Editorial

Finding the Therapeutic Sweet Spot
Using Naturally Occurring Human Variants to Inform Drug Design

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Human genetic research can pinpoint drug targets by iden-tifying complete loss-of-function mutations affecting a human gene product that, in turn, underlie a favorable phe-notype.[1] Most small-molecule oral drugs, monoclonal anti-bodies, or RNA-based strategies act by inhibiting a selected molecular target, thereby pharmacologically mimicking the naturally advantageous genetic deficiency. The fields of ath-erosclerosis and lipoprotein biology have several examples of drugs whose raison d'être is to impersonate a naturally occur-ring, genetically determined beneficial phenotype.

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For instance, 3 new classes of agents approved in the United States reduce low-density lipoprotein (LDL)-cholesterol lev-els through nonstatin mechanisms.[2,3] These include (1) an oral inhibitor of microsomal triglyceride transfer protein (MTP), namely lomitapide (Juxtapid; Aegerion); (2) an injectable anti-sense oligonucleotide against apolipoprotein (apo) B, namely mipomersen (Kynamro; ISIS-Genzyme); and (3) monoclonal antibodies that impede the activity of proprotein convertase subtilisin kexin type 9, such as evolocumab (Repatha; Amgen) and alirocumab (Praluent; Sanofi-Regeneron). In all 3 cases, drug targets were identified by studying natural human mono-genic deficiency states of LDL-cholesterol.[1–3]

The story behind the proprotein convertase subtilisin kexin type 9 inhibitors began >10 years ago, when uncommon het-erozygous loss-of-function variants were seen in individuals with low LDL-cholesterol levels and lifelong protection against atherosclerosis.[4] Rare heterozygous gain-of-function variants were earlier shown to cause the opposite phenotype, namely familial hypercholesterolemia (FH).[5] Subsequent drug devel-opment to target proprotein convertase subtilisin kexin type 9 was swift and competitive. Despite no long-term outcome data, these agents were recently approved because they so effectively

reduce LDL-cholesterol apparently safely and seem to reduce atherosclerosis events, at least in the short term.[6,7]

Targets of the MTP inhibitor and apoB antisense agent were identified >20 years ago by mapping the causative genes in families with extreme syndromic LDL deficiency states, namely abetalipoproteinemia and homozygous familial hypo-betalipoproteinemia (FHBL), respectively.[8] The development programs for both agents have been complicated. Both drugs reduce LDL in relative terms, but each has adverse effects: steatorrhea and hepatic fat accumulation for lomitapide, and injection site reactions and systemic flu-like symptoms in the case of mipomersen. Despite these negative attributes, the US Food and Drug Administration in December 2012 approved both lomitapide and mipomersen as adjunctive to a low-fat diet and other lipid-lowering therapies including apheresis for adult patients with homozygous FH.[2]

A question arising from these experiences is whether the benefit:risk ratio of next-generation agents could be enhanced through insights gained via deeper phenotypic analysis of individuals carrying different variants within the same gene. Rather than focusing on nonsense or splicing variants that completely obliterate expression and activity of the gene prod-uct, could more subtle functional changes seen with some mis-sense mutations provide additional data to inform rational drug design? For instance, could the position of a missense variant within the coding sequence in a phenotypically well-character-ized individual highlight a functional domain for developing smarter, more focused therapies when compared with com-plete abolition of function? Can more intricate functions be discerned through comprehensive molecular and phenotypic evaluation of new patients with rare monogenic disorders?

A step in this direction is reported in the current issue of *Circulation Cardiovascular Genetics*.[9] Walsh et al[9] studied a novel homozygous *MTTP* gene mutation causing a missense variant in MTP, namely p.D169V, in a 4-month-old Turkish child with abetalipoproteinemia. The mutation was found in a region encoding a domain that has heretofore had few mutations reported. The authors' extensive analyses provide new insights into understanding the normal structure–function of MTP, which in turn might help direct future drug development efforts.

Abetalipoproteinemia

Abetalipoproteinemia, alias Bassen–Kornzweig syndrome (MIM 200100 and 157147), has an estimated prevalence of <1 in 1 million.[8] Abetalipoproteinemia is characterized by the absence of plasma apoB-containing lipoproteins, including chylomicrons, very low density lipoprotein, and LDL, together with systemic findings, such as fat malabsorption, abnormal red blood cells (acanthocytosis), prolonged prothrombin time (high international normalized ratio), atypical retinitis

pigmentosa, spinocerebellar ataxia, and myopathy.[8] Fat content of hepatocytes and enterocytes is increased. Deficiency of fat soluble vitamins results from impaired vitamin absorption and transport and underlies most of the systemic manifestations.[8] Homozygous FHBL is phenotypically similar to abetalipoproteinemia, except that obligate heterozygote FHBL parents have approximately one-third plasma concentration of LDL-cholesterol and apoB, whereas obligate heterozygote parents of abetalipoproteinemia patients have normal lipid profiles.[8] Family-based serological studies identified *APOB* as the likely causative gene in FHBL,[10] whereas family-based molecular and candidate gene studies mapped *MTTP* as the causative gene for abetalipoproteinemia.[11] Treatment for both conditions includes lifelong dietary fat restriction and large doses of oral fat soluble vitamins.[8] Understanding the causes for the deficiency of LDL-cholesterol in abetalipoproteinemia and FHBL provided the initial motivation for development of agents to target MTP and apo B, respectively, to reduce elevated LDL-cholesterol levels.

Microsomal Triglyceride Transfer Protein

MTP, isolated from liver and intestinal microsomes, is a heterodimer comprised of protein disulphide isomerase (PDI), a 55-kDa multifunctional protein and a large M subunit with apparent molecular weight of ~97 kDa. MTP catalyzes transfer of triglyceride, cholesteryl ester, and phospholipids[11] to apoB in the early assembly stages of lipoprotein precursor particles. MTP also prevents intracellular degradation of nascent apoB-containing lipoprotein particles. Although MTP has not been crystallized, modeling of the M subunit indicates 3 functional domains: the N-terminal β-barrel domain likely has phospholipase activity, whereas the central α-helical domain and C-terminal domains have lipid transfer activity.[9] Abetalipoproteinemia-causing variants in the *MTTP* gene affect the ability of MTP to support assembly and secretion of apoB-containing lipoproteins.[8,11–13]

Current State of MTP Inhibition

Several MTP inhibitors lowered plasma LDL-cholesterol in animals and humans, but development programs were discontinued because of adverse effects.[14] Lomitapide (formerly BMS-201038) was initially evaluated open-label in 6 homozygous FH patients on a low-fat diet, and after 4 weeks, reduced plasma LDL-cholesterol by 50.9% at the highest dose (1.0 mg/kg body weight/d). Significant increases in plasma aminotransferase levels and hepatic fat content were reversible 14 weeks after cessation of therapy.[15] Lomitapide was then studied in an open-label phase 3 trial, using a dose-ranging regimen (maximum 60 mg/d >26 weeks), in homozygous FH patients on standard of care including lipoprotein apheresis.[16] With a median dose of lomitapide 40 mg/d, plasma LDL-cholesterol fell by 50% at week 26 among the 29 enrolled patients and by 38% in 23 subjects at 78 weeks of treatment. Plasma aminotransferases increased at least 5-fold in 4 patients and resolved after dose reduction. Gastrointestinal symptoms occurred in 80% of patients, accounting for 3 withdrawals. Hepatic fat content increased from 1.0% at baseline to 8.6% at week 26, but remained stable to 78 weeks. The effect on

LDL-cholesterol was thus offset somewhat by hepatosteatosis. A patient with severe hypertriglyceridemia took lomitapide for 13 years, with good prophylaxis against acute pancreatitis although the fatty liver progressed to steatohepatitis and eventually to fibrosis.[17] In this context, studying new human variants in MTP, such as the one reported by Walsh et al[9] may help refine understanding of MTP function that could help inform the design of next-generation drugs.

Implications of a New Human Variant for Targeting MTP

The studies by Walsh et al[9] of the rare MTP p.D169V variant showed that (1) cells expressing normal quantities of p.D169V had significantly lower amounts of secreted B-48; (2) p.D169V localized to the endoplasmic reticulum and interacted with the N terminus of apoB; (3) p.D169V had no triglyceride or phospholipid transfer activity; (4) p.D169V did not interact with the PDI subunit; (5) p.D169V is potentially a part of a salt bridge that involves residues K187 and K189; (6) mutagenesis of the K187 and K189 residues to leucines had similar effects on PDI-binding, lipid transfer, and apoB secretion as did the natural p.D169V variant; and (7) restoration of the charges at residues 169, 187, and 189 using other mutants restored PDI-binding, lipid transfer, and apo B secretion. The microdomain containing D169 interacts with apoB may be responsible for MTP's chaperone activity. The authors concluded that the abetalipoproteinemia mutation MTP p.D169V is pathogenic because it disrupts an internal salt bridge and proposed that future MTP inhibitors could avoid targeting the N-terminal region of MTP as a means of mitigating the adverse effects by reducing synthesis of apoB-containing lipoproteins without causing steatosis.

Functional characterization and modeling of MTP's N terminus suggest that interaction with β-strands 7 to 9 may disrupt PDI binding and inhibit lipid transfer activities, resulting in steatosis similar to that seen with lomitapide.[9] Walsh et al[9] speculate that β-strands 1 to 3 and helix NH1 may reduce apoB-MTP binding without affecting lipid transfer activity and could thus represent a preferred target subdomain. Also, they suggest that small-molecule inhibitors or mutations in apoB that target MTP's chaperone activity without affecting MTP's lipid transfer activity would reduce apoB secretion. This is because some lumenal lipid droplet content could still fuse with nascent apoB-containing lipoproteins and be secreted, thus reducing steatosis. Alternatively, because inhibiting MTP's chaperone activity may also cause steatosis, it would be important to remain vigilant for new human missense mutations that affect only MTP's chaperone activity.

Looking to the Future

Lomitapide is a helpful adjunctive therapy for some patients with homozygous FH. Its benefit:risk ratio is probably favorable for these patients, with reduction of atherosclerosis risk offsetting hepatic and gastrointestinal side effects. However, for other patients, its benefit:risk ratio is less obvious. There is strong rationale for new strategies that target MTP with minimal side effects. Newer approaches include RNA interference using microRNA-30c, a potent repressor of MTP,[18] an MTP

antisense oligonucleotide,[19] and intestine-specific MTP inhibition.[20] The data from Walsh et al[9] provide a hint that targeting specific functional domains within MTP may also enhance the sweet spot by reducing both LDL and risk of adverse effects.

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