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

John R. Burnett, MD, PhD; Robert A. Hegele, MD

Human genetic research can pinpoint drug targets by identifying complete loss-of-function mutations affecting a human gene product that, in turn, underlie a favorable phenotype.1 Most small-molecule oral drugs, monoclonal antibodies, or RNA-based strategies act by inhibiting a selected molecular target, thereby pharmacologically mimicking the naturally advantageous genetic deficiency. The fields of atherosclerosis and lipoprotein biology have several examples of drugs whose raison d’être is to impersonate a naturally occurring, genetically determined beneficial phenotype.

The story behind the proprotein convertase subtilisin kexin type 9 inhibitors began >10 years ago, when uncommon heterozygous gain-of-function variants with low LDL-cholesterol levels and lifelong protection against atherosclerosis were identified >20 years ago by mapping the causative genes in families with extreme syndromic LDL deficiency states, namely abetalipoproteinemia and homozygous familial hypobetalipoproteinemia (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 product, could more subtle functional changes seen with some missense 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-characterized individual highlight a functional domain for developing smarter, more focused therapies when compared with complete 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 al9 studied a novel homozygous MTP 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.

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
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 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. Abetalipoproteinemia-causing variants in the MTP gene affect the ability of MTP to support assembly and secretion of apoB-containing lipoproteins.

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. 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. 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. 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. In this context, studying new human variants in MTP, such as the one reported by Walsh et al, 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 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. Walsh et al 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 luminal 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 mis-sense 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:risks ratio is probably favorable for these patients, with reduction of atherosclerosis risk offsetting hepatic and gastrointestinal side effects. However, for other patients, its benefit:risks 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, an MTP
antisense oligonucleotide, and intestine-specific MTP inhibition. The data from Walsh et al 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.

**Sources of Funding**

Dr Burnett was supported by a Practitioner Fellowship from the Royal Perth Hospital Medical Research Foundation. Dr Hegele was supported by the Jacob J. Wolfe Distinguished Medical Research Chair, the Martha G. Blackburn Chair in Cardiovascular Research, and operating grants from the Canadian Institutes for Health Research (MOP-13430, MOP-79523), the Heart and Stroke Foundation of Ontario (G-15-0009214) and Genome Canada through Genome Quebec (award 4530).

**Disclosures**

Dr Hegele is on the speaker’s bureaus and consults for Aegerion, Amgen, Lilly, Pfizer, Sanofi-Regeneron, and Valeant Pharmaceuticals. Dr Burnett reports no conflicts.

**References**


Finding the Therapeutic Sweet Spot: Using Naturally Occurring Human Variants to Inform Drug Design
John R. Burnett and Robert A. Hegele

Circ Cardiovasc Genet. 2015;8:637-639
doi: 10.1161/CIRCGENETICS.115.001219

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circgenetics.ahajournals.org/content/8/5/637

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Cardiovascular Genetics can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Cardiovascular Genetics is online at:
http://circgenetics.ahajournals.org/subscriptions/