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EDITORIALS

ABCG8 D19H polymorphism: A basis for the genetic prediction of cholesterol gallstone disease

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Cholesterol gallstone disease is one of the most common digestive diseases, with an overall prevalence of 10–20%. Although a common and economically-relevant problem in developed countries, its pathogenesis remains undefined and is the subject of ongoing investigation.[1] Cholesterol gallstone disease appears to be influenced by both genetic predisposition and environmental factors,[2] and ethnic and geographical differences have indeed been found.[3] Currently, there is active investigation regarding cholesterol gallstone susceptibility genes (Lith genes), as these genes have been found not only to be useful in treating gallstone disease, but also in diagnosing a 'prestone' state in patients.[4] Recently, as the result of a genome-wide association scan, cholesterol transporter adenosine triphosphate-binding cassette (ABC) G8 was identified as a susceptibility factor for human cholesterol gallstone disease.[5] Buch *et al*.[5] showed that single-nucleotide polymorphism (SNP) A-1791411 in ABCG8 encoded the variant rs11887534 (D19H), and that the association of ABCG8 D19H with cholesterol gallstones was present in Germans and Chileans after adjusting for body mass index, sex, and age. The overall odds ratio for gallstone disease among D19H carriers in Germans and Chileans was 2.2 (95% confidence interval [CI]: 1.8–2.6).

ABC transporters are transmembrane proteins that facilitate the transport of specific substrates across the membrane in an ATP-dependent manner. Eukaryotic ABC transporters have been subdivided into either 'full' or 'half' transporters and into seven subgroups (A–G), based on sequence similarity and domain organization.[6] Among these ABC transporters, ABCG5 and ABCG8 represent apical (canalicular) membrane sterol export pumps that promote the active efflux of cholesterol from hepatocytes into bile. ABCG5 and ABCG8 proteins form functional heterodimers, localized in the apical membranes of enterocytes and canalicular membranes of hepatocytes; they act as efficient exporters of cholesterol into bile.[7] The subcellular localization of ABCG5 and ABCG8 has been shown to change, with predominantly intracellular localization at baseline and predominantly apical plasma membrane localization following treatment with model bile or ligands for liver X receptor-α.[8] Additionally, ABCG8 has been detected in the epithelia of normal human gallbladders. The expression pattern is diffuse, but with focal areas of increased expression in the apical poles of cells. Of note, increased ABCG8 expression has been found in diseased gallbladders of patients with cholesterol gallstones (Dr Jai Hoon Yoon and Dr Ho Soon Choi, pers. comm., 2008).

Cholesterol gallstones develop when bile contains excessive amounts of cholesterol and insufficient amounts of bile salts. As mentioned previously, the presence of the D19H mutant allele of the *ABCG8* gene is associated with cholesterol gallstones,[5] suggesting that the mutated allele might confer more efficient transport of cholesterol into bile, in turn causing cholesterol supersaturation. Other studies have identified a variety of polymorphisms in ABCG8 (A632V, T400K, D19H, and C54Y) and in ABCG5 (Q604E) that have been linked to baseline plasma cholesterol levels, cholesterol absorption, or responsiveness to dietary intervention. In addition, genetic variations in the *ABCG8* gene (D19H and T400K) might increase the risk of gallstone disease in certain populations.[9]

These sex- and population-specific gene polymorphisms, and the interactions between sex, genes, and diet also need to be considered in studies of polymorphisms of Lith genes. Hubacek *et al*.[10] reported that none of the polymorphisms examined, including ABCG5 Q604E, ABCG8 D19H, and ABCG8 A632V, were related to plasma lipid levels in patients after 'evolutionary' dietary changes from a traditional, high-fat Eastern European diet to a lower-fat diet based on nutritional advice. Yet when a subanalysis of male participants was performed, the ABCG8 T400K wild-type allele carriers exhibited a greater decrease in plasma total cholesterol and low-density lipoprotein cholesterol (LDL-C) than mutant allele carriers.[10] Wang *et al*.[11] suggested that the T400K polymorphism in ABCG8 might be associated with gallstone disease in Chinese males by revealing a possible association between this transporter gene polymorphism and gallstone formation. Wang *et al*.[11] examined five common polymorphisms in the *ABCG5* (Q604E) and *ABCG8* (D19H, Y54C, T400K, A632V) genes in 287 patients with gallstone disease. The relative risk of gallstone formation was 2.31 (95% CI: 1.12–4.76) for males carrying the K400 allele of ABCG8 T400K. Katsika *et al*.[12] demonstrated that twins carrying a heterozygous or homozygous ABCG8 D19H genotype have a significantly increased risk of gallstone disease. This result confirmed the ABCG8 D19H genotype as a major risk factor for gallstone disease in Swedish twins. Additionally, Chen *et al*. suggested that the D19H polymorphism of ABCG8 could be considered a susceptible gene marker by revealing an increased likelihood of developing high cholesterol and LDL-C with the D19H polymorphism in Taiwanese consuming an ordinary Chinese diet.[13]

In this issue of *Journal of Gastroenterology and Hepatology*, Srivastava *et al*.[14] report that the ABCG8 D19H (rs11887534) variant, DH genotype, and H allele increase susceptibility to cholesterol gallstone disease in a north Indian population. They found that the risk of cholesterol gallstone disease due to the ABCG8 D19H variant was more prominent in females. Therefore, when investigating polymorphisms in ABCG5/ABCG8 transporters linked to cholesterol gallstone diseases, it is vital to consider population- and sex-specific differences. As such, ongoing studies are gradually revealing the unique epidemiological and genetic distribution of various populations. However, the mechanism by which cholesterol molecules are effluxed from hepatocytes and gallbladder epithelial cells into bile still represents an unsolved mystery. Even the largest epidemiological surveys did not find any

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relationship between the total plasma cholesterol level and gallstones, but most confirmed the findings of the original studies, namely an association of high-density lipoprotein levels[15] and hypertriglyceridemia[16] with cholesterol gallstone formation.

Gallstone susceptibility might be influenced by a number of factors, including genetic predisposition involving multiple genes and gene–gene interactions, plus interactions with a 'lithogenic' environment; the latter involves such factors as diet, obesity, weight loss, certain drugs, and multiple pregnancies. A combination of these factors might lead to gallstone formation if a threshold of susceptibility is reached, since each susceptibility allele only confers a modest increase in risk.[17] However, the D19H substitution of the *ABCG8* gene resulted in a completely different situation: low serum cholestanol, sitosterol, and campesterol levels, suggesting limited sterol absorption,[18] causing cholesterol supersaturation in bile and promoting the formation of cholesterol gallstones. Srivastava *et al*. report negligible differences between the wild-type and polymorphic ABCG8 protein structures, and suggest that the effect of this SNP might be mainly due to a change in charge, rather than a change in the 3-D structure of the protein.

The article by Srivastava *et al*. brings up interesting questions regarding the susceptibility mechanisms of cholesterol gallstone disease. First, how can genes that regulate cholesterol absorption and excretion in bile regulate cholesterol saturation in the gallbladder lumen? Second, what are the functional interactions with sex and environmental factors, such as diet? Another potential mechanism for gallstone disease susceptibility is 'inflammation'; studies in mouse models reveal a role for the inflammatory process in gallstone formation.[19] How does inflammation interact with gene polymorphisms associated with gallstone susceptibility? Finally, sphingolipid biosynthetic pathways,[20] and obesity and its comorbid conditions that comprise the metabolic syndrome,[21] are being investigated as susceptible factors in the development of cholesterol gallstone disease. How does the D19H polymorphism of ABCG8 interact with these factors in promoting cholesterol gallstones?

In conclusion, the results of the population-specific study reported by Srivastava *et al*. confirm that the DH genotype and H allele of the ABCG8 D19H polymorphism are associated with a risk of gallstone susceptibility in a north Indian population. Further population- and gene-specific ABCG8 polymorphism investigations should be performed through large-scale studies to define its role in the development of human cholesterol gallstone disease. We can anticipate that this ABCG8 polymorphism could become a potential marker that will aid in efforts in preventing cholesterol gallstone formation.

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