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Commentary

The iPSC Awakens ANGPTL3 in Tangier Disease

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Patients with genetic mutation of ATP-binding Cassette Transporter A1 (ABCA1), commonly known as Tangier Disease (TD), can be characterized by elevated levels of plasma triglycerides (hypertriglyceridemia) and low plasma high-density lipoprotein (HDL) levels. With current HDL therapies continuously failing in clinical trials, there is no cure for TD other than maintaining a very low fat diet. The lipid phenotype in TD is largely contributed by the defect in ABCA1 in the liver. Our understanding of human hepatic ABCA1 expression being crucial in the maturation towards HDL is largely characterized in healthy conditions. However, the direct defect incited by ABCA1 loss of function (LOF) mutations in hepatocytes in TD has never thoroughly been explored, as availability of human TD liver tissue for research is extremely scarce. Nevertheless, mouse models mimicking TD (*i.e. Abca1*$^{-/-}$) or by specifically deleting hepatic ABCA1 have been used to gain insight into potential mechanism of altered lipid metabolism, although translational relevance to humans remains uncertain. The work presented in this issue of *EBioMedicine* by Bi et al. (2017–in this issue), provides a valuable and translatable model in studying hepatocyte-specific function and mechanisms of human TD generated from induced pluripotent stem cells (iPSCs).

ABCA1 is the rate-limiting step for the biogenesis of HDL mediated through cholesterol efflux. Specific deletion of *Abca1* in the mouse liver leads to an ~75% reduction in circulating HDL (Timmins et al., 2005), highlighting the crucial role of hepatic ABCA1 in maintaining plasma HDL levels. ABCA1 deficiency in hepatocytes also results in elevated levels of plasma triglycerides (TG). As discussed by Timmins et al., this can be via two mechanisms: 1) An overproduction of TG-enriched VLDL particles in the liver (Shelness and Sellers, 2001) and 2) decreased TG clearance, due to reduced LPL activity. The factors that affect the overproduction of VLDL are complex and depend on the availability of lipid substrates and protein co-factors to load lipids into VLDL particles. VLDL biogenesis occurs when apolipoprotein B (apoB) becomes lipidated by microsomal triglyceride transfer protein in the endoplasmic reticulum before lipids are added to form VLDL2. When TG availability is high in the Golgi apparatus, VLDL2 can further undergo maturation into VLDL1 (TG-enriched) particles, which is then mobilized into circulation. Generally, phosphoinositide 3-kinase (PI3K) acts as a brake to limit this second step of maturation into VLDL1. However, the absence of ABCA1 seems to reduce PI3K activity (Chung et al., 2010). What functional interactions exist between ABCA1 and PI3K in hepatocytes remains undetermined and requires further testing *in vitro*. Elevated plasma levels of TG in people with TD and mice lacking *Abca1* are associated with increased expression and secretion of ANGPTL3 from the liver. ANGPTL3 regulates the activity of lipoprotein lipase (LPL) *in vivo* and is the rate limiting enzyme for TG hydrolysis into free fatty acids (FFAs), which are then taken up by peripheral tissues such as the adipose tissue and skeletal muscle. However, the distribution of VLDL1 into these peripheral tissues does not seem to be present in patients of TD and *Abca1*-deficient mice, hence reduced clearance of VLDL1.

To understand whether these phenotypes and mechanisms are at play in hepatocytes of TD patients, Bi et al., generated iPSCs from peripheral blood mononuclear cells taken from control and TD subjects, and differentiated them into hepatocyte-like cells (HLCs) (Bi et al., 2017–in this issue). As expected, TD HLCs displayed defective cholesterol efflux and nascent HDL formation, along with elevated TG secretion. This inverse association between HDL and TG levels mimics the phenotypes found in TD patients and *Abca1*-deficient mice, and shows that iPSCs can be a powerful and translatable tool to study the function and mechanism of rare diseases. Taking advantage of this technique, the authors explored the transcriptome of TD and control HLCs. Gene Ontology (GO) lipid terms revealed the differential expression of five genes including *ANGPTL3*. Consistently, ANGPTL3 was secreted by TD HLCs and higher plasma levels are observed in people with TD. While further work is required to understand the contribution of the ANGPTL3/LPL axis to increased TGs in people with TD, ANGPTL3 may represent an attractive target to control plasma TG levels in people with TD. Interestingly, Regeneron Pharmaceuticals has developed a neutralizing monoclonal antibody (REGN1500; Evinacumab) to ANGPTL3, which results in enhanced LPL activity, dramatically dropping TG and cholesterol levels in the plasma (Gusarova et al., 2015). Although REGN1500 reduces cholesterol levels, HDL levels also seem to be reduced, which reflects the LOF mutations of ANGPTL3 observed in people (Musunuru et al., 2010). The decrease in HDL is likely not a concern as preclinical mouse models carrying a LOF mutation in *Angptl3* have significantly smaller atherosclerotic lesions (Ando et al., 2003). Thus, while HDL is losing traction as therapy with its growing collection of failed clinical trials, novel approaches targeting TGs and clearance of

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TG-lipoproteins appear to be more effective. As such, REGN1500 is currently in phase 2 clinical trials to target dyslipidemia (France, 2016). The clinical relevance of the findings in TD by the Rader group presented in this issue of *EBioMedicine* (Bi et al., 2017–in this issue), while requiring additional research, may unlock a novel therapeutic strategy by targeting ANGPLT3 in these people. Thus, as ANGPTL3 is expressed in the liver, using TD HLCs generated from iPSCs could be key when paired with other experimental models to understand whether the utility of ANGLTP3 targeting therapies such as REGN1500 can be effectively used to treat the abnormal and detrimental lipid profile present in people with TD.

Conflict of Interest

The authors declare no conflicts of interest.

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