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Genetic polymorphism of sterol transporters in children with future gallstones

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ABSTRACT

Background & aims: Gallstone disease is related to hypersecretion of cholesterol in bile, and low serum phytosterol levels. We examined how genetic polymorphisms of sterol transporters affect childhood cholesterol metabolism trait predicting adult gallstone disease.
Patients and methods: In retrospective controlled study, we determined *D19H* polymorphism of *ABCG8* gene, genetic variation at *Niemann-Pick C1-like 1* (*NPC1L1*) gene locus (rs41279633, rs17655652, rs2072183, rs217434 and rs2073548), and serum cholesterol, noncholesterol sterols and lipids in children affected by gallstones decades later ($n = 66$) and controls ($n = 126$).
Results: In childhood, phytosterols were lower (9.7%–23.4%) in carriers of risk allele *19H* compared to *19D* homozygotes. Lowest campesterol/cholesterol tertile consisted of 1.9-times more future gallstone subjects, and 3.7-times more *19H* carriers than highest one. Campesterol/cholesterol-ratio was highest in *19D* homozygote controls, but ~11% lower in gallstone *19D* homozygotes and ~25% lower among gallstone and control carriers of *19H*. Gallstone subjects with alleles *CC* of rs41279633 and *TT* of rs217434 of *NPC1L1* had ~18% lower campesterol/cholesterol-ratio compared to mutation carriers.
Conclusions: Risk trait of cholesterol metabolism (low phytosterols) in childhood favouring cholesterol gallstone disease later in adulthood is influenced by risk variant *19H* of *ABCG8* and obviously also other factors. *NPC1L1* variants have minor influence on noncholesterol sterols.

1. Introduction

Adult gallstone disease represents a prevalent and costly health problem. The vast majority – over 90% – of gallstones in adults are cholesterol stones in the Western world [1–4]. Although the cholesterol gallstone disease has a multifactorial etiology consisting of genetic and environmental factors, the adults predisposed to the gallstone disease share as a common feature increased biliary output of cholesterol [5–7].

Heterodimeric sterol transporter ABCG5/G8 determines biliary secretion of cholesterol and plant sterols from the hepatocyte into the biliary canaliculus and their efflux out of the enterocyte into the intestinal lumen [8,9]. Niemann-Pick C1-like 1 (NPC1L1) protein has an essential role in intestinal cholesterol up-take into the enterocytes, and, additionally in humans, in the retention of biliary cholesterol by the hepatocytes [10]. Consequently, the biliary secretion of cholesterol and plant sterols is determined by the balance between their efflux across the hepatocyte canalicular membrane governed by these transporters [11]. The adult gallstone disease has been related to *ABCG8* gene polymorphism *D19H* (rs11887534) in several studies [5,12]. However, Krawczyk et al. suggested that

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ABCG8 polymorphism did not fully explain the sterol metabolic trait of the gallstone carriers [5]. Theoretically, decreased expression or function of the hepatic NPC1L1 transporter (*e.g.*, by genetic loss-of-function variants), or its increased expression at the brush border of the enterocytes are both likely to increase biliary cholesterol secretion and the propensity for gallstone formation [13]. Overall, results concerning the relationship between the cholesterol gallstone disease and polymorphism of *NPC1L1* have remained controversial [14,15]. Interestingly, Lauridsen et al. showed in their large population-based study that genetic variation in *NPC1L1* that is associated with lowered cholesterol absorption is also associated with a reduced risk of ischaemic vascular disease, and reduction in serum LDL-cholesterol, but with a concomitant rise in the incidence of gallstone disease [15].

Among the serum noncholesterol sterols, cholestanol (a derivative of endogenous cholesterol), and phytosterols campesterol and sitosterol reflect positively the absorption efficiency of cholesterol [16–20]. Opposite to that, the cholesterol precursor sterols, cholestenol, lathosterol, and desmosterol, mirror whole-body cholesterol synthesis [16,18,20], and also the activity of hepatic hydroxy-methyl-glutaryl-CoA reductase [21,22]. Thus, determination of cholestanol, the cholesterol precursor sterols and phytosterols in serum provides a signature of cholesterol metabolism, which evaluated together with the risk mutations of the genes encoding the major canalicular sterol transporters helps to assess the etiopathogenesis of the gallstone disease. Our previous results indicated that cholesterol metabolism trait characterized by low serum levels of surrogate markers of cholesterol absorption precedes adult gallstone disease already in childhood [7]. Recent data from morbidly obese subjects suggest that low serum plant sterols among patients with gallstones indicate potentially inherited alterations in intestinal absorption and biliary transport of sterols [23].

It has remained unexplored (I) how *D19H* of *ABCG8* and polymorphisms at *NPC1L1* locus modify metabolism of cholesterol in childhood, and (II) to what extent are these genetic polymorphisms related to the childhood cholesterol metabolism trait predicting the adult gallstone disease.

To this end, we performed a retrospective controlled study, in which we evaluated how *D19H* polymorphism of the *ABCG8* gene and genetic variation at the *NPC1L1* gene locus affect metabolism of cholesterol, plant sterols and lipids in a pediatric cohort affected by the gallstone disease decades later in adulthood, and compared the results with the age-, gender- and body mass index (BMI) matched control subjects. The study subjects were participants of the prospective Cardiovascular Risk in Young Finns Study [24–26].

2. Subjects and methods

2.1. Subjects and ethics

The Cardiovascular Risk in Young Finns Study is a multicenter follow-up study of Finnish children and adolescents [24–26]. The first cross-sectional survey was conducted in 1980, when 3596 participants, ages 3, 6, 9, 12, 15 and 18 years, were randomly chosen from the five geographic study areas of Finland through the national population register. At the study inclusion, determination of serum lipids, dietary survey and collection of serum samples was performed. In the present study, the participants were followed up based on the unique personal identification codes through the Care Registers for Social Welfare and Health Care until 2012 for the diagnosis of gallstone disease and regular medications. Gallstone disease was defined as a hospital discharge diagnosis that included ICD-10 codes K80.0–K80.8 as either a primary or secondary code. In Finland, the hospital discharge register includes all hospitals. Both primary and secondary codes were used to ensure capture of all gallstone disease patients. For each case, who developed gallstone disease ($n = 95$) two unaffected controls ($n = 190$) were matched for BMI, age and sex recorded at the start of the follow-up in 1980. For the purposes of the present study, the respective BMI-values in year 1983 were registered. The frozen serum samples of the patients and controls obtained at the first survey in 1980 were retrieved for measurement of cholesterol and non-cholesterol sterols, and the preliminary results of these data without knowledge of the genetic data have been reported earlier [7]. Of these cases and controls, genome-wide genotyping data was available in 66 cases and 126 controls. Data of *NPC1L1* gene variants was missing in one of the gallstone patients. All the analyses were repeated after excluding subjects taking lipid-lowering medications at some point during the follow-up (one for the gallstone and two for the control cohort) with essentially similar results.

Consumption of vegetables (excluding potatoes), fruits and juices, meat meals and fish meals had been recorded in year 1980 and classified: 1 = once a day or more frequently, 2 = almost every day, 3 = twice a week, 4 = once a week, 5 = once or twice a month and 6 = seldom or never.

All subjects volunteered to the study and provided their written informed consent. The study followed up the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the human research committee. The Ethics Committee of the Turku University Central Hospital had accepted the study protocol.

2.2. Biochemical determinations and DNA analysis

Standard enzymatic methods were used for determination of serum total cholesterol, HDL cholesterol and serum triglyceride concentrations, and that of LDL cholesterol was calculated. Details of the methods have been described previously [26].

Serum squalene, cholesterol and non-cholesterol sterols were measured by gas-liquid chromatography (GLC) using a 50-m Ultra 2 capillary column (Agilent Technologies, Wilmington, Del) (for detailed method description see Ref. [27]). The procedure uses 5α-cholestane as internal standard, and it measures the serum concentrations of squalene, cholesterol, cholestanol, cholestenol, desmosterol, lathosterol, campesterol, sitosterol, stigmasterol and avenasterol, in this order of retention time. The serum levels of major lipoproteins, which transport non-cholesterol sterols, particularly LDL, vary with age. For this reason, the values were calculated as ratios to cholesterol measured in the same GLC run, expressed as 100× mmol/mol of cholesterol, and given in the text as ratios. Serum cholestenol, lathosterol, and desmosterol ratios are called surrogate or relative synthesis markers, and the ratios of cholestanol, campesterol, sitosterol and avenasterol are called surrogate or relative markers of cholesterol absorption efficiency. Calculation of synthesis marker/absorption marker ratios, *e.g.*, lathosterol/sitosterol reflected whole-body cholesterol metabolism [28].

Five single nucleotide polymorphisms (SNPs) in the *NPC1L1* locus were genotyped in this study because they are related to serum cholesterol levels: −18C > A (rs41279633), −133A > G (rs17655652), 1679C > G or L272L (rs2072183), 27621T > C or V1296 V (rs217434) and g-762T > C (rs2073548).

Genotyping of all SNPs was performed using Illumina Human 670 K BeadChip. Genotypes were called using the Illumina clustering algorithm [29] and imputation was performed using SHAPEIT v1 [30] and IMPUTE2 [31] software and the 1000G Phase I Integrated Release Version 3 as a reference panel [32]. Two of the candidate SNPs analyzed in this study were directly genotyped (rs17655652 and rs217434), the other four were imputed. Both genotyped SNPs were in Hardy-Weinberg equilibrium ($p > 0.05$). Since the values of imputed genotypes account for the uncertainty