, that plasma cholesterol levels are abnormally low (i.e., <5th percentile for age and sex) in patients with cholesterol biosynthetic disorders such as Smith-Lemli-Opitz syndrome (106). However, it has only recently become apparent that a HMGCR variant (rs384662) within intron 13 is associated with modest reductions in plasma LDL-C levels (comparable to ABG8 LD-Lowering variant) in the population at large (Table 3). This discovery originated with a GWAS, which identified association among three HMGCR variants in strong LD with the intron 13 variant and LDL-C levels in Kosraen Polynesians (107). Subsequent analyses replicated the association in three white (107), two Japanese (108) and the GLGC cohort populations In the Japanese, the intron 13 variant is also associated with increased risk of myocardial infarction (Table 3).

It turns out that the intron 13 variant rs384662 modulates splicing efficiency of HMGCR pre-mRNA, culminating in the production of fewer naturally occurring nonfunctional transcripts (i.e., minus exon 13 and catalytic residues). Hence, in vivo this variant is envisaged to
increase HMGCR activity and cholesterol synthesis, producing a compensatory reduction in LDL-C uptake to maintain cellular cholesterol homeostasis (107).

NEW INSIGHTS INTO APOB-CONTAINING LIPOPROTEIN ASSEMBLY AND SECRETION

In the sections that follow, we provide an update on apoB and the microsomal triglyceride transfer protein (MTP), while acknowledging that the assembly of apoB-containing lipoproteins serves two complementary functions: (i) the delivery of lipids to distant cells and (ii) the removal of excess lipids that might otherwise lead to disease (e.g., liver cirrhosis). Another section highlights a potentially new function for angiopoetin-like protein 3 (ANGPLT3) and reports on the association of a DOCK7/ANGPLT3 variant with both LDL-C and triglyceride (TG) levels. Other sections focus on the intracellular trafficking of nascent chylomicron and VLDL. The last section summarizes the emerging evidence that the tribbles homolog 1 (Trib1) increases VLDL production through enhanced de novo lipogenesis. Although, helpful for understanding the hypcholesterolemia that develops in familial hypobetalipoproteinemia (FHBL) and the hypocholesterolemia that develops in de novo lipogenesis. Although, helpful for understanding the hypcholesterolemia that develops in apoB-specific familial hypobetalipoproteinemia (FHBL) and ANGPLT3-specific combined hypolipidemia, it is beyond the scope of this review to cover the array of molecular processes regulating presecretory degradation of nascent apoB-containing lipoproteins (109-111).

**ApoB structures and lipoprotein production**

The specific structures required to initiate the cotranslational assembly of apoB-containing lipoproteins (Fig. 3) are formed by the N-terminal region (βA1) of apoB (amino acids 1-782; apoB17) (112-114), whereas sequences beyond apoB19.5/22 (i.e., amino acids 884/1000) supply the lipid binding structures for forming a stable, secretion-competent, lipoprotein particle (115, 116). These sequences include amphipathic β-strand and α-helix structures in the B1 (apoB20-apoB41; amino acids ~908 to ~1860) and α2 (apoB42-48; amino acids ~1900-2152) domains of apoB, respectively, and in particular sequences between apoB32 and apoB41 (~1450-1860), which have a marked ability to recruit triglyceride into assembling lipoproteins (117). The amphipathic β-strand structures formed by apoB37-41 (amino acids 1694-1880) and apoB56-78-82 (amino acids 2571-4000) also appear to irreversibly anchor apoB to the lipid cores of VLDL and LDL (114, 118).

**Table 3. “Functional” HMGCR variant associated with increased LDL-C levels**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sample (Reference)</th>
<th>Typing Variant (Proxy)a</th>
<th>Positionb (Effect)</th>
<th>Allele (MAF)</th>
<th>Effect on LDL-C</th>
<th>Other Associated Traits</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMGCR</td>
<td>2,946 inhabitants of Micronesian island of Kosrae, plus1464 type 2 diabetes cases plus 1,467 controls (Diabetes Genetics Initiative data) (107)</td>
<td>1) rs3846662</td>
<td>c.1722+45A&gt;G</td>
<td>G(0.40)</td>
<td>G/G Kosraens +11.6 mg/dl versus A/A (P &lt; 2 × 10^-6 for combined dataset, P &lt; 1 × 10^-5).</td>
<td>Total cholesterol (combined dataset, P &lt; 2 × 10^-6).</td>
</tr>
<tr>
<td>GLGC (52)</td>
<td>2 Japanese population studies (n = 2,686 and n = 2,110) (108)</td>
<td>1) rs12916 (rs384662)</td>
<td>c.*372T&gt;C</td>
<td>C(0.39)</td>
<td>+2.45 mg/dl per copy (Z = 14.079, P = 5 × 10^-45)</td>
<td>Total cholesterol +2.84 mg/dl per copy (Z = 14.365, P = 9 × 10^-45).</td>
</tr>
</tbody>
</table>

a Independent variants/associations are numbered. "Proxy" variant is in strong linkage disequilibrium with genotyped variant.

b Relative to the initiating ATG or stop codon (*).

c Minor allele is listed with frequency (MAF) in study population. 95% CI, 95% confidence interval; OR, odds ratio.
manifestations that may develop for any given apoB mutation. We also note that more apoB missense mutations within the apoB-lipoprotein-initiating domain (after case studies) may emerge as patients with fatty liver and/or hypocholesterolemia are screened for such variants.

**CASE STUDY 1.** A Caucasian male infant was hospitalized at 4 months of age for investigation of failure to thrive with diarrhea, intolerance to fat-rich meals, and marked hypocholesterolemia (Table 4). Molecular analyses confirmed his diagnosis (Table 4), identifying two different mutations, both of which almost certainly blocked the initiation of apoB-containing lipoprotein assembly in all cell types. At 10 months, he was treated with medium-chain triglycerides, resulting in weight gain and decreased steatorrhea. However, he developed micronodular cirrhosis, portal hypertension, and esophageal varices by age 11, plus severe neurological deficiencies that included ataxia, dysarthria, severe impairment of position and vibratory sensation, and absent deep tendon reflexes. His neurological symptoms were stabilized by intramuscular and intravenous vitamin E therapy. However, in his last three years of life he suffered seizures, a cerebrovascular accident, recurrent massive gastrointestinal hemorrhages, and finally cardiac arrest at age 18.

**CASE STUDY 2.** A 4-year-old Tunisian girl was born from consanguineous parents (121). She had a long history of diarrhea, intolerance to fat-rich meals, and marked hypolipidemia (Table 4). Histological and biochemical analyses revealed fat-laden enterocytes, resulting from a homozygous mutation that removed >95% of apoB’s lipid binding structures. Her obligate heterozygote parents had greatly reduced total cholesterol and LDL-C levels (Table 4), potentially attributable to either decreased production or increased catabolism of VLDL-apoB100, LDL-apoB100, or both (122–124). The parents did not clinically manifest fat malabsorption. However, when Hooper et al. (125) formally tested the postprandial fat responses of other similarly asymptomatic, heterozygote, FHBL subjects (apoB100/apoB6.9, n = 3; apoB100/apoB25.8, n = 1; apoB100/apoB40.3, n = 2), their plasma triglyceride and apoB-48 levels peaked earlier than in the controls (n = 10), and these peaks were markedly reduced in magnitude. Thus, these findings support the anecdotal reports of intestinal manifestations suggestive of mild fat malabsorption in apoB100/apoB6.7 (126) and apoB100/B8.2 (127) individuals, when they refrained from their fat-restricted diet. By contrast, normal postprandial fat-absorption responses were found in heterozygous apoB48.4 and apoB76 FHBL subjects (128, 129), implying that only those FHBL heterozygotes with apoBs shorter than apoB-48 have a reduced capacity to assemble dietary lipids into chylomicrons.

**CASE STUDY 3.** A 21 year-old Caucasian woman from consanguineous parents was investigated because of chronic fatty diarrhea (130). Inspection of jejunal and ileal biopsies revealed accumulation of fat vacuoles. Acanthocytes were present, but the patient displayed neither neuropathy nor retinopathy. Laboratory analyses revealed trace amounts of plasma apoB27.6 associated with lipoprotein
TABLE 4. Monogenic disorders of lipoprotein assembly and secretion: representative lipid and lipoprotein levels

| Disorder/Gene/CS (Reference) | Subject (Age)
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mutation State</td>
</tr>
<tr>
<td></td>
<td>TC, LDL-C, TG, HDL-C, ApoB, TC:HDLC ratio</td>
</tr>
</tbody>
</table>

FHBL/APOB/CS1 (120)  
Proband (15)  
Mother (40)  
Father (45)  
Brother (14)  
Proband (4)  
Mother (27)  
Father (40)  
Brother (1)  
Proband (21)  
Mother  
Brother  
Father  
Brother  
Proband (57)  
Son  
Son  
Proband (48)  
Proband (10)  
Proband (55)  
Father (37)  
Brother (6)  
Proband (77)  
Son  
Proband (15)  
Brother (37)  
Daughter (26)  
Proband (c1)  
Mother  
Father  
Sister  
Proband (13)  
Mother (36)  
Father (37)  
Brother (2)  
Proband (10)  
Mother (39)  
Father (43)  
Brother (1)  
Proband (10)  
Mother  
Father  
Sister  
Proband (2)  
Mother (37)  
Father (35)  
Brother (5)  
Proband (23)  
Familial Combined Hypolipidemia/APOB/ANGPTL3 (170)  
Familial Combined Hypolipidemia/APOB/ANGPTL3 (170)  
Familial Combined Hypolipidemia/APOB/ANGPTL3 (170)  
Familial Combined Hypolipidemia/APOB/ANGPTL3 (170)  
Familial Combined Hypolipidemia/APOB/ANGPTL3 (170)  
Familial Combined Hypolipidemia/APOB/ANGPTL3 (170)  
Familial Combined Hypolipidemia/APOB/ANGPTL3 (170)  
Familial Combined Hypolipidemia/APOB/ANGPTL3 (170)  
Familial Combined Hypolipidemia/APOB/ANGPTL3 (170)  
Familial Combined Hypolipidemia/APOB/ANGPTL3 (170)  
Familial Combined Hypolipidemia/APOB/ANGPTL3 (170)  

**Notes:**  
- Age in years.  
- Predicted effect of mutations is specified in parentheses.  
- Levels ~0 were undetectable; "NA" indicates that measurements were not available.  
- TC: HDL-C ratio in CMD and ANGPTL3-specific familial combined hypolipidemia > TC:HDL-C ratio in APOB-specific FHBL.  
- CS, case study. Proband are highlighted in bold.
particles sized between normal LDL and HDL, plus a homozygous splice site mutation compatible with the observed truncated apoB species (Table 4). As seen in other heterozygote apoB-specific FHBL individuals, her mother, brother, and sister were clinically well and had less than half the expected plasma concentration of cholesterol, triglycerides, and apoB (Table 4). The patient received vitamin A and E supplementation, which may have contributed to her developing liver fibrosis two year later (130).

**Case study 4.** The proband, a 57-year-old Japanese mother homozygous for an apoB mutation predicted to truncate apoB after amino acid 1755 (apoB38.7), was referred for evaluation of a liver mass (131). She had no history of diabetes or steatorrhea, and plasma vitamin E levels were within the normal range. However, she was severely hypocholesterolemic (Table 4). Blood studies showed she was anemic, with blood film acanthocytosis. She also had proteinuria, extensive calcification of the major arteries, and cholelithiasis (see case study 6). Her medical history was significant for asthma, night blindness, and type II diabetes, all diagnosed at age 36 and managed by diet and insulin. At age 48, she had a retinal hemorrhage attributed to hemorrhagic glaucoma, resulting in right eye blindness, and at age 54, she developed hemoptysis secondary to bronchiectasis. Retinal pigmentation, hard exudates, and extensive photocoagulation scars were noted in both optic fundi. Neurological examination revealed no abnormal pyramidal, cerebellar, or posterior column abnormalities but she did have paraesthesia in both hands (“stocking-glove” type hypoaesthesia, absent deep tendon reflexes in the lower extremities, and positive Romberg’s sign).

**Case study 5.** Homozygous FHBL was diagnosed in a 48-year-old Caucasian mother (three normal pregnancies) following routine cholesterol screening (132). She denied any unusual dietary habits, symptoms of fat malabsorption, night blindness, or neurological or visual impairment. She was found to have a homozygous nonsense mutation that retained the apoB sequences required to form triglyceride-rich lipoproteins (Table 4) and the anchoring of apoB to its lipid core (114, 118). Accordingly, in marked contrast to her severe hypercholesterolemia, her plasma triglyceride, apoA1, HDL-C and vitamin E levels were within the normal range.

**Case study 6.** A 9-year-old patient presented with epigastric pain, cholestatic jaundice, and acute cholecystitis (133). Liver ultrasound revealed grossly dilated common bile ducts due to obstruction by a gallstone, with many more within her gallbladder. Laboratory analyses led to a diagnosis of heterozygous apoB-specific FHBL (Table 4) in her and her clinically well mother. This case study reminds us that gallstone disease is not uncommon in heterozygous apoB-specific FHBL patients (apoB90/40 (134); apoB100/apoB46 (135); apoB100/83 (136)) and that it may relate, at least in part, to their near-normal levels of intestinal cholesterol absorption (i.e., 47.5 ± 6.3 versus 53.8 ± 11.7% in controls (137, 138)).

**Case study 7.** A 77-year-old proband was investigated for marked hypercholesterolemia following routine cholesterol screening (139). Laboratory analysis revealed that he and his son were heterozygous for apoB70.5 and homozygous for apoE2 (Table 4). Moreover, they had the classical features of dysbetalipoproteinemia superimposed onto heterozygous apoB-specific FHBL. Thus, their fasting plasma samples contained apoB48 in the triglyceride-rich fraction plus β-migrating VLDL, indicative of impaired clearance of apoE-containing lipoproteins (Fig. 5).

A full understanding of the effects of apoB truncations on organ cholesterol homeostasis is hampered by incomplete (by contemporary standards) phenotypic and genetic characterization of apoB-specific FHBL patients. This is unfortunate because it is clear that such mutations (i) affect lipid homeostasis in multiple cell types (e.g., cardiomyocytes (140), granulocyte cells (141), T cells (142), renal tubular epithelial cells (143)) and could contribute to multiple cell phenotypes (case study 5); (ii) may coexist with another inherited disorder (especially within consanguineous pedigrees); and (iii) may be influenced by the ever-present individual variation in compensatory mechanisms, as well as different apoE genotypes (case study 7 (144), Fig. 5). In this respect, three reports have shown that VLDL production is reduced in apoE deficiency (145, 146) and apoE2 homozygosity (147). However, in vitro data have indicated that the expansion of the neutral lipid core of VLDL in the Golgi apparatus promotes apoE binding (148-150) and that this apolipoprotein is not required for VLDL maturation (149).

Arguably, insights into the molecular consequences of apoB-specific FHBL are best gleaned from model systems. Of these, the human apoB38.9 mouse model provides important data (151). In these mice, hepatic cholesterol levels were normal, despite impaired hepatic lipid export into the circulation via VLDL. However, hepatic ABCG5/8 mRNA levels were increased, suggesting that cholesterol efflux into the bile was enhanced (Fig. 1). Additionally, mRNAs levels of cholesterol synthetic genes were decreased, as was hepatic cholesterol synthesis. LDLR mRNA was also decreased, indicative of reduced hepatic cholesterol uptake. Hence, it seems that the apoB38.9 mice called upon multiple mechanisms to maintain hepatic cholesterol homeostasis. However, and in keeping with the human situation, where fatty liver is a common complication of apoB-specific FHBL (152, 153), these mice were less successful in dealing with their reduced capacity to export triglycerides via VLDL (154); both the heterozygous and homozygous animals developed fatty liver.

Indeed, a diagnosis of apoB-specific FHBL should be considered whenever a patient is found to have fatty liver and LDL-C levels <75 mg/dl. In our series of 110 such patients, 51 had apoB100-truncating mutations (152, 153) and 6 had heterozygous missense variants (A31P, G275S, L324M, R463W [n = 3]) within the apoB lipoprotein-initiating domain (Fig. 3). In fact, the R463W mutation was originally identified in the homozygous state in a 47-year-old Christian-Lebanese woman (155) following detection of marked hypercholesterolemia during a routine lipid screen (Table 4), and it was shown to block the assembly/secretion of both apoB48- and apoB100-containing lipoproteins in...
in vitro (155). In vivo, R463W patients accumulate fat in their intestinal enterocytes and exhibit a blunted postprandial rise in blood lipid levels (156), despite their apparent absence of clinically significant intestinal fat malabsorption (153, 155, 156).

**Multiple common APOB variants modulate LDL-C levels**

The GLGC study (Table 5) (32) lends support to the two propositions put forward by Benn et al. (157). First, relatively common NS coding sequence variants predicted to alter the structure of the apoB-lipoprotein-initiating domain (e.g., T1171 in mature protein) contribute to the population variance in apoB, total, and LDL-cholesterol levels. Second, an additional set of independent apoB alleles modulate LDL-C levels, potentially by increasing apoB transcription. The GLGC results also indicate that causative apoB variants have a larger impact on LDL-C levels than common NPC1L1 and ABCG8 lesions.

TABLE 5. Multiple common genetic determinants influencing LDL-C levels through the ApoB-lipoprotein assembly and secretion axis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sample (Reference)</th>
<th>Typed Variant (Proxy)</th>
<th>Position (Effect)</th>
<th>Allele (MAF)</th>
<th>Effect on LDL-C</th>
<th>Other Associated Traits</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOB</td>
<td>Prospective study of 9,185 Danish individuals (157)</td>
<td>1) rs13561177 c.293C&gt;T (T71H)</td>
<td>T/T (0.33)</td>
<td>T/T +11.69 mg/dl versus C/C, (P &lt; 0.001)</td>
<td>Total cholesterol, apoB</td>
<td>Total cholesterol, apoB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) rs10199768 c.2817–1293&gt;C&gt;A</td>
<td>C (0.30)</td>
<td>C/A – 3.09 mg/dl versus T/4, (P &lt; 0.01)</td>
<td>Total cholesterol, apoB</td>
<td>Total cholesterol, apoB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3) rs693 c.7545C&gt;T (T2488T)</td>
<td>T (0.48)</td>
<td>T/T + 9.67 mg/dl versus C/C, (P &lt; 0.001)</td>
<td>Total cholesterol, apoB</td>
<td>Total cholesterol, apoB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4) rs1801701 c.10913G&gt;A (R3611Q)</td>
<td>A (0.09)</td>
<td>G/A + 4.25 mg/dl versus G/G, (P &lt; 0.01)</td>
<td>Total cholesterol, apoB</td>
<td>Total cholesterol, apoB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5) rs1042031 c.12541G&gt;A (E4154K)</td>
<td>A (0.17)</td>
<td>A/A – 6.96 mg/dl versus G/G, (P &lt; 0.05)</td>
<td>Total cholesterol, apoB</td>
<td>Total cholesterol, apoB</td>
</tr>
<tr>
<td>GLGC</td>
<td>(32)</td>
<td>1) rs13561177 c.293C&gt;T (T71H)</td>
<td>T (0.30)</td>
<td>+ 0.05 mg/dl per copy (Z = 22.7, P = 4 × 10⁻¹¹)</td>
<td>Total cholesterol, +4.16 mg/dl per copy (Z = 29.003, P = 4 × 10⁻¹⁶), Triglyceride (Z = 6.25, P = 4 × 10⁻¹⁰)</td>
<td>Total cholesterol, Z = 19.518, (P = 8 × 10⁻¹⁰) Triglyceride (Z = 8.382, P = 5 × 10⁻⁷)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) rs10199768 c.2817–1293&gt;C&gt;A</td>
<td>A (NA)</td>
<td>Z = 21.388 (P = 2 × 10⁻¹⁰)</td>
<td>Total cholesterol, apoB</td>
<td>Total cholesterol, apoB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3) rs693 c.7545C&gt;T (T2488T)</td>
<td>T (NA)</td>
<td>Z = 20.022 (P = 4 × 10⁻⁹)</td>
<td>Total cholesterol, apoB</td>
<td>Total cholesterol, apoB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4) rs1801701 c.10913G&gt;A (R3611Q)</td>
<td>A (NA)</td>
<td>Z = 6.838 (P = 8 × 10⁻¹²)</td>
<td>Total cholesterol, apoB</td>
<td>Total cholesterol, apoB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5) rs1042031 c.12541G&gt;A (E4154K)</td>
<td>A (NA)</td>
<td>Z = -9.982 (P = 2 × 10⁻²⁵)</td>
<td>Total cholesterol, apoB</td>
<td>Total cholesterol, apoB</td>
</tr>
<tr>
<td>ANGPT1</td>
<td>GLGC (32)</td>
<td>1) rs3850634 ~13 kb upstream (intrinsic DOCK7)</td>
<td>G (0.32)</td>
<td>-1.59 mg/dl per copy (Z = -8.796, P = 3 × 10⁻¹⁰)</td>
<td>Total cholesterol, apoB</td>
<td>Total cholesterol, apoB</td>
</tr>
<tr>
<td>SORT1</td>
<td>1,132 Jamaicans of African ancestry (210)</td>
<td>1) rs12740374 ~35 kb downstream</td>
<td>G (0.26)</td>
<td>β = 0.25, P = 4.6 × 10⁻⁸</td>
<td>Total cholesterol, apoB</td>
<td>Total cholesterol, apoB</td>
</tr>
<tr>
<td></td>
<td>Japanese population study (n = 21,010) (211)</td>
<td>G (0.08)</td>
<td>-4.7 mg/dl per copy (P = 3 × 10⁻¹¹)</td>
<td>Total cholesterol, apoB</td>
<td>Total cholesterol, apoB</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GLGC (32)</td>
<td>G (0.22)</td>
<td>-5.65 mg/dl per copy (Z = 27.854, P = 1 × 10⁻¹⁰)</td>
<td>Total cholesterol, apoB</td>
<td>Total cholesterol, apoB</td>
<td></td>
</tr>
<tr>
<td>TRIB1</td>
<td>Danish population study (n = 50,309) (215)</td>
<td>1) rs2954029 ~40 kb downstream</td>
<td>T (0.48)</td>
<td>T/T – 4.25 mg/dl versus A/A (P &lt; 0.001)</td>
<td>Total cholesterol, apoB</td>
<td>Total cholesterol, apoB</td>
</tr>
<tr>
<td></td>
<td>Japanese population study (n = 21,010) (211)</td>
<td>G (0.48)</td>
<td>-1.7 mg/dl per copy (β = -0.04, P = 1 × 10⁻⁷)</td>
<td>Total cholesterol, apoB</td>
<td>Total cholesterol, apoB</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GLGC (32)</td>
<td>G (0.47)</td>
<td>Z = -11.176, (P = 5 × 10⁻²⁰)</td>
<td>Total cholesterol, apoB</td>
<td>Total cholesterol, apoB</td>
<td></td>
</tr>
</tbody>
</table>

* Independent variants/associations are numbered. "Proxy" variant is in strong linkage disequilibrium with genotyped variant.

a Relative to the initiating ATG.

b Minor allele is listed with frequency (MAF) in study population.

c APOB mutations are numbered according to Benn et al. (157) rather than current Human Genome Variation Society nomenclature.

NA, not available.
Update on the role of MTP function in cholesterol homeostasis

MTP mutations are the major, if not sole, cause of the rare recessive condition abetalipoproteinemia (ABL; [OMIM #200100], Fig. 3) (158). This condition is biochemically characterized by exceedingly low plasma levels of cholesterol plus triglyceride, as well as a total absence of all apoB-containing lipoproteins (Table 4). Its differential diagnosis from homozygous apoB-specific FHBL includes “normal” lipid levels in the parents, and exceedingly low plasma triglyceride levels. For information regarding the full spectrum of MTP mutations in ABL, the clinical manifestations, and management of this debilitating disorder, see Refs. 159–161. Here, we review the role of MTP in governing cholesterol homeostasis, and we note that the GLGC study detected no genome-wide significant associations between MTP variants and LDL-C levels (32).

The role of apoE in delivering cholesterol to specific body sites in ABL is largely unknown. An early study involving 9 patients with clinical features of this condition and 50 controls (162) revealed that the patients had increased concentrations of both plasma apoE (44.8 ± 8.2 µg/ml versus 36.3 ± 11.1 µg/ml, P < 0.025) and an apoE cholesterol-enriched HDL subfraction (~1.5-fold) that had the capacity to compete with 125I-LDL for specific binding sites on the surface of cultured human skin fibroblasts. Moreover, the apoE genotype status of these patients was not determined (Fig. 5). Subsequently, an analysis of the plasma decay curves of injected radiolabeled autologous lipoproteins (125I-ApoAI and 125I-ApoE) in the HDL density range of 2 unrelated ABL patients and 22 control individuals revealed that the overall catabolism of apoE was increased by ~25% in the patients, as was apoE production. Moreover, the catabolism of apoAI in apoE-containing HDL occurred at a faster rate than that in HDL without apoE, potentially explaining the lower plasma apoAI levels in ABL patients (163). Thus, quantitatively, neither the catabolic nor the production rate of apoE in ABL is impaired, despite the lack of apoB-containing lipoprotein assembly.

Two studies suggest that cholesterol biosynthesis may be increased in ABL, although note that these analyses were performed on individuals with no confirmatory molecular diagnosis. In the first study, cholesterol synthesis was increased by ~2-fold compared with aged-matched controls (164), with the kinetic data conspicuously indicating that the increased synthesis was largely “driven” by enhanced fecal loss of intestinal cholesterol (i.e., both dietary and biliary). In the second study, Kuriyama et al. (165) showed that their patient had nearly 2-fold higher blood levels of dolichols (synthesized through the cholesterol biosynthetic pathway) than control individuals. Additionally, the dolichols were largely (~80%) associated with HDL in both the patient and control subjects.

Recent studies on two different mouse models of MTP deficiency also contribute to our understanding of the different ways that the MTP:PDI heterodimer may influence organ cholesterol levels (166, 167). Xie et al. (167) produced mice with (conditional) intestine-specific deletion of the major, but not minor, Mtpg species (168). They found that mean cholesterol absorption was reduced by 70% in the knockout animals and that enterocyte Mtpg mRNA levels were positively correlated with cholesterol absorption (r² = 0.69). They inferred from their studies that, in mice, as much as 30% of intestinal cholesterol reached the circulation via an apoB/MTP-independent mechanism. Of potential significance, they also found that enterocytes from their Mtpg-deficient mice contained control levels of cholesterol and, not surprisingly, increased HMGCR and Acat2 mRNA levels. Liver HMGCR mRNA was also increased (by ~4-fold) and ex vivo hepatic cholesterol synthesis was similarly elevated. Despite these increases, their mean liver cholesterol content was marginally decreased (~10%), most likely as a result of increased VLDL secretion and cholesterol efflux into bile, given that hepatic ABCG5/8 mRNA were increased. In the second study, Iqbal et al. (166) first showed that the livers of mice deficient for both intestinal and hepatic Mtpg contained 132% more unesterified cholesterol than did control animals and, unexpectedly, a 90% reduction (rather than increase) in cholesteryl esters. Next, they established that the MTP:PDI heterodimer very likely enhances cellular cholesterol esterification through its transfer of ACAT reaction products (i.e., cholesteryl esters) from their site of synthesis onto assembling apoB lipoproteins, leading them to speculate that ACAT-product inhibition may serve to prevent excessive accumulation of neutral lipids in ER membranes and thus to maintain cellular integrity. On the other hand, they noted that the observed increase in free cholesterol in their Mtpg-deficient animals could explain the toxicity associated with pharmacological inhibition of MTP activity.

In summary, both humans (164, 165, 169) and mice (166, 167), data indicate that the levels of blood/tissue cholesterol/cholesteryl esters in ABL reflect both the classically taught defective production of apoB-containing lipoproteins, which restricts cholesterol absorption (dietary and biliary-derived), as well as enhanced biliary cholesterol secretion and de novo cholesterol biosynthesis in multiple cell types. It should be emphasized, however, that point-for-point comparison of mouse and human is unlikely ever to be achieved, and some important differences may exist.

ANGPTL3-specific familial combined hypolipidemia

In both man (170) (Table 4) and mouse (171, 172), compound heterozygous/homozygous angiopoietin-like 3 (ANGPTL3) mutations have been associated with a low LDL-C phenotype. The human study involved exome sequencing of two siblings from a large European family exhibiting a combined hypolipidemia phenotype of extremely low plasma cholesterol, LDL-C, HDL-C, and triglyceride levels (Table 4). Adopting an analysis strategy that considered the combined hypolipidemia phenotype in this pedigree to be autosomal recessive in inheritance led to identification of a single bi-allelically mutated gene, ANGPTL3 (Table 4). However, close inspection of the lipid profiles in this family suggests an additive (rather than recessive) genetic model for both the low LDL-C and
triglyceride traits, prompting Kathiresan and colleagues (170) to remind us that hepatic (171, 173) and secreted (174) ANGPTL3 perform distinct functions. In the liver, it acts to promote the production/secretion of apoB-containing lipoproteins whilst inhibiting lipoprotein clearance; the ANGPTL3 mutation carriers in the Kathiresan family displayed decreased rates of VLDL-apoB production (compound heterozygotes: 8.6 ± 0.1 mg/kg/day; heterozygote:16.4 ± 4.9 mg/kg/day; no mutations: 26.9 ± 4.6 mg/kg/day, P = 0.001, additive model) and elevated fractional catabolism of LDL-apoB (compound heterozygotes: 0.52 ± 0.013 pools/h; heterozygote 0.045 ± 0.01 pools/h; no mutations: 0.027 ± 0.004 pools/h, P = 0.005, additive model). In comparison, in the circulation ANGPTL3 inhibits the activities of lipoprotein lipase (175, 176) and endothelial lipase (177) and modulates triglyceride and HDL-C levels.

Importantly, two population-based studies support the ANGPTL3/LDL-C connection. Thus, sequencing of ANGPTL3 in Dallas Heart Study participants (n = 3,551) showed that carriers of frame shift mutations (n = 12) had lower LDL-C levels than did noncarriers (median 77.5 mg/dl versus 104 mg/dl in noncarriers, P = 0.03). Additionally, plasma triglyceride levels were lower (median 72 mg/dl versus 96 mg/dl, P = 0.08). In the GLGC sample, the DOCK7/ANGPTL3 variant rs2131925 displayed robust association with plasma cholesterol, LDL-C, and triglyceride (Table 5).

Chylomicron retention disease

Chylomicron retention disease (CMRD; also known as Anderson disease) ([OMIM #246700], Table 4) is a rare autosomal recessive disorder caused by mutations of SARA2. It typically presents in infancy with failure to thrive in association with severe fat malabsorption (178–183). Biochemically, CMRD is characterized by a selective absence of apoB48 in plasma, very low levels of total cholesterol, LDL-C, and HDL-C, plus substantially reduced apoB100 and A1 (Table 4). Plasma triglyceride levels, in comparison, tend to fall within the normal range, which differs from ABL and the genetically severe forms of hereditary hypertriglyceridemia (184, 185). Golgi complex examination on total cholesterol and LDL-C expression on total cholesterol and LDL-C. For an up-to-date description of the subclinical phenotypes found in CMRD patients, see Refs. 185 and 186.

The cause of the exceptionally low plasma cholesterol levels in CMRD is still not understood. An early study (187), performed on six CMRD children (mean age 8 ± 3 years), two to three years after their placement on a diet restricted in long-chain triglycerides and supplemented with medium-chain triglycerides, revealed that they manifested low levels of both LDL-C (38.0 ± 19.4 versus 110.8 ± 20.5 mg/dl) and HDL-C (12.8 ± 2.3 versus 59.0 ± 11.3 mg/dl). By contrast, VLDL-C (27.3 ± 11.0 versus 13.0 ± 5.7 mg/dl) and plasma triglyceride (155 ± 38 versus 65 ± 17 mg/dl) levels were increased. This increase was ascribed to their substantially decreased postheparin LPL and hepatic lipase activities, as they had larger VLDL than the control children. In comparison, the CMRD children had smaller LDL, and these contained more (2.5-fold) triglyceride and less (2.5-fold) cholesteryl esters. Their HDL particles were also smaller (i.e., HDL₃ size) and contained decreased (3.4-fold) amounts of cholesteryl esters. Importantly, the molecular diagnosis of many of these patients was confirmed in a subsequent study (188).

Another challenge in understanding the pathogenesis of CMRD is why functional compensation for Sar1b deficiency is not provided by Sar1a, a protein with which it shares tissue and subcellular distribution, as well as ~90% amino acid homology (188–190). It is apparent from structural studies that most of the amino acid differences between the two Sar1 isoforms reside on one surface of these two small GTPases at some distance from their GDP/GTP binding sites (188, 190). Moreover, Sar1 exchanges GDP for GTP on the cytosolic side of the ER membrane, and this exchange initiates COPII-coated vesicle assembly (191). Sar1-GTP hydrolysis also regulates assembling COPII-vesicle fission, along with its captured cargo (192, 193).

On the basis of structural (194, 195), organ bath (187), histological (179), and of course, clinical data, the current thinking is that Sar1b is required to promote the transport of nascent chylomicrons out of the ER in specialized COPII-transport carriers. Moreover, CMRD patients homozygous for SARA2 null mutations (described in two families) may produce few, if any, of these putative specialized COPII-transport carriers, whereas patients with mutations affecting the GDP/GTP binding site of Sar1b (majority of affected families) may retain some ability to initiate the assembly of such carriers at the site of chylomicron production but not their fission. As such, they also lead to retention of precursor chylomicrons in the ER.

Sortilin: post-ER trafficking of ApoB-containing lipoproteins

Sortilin, the archetypal member of a mammalian family of receptors defined by a unique vacuolar protein-sorting 10 domain (196), contains a single transmembrane and a short cytoplasmic domain composing the typical motifs that mediate intracellular trafficking events (197–200) and endocytosis (201–203). Its RNA is expressed at high levels in several tissues and cell types, including skeletal muscle, heart, and adipose tissue, but it is expressed at relatively low levels in the liver (204).

Two groups have investigated how human genetic variation at the chromosome 1p13 locus (205, 206), specifically, Sortilin 1 (Sort1), might influence plasma LDL-C levels (Table 4). Rader and colleagues (206) showed that in mice Sort1 overexpression and knockdown decreased and increased, respectively, total plasma cholesterol and LDL-C levels, which, at least in part, were attributable to changes in VLDL-apoB100 secretion. Independently, the Nykaer group (205) presented evidence that sortilin interacts with apoB100 in the medial- to trans-Golgi compartment, presumably to determine the fate of nascent apoB100-containing lipoproteins: presecretory degradation or secretion (Fig. 3) (109–111).

Paradoxically - and important to resolve - is why the Nykaer (205) and Rader (206) groups observed opposite effects of Sort1 expression on total cholesterol and LDL-C
levels. There is reason to think that part of the answer may reside in the different targeting strategies used by the two groups to “knock out” Sort1, especially as, contrary to expectation, both Sort1-deficient mice were born viable and showed no gross abnormalities (207, 208). In the “Rader” mice, two 5′ sites were targeted (i.e., intron 1/exon 2, which encodes the N-terminus of the Vps 10 domain; plus intron 3) (208), and knockout was deemed successful based on the absence of detectable sortilin in cell lysates from the brain, liver, lung, and testis of the knockout animals. In the “Nykjaer” mice, inactivation was “achieved” by targeting exon 14, thereby disrupting the reading frame of Sort1 after amino acid 3564, leaving open the possibility that their mice may produce a C-terminally truncated form of Sort1.

Consistent with their knockout animals, the Rader group found that liver-specific Sort1 knockdown in chow-fed “humanized” mice (i.e., Apob48-null/high-apoB100 expressers) increased total plasma cholesterol (46%) and LDL-C (−220%) levels (206). Similar, but less dramatic, results were seen in other mouse models (e.g., Apobec1−/−/Ldr−/−), but sadly, no data were presented for apoB48-only mice, which could have served as a useful control given the data from Nykjaer and colleagues (205) that sortilin may only interact with apoB100. On the basis of ex vivo measurements, the observed dyslipidemia in the “Rader” Sort1−/− mice was ascribed to increased VLDL secretion.

Important differences in study design could also explain the reported diametrically opposed effects of high-level Sort1 expression on plasma LDL-C levels. Rader and colleagues (206) used “humanized” apoB100-only transgenic mice models (i.e., comparable plasma cholesterol to man through increased apoB-lipoprotein assembly), while the Nykjaer group (205) used wild-type mice that express both apo48 and 100 in liver and have ∼4× lower plasma cholesterol levels than man. The two groups also used different adenoviruses to deliver Sort1. Focusing on the Rader experiments, Sort1 was delivered to chow-fed mice via an adenovirus vector known to appropriately target genes for specific expression in the liver. Compared with the “humanized” mice receiving the null adenovirus construct, Sort1 overexpression led to dramatic drops in total plasma cholesterol: 70% and 46% at weeks 2 and 6, respectively. These reductions were paired with a 73% reduction in LDL-C (at the 2-week time point) and markedly fewer medium-small and very small LDL particles, with the peak LDL-particle size increasing from 20.9 nm to 22.0 nm (P = 0.05). The Sort1 construct also induced reductions in total plasma cholesterol and LDL-C in other mice models (e.g., Apobec1−/−/APOB100−/−/Ldlr−/−, −44%, −70%; Apobec1−/−/Ldlr−/−, −26%, −29%) at the 2-week time point. Moreover, in all mouse models examined, high-level Sort1 expression decreased VLDL secretion (range 30-70%). Potentially pertinent, LDL-C uptake was increased in HEK 293 cells transiently transfected with Sort1 (209).

Sort1 genetics. Sort1 resides in a 98 kbp genomic interval containing multiple SNPs that associate with both reduced plasma LDL-C levels (Table 5) and a reduced risk of myocardial infarction in multiple populations, including Europeans (32), Africans (210), and Japanese (211). However, SNPs displaying the strongest association reside at some considerable distance from the 3′ end of Sort1 (Table 5) and actually closer to MYBPHL, PSRC1, and CELSR2, which, respectively, encode myosin binding protein H-like protein, a microtubule destabilizing protein and a non-classic-type cadherin. Consequently, >1 causal variant(s) may underlie the LDL-C and myocardial infarction association signals, with the causative allele(s) impacting differently, both quantitatively and qualitatively, and in a cell-specific fashion to influence these two phenotypes. In two independent series of liver samples, the Rader group (206) found that the rare allele at tagging SNP rs646776 (Table 5) was associated with ∼10-fold higher levels of PSRC1 and SORT1 mRNA (P values for PSRC1: 2 × 10−271, 9 × 10−7; SORT1: 2 × 10−380, 1 × 10−41), a finding that both prompted and is consistent with their rodent Sort1 study results. In comparison, no SNP and transcript associations were detected in adipose tissue.

Trib1: primary role in hepatic lipogenesis modulates VLDL production

Trib1, a homolog of Drosophila tribbles, is evolutionarily conserved in mouse and humans (212). It expression is ubiquitous and certainly includes the liver (213) and coronary arteries (214). Indeed, preliminary data indicate that Trib1 expression is elevated in the coronary arteries of patients with advanced CHD (214).

Drosophila tribbles regulates String activity and, hence, mitosis during ventral furrow formation (212). Much more interesting, however, for the present discussion, is the Trib1 modulation of mouse liver levels of mRNA encoding three key enzymes of lipogenesis (acytetyl-CoA carboxylase, fatty acid synthase, and stearoyl-CoA desaturase 1) and of plasma cholesterol and triglyceride concentrations (213). Specifically, when Burkhhardt et al. (213) induced high-level Trib1 expression in mice livers (i.e., using the appropriate AAV8 adenovirus vector), plasma cholesterol and triglyceride levels fell by ∼45% and ∼57%, respectively. Similar decreases were also observed in Ldlr−/− animals (27% and 34%), as well as in humanized, transgenic apoB100 mice on a background of LDLR-haploinsufficiency (25% and 26%). Furthermore, in these mice, the Trib1-induced reductions in cholesterol and triglyceride were paired with reduced apoB (23%), indicative of an overall reduction in the number of plasma apoB-containing lipoproteins. In comparison, Trib1-deficient mice had elevated plasma cholesterol (54%) and triglyceride (33%) levels and increased numbers of circulating VLDL and LDL particles. Importantly, the triglyceride phenotype was largely reversed by high-level hepatic Trib1 expression (via use of adenovirus construct), suggesting that the elevated triglyceride levels in the Trib1-deficient animals was specifically attributable to the lack of hepatocyte Trib1 mRNA. Moreover, restoration of Trib1 expression decreased VLDL-triglyceride production to control levels.
The association of mouse Trib1 overexpression or under-expression with plasma cholesterol and triglyceride levels is clear-cut, but the underlying mechanisms are much less so. The data imply modulation of lipogenic gene expression influences the amounts of lipid available for VLDL secretion (Fig. 3), but understanding the relative importance of each enzyme affected, and how, must await further research.

Downstream TRIB1 variants associated with decreased LDL-C. Table 5 shows that the variants assigned to TRIB1 reside at some distance from its 3′ end (32, 211, 215). However, results in mice make this assignment plausible, especially as the variant (or allele[s] in LD) is associated with both plasma cholesterol and triglyceride levels. Indeed, in a Japanese study, the estimated effect on LDL-C and triglyceride levels returned exactly the same standardized coefficient (0.04) (211). However, the demonstration that TRIB1 mutations cause either combined hyperlipidemia or FHBL (gain-of-function) in non-apoB/PCSK9/ANGPTL3 patients would help reinforce the importance of TRIB1 in the lipid arena.

RECEPTOR-MEDIATED CATABOLISM OF LDL-PARTICLES

All known causes of monogenic hypercholesterolemia, whether it be autosomal dominant (ADH) or recessive hypercholesterolemia (ARH) involve mutations that block cellular cholesterol uptake via the LDLR pathway (Fig. 4, Tables 6, 7). Starting with familial hypercholesterolemia (FH or ADH-1, [OMIM #606945]), five major classes of LDLR mutations can be discerned through functional assays that probe the integrity of production and the individual cellular properties of the encoded gene product (216–219). These are (i): production of the 893 amino acid protein; (ii) its transport to the cell surface; (iii) ligand binding at neutral pH; (iv) delivery of LDLR (constitutive endocytosis) and LDLR/ligand complexes to endosomes, where acidic conditions and low free calcium concentration promote ligand release (220); and (v) recycling of LDLR to the cell surface for further rounds of lipid uptake. The all-important mechanisms regulating the egress of lipoprotein-released cholesterol from late...
endosomes and lysosomes to other cell compartments (221–223) are beyond the scope of this review.

One of the following sections provide a critique of what is and isn’t known about the binding of LDL-apoB to the LDLR; the identities of mutations known to cause familial defective apoB (FDB or ADH-2, [OMIM #144010]); and the common NS coding sequence variants that might regulate LDL-C levels through effecting an LDL-apoB100:LDLR interaction(s). Another section covers the ARH gene product LDLR-associated protein 1 (LDL-RAP1), a clathrin-associated sorting protein that ushers, in a cell-specific fashion, LDLR-LDL complexes into clathrin-coated structures (Fig. 4), describing the clinical and phenotypic (Table 6) presentation of this rare recessive

### TABLE 6. Monogenic disorders of LDL-cholesterol clearance: representative phenotypes

<table>
<thead>
<tr>
<th>Reference</th>
<th>Disorder (Gene)</th>
<th>Mutation*</th>
<th>N</th>
<th>Age (years)</th>
<th>TC (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>CHD (%)</th>
<th>Comment/Differential Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>(253)</td>
<td>ADH-1(LDLR)</td>
<td>Hm-null</td>
<td>14</td>
<td>13 ± 12</td>
<td>826 ± 125</td>
<td>768 ± 125</td>
<td>31 ± 7</td>
<td>129 ± 37</td>
<td>71</td>
<td>ADH-1 Hm-null most severe. ARH</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hm-defective</td>
<td>26</td>
<td>28 ± 16</td>
<td>660 ± 108</td>
<td>604 ± 106</td>
<td>31 ± 9</td>
<td>114 ± 46</td>
<td>62</td>
<td>similar to ADH-1 Hm-defective.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ARH p.P22X and/or p.H144QpX26</td>
<td>42</td>
<td>31 ± 13</td>
<td>629 ± 95</td>
<td>566 ± 94</td>
<td>44 ± 8</td>
<td>97 ± 34</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>(300)</td>
<td>ADH-1(LDLR)</td>
<td>Ht-null</td>
<td>43</td>
<td>56 ± 13</td>
<td>432 ± 64</td>
<td>351 ± 64</td>
<td>54 ± 15</td>
<td>138 ± 61</td>
<td>42</td>
<td>Hi-defective is less severe than Ht-null</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ht-defective</td>
<td>13</td>
<td>54 ± 15</td>
<td>354 ± 51</td>
<td>277 ± 45</td>
<td>52 ± 14</td>
<td>128 ± 62</td>
<td>15</td>
<td>(lipids and CHD incidence).</td>
</tr>
<tr>
<td>(378)</td>
<td>ADH-1(LDLR)</td>
<td>Ht-null</td>
<td>48</td>
<td>42 ± 14</td>
<td>404 ± 86</td>
<td>326 ± 84</td>
<td>51 ± 16</td>
<td>116 ± 27</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ht-defective</td>
<td>62</td>
<td>43 ± 19</td>
<td>379 ± 78</td>
<td>296 ± 79</td>
<td>55 ± 14</td>
<td>118 ± 13</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>(380)</td>
<td>ADH-1(LDLR)</td>
<td>Ht-null</td>
<td>172</td>
<td>11 ± 31</td>
<td>247 ± 49</td>
<td>247 ± 49</td>
<td>73 ± 49</td>
<td>73 ± 49</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ht-defective</td>
<td>202</td>
<td>11 ± 31</td>
<td>298 ± 22</td>
<td>298 ± 22</td>
<td>71 ± 22</td>
<td>71 ± 22</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>(381)</td>
<td>ADH-1(LDLR)</td>
<td>Ht p.R3500Q</td>
<td>11</td>
<td>9 ± 5</td>
<td>305 ± 15</td>
<td>237 ± 5</td>
<td>51 ± 13</td>
<td>75 ± 62</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ht p.R3500Q</td>
<td>124</td>
<td>10 ± 5</td>
<td>258 ± 19</td>
<td>193 ± 19</td>
<td>72 ± 19</td>
<td>72 ± 19</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ht p.R3500Q</td>
<td>2</td>
<td>59 ± 6</td>
<td>391 ± 18</td>
<td>327 ± 5</td>
<td>56 ± 15</td>
<td>NA</td>
<td>50</td>
<td>2 families.</td>
</tr>
<tr>
<td></td>
<td>ADH-3(PCS)9</td>
<td>Ht p.S127R</td>
<td>18</td>
<td>34 ± 14</td>
<td>365 ± 58</td>
<td>277 ± 58</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>2 families.</td>
</tr>
<tr>
<td>(382)</td>
<td>ADH-3(PCS)9</td>
<td>Ht p.D374Y</td>
<td>6</td>
<td>24 ± 14</td>
<td>416 ± 97</td>
<td>350 ± 98</td>
<td>48 ± 13</td>
<td>199 ± 47</td>
<td>0</td>
<td>2 families.</td>
</tr>
<tr>
<td>(383)</td>
<td>ADH-3(PCS)9</td>
<td>Ht p.D374Y</td>
<td>13</td>
<td>21 ± 15</td>
<td>326 ± 112</td>
<td>425 ± 159</td>
<td>46 ± 15</td>
<td>151 ± 44</td>
<td>46</td>
<td>4 families.</td>
</tr>
<tr>
<td>(390)</td>
<td>ADH-3(PCS)9</td>
<td>E207K/p.R3500Q</td>
<td>11</td>
<td>10 ± 10</td>
<td>649 ± 58</td>
<td>583 ± 34</td>
<td>NA NA</td>
<td>NA</td>
<td>100</td>
<td>Mutations have an additive effect.</td>
</tr>
<tr>
<td>(301)</td>
<td>ADH-3(PCS)9</td>
<td>p.A408H/p.R3500Q</td>
<td>12</td>
<td>12 ± 12</td>
<td>340 ± 62</td>
<td>162 ± 46</td>
<td>54 ± 15</td>
<td>154 ± 15</td>
<td>0</td>
<td>4 families.</td>
</tr>
<tr>
<td>(302)</td>
<td>ADH-3(PCS)9</td>
<td>p.A696H/p.R3500Q</td>
<td>6</td>
<td>27 ± 16</td>
<td>509 ± 111</td>
<td>446 ± 110</td>
<td>38 ± 13</td>
<td>111 ± 58</td>
<td>0</td>
<td>4 families.</td>
</tr>
<tr>
<td>(343)</td>
<td>ADH-3(PCS)9</td>
<td>p.A228T/p.R496W</td>
<td>1</td>
<td>35 ± 5</td>
<td>580 ± 518</td>
<td>518 ± 50</td>
<td>50 ± 6b</td>
<td>50 ± 6b</td>
<td>100</td>
<td>Mutations have an additive effect.</td>
</tr>
<tr>
<td></td>
<td>ADH-3(PCS)9</td>
<td>p.A408H/p.R496W</td>
<td>1</td>
<td>62 ± 8</td>
<td>487 ± 405</td>
<td>405 ± 405</td>
<td>70 ± 61</td>
<td>70 ± 61</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Age and lipid levels are means ± standard deviation where applicable. Hm, homozygous; Ht, heterozygous.

* Effect of mutation on protein is given.

** A subset of individuals with receptor-null alleles had greater mean carotid intima-media thickness, a marker for atherosclerosis and future cardiovascular outcome, than those with receptor-defective alleles (difference, 0.020 ± 0.01 mm; P = 0.01) (379).

Incidence of CHD relates to family rather than to individual.

Level measured while patient treated with lipid-lowering medication.

Father died of early myocardial infarction.

Individuals heterozygous for both mutations.

### TABLE 7. Influence of APOE and APOB polymorphisms upon LDL-C levels in heterozygous ADH-1 patients

<table>
<thead>
<tr>
<th>Reference</th>
<th>Age Group*</th>
<th>Modifier</th>
<th>N</th>
<th>LDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(253)</td>
<td>Children</td>
<td>e2e3</td>
<td>13</td>
<td>205 ± 30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>e3e3</td>
<td>36</td>
<td>254 ± 48</td>
</tr>
<tr>
<td>(254)</td>
<td>Adults</td>
<td>e2e2/e2e3</td>
<td>47</td>
<td>258 ± 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>e3e3</td>
<td>425</td>
<td>282 ± 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>e2e4/e3e4/e4e4</td>
<td>98</td>
<td>309 ± 7</td>
</tr>
<tr>
<td>(252)</td>
<td>Adults</td>
<td>e2e2/e2e3</td>
<td>288</td>
<td>299 ± 11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>e3e3</td>
<td>311</td>
<td>311 ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>e3e4/e4e4</td>
<td>313</td>
<td>313 ± 8</td>
</tr>
<tr>
<td>(254)</td>
<td>Adults</td>
<td>APOB c.516 C/C</td>
<td>392</td>
<td>280 ± 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>APOB c.516 T/T</td>
<td>162</td>
<td>291 ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>APOB c.516 T/T</td>
<td>16</td>
<td>351 ± 16</td>
</tr>
</tbody>
</table>

* Children < 15 years old.

** Total number of study individuals=288.

Biological and genetic classification of LDL dyslipidemias 1903
### TABLE 8. Lipoprotein clearance: genetic determinants affecting LDL- and total-cholesterol levels

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sample (Reference)</th>
<th>Typel Variant (Proxy)</th>
<th>Position (Effect)</th>
<th>Allele (MAF)</th>
<th>Effect on LDL-C</th>
<th>Other Associated Traits/Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>APOE</strong></td>
<td>1,943 white participants in longitudinal CARDIA study (258) Prospective study of 2767 white-British men (384) Meta-Analysis of 8 individual European GWAS (385) GLGC (32, 377)</td>
<td>1) rs35136575</td>
<td>~27 kb downstream</td>
<td>G (0.26)</td>
<td>-8.66 mg/dl G/G versus C/C (P=0.001)</td>
<td>ApoB, +11 mg/dl G/G versus A/A (P &lt; 0.01); Triglyceride, +10.6 mg/dl G/G versus A/A (NS) Triglyceride, $\beta = 0.042^2$ $P = 6 \times 10^{-7}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) rs4420638 (rs429358)</td>
<td>~10 kb downstream</td>
<td>G (0.19)</td>
<td>+20.5 mg/dl G/G versus A/A (P&lt;0.01) (versus e2 and e3 carriers)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) rs4420638 (rs429358)</td>
<td>~10 kb downstream</td>
<td>G (0.18)</td>
<td>$\beta = 0.056^2; P = 2 \times 10^{-4}$ (versus e2 and e3 carriers)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) rs4420638 (rs429358)</td>
<td>~10 kb downstream</td>
<td>G (0.17)</td>
<td>+7.14 mg/dl per copy ($Z = 25.801, P = 9 \times 10^{-6}$) (versus e2 and e3 carriers)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prospective study of 2767 white-British men (384)</td>
<td>3) APOE e2/3/e4 genotype</td>
<td>NA</td>
<td>NA</td>
<td>+17 mg/dl e3/e3 versus e2 carriers; +5 mg/dl e4 carriers versus e3/e3 (P&lt;0.01)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10,035 men and 12,134 women in EPIC study (386)</td>
<td>3) APOE e2/3/e4 genotype</td>
<td>NA</td>
<td>NA</td>
<td>Men: e4/e4, +7.8 mg/dl versus e3/e3 and +30.9 mg/dl versus e2/e2 (P&lt;0.001)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3) APOE e2/3/e4 genotype</td>
<td>NA</td>
<td>NA</td>
<td>Women: e4/e4, +23.2 mg/dl versus e3/e3 and +57.9 mg/dl versus e2/e2 (P&lt;0.001)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>968 Canadians of South Asian, Chinese or European descent (387)</td>
<td>3) APOE e2/3/e4 genotype</td>
<td>NA</td>
<td>NA</td>
<td>e4 carriers, +35 mg/dl (South Asians), +17 mg/dl (Chinese), +22 mg/dl (Europeans) versus e2 carriers (P&lt;0.01)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,507 postacute coronary syndrome patients randomized to pravastatin or atorvastatin treatment (388)</td>
<td>3) APOE e2/3/e4 genotype</td>
<td>NA</td>
<td>NA</td>
<td>Pravastatin: e2 carriers, 53.8% reduction in LDL-C versus 48.1% e3/e3 and 46.4% e4 carriers (P=0.0004), e2 carriers, 22.1% reduction in LDL-C versus 21.8% e3/e3 and 16.6% e4 carriers (P=0.0004), Mean baseline LDL-C levels: e2 carriers, 100.0 ± 2.1 mg/dl; e3/e3, 115.9 ± 1.1 mg/dl; e4 carriers, 118.7 ± 1.7 mg/dl (P = 1.5 × 10^{-6})</td>
<td></td>
</tr>
<tr>
<td><strong>LDLR</strong></td>
<td>German population-based cohorts (total n = 8819) (273) GLGC (32)</td>
<td>1) rs2228671</td>
<td>~94 kb upstream</td>
<td>A (0.45)</td>
<td>-7.34 mg/dl per copy ($P = 1.5 \times 10^{-10}$)</td>
<td>Total cholesterol, $Z = 20.89, (P = 7 \times 10^{-10}$)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1) rs6511729 (rs2228671)</td>
<td>c.81C&gt;T (C27C)</td>
<td>T (0.11)</td>
<td>-7.69 mg/dl per copy ($Z = -23.004, P = 4 \times 10^{-15}$)</td>
<td>Total cholesterol, $Z = 9.046, (P = 1 \times 10^{-4}$)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) rs688</td>
<td>c.1773G&gt;T (N591N)</td>
<td>T</td>
<td>Z = 11.342 ($P = 8 \times 10^{-8}$)</td>
<td>Total cholesterol $-1.22 \text{ mg/dl per copy (}P = 4 \times 10^{-15}$)</td>
</tr>
</tbody>
</table>
TABLE 8. Continued.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sample (Reference)</th>
<th>Typed Variant (Proxy)</th>
<th>Position/Effect</th>
<th>Allele (MAF)</th>
<th>Effect on LDL-C</th>
<th>Other Associated Traits/Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCSK9</td>
<td>1,750 African American participants in longitudinal CARDIA study (389)</td>
<td>rs28362263</td>
<td>c.1327G&gt;A (A443T)</td>
<td>A (0.10)</td>
<td>−9.2 mg/dl in carriers versus noncarriers (baseline levels) (P &lt; 0.01)</td>
<td>Total cholesterol −10.3 mg/dl in carriers versus noncarriers (P &lt; 0.01)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs28362286</td>
<td>c.2057C&gt;A (G679X)</td>
<td>A (&lt;0.01)</td>
<td>−33 mg/dl in carriers versus noncarriers (baseline levels) (P &lt; 0.01)</td>
<td>Total cholesterol −38.3 mg/dl in carriers versus noncarriers (P &lt; 0.01)</td>
</tr>
<tr>
<td></td>
<td>3,363 Black participants in ARIC study (390)</td>
<td>rs28362286</td>
<td>c.1327G&gt;A (A443T)</td>
<td>A (0.10)</td>
<td>−9.2 mg/dl in carriers versus noncarriers (baseline levels) (P &lt; 0.01)</td>
<td>Total cholesterol −43 mg/dl in carriers versus noncarriers (P &lt; 0.01)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs67608943</td>
<td>c.2037C&gt;A (C679X)</td>
<td>A (&lt;0.01)</td>
<td>−33.7 mg/dl in carriers versus noncarriers (baseline levels) (P &lt; 0.01)</td>
<td>Total cholesterol −38.3 mg/dl in carriers versus noncarriers (P &lt; 0.01)</td>
</tr>
<tr>
<td></td>
<td>3,363 Black participants in ARIC study (390)</td>
<td>rs28362286</td>
<td>c.1327G&gt;A (A443T)</td>
<td>A (0.10)</td>
<td>−9.2 mg/dl in carriers versus noncarriers (baseline levels) (P &lt; 0.01)</td>
<td>Total cholesterol −43 mg/dl in carriers versus noncarriers (P &lt; 0.01)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs67608943</td>
<td>c.2037C&gt;A (C679X)</td>
<td>A (&lt;0.01)</td>
<td>−33.7 mg/dl in carriers versus noncarriers (baseline levels) (P &lt; 0.01)</td>
<td>Total cholesterol −38.3 mg/dl in carriers versus noncarriers (P &lt; 0.01)</td>
</tr>
<tr>
<td></td>
<td>1,872 Italian patients with premature MI and 1,865 controls (392)</td>
<td>rs11591147</td>
<td>c.137G&gt;T (R46L)</td>
<td>T (0.01)</td>
<td>−21.2 mg/dl in carriers versus noncarriers (P = 2 × 10^-4)</td>
<td>Total cholesterol, G/G +15% versus A/A (P = 0.002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs11591147</td>
<td>c.137G&gt;T (R46L)</td>
<td>T (0.01)</td>
<td>−12 mg/dl per copy, (P = 1.6 × 10^-7)</td>
<td>Total cholesterol, G/G +15% versus A/A (P = 0.002)</td>
</tr>
<tr>
<td></td>
<td>6,882 healthy American women (age &gt; 45 years) (393)</td>
<td>rs11591147</td>
<td>c.137G&gt;T (R46L)</td>
<td>T (0.02)</td>
<td>−21 mg/dl in carriers versus noncarriers (P = 2 × 10^-4)</td>
<td>Total cholesterol, G/G +15% versus A/A (P = 0.002)</td>
</tr>
<tr>
<td></td>
<td>9,524 white participants in ARIC study (390)</td>
<td>rs11591147</td>
<td>c.137G&gt;T (R46L)</td>
<td>T (0.02)</td>
<td>−13 mg/dl in carriers versus noncarriers (P &lt; 0.0001)</td>
<td>Total cholesterol, G/G +15% versus A/A (P = 0.002)</td>
</tr>
<tr>
<td></td>
<td>1,828 white participants in longitudinal CARDIA study (389)</td>
<td>rs11591147</td>
<td>c.137G&gt;T (R46L)</td>
<td>T (0.02)</td>
<td>−15 mg/dl in carriers versus noncarriers (P &lt; 0.0001)</td>
<td>Total cholesterol, G/G +15% versus A/A (P = 0.002)</td>
</tr>
<tr>
<td></td>
<td>1,828 white participants in longitudinal CARDIA study (389)</td>
<td>rs11591147</td>
<td>c.137G&gt;T (R46L)</td>
<td>T (0.02)</td>
<td>−15 mg/dl in carriers versus noncarriers (P &lt; 0.0001)</td>
<td>Total cholesterol, G/G +15% versus A/A (P = 0.002)</td>
</tr>
<tr>
<td></td>
<td>Meta-analysis of three Danish cohorts (n = 45,699) (394)</td>
<td>rs11591147</td>
<td>c.137G&gt;T (R46L)</td>
<td>T (0.02)</td>
<td>−17 mg/dl in carriers versus noncarriers (P &lt; 0.01)</td>
<td>Total cholesterol up to 9% lower in carriers versus noncarriers (P = 2 × 10^-5). Early-onset MI OR = 0.67 [95% CI 0.46-0.97], (P = 0.036)</td>
</tr>
<tr>
<td></td>
<td>1,828 white participants in longitudinal CARDIA study (389)</td>
<td>rs11591147</td>
<td>c.137G&gt;T (R46L)</td>
<td>T (0.02)</td>
<td>−15 mg/dl in carriers versus noncarriers (P &lt; 0.0001)</td>
<td>Total cholesterol, G/G +15% versus A/A (P = 0.002)</td>
</tr>
<tr>
<td></td>
<td>401 African Caribbeans belonging to seven multigenerational pedigrees plus replication in 1,750 African-Caribbean men (395)</td>
<td>rs7517090</td>
<td>g.523+2637 G&gt;A</td>
<td>A (0.20)</td>
<td>Z = 7.008, (P = 4 × 10^-12)</td>
<td>Total cholesterol, Z = 6.182, (P = 6 × 10^-14)</td>
</tr>
<tr>
<td></td>
<td>7) rs11206510 (R46L)</td>
<td>c.2009G&gt;A (E670G)</td>
<td>G</td>
<td>G</td>
<td>Z = 6.182, (P = 6 × 10^-14)</td>
<td>Total cholesterol, Z = 6.182, (P = 6 × 10^-14)</td>
</tr>
<tr>
<td></td>
<td>8) rs2479409</td>
<td>c.2009G&gt;A (E670G)</td>
<td>G</td>
<td>G</td>
<td>Z = 1.96 mg/dl per copy, (Z = 0.136, P = 4 × 10^-4)</td>
<td>Total cholesterol, Z = 7.046, (P = 2 × 10^-14)</td>
</tr>
<tr>
<td></td>
<td>9) rs516499</td>
<td>c.1682 -363A&gt;G</td>
<td>G</td>
<td>G</td>
<td>Z = 7.046, (P = 2 × 10^-14)</td>
<td>Total cholesterol, Z = 7.046, (P = 2 × 10^-14)</td>
</tr>
</tbody>
</table>
### TABLE 8. Continued.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sample (Reference)</th>
<th>Position (Proxy)</th>
<th>Effect on LDL-C</th>
<th>Other Associated Traits/Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDOL</td>
<td>GLGC (32)</td>
<td>rs3757354</td>
<td>−1.13 mg/dl per copy</td>
<td>(Z = −3.985, P = 3 × 10⁻⁶)</td>
</tr>
</tbody>
</table>

**a** Independent variants/associations are numbered. “Proxy” variant is in strong linkage disequilibrium with genotyped variant (r²/H11091 0.8, unless indicated otherwise).

**b** Relative to the initiating ATG.

**c** Minor allele is listed with frequency (MAF) in study population.

**d** rs429358 C allele = APOE/H9255 4 allele. rs4420638 in linkage disequilibrium with rs429358 (r² = 0.967) (377). APOE/H9255 2 equivalent variant (rs7412) not genotyped in GLGC.

**e** Represents the change in lipid level (natural log) per effect allele.

**f** rs35136575, rs11591147, rs28362263, rs28362286, and rs7417090 not surveyed in GLGC.

**g** Variant not found in European populations.

**h** Signal attributed to R46L (377). Linkage disequilibrium between alleles, r² = 0.10.

5% CI, 95% confidence interval; HR, hazard ratio; NS, not significant; OR, odds ratio.

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The fourth section focuses on determinants of PCSK9-regulated recycling of LDLR to the plasma membrane (Tables 6, 8), a process that is defective in ADH-3 ([OMIM #607787], Table 6) and enhanced in PCSK9-defective FHBL (Table 9). We also point out differences in lipid phenotypes that aid the differential diagnosis of this seemingly rare condition, apoB-specific FHBL, and ANGPTL3-combined hypolipidemia (Tables 4, 9). The final section touches upon the inducible degrader of LDLR (IDOL), an E3 ubiquitin ligase that stimulates LDLR degradation through ubiquitination of its C-terminal domain, thereby limiting the cellular uptake of LDL and VLDL.

**Cataloguing LDLR mutations in ADH-1: cautionary tales and update**

The large number (n = 1100+) of LDLR variants found in ADH-1 patients prompted the development of a database (http://www.ucl.ac.uk/ldlr) (224) that contains information regarding their segregation in families, geographic origins, and predicted effects on encoded gene products (224). Importantly, the database now holds the same information for the rather fewer PCSK9 and LDLRAP1 variants found in ADH-3 and ARH patients, respectively (225).

The ADH1:3/ARH database also provides information on mutational heterogeneity across specific populations and communities. In countries such as France (226) and Holland (227), >100 different LDLR mutations cause ADH-1, whereas in other countries, only one or two mutations are responsible (228–230). For example, in Ashkenazi Jews, the LDLRc.652-654 del GGT mutation (G197del mutation in mature protein) is by far the most common cause of ADH-1 (230). In fact, genetic analyses indicate that the most recent common ancestor bearing this mutation dates back to the start of the 14th century at the founding of the Jewish community in Lithuania (230). Similarly, most (>80%) Lebanese ADH-1 patients have the c.2043 C>A (Cys660X; known as Lebanese allele) mutation (228).

It is important to emphasize that certain sequence variants entered into the ADH1:3/ARH database will have no pathogenic consequences, despite their cosegregation with hypercholesterolemia. For example, a sequence variant in the donor splice region of intron 14 (c.2140 +5 G>A) was originally deemed to be pathogenic, as it occurred in a number of ADH-1 patients from different backgrounds (e.g., Dutch, Austrian, Canadian) (231). However, subsequent studies revealed that it was present at comparable frequencies in ADH patients from the Simon Broome Register and healthy white men from the Northwick Park Heart Study II (0.022 versus 0.013, P = 0.23) (232). Moreover, RNA analysis on mononuclear cells from an ADH patient revealed that it had no effect on LDLR pre-mRNA splicing (233), whereas DNA sequencing showed that some ADH patients with this so-called mutation had, on the same chromosome, real pathogenic LDLR mutations (234). Thus, although the ADH1:3/ARH database is an extremely valuable tool for those interested...
in the molecular genetics of monogenic hypercholesterolemia, in vitro (e.g., fibroblasts and/or lymphocytes from affected patients), or solid phase binding assays (218) should be performed on variants contained therein to demonstrate their pathogenic effects.

The relatively new multiplex ligation-dependent probe amplification assay technique has greatly facilitated the identification of LDLR deletions/insertions and rearrangements in ADH-1 patients (226, 235–240). In a Polish sample, this technique revealed that duplication of exons 12 and 16 were the second most common cause of ADH-1, affecting 4.2% of the sample (226). As such, screening for large rearrangements of the LDLR is now recommended for the molecular diagnosis of ADH-1.

One of the most interesting observations on the molecular pathology of LDLR mutations concerns the pathogenic effects of apparently silent coding sequence variants, which can be easily overlooked. For instance, screening a series of patients with a definite clinical diagnosis of monogenic hypercholesterolemia led to the identification of two single-nucleotide substitutions, c.621 C>T (G186G in mature protein) and c.1216 C>A (R385R in mature protein) (241, 242), which were correctly predicted in silico to be pathogenic. Thus, the c.621 C>T transition introduces a new donor splice site, resulting in formation of a truncated product (241). Hence, it is possible that in silico analysis is also correct in predicting that other silent LDLR coding sequence variants [i.e., c.1813 C>T (L605L) and c.2140 G>C (E714E)] also cause ADH-1 through aberrant pre-mRNA splicing (226). Furthermore, we now appreciate that even mutations predicted to lead to an amino acid substitution may actually cause ADH-1 through aberrant splicing. One such example involves the c.2389 G>T transversion (last nucleotide of exon 16; V776L). Examining patients’ blood mononuclear cells revealed they produced an abnormal mRNA devoid of exon 16 (233), demonstrating the importance of functional analyses to establish the molecular mechanism by which pathogenic mutations exert their effects.

Finally, Civeira et al. (243) have highlighted the importance of screening for LDLR mutations in people who have personal and family lipid profiles indicative of familial combined hyperlipidemia (FCHL). In their series of 143 unrelated patients, which they acknowledged had an inherent selection bias toward those with more severe lipid abnormalities and therefore might not be representative of FCHL patients in the general population, the frequency of LDLR mutations was 19.6%. “FCHL” patients with a LDLR mutation (n = 28) had higher mean total cholesterol, LDL-C, and apoB and nonsignificantly lower triglyceride levels (e.g., cholesterol: 357 ± 54 versus 318 ± 56 mg/dl, P = 0.001; triglyceride 248 (217–361) versus 301 (226–464) mg/dl, P = 0.059) than those who did not. Thus, 41.7% of the 48 patients (i.e., 20) in their series with a total cholesterol or apoB >335 and 185 mg/dl, respectively, had a LDLR mutation, compared with 8.8% (i.e., 8/91) who had values below these levels. Although there is no formal proof that all of LDLR mutations found by Civeira et al. (243) were pathogenic, their study clearly demonstrates that screening for LDLR mutations should be performed in patients with a clinical diagnosis of FCHL who have very high total cholesterol and apoB levels.

Genotype-phenotype correlations in ADH-1. One of the most challenging issues in ADH-1 pathology is defining
the relationship among causative mutations, the associated biochemical disturbance (e.g., elevated LDL-C), and clinical/preclinical phenotypes, such as premature CHD, tendon xanthomas, and asymptomatic atherosclerosis. The problem arises in large part from the low frequency of each individual causative mutation encountered in genetically heterogeneous populations combined with patient ascertainment bias. One simple approach for overcoming this problem is to subdivide mutations into two broad categories: null alleles (also designated “LDLR-negative”) mutations that are expected to completely abolish LDLR functions and defective alleles (designated LDLR-defective mutations; i.e., variable, residual LDLR functional activities). Using these criteria, we compared LDL-C levels in both homozygous and heterozygous Italian patients (244). For homozygous patients, the different impact of mutation type was clearly discernible (Table 6) (245), with the LDLR-null patients having markedly higher LDL-C levels. Similarly, the heterozygous LDLR-negative subjects had higher LDL-C levels and a 2-fold higher prevalence of tendon xanthoma and CHD than did the LDLR-defective patients. Likewise, heterozygous Spaniards with a null allele (n = 269; mean age 43 years) had ~50 mg/dl higher LDL-C and ~2-fold increased prevalence of tendon xanthoma and CHD than did LDLR-defective patients (n = 162, mean age 46 years). Additionally, in this population, null allele ownership was documented to be associated with more advanced femoral atherosclerosis (246).

Intriguingly, data suggest that the differential effects of LDLR-negative and defective alleles on LDL-C levels are diminished by the β-thalassemia trait (247). Sardinians with this trait and a null LDLR allele (i.e., Fs572 mutation) had lower LDL-C than those with just the Fs572 mutation (222.7 ± 41.8 mg/dl versus 319.0 ± 64.2 mg/dl, P < 0.001). Additionally, the one ADH-1 Sardinian with this null mutation and full-blown β-thalassemia had the lowest plasma LDL-C of all (100.9 mg/dl). Similar findings were also evident when the analyses were extended to include LDLR-defective patients. Allelic heterogeneity, however, precluded an accurate estimate of the effect of the β-thalassemia trait on LDL-C levels in these patients.

Considering the biological basis of the thalassemia-LDLR interaction, two reports implicate high-erythropoietic activity, with the authors concluding that substantial amounts of cholesterol are utilized during active erythropoiesis (248, 249). Shalev et al. (249) observed that patients with chronic anemia associated with high-erythropoietic activity (e.g., thalassemia intermedia) had significantly lower plasma total cholesterol, LDL-cholesterol, and HDL-cholesterol levels (80 ± 19, 35 ± 14, 31 ± 10 mg/dl) than did patients with low-erythropoietic activity anemias (139.75 ± 15.86, 82.33 ± 17.85, 49.38 ± 12.86 mg/dl) and healthy controls (156.80 ± 35.69, 83.10 ± 32.52, 51.95 ± 10.88 mg/dl). Moreover, whereas all patients with chronic anemia and increased erythropoietic activity had hypocholesterolemia, none with low erythropoietic activity did. Hence, it is rather disappointing, particularly given the relatively high incidence of hemoglobinopathies in Tunisia (250), that the unexpectedly mild lipid phenotype in their heterozygous Tunisian ADH-I patients (total and LDL-cholesterol: 272.2 ± 54.1 and 216.6 ± 42.5 mg/dl, Table 6) was not investigated in terms of their thalassemia status, especially as homozygous Tunisian ADH-I patients have the expected severe phenotype (total and LDL-cholesterol levels of 677.5 ± 120.7 and 623.0 ± 119.5 mg/dl, respectively) (251).

As highlighted in Table 7, apoE isoforms e2 (rs7412) and e4 (rs429358) may influence the effect of LDLR mutations on LDL-C levels in both children and adults (252–254), consistent with structural (255, 256), functional (257) and nonADH-1 genetic (Table 8) data. Thus, in (apparently unrelated) heterozygous ADH-I French-Canadian children with the same 15 kb+ deletion null allele, those with an apoE2/3 genotype had markedly lower total plasma cholesterol, LDL-C, and apoB levels than apoE3 homozygotes, whereas LDL-C levels in children from the general population who had an apoE2/3 genotype were only decreased by 17.4 mg/dl (253). The ADH-I adult studies also point in the same direction as young children studies, albeit with a more modest LDL-C lowering effect (252, 254). Conversely, e4 allele (rs429358) ownership in the adult ADH-1 cohorts was associated with elevated LDL-C: 26.7 mg/dl in the Italians and 2 mg/dl in the Finnish (Table 7), consistent with population data (Table 8). By analogy, variants at the apoE/C1/C4/C2 gene cluster, other than the e2 and e4 alleles, are also likely to influence LDL-C levels in ADH-1 patients (Table 8); including one (i.e., rs35136575) modulating apo gene expression, given its location in an hepatic apoE/C1/C4/C2 control region (258).

Intriguingly, not only the magnitude but also the very direction of the effect of specific apoE isoforms on plasma cholesterol levels in response to a high-fat, Western type diet is likely to depend on a number of factors, including their interactions with prevailing levels of proteoglycans (259–261), LDLR-related protein 1 (262), and LDLR at the hepatic cell surface. Supporting evidence for this proposition derives from the study of transgenic apoE and Ldlr mice (257, 263, 264) in which native apoE was replaced by human apoE isoforms and an endogenous murine Ldlr allele was replaced by a human LDLR minigene to produce mRNA transcripts with a longer half-life. When exposed to a Western-style diet, apoE4/4 mice expressing ~2-3-fold higher levels of LDLR developed higher plasma cholesterol concentrations than those with baseline LDLR expression (187 ± 19 versus 124 ± 8 mg/dl), whereas apoE3/3 littermates displayed the expected decrease (72 ± 8 versus 114 ± 12 mg/dl; P < 0.0001 for LDLR × Apo isoform interaction). Similarly, in an independent experiment (263), the dyslipidemia of chow-fed apoE2/2 mice (plasma cholesterol 268 ± 12 mg/dl; triglyceride 157 ± 22 mg/dl) was normalized (plasma cholesterol 83 ± 4 mg/dl; triglyceride 40 ± 5 mg/dl) by constitutively high LDLR expression. Additionally, the cholesterol-elevating effect of the Western-style diet (cholesterol 546 ± 30 mg/dl) was ameliorated (172 ± 43 mg/dl). Through follow-up analyses, Maeda and colleagues (257) were able to conceptualize the counter-intuitive apoE4/4 results in terms of
glycan than either apoE/H92552 or apoE/H92553 (Fig. 5A) (261), it is poorly internalized and recycled (265). In the face of constitutively elevated LDLR expression, the effect is to reduce apoE availability for transfer to nascent chylomicrons and hepatic sequestration of apoE, chylomicron- and VLDL- remnants on the surface of hepatocytes, and their subsequent internalization. In essence, although apoE4 has a greater affinity for the LDLR and heparan sulfate proteoglycan than either apoE3 or apoE2 (Fig. 5A) (261), it is poorly internalized and recycled (265). In the face of constitutively elevated LDLR expression, the effect is to reduce apoE availability for transfer to nascent chylomicrons and...
VLDL (either directly or indirectly via HDL) that ultimately enables the cellular uptake of cholesterol-rich lipoproteins (i.e., LDL and remnant particles), for example, by the LDLR and LRP1 (Fig. 5B). ApoE2 and rE3, on the other hand, with their reduced affinity for the LDLR (Fig. 5A) are compatible with elevated LDLR expression reducing plasma cholesterol levels. The big question, of course, is how this robust model translates in man and, in particular, whether the relatively low plasma cholesterol levels seen in most (~95%) apoe2/2 individuals (Table 8) is mechanistically linked to the increased availability of this isoform for transfer to circulating lipoproteins in spite of its lower affinity (i.e., on human fibroblasts; 8.5 ± 1.0% of apoE3 (266) for the LDLR). Clearly, detailed in vivo kinetics will be crucial.

The GLGC study also substantiates our observation that a particular APOB allele (or allele in LD) has a LDL-C elevating effect in people with LDLR mutations (254). We found that heterozygote ADH-1 patients with the rare allele at SNP site rs934197 (-516 C>T), which is in strong LD with the T71I variant (Table 5), had higher LDL-C levels than noncarriers; specifically, 11 mg/dl in the heterozygous apoB ADH-1 patients and a staggering 71 mg/dl hike in the apoB homozygotes (Table 8), a result that warrants further clinical and laboratory investigations.

Finally, it is important to appreciate the data from Tby-jaerg-Hansen et al. (267) that suggest the effect of LDLR mutations on LDL-C levels in patients seen within a clinical environment may be overestimated. On genotyping 9,255 adults from the general Danish population for the three LDLR mutations [W23X, W66G, and W556S (Class 2A, Fig. 3) that explain >40% of all clinical cases of ADH-1 in Denmark], the heterozygous carriers (n = 6) had, respectively, 112 mg/dl and 147 mg/dl higher total plasma cholesterol and LDL-C levels than those with the same mutations in ADH-1/CHD patients (3/948) increased values by 158 mg/dl and 162 mg/dl, respectively. Furthermore, ADH-1 patients (28/63) with a clinical diagnosis of monogenic hypercholesterolemia had increases of 189 mg/dl and 205 mg/dl.

**Mechanistic insights.** Conventional wisdom is that the LDLR primarily serves to mediate the uptake of extracellular lipoproteins, primarily LDL, and that disruption of this uptake explains the higher plasma LDL-C levels in LDLR-negative patients compared with those with a LDLR-defective allele. However, it now appears that this difference is not solely ascribed to failure of apoB100-mediated uptake of lipoprotein particles; it also includes significant contributions from both increased net secretion of VLDL and decreased clearance of VLDL catabolic by-products (268–271).

In outline, metabolic studies have established that VLDL-apoB100 production/secretion is increased in LDLR-negative patients (270, 271) and that in mice, the LDLR preferentially directs small, poorly lipidated apoB particles to a post-ER compartment for degradation (268, 269). Thus, Tremblay et al. (271) showed that the production/secretion of VLDL-apoB100 was increased by 50% and 109%, respectively, in six heterozygote patients and one homozygote individual that had the same LDLRnull allele. Likewise, Millar et al. (270) found a 68.8% increase in heterozygous LDLR-negative patients with the Lebanese C660X mutation (Class 2A, Fig. 4) but not in LDLR-defective patients (increase 21.2%; P = 0.48, for difference from controls). Likewise, compared with control livers, the livers from homozygous LDLR−/− and C678Y (LDLR retained in ER) mice have increased net secretion of apoB48- and apoB100-containing lipoproteins (268), whereas hepatocytes expressing LDLR mutant Y807C, which does reach the cell surface, do not. In fact, through a series of cleverly designed experiments that employed a LDLR mutant (i.e., II40D) defective in apoB, but not apoE, binding and internalization (Class 3A, Fig. 3), Blaisole et al. (268) reached the conclusion that the LDLR regulates VLDL-apoB secretion through selective (re)uptake of (VLDL-bound) apoE (Fig. 5). Moreover, this uptake and that of β-VLDL (272), in contrast to the LDL-apoB100 ligand, is mediated via a VLDL-induced 80S-FDNPV-807-independent internalization mechanism(s).

**Common LDLR variants associated with LDL-C levels.** From the perspective of increased understanding of how LDLR transcript functionality is regulated, two independently segregating SNPs are noteworthy (Table 8). In multiple German and British population samples, the rare allele at SNP site rs2228671 displays association with decreased LDL-C (273) and CHD risk. Moreover, because adjustment for LDL-C levels by logistic regression and Mendelian randomization models abolished the rs2228671 and CHD signal, the data point to a link between the rare allele at this SNP site [or allele(s) in LD] and a lifelong reduction in LDL-C levels and CHD risk. In the GLGC cohort, the rs6511720 rare allele (which tags rs2228671) also displayed strong association with decreased LDL-C levels, with the estimated effect size being larger than those observed for APOB, SORT1, and TRIB1 variants (Tables 5, 8).

The story of the second LDLR variant (rs688) started with an in silico prediction that the rare allele at this SNP site neutralizes a putative exonic splicing enhancer within exon 12. Estus and colleagues (274, 275) then went on to show that the splicing efficiencies of minigene-derived transcripts containing the rare allele were, relative to common allele bearing transcripts, decreased by ~9 and 15% in both HepG2 and SH-SY5Y (neuroblastoma) cells. Additionally, in human tissue samples, the rare variant was associated with less efficient exon 12 splicing: in female, postmortem liver and male anterior cingulate region of brain, the decreases were 8.6% (P = 0.024) and 8% (P = 0.041), respectively. Consistent with a potential reduction in LDLR protein production, the GLGC study associates the rs688 rare allele with increased LDL-C (32) (Table 8), whereas Estus and colleagues (275) present evidence of association with Alzheimer disease in elderly men [recessive model, OR 1.49, (95% CI 1.13-1.97), P = 0.005] but not in women, leading them to speculate that the rare rs688 allele decreases exon 12 splicing efficiency.
in vivo, leading to reduced functional LDLR and apoE clearance, increased amyloid pathology, and risk of Alzheimer disease.

**Binding of LDL-apoB100 to LDLR**

General acceptance that an apoB100-LDLR interaction(s) initiates the uptake of LDL-C stands in marked contrast to our level of ignorance over the precise molecular contacts that apoB100 makes with this receptor. Therefore, critical review of exactly what is known seems timely.

Early antibody studies mapped potential binding sequences to amino acids 2835-4189 (276–278), whereas comparisons with the apoE-LDLR-ligand binding sequence (Fig. 5) suggested two prime candidate regions: peptides A, amino acids 3147-3157 and B, 3359-3367 (279). However, despite extensive sequencing of the exonic region encoding these two apoB regions, no LDL-C raising mutations/variants have been identified in either region. Additionally, data from mice regarding the functional importance of peptide B in vivo are equivocal. Promisingly, Boren et al. (280) found that mutant human recombinant LDL containing amino acid changes in peptide B of the putative LDL-apoB100 binding region (i.e., R3359S, R3362S, K3363A, R3364S, and R3367S) competes far less efficiently with labeled human plasma LDL for binding to human fibroblasts in vitro than similarly derived control recombinant human LDL. However, data regarding the effects of the defective mutant LDL binding on plasma LDL-C levels in their mice were conspicuous by their absence. Moreover, even the in vitro data could conceivably be explained by the introduced mutations perturbing LDLR binding residues/motifs outside the hypothetical peptide B region. The more recent mouse studies of Johnson et al. (281) serve only to underscore the current ignorance of the apoB100-LDLR binding mechanism. They showed that LDLr+/−/ApoBec1−/− mice expressing recombinant apoB100 with two foreign peptide sequences in place of peptides A and B had lower, rather than the expected higher, LDL-C than mice expressing control, recombinant apoB100. Investigations revealed that the mutant apoB mice were secreting abnormally large VLDL particles overendowed with apoE, which were cleared more rapidly from the circulation than control VLDL-apoB100 through an apoE-ERP-mediated mechanism, a salutary reminder to always consider the secretary as well as uptake pathways when studying LDL-C levels.

Immuoelctron microscopic analyses of human LDL (282) indicate that peptide A and B (amino acids 3147-3157 and 3359-3367) reside on one side of the LDL hemisphere close to where apoB100 amino acid residues 4275-4440 cross the ~620A long, wedge-shaped, kinked ribbon (apoB1.5-89; amino acid residue 71-4050) encircling LDL. Subsequent competitive binding assays involving labeled human LDL-apoB100 and LDL containing recombinant apoB substantiate these data and indicate apoB amino acids R3500 and W4369 (apoB96.3) interact to facilitate normal apoB100-LDLR binding (280, 283). Moreover, LDLs containing C-terminal truncated forms of apoB retaining the hypothetical LDLR ligand binding peptides A and B, such as apoB77 and apoB95 (i.e., truncated at residues 3497 and 4329), compete more avidly for LDLR binding than full-length apoB100. Hence, the extremely low LDL-C levels seen in apoB-specific FHBL patients with C-terminal truncated apoBs in the range of ~apoB77-95 (284, 285) may be attributable, at least in part, to enhanced LDL clearance.

Very recently, electron cryomicroscopy analyses (118) have suggested that the apoB100 structures that interact with the LDLR are stabilized by the cholesteryl-ester rich core of LDL and that they include a region formed by amino acids ~2050-2600 (apoB45.2-57.3), which bind to the LDLR β-propeller (Fig. 5B), as well as apoB structure(s) within one of its β-sheet domains (i.e., residues 1000-2074 or 2600-4000). In summary, the LDL-apoB100:LDLR interaction(s) seems considerably more complex and extensive than originally thought.

**APOB mutations, defective LDLR binding and ADH-2.**

Grundy and colleagues were the first to report the association between moderate hypercholesterolemia, defective LDLR binding, and the apoB100 R3500Q mutation (286). Specifically, they identified a heterozygous index patient (LDL-C, 215 mg/dl) who cleared his own LDL from the circulation ~2-fold (P < 0.001) less efficiently than control LDL and then showed that the patient’s LDL in vitro was ~90% less effective in competing with normal LDL for cellular uptake and degradation. Moreover, the patient’s LDL had reduced capacity to stimulate intracellular cholesteryl ester synthesis. Similar, but numerically unspecified, in vitro results were obtained from four other family members, all of whom possessed the apoB100 R3500Q allele. In comparison, blood relatives with “normal” LDL binding (range not specified) did not. Subsequent studies have confirmed that the R3500Q allele(s) is a major cause of moderate to severe hypercholesterolemia in people of European descent (Table 6) and that it confers increased risk of premature atherosclerotic disease (226, 227, 235, 238, 287–292). In Asian populations, the recurrent R3500W mutation (293, 294) is reported to be the principle cause of ADH-2.

In the last year, it has emerged that ~12% of the Old Order Amish residents of Lancaster County, Pennsylvania, have the R3500Q mutation (295) and that it is likely to have been introduced into this community at the end of the 1700s by a single Swiss ancestor. Today, Switzerland has one of the highest frequencies (0.41%) of the R3500Q allele in Europe (296). Returning to the Amish community, Shen et al. (295) performed a GWAS to identify variants associated with high LDL-C levels and coronary artery calcification in 841 asymptomatic individuals (age 43.7 ± 13.9 years) and found a cluster of 65 SNPs spanning a 12-megabase region near APOB displaying strong association with LDL-C levels. Subsequent genotyping for the R3500Q (rs5742904) mutation disclosed that it was in near-complete LD with the SNP alleles that had displayed the strongest association with LDL-C. Moreover, combining this GWAS sample with another 663 Amish revealed that it accounted for 26% of the variation in age-
sex-adjusted LDL-C levels in the population (Table 6). Moreover, R3500Q carriers had 4.41-higher odds of having radiologically detectable coronary artery calcification. In a control analysis, all other SNP associations (n = 65) with LDL-C concentrations disappeared when APOB R3500Q was considered as a covariate. Nonetheless, because many of the associating SNPs are in high LD (i.e., many carriers of the R3500Q mutation share a common extended haplotype potentially originating from a Celtic ancestor some 6,000-7,000 years ago (296)), it would have been nearly impossible without independent genetic (227, 293, 294) and supporting functional (280, 283) data to conclude that the R3500Q mutation (rather than a mutation(s) in LD) is the cause of moderate hypercholesterolemia in the Old Order Amish and other populations.

Boren et al. (280) showed that LDL containing recombinant apoB100 with a glutamine, lysine, or glycine at amino acid 3500 bound the LDLR ~80% less efficiently than recombinant apoB100-R3500 in vitro. R3500, however, is unlikely to be directly involved in LDLR binding (or be the major determinant of LDLR binding) because LDL containing recombinant apoB80 R3500Q and apoB77 (truncated at amino acid 3497) displayed enhanced LDLR binding (280). Instead, it is envisaged that amino acid residue R3500 is required to attain the conformation of the yet undefined and potentially multiple apoB100 motifs that bind to the LDLR.

It is also now known that 2-10% of patients with a clinical diagnosis of FH have the apoB R3500Q mutation (289, 297, 298), including some who also have a LDLR mutation (299–301). Compound heterozygote ADH-1/2 patients tend to have higher LDL-C, more extensive xanthomatosis, and more severe premature CHD than heterozygote ADH-1 and homozygote ADH-2 patients (Table 6). Their phenotype, however, appears to be less severe than that seen in homozygous ADH-1 (300).

It has emerged that some people with familial ligand-defective apoB100 due to R3500 and R3531 mutations have plasma cholesterol levels within the normal range (302–306), despite defective LDL binding and accumulation of the mutant allotype in blood. In one study, Pullinger et al. (304) showed by using a monoclonal antibody and dynamic light scattering that the mass ratio of Gln3500 to Arg3500 in the LDL of heterozygote apoB100R3500Q patients was ~73:27 and that in vitro the mutant LDL had <10% of the normal affinity for the LDLR. In comparison, the mass ratio of Cys3531 to Arg3531 was 58:42 for heterozygote apoB100R3531C individuals, and the mutant LDL displayed 27% of normal affinity for the LDLR. Analyzing the lipid profiles of R3531C family members revealed a wide range of total cholesterol and LDL-cholesterol values (age- and sex-adjusted cholesterol and LDL-C: 152-326 and 68-238 mg/dl, respectively), with the average LDL-C increase (i.e., 32 mg/dl or 16%) being more modest than that observed for the R3500Q mutation (Table 6). In a second study, five heterozygote individuals [i.e., R3480P (n = 4); P3480W (n = 1)] from the Danish general population (n = 9,255) with ligand-defective apoB100 showed by guest, on July 19, 2013www.jlr.org Downloaded from
began this story with a full clinical and biochemical description of two Japanese siblings who had lipid profiles and clinical symptoms (e.g., multiple xanthomas since childhood) similar to those in homozygous ADH-1 patients, despite evidence of normal functioning LDLR. Specifically, their cultured skin fibroblasts synthesized normal quantities of fully active LDLR protein, and these were normally downregulated with LDL or cholesterol loading. Moreover, genetic markers at the LDLR locus did not segregate with the hypercholesterolemia. Whole-body in vivo turnover studies, however, returned convincing evidence that the elevated LDL-C in these two siblings was not attributable to increased de novo cholesterol synthesis but, rather, to impaired LDL catabolism. This conclusion was subsequently substantiated by 125I-LDL turnover studies and a biodistribution and uptake analysis of 59Fe(technetium)-labeled LDL in three Sardinian ARH patients (309). Thus, these patients had a marked reduction in the fractional catabolic rate of LDL (patients versus five controls: 0.19 ± 0.1 and 0.36 ± 0.03 pools/day, P < 0.001), plus a significant increase in LDL production (20.7 ± 4.4 versus 14.0 ± 2.4 mg/kg/day, P < 0.01), although this increase is lower than that typically seen in homozygous ADH-1 patients. These changes were paired with a severe reduction in hepatic LDL uptake similar to that observed in ADH-1 homozygotes and decreased LDL uptake by the kidney and spleen (but not heart), findings which prompted Zuliani et al. (309) to speculate that the ARH lipid phenotype was caused by selective reduction in hepatic LDL uptake. Subsequently, Norman et al. (310) showed that the defect resided in a component of the endocytic machinery required for LDLR and LDLR-LDL complexes uptake through clathrin-coated pits; specifically, they found that LDLR was present on the cell surface of EBV-immortalized lymphocytes prepared from ARH patients and that the receptor bound LDL normally but was unable to internalize it.

It turns out that LDLRAP1 is required for the hepatic uptake of LDLR-LDL-apoB100 complexes but not of LDLR-VLDL-remnant complexes, which are internalized by an 862FDNPPV867-independent mechanism (Fig. 4). Thus, when Jones et al. (311) fed ldlr−/− and ldlrap1−/− mice a high sucrose diet (to stimulate hepatic VLDL production), the ldlrap1P−/− animals developed less pronounced hyperlipidemia due to preservation of the LDLR-dependent clearance of VLDL remnants. This phenomenon in humans could explain the development of a relatively mild LDL-C phenotype in ARH patients compared with their compatriots with homozygous/compound heterozygous LDLR-null alleles (Table 6), especially as the LDL phenotypes of ARH and homozygous ADH-1 patients with LDLR-defective mutations are superimposable (Table 6).

As to the role of LDLRAP1 in mediating LDL uptake in only certain cell types, it seems noteworthy that ARH typically manifests in children as large tendon xanthomata, either planar or tiberosus (310, 312–318), suggesting that LDLRAP1 is dispensable for LDLR activity in macrophages.

**Genetics of autosomal recessive hypercholesterolemia.** Current studies indicate that in most parts of world autosomal recessive hypercholesterolemia (ARH) is likely to be an exceedingly rare condition (314–322). One exception is Sardinia, where the disease has an estimated frequency of 1:40,000, accounted for by two mutations (W22X and c.432 ins A) (313). In middle-aged people from this population, ARH carriers (1:143 individuals) had similar LDL-C levels to noncarriers (141.0 ± 0.41 versus 137 ± 0.41 mg/dl, P = 0.19) and a comparable rate of myocardial infarction (323). However, it should be noted that the study of only 3,410 islanders limited the power to detect small effect size and that in the GLGC sample a common LDLRAP1/Tmem57 variant displayed association with LDL-C levels (Table 8). However, whether the actual allele(s) causing this association signal is mediating its LDL-lowering effect through LDLRAP1 or Tmem57 is not known.

**Returning internalized LDLR to the plasma membrane**

As depicted in Fig. 4, PCSK9 binds hepatic LDLR, promoting its degradation and thereby impairing LDL/VLDL uptake by this receptor. This discovery has culminated in the development of a PCSK9-binding antibody that mimics the epidermal growth factor (EGF)A domain of the LDLR, which in vivo serves to effectively remove PCSK9’s ability to bind and promote LDLR degradation and significantly reduce LDL-C by 20-50% in monkeys and 40% in “humanized” mice (324). The mechanics of PCSK9/LDLR binding and intracellular degradation have been reviewed quite recently (325–327) and are not covered in depth here. Instead, we focus on correlations between PCSK9, de novo cholesterol biosynthesis, and LDL-C levels in humans (328, 329), as well as the growing consensus that PCSK9 participates in VLDL production/net secretion (330).

In humans, plasma PCSK9 levels normally display a diurnal rhythm that closely parallels that of cholesterol synthesis, whereas total plasma cholesterol levels remain relatively stable during these oscillations (328, 329). However, depleting hepatic cholesterol and prolonged fasting obliterates this natural rhythm. Thus, in healthy individuals consuming three standardized meals per day, plasma PCSK9 and lathosterol (measure of cholesterol synthesis) levels are strongly correlated, reaching a nadir between 3 and 9 PM and a peak at 4.30 AM (329). By contrast, short-term (12 h) depletion of hepatic cholesterol by cholestyramine induces parallel increases in plasma PCSK9 and cholesterol synthesis, which remain elevated for 2-3 days after cessation of these bile acid sequestrants (329), suggesting that changes in a hepatic regulatory pool(s) of cholesterol contribute to the regulation of plasma PCSK9 levels, a result substantiated by the finding that statins also cause a sustained increase in plasma PCSK9 levels (331). Conversely, during prolonged fasting, cholesterol synthesis and PCSK9 levels fall, reaching nadirs at 32 and 36 h, respectively (328), and somewhat unexpectedly, LDL-C levels steadily rise (~20 mg/dl from fed state), reaching a peak at 32 h, when de novo cholesterol synthesis is at its lowest. This paradoxical rise in LDL-C levels, given the observed reduction in both cholesterol synthesis and LDLR...
degradation (via reduced PCSK9), suggests that prolonged fasting also reduces LDLR activity and thus LDL-C uptake and that this is mediated at least in part by the SREBP-2 transcriptional route, which also decreases PCSK9 and cholesterol biosynthetic gene expression (328, 332).

A recent study by Herbert et al. (330) substantiates the in vivo evidence that PCSK9 gain-of-function mutations increase the net secretion of VLDL (333). In brief, they produced and analyzed transgenic mice lines expressing wild-type human PCSK9 and PCSK9/D374Y (Fig. 4) at levels comparable to endogenous Psk9 and with the same tissue specificity. On a chow diet, the wild-type Psk9 mice had, compared with their BAC-negative littermates, reduced hepatic levels of LDLR, increased plasma cholesterol (∼1.8-fold, mostly in LDL fraction), and a comparable rate of triglyceride secretion. The livers of PCK9/D374Y mice contained even less LDLR, and they developed more pronounced hypercholesterolemia (cholesterol increase, 2.7-fold). Additionally, they secreted ∼2-fold more triglyceride-rich lipoproteins into the circulation than the control animals. This result complements two earlier studies: the first study showed that hepatocytes from Psk9-deficient mice ex vivo secreted fewer apoB-containing (∼33%) and apo100-containing (25%) lipoproteins than their wild-type litter mates (334), and the second study showed that these same mice secreted fewer chylomicrons and had attenuated postprandial hypertriglyceridemia (335).

Genetics of PCSK9. Ten gain-of-function mutations have been described in ADH-3: two (S127R, D129G) residing in its prodomain (amino acids 31-152), seven (R215H, F216L, R218S, R357H, D374Y, D374H, N425S) within the catalytic domain (amino acids 153-451), and one to three (N425S, R218S, R357H, D374Y, D374H, N425S) within the catalytic domain (amino acids 452-692) (333). In vitro assays have provided plausible genotype-phenotype correlations (337, 338). Thus, the S127R mutation in vitro significantly reduces autocleavage of the PCSK9 prodomain in the ER, a prerequisite for its subsequent trafficking and secretion, whereas PCSK9/D374Y undergoes normal rates of autocleavage and secretion (338). Moreover, S127R is less potent than D374Y in reducing cellular LDL uptake (LDL-uptake EC50: wild-type, 56 ± 10, S127R, 13 ± 5.8; D374Y, 2.2 ± 0.4 nM) (339). Thus, the combination of reduced PCSK9-S127R secretion and of potency in blocking LDL-C uptake could well explain the reduced penetrance (0.94) of the S127R mutation (339), whereas the normally secreted PCSK9-D374Y with its higher potency accords with the unusually severe hypercholesterolemia observed in ADH-3 patients with this particular mutation (Table 6). Thus, while some heterozygote ADH-3 patients have plasma cholesterol and LDL-C levels comparable to homozygous LDLR-null allele patients, others, consistent with in vitro assay results (337), have levels more similar to heterozygote ADH-1 patients with LDLR-defective alleles (Table 6). Finally, it should be noted that even though large-scale mutation screening projects have indicated that gain-of-function PSCK9 mutations are a rare (<1%) cause of ADH (245, 307, 340-342), such mutations can occur in heterozygote ADH-1 patients with a particular severe phenotype (Table 6), and these patients may respond better to aggressive LDL-C lowering treatment than homozygous LDLR-null allele patients (343).

It turns out that 11% of the African American subjects in the Dallas Heart Study with low plasma LDL-C levels harbor one of two PCSK9 nonsense mutations (i.e., Y142X, Tables 8, 9; C679X, Table 8) compared with ∼1.8% of medically unselected but ethnically and geographically matched individuals (344). The Y142X allele may contribute to FHBL in this group, insofar as it segregates with the “the LDL-C <75 mg/dl trait” (344, 345); however, internationally, the picture is less clear. Thus, in an Italian “FHBL” family with a similar truncating mutation (Ala68fsLeu82X) three of the four mutation-carrying family members had “raised” (∼85 mg/dl, ∼10th percentile) LDL-C levels, albeit possibly confounded by coexisting obesity (346) (Table 9). On the other hand, the sister and daughter of a 49-year-old Frenchman (LDL-C 16 mg/dl) with the dominant-negative double-mutant PCSK9 R104C/V114A had LDL-C levels of 57 mg/dl and 58 mg/dl, respectively (347). Thus, it seems that a preliminary diagnosis of PCSK9 deficiency (rather than apoB-specific FHBL) should be considered when a proband with very low LDL-C levels has family members with moderate rather than very low LDL-C levels and relatively high cholesterol:HDLC ratios (Tables 4, 8). Plasma PCSK9 measurements may also be helpful. Indeed, despite the fact that plasma PCSK9 levels explain <10% of the population variance in LDL-C levels, recent data have shown that certain loss-of-function mutations are associated with lower PCSK9 concentrations (348). For example, Dallas Heart Study participants with an Y142X/C679X or R46L mutation had, respectively, 60% and 34% lower plasma PCSK9 levels than those had participants with no mutation (P < 0.0001).

At the population level, the two African mutations/polymorphisms (i.e., Y142X and C679X) have remarkably similar effects on LDL-C levels (Table 8), the average drop being ∼35-38 mg/dl compared with ∼20 mg/dl in Europeans/Americans with the R46L allele (Table 8). Encouragingly, the GLGC has now replicated the evidence for association between the rare alleles at SNP sites rs505151 (E670G) and rs11206510 (upstream) and LDL-C levels (Table 8), and perhaps more importantly, it has provided a platform for the first time on which to compare their potential effects on LDL-C levels relative to all other associated common variants across the entire genome in a single population, even for those genes with no prior connection to sterol and LDL metabolism (Table 10).

Moderate impact of IDOL-mediated degradation of LDLR on LDL-C levels

In 2009, Zelcer et al. (349) showed that the sterol-responsive nuclear receptor liver X receptor (LXR) helps maintain cellular cholesterol homeostasis not only by promoting cholesterol efflux but by also suppressing cellular LDL uptake through transcriptional induction of IDOL expression. In brief, they (349) demonstrated that activated LXR increased IDOL mRNA levels in specific cell types and that in wild-type

but not \( Ldbr^- \) mice, (adenovirus-mediated) high-level hepatic expression of \( \text{IDOL} \) markedly reduced LDLR protein levels, and increased plasma concentrations of total and unesterified cholesterol (\( \sim 2\)-fold), LDL-C (\( \sim 6\)-fold), and apoB (\( \sim 3\)-fold). In comparison, PCSK9 levels were not altered. Notably, however, the totality of the data suggests that the LXR-IDOL pathway is more active in peripheral cells, such as macrophages and adrenal cells, than in hepatocytes, which may explain why in humans the estimated size effect of the rare allele at SNP site rs3757354 on LDL-C levels is considerably smaller than those observed for PCSK9, apoB, and LDLR (Table 8).

### CONCLUDING COMMENT

In the past five years, our understanding of the biological and genetic bases of low and high LDL levels has been advanced by the acquisition of new insights into noncholesterol sterol and cholesterol absorption and elimination, apoB-containing lipoprotein production and intracellular trafficking, and the fine regulation of cellular cholesterol uptake via the LDLR. Genetic (e.g., in-depth sequence) analyses of patients with monogenic dyslipidemias and of individuals with plasma LDL-C levels at the extreme of the population distribution, as well as the identification of pathogenic mutations in diverse populations, have established important genotype-phenotype correlations. Independently, large GWAS studies have not only confirmed the role of genes previously known to affect plasma LDL-C levels but, more importantly, also identified a large set of previously unsuspected genes. Their impact on cholesterol metabolism and LDL-C levels is under active investigation. Indeed, recently developed mouse models have begun to provide some fascinating new knowledge about the cellular mechanisms contributing to the pathophysiology of cholesterol and apoB-containing lipoprotein diseases, thereby suggesting new molecular targets for hyperlipidemic drugs. In the immediate future, new DNA sequencing technologies, such as exome sequencing, will provide a very powerful tool for identifying the causative mutations in those familial conditions of low and high LDL levels that are not explained by the usual suspects. The overall picture to emerge is that circulating LDL levels result from the output of a complex array of cellular activities and that even in people with conventional, straightforward, Mendelian clinical conditions of LDL (e.g., ADH and FHBL) metabolism, these conditions have a complex genetic basis. A major challenge will be to integrate the effects of lifestyle choices (e.g., diet, obesity, smoking) and epigenetic variation, often neglected in the study of genotype-phenotype correlations, with knowledge of DNA sequence determinants to arrive at a deeper understanding of the cause, course, treatment, and prevention of LDL dyslipidemias.

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