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. 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.

For instance, 3 new classes of agents approved in the United States reduce low-density lipoprotein (LDL)-cholesterol levels through nonstatin mechanisms. These include (1) an oral inhibitor of microsomal triglyceride transfer protein (MTP), namely lomitapide (Juxtapid; Aegerion); (2) an injectable antisense 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 monogenic deficiency states of LDL-cholesterol.

The story behind the proprotein convertase subtilisin kexin type 9 inhibitors began >10 years ago, when uncommon heterozygous loss-of-function variants were seen in individuals with low LDL-cholesterol levels and lifelong protection against atherosclerosis. Rare heterozygous gain-of-function variants were earlier shown to cause the opposite phenotype, namely familial hypercholesterolemia (FH). Subsequent drug development 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. 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 hyperbetalipoproteinemia (FHBL), respectively. 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.

A question arising from these experiences is whether the benefit:risk ratio of next-generation agents could be enhanced through insights gained via a 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. Walsh et al 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 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. 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, and hepatic steatosis. Abetalipoproteinemia may be inherited in an autosomal recessive manner or occur sporadically. The disease results from a defect in the microsomal triglyceride transfer protein (MTP) that is required for the assembly and secretion of lipoproteins in the liver and intestine. Mutations in the MTP gene (MTTP, chromosome 7q36.2) result in reduced synthesis and secretion of lipoproteins, leading to a deficiency of apoB-containing lipoproteins.

**Atherosclerosis**

Atherosclerosis is a chronic inflammatory disease that primarily affects the arteries. It is characterized by the accumulation of lipid-laden plaques in the arterial wall, leading to narrowing of the arterial lumen. Atherosclerotic plaques can rupture, leading to acute events such as myocardial infarction or stroke. The disease is closely associated with dyslipidemia, insulin resistance, obesity, and other metabolic factors. The main components of atherosclerotic plaques include cholesterol-rich lipid-laden macrophages, fibrous tissue, and smooth muscle cells. The process of atherosclerosis involves the interaction of multiple factors, including inflammation, immune response, and cellular metabolism.

**LDL-cholesterol**

LDL-cholesterol, also known as low-density lipoprotein cholesterol, is a type of cholesterol that is carried in the blood attached to a protein known as an apoprotein. LDL-cholesterol is a major risk factor for atherosclerosis and cardiovascular disease. It is often referred to as "bad cholesterol" because it contributes to the formation of plaque in the arteries, which can lead to heart attacks and strokes.

**MTP**

Microsomal triglyceride transfer protein (MTP) is an enzyme that is involved in the assembly and secretion of lipoproteins. Mutations in the MTP gene can lead to a deficiency of apoB-containing lipoproteins, resulting in abetalipoproteinemia.

**Missense variant**

A missense variant is a genetic variation that results in the substitution of one amino acid for another in a protein. This type of variation can be either benign or pathogenic, depending on the location and effect of the substitution on the protein function.

**Phenotypic**

Phenotypic refers to the observable characteristics of an individual or population, which can be influenced by both genetic and environmental factors. In the context of genetic research, phenotypic analysis involves studying how different genetic variants affect an individual's physical and functional traits.
pigmentosa, spinocerebellar ataxia, and myopathy. 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. 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. Family-based serological studies identified APOB as the likely causative gene in FHBL, whereas family-based molecular and candidate gene studies mapped MTTP as the causative gene for abetalipoproteinemia. Treatment for both conditions includes lifelong dietary fat restriction and large doses of oral fat soluble vitamins. 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, cholesterol, and phospholipids to apoB in the early assembly stages of lipoprotein precursors. 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 MTTP gene affect the ability of MTP to support assembly and secretion of apoB-containing lipoproteins. 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 activity, 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 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, 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.

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References

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