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**NAFLD Phenotype in Patients With V-ATPase Proton Pump Assembly Defects**

Nonalcoholic fatty liver disease (NAFLD) is a very common chronic liver disease marked by hepatic fat accumulation. This might trigger inflammation and liver cell injury, a condition known as nonalcoholic steatohepatitis. Nonalcoholic steatohepatitis may promote fibrogenesis and evolve toward cirrhosis and hepatocellular carcinoma, and is considered the phenotypic extension of NAFLD.

There is considerable heterogeneity in NAFLD. Most patients carry several features of the metabolic syndrome such as insulin resistance. However, there are well-documented NAFLD cases without metabolic syndrome. For these cases of secondary NAFLD, the mechanism of steatogenesis appears to be independent of insulin resistance and adipose tissue expansion. Secondary causes of NAFLD are important to recognize because their natural history, associated symptoms, and overall management differ from that of metabolic syndrome–associated NAFLD. Known inborn errors of metabolism that cause NAFLD are hypoor abetalipoproteinemia, lysosomal acid lipase deficiency, and Wilson disease.

Lesser-known inborn errors of metabolism that can cause liver injury are primary glycosylation defects or congenital disorders of glycosylation (CDGs). Glycosylation is the attachment of a glycan, composed of monosaccharides, to a protein or lipid and is a complex and highly regulated form of post-translational modification. The process starts in the endoplasmic reticulum (ER), where the glycan buildup and en bloc transfer to the nascent protein or lipid take place. If correctly folded, the newly formed glycoprotein travels to the Golgi apparatus (or simply Golgi). In the Golgi, modification of the glycan takes place, which results in the mature glycoprotein, ready to be transported to other cellular compartments or secreted into the bloodstream.

To date, more than 100 CDGs have been discovered and this number is expanding rapidly. The majority of defects are inherited in an (X-linked) autosomal-recessive pattern and the group as a whole presents a complex heterogeneous phenotype. As the main site for protein synthesis, the liver frequently is affected in CDG and increased aspartate aminotransferase and alanine aminotransferase serum levels are a common finding.

Recently, we identified 3 novel CDG subtypes in 23 patients, ranging from neonates to adults, from 14 families across Europe. Patients with mutations in TMEM199 (MIM: 616829), CCDC115 (MIM: 616828), and ATP6AP1 (MIM: 300972) presented with a NAFLD phenotype including steatosis on liver biopsy and increased liver enzymes. Low serum ceruloplasmin and increased hepatic copper concentrations were present in selected patients, without meeting the Wilson disease criteria. Nonhepatic symptoms such as hypercholesterolemia (for all defects), neurodegenerative symptoms (CCDC115 and ATP6AP1 deficiency), and hypogammaglobulinemia (ATP6AP1 deficiency) are also present. Various other nonhepatic symptoms can be found (Supplementary Table 1). The families were identified based on their abnormal protein glycosylation pattern through a straightforward biochemical assay. The severity of symptoms varied: some patients had isolated mild increases of transaminase levels whereas others developed end-stage liver disease necessitating liver transplantation.

TMEM199, CCDC115, and ATP6AP1 most likely function in the assembly of the vacuolar H\(^+\) adenosine triphosphatase, or V-ATPase. The V-ATPase is the main proton pump of the secretory pathway for acidification of the Golgi and lysosomes. It also is involved in a large number of additional cellular functions such as protein trafficking and membrane fusion. The V-ATPase consists of 14 core units divided between 2 domains: a transmembrane V0 domain and a cytosolic V1 domain. The V1 domain provides the energy for the V0 domain to translocate protons. The V0 domain consists of a proteolipid ring (made from c, c’, and c” subunits) and a cage structure made from subunit a. An additional d subunit provides the link between the V0 and V1 domains.

TMEM199 and CCDC115 are located in the ER-to-Golgi intermediate complex (ERGIC), and in coatamer protein complex I and II (COPI and COPII) vesicles in HeLa- and primary hepatoma cells. TMEM199, 208–amino acids small, is a transmembrane protein and shares a Vma12 domain that is conserved throughout species. CCDC115 is a 181–amino acid small protein with 2 coiled-coil domains. A recent immunoprecipitation study and large proteome-wide interaction studies have shown an interaction of TMEM199 with CCDC115. ATP6AP1 is located on the X chromosome and encodes the 470–amino acid long Ac45 protein. Ac45 is a V-ATPase accessory protein and is located in the ER and ERGIC. In yeast, it interacts with the c’ subunit of the proteolipid ring of the V-ATPase complex.

Mutations in all 3 proteins lead to impaired N- and O-glycosylation with a pattern compatible with defects in Golgi homeostasis. Impaired expression of TMEM199, CCDC115, and ATP6AP1 likely results in impaired functioning of the lysosomal compartment, similar to knockdown of the V-ATPase.

Yeast studies with homologs of TMEM199, CCDC115, and ATP6AP1 have shown that during assembly of the V0 domain, Vph2p (yeast homolog of TMEM199) and Vma22p (homolog of CCDC115) stabilize the cage structure. Voa1p (homolog of ATP6AP1) and Vma21p (homolog of VMA21, a fourth protein) stabilize the proteolipid ring and chaperone the V0 domain from the ER to the Golgi.
These data lead to a model that places these proteins in the assembly pathway of the V-ATPase V0 domain. CCDC115 and TEMEM199 stabilize the a-subunit during assembly and VMA21 and cleaved Ac45 stabilize the c-ring and guide the V0 domain (or the holocomplex with the V1 domain) to the Golgi. VMA21, and possibly CCDC115 and TEMEM199, then are transported back via COPI vesicles. However, this model largely is based on yeast studies, and functional evidence in human beings for involvement of CCDC115 and TEMEM199 in ER-to-Golgi transport currently is lacking.

Our hypothesis is that defects in V-ATPase assembly factors lead to abnormal glycosylation because they impair ER-to-Golgi protein trafficking. Indeed, V-ATPase assembly factors co-localize with COPI, COPII, and ERGIC markers, indicating a function in early protein transport. In addition, serum proteins from patients with a V-ATPase assembly factor defect have a specific glycosylation pattern, indicative for abnormal Golgi protein trafficking.

Impaired protein trafficking also could explain the NAFLD phenotype because emerging insights in ER-to-lipid droplet protein trafficking show that knockdown of the COPI machinery results in intracellular triglyceride accumulation. However, further research is required to determine if the phenotype is dependent on defects in the V-ATPase assembly proteins, the function of the V-ATPase, impaired glycosylation, or a factor yet undetermined.

To conclude, V-ATPase assembly protein deficiencies are a novel group of inborn errors of metabolism that have to be considered in the differential diagnosis of NAFLD not associated with the metabolic syndrome. The hepatic phenotype ranges from mild impairment to end-stage liver disease necessitating liver transplantation. We propose a multifaceted etiology based on abnormal Golgi apparatus homeostasis. Screening can be performed easily via glycosylation analytics. Better awareness of these genetic defects will improve patient outcome and provide new insights in the etiology of NAFLD.

**References**


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Author contributions
Jos C. Jansen and David Wolthuis drafted the manuscript, tables, and figures; Monique van Scherpenzeel reviewed the manuscript; and Vlad Ratziu, Joost P. H. Drenth, and Dirk J. Lefeber supervised, designed, and reviewed the manuscript.

Conflicts of interest
These authors disclose the following: Vlad Ratziu serves as a Scientific Advisor for Phenex Pharmaceuticals AG, Galmed Pharmaceuticals Ltd, Genfit SA, and Tobira Therapeutics, Inc; and Joost P. H. Drenth has served on the advisory boards of AbbVie, Gilead, and Intercept. The remaining authors disclose no conflicts.

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**Supplementary Table 1. Extrahepatic Symptoms of V-ATPase Assembly Factor Deficiencies**

<table>
<thead>
<tr>
<th>Extrahepatic symptoms</th>
<th>TMEM199-CDG</th>
<th>CCDC115-CDG</th>
<th>ATP6AP1-CDG</th>
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<td>±</td>
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<td>Hematologic symptoms</td>
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