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Brief Communication

The combination of single nucleotide polymorphisms rs6720173 (*ABCG5*), rs3808607 (*CYP7A1*), and rs760241 (*DHCR7*) is associated with differing serum cholesterol responses to dairy consumption

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Abstract

Existing evidence on influence of genetic architecture on serum cholesterol responsiveness to dietary interventions focuses on individual SNPs and single nutrients. We associated the combination of *ABCG5* rs6720173-C, *CYP7A1* rs3808607-TT, and *DHCR7* rs760241-GG genotypes with lower LDL-cholesterol concentrations relative to the combination of rs6720173-GG, rs3808607-G, and rs760241-A genotypes (-0.37 ± 0.12 ($n = 9$) vs. $+0.38 \pm 0.14$ mmol/L ($n = 7$), $p = 0.0016$) following a blended dairy (3 servings/day for 4 weeks) intervention.

Key words: dairy, cholesterol, nutrigenetics, SNP combination

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Introduction

In the past few years, genome-wide association studies (GWAS) have successfully identified numerous single nucleotide polymorphisms (SNPs) that are linked to the variability in intrinsic cholesterol concentrations (Teslovich et al. 2010; Willer et al. 2008). Frequent criticisms of these studies are that most of them do not consider possible interactions with dietary intakes and that the variants discovered have small effect sizes. On the other hand, to date, the majority of gene by diet interaction studies documenting responsiveness of circulating cholesterol concentrations to dietary interventions have focused on individual SNPs and single nutrients (Abdullah et al. 2015), which, in quest of better personalized dietary advice, may not realistically reflect the nature of mechanisms of action in the body or typical eating habits of populations.

Recently, we have associated three common SNPs, rs6720173 in the cholesterol transport gene *ABCG5*, rs3808607 in the bile acid synthesis gene *CYP7A1*, and rs760241 in the cholesterol synthesis gene *DHCR7*, individually, among several other cholesterol metabolism related genes and SNPs, with varying responses of serum cholesterol to a recommended level of a blended dairy product intake (Abdullah et al. 2016). However, the cholesterol response is a complex phenotype that is likely to involve a synergistic act of numerous genes implicated in cholesterol absorption, biosynthesis, transport, and excretion (Desmarchelier et al. 2016; Desmarchelier et al. 2014). Therefore, we investigated the impact of the combination of *ABCG5* rs6720173, *CYP7A1* rs3808607, and *DHCR7* rs760241 to identify genotype sets that are associated with significant variability in the cholesterol responsiveness to controlled intakes of dairy products.

Materials and Methods

Study population

Normocholesterolemic individuals ($n = 137$) aged 18-69 years were recruited from Winnipeg, Manitoba ($n = 68$), and Quebec City, Quebec ($n = 69$), Canada, as has been previously detailed (Abdullah et al. 2016). The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the research ethics boards of the collaborating institutes. All subjects provided written informed consent prior to taking part in the study ([Clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01444326), NCT01444326).

Study design and dietary protocol

This was a secondary analysis of gene by diet interaction from a dual-centre, randomized, free-living, crossover clinical trial designed to investigate impacts of a recommended dairy intake (Health Canada 2011) on established risk factors for cardiovascular disease (CVD) in a healthy Canadian population. The study was conducted at the clinic units of the Richardson Center for Functional Foods and Nutraceuticals (RCFFN), University of Manitoba, and the Institute of Nutrition and Functional Foods (INAF), Laval University. Dairy products, including 375 ml/d of 1% milk fat (M.F.) milk, 175 g/d of 1.5% M.F. creamy stirred fruit-flavored yogurt, and 30 g/d of 34% M.F. cheddar cheese, and dairy-free products, including calorie-matched 100% fruit and vegetable juices, cashews, and study-made chocolate chip cookies, were consumed for periods of 28 days each on a prudent background diet with a median of 31-day washout period between study phases. Subjects were provided with the study products on a weekly basis, and completed dietary checklists and questionnaires regularly throughout the study time, as previously detailed (Abdullah et al. 2016).

Blood sampling and serum cholesterol analysis

Serum samples were collected once at screening and twice within the last week (days 22 and 28) of each dietary phase, after an overnight fast. Following centrifugation at 3000 rpm and 4°C for 20 min, aliquots of serum, plasma, buffy coat, and erythrocyte fractions were separated and immediately stored at –80°C until analysis. Serum total cholesterol and HDL-cholesterol were determined by automated enzymatic methods on a Roche/Hitachi Modular analyzer (Roche Diagnostics, Canada). LDL-cholesterol was calculated according to Friedewald formula (Friedewald et al. 1972).

Choice of candidate SNPs and genotyping

Selection of the *ABCG5* rs6720173, *CYP7A1* rs3808607, and *DHCR7* rs760241 to be investigated in combination stemmed from our findings of a previous gene by diet interaction analysis (Abdullah et al. 2016), where among 22 SNPs in 13 candidate genes (*ABCA1*, *ABCG5*, *ABCG8*, *ACAT2*, *APOE*, *CETP*, *CYP7A1*, *DHCR7*, *LDLR*, *LSS*, *PCSK9*, *SCAP*, *SREBF2*), these three SNPs were individually associated with differing cholesterol concentrations following dairy intake. The original selection of the 13 genes and 22 SNPs was based on a literature search (PubMed) for cholesterol metabolism related genes whose SNPs have been shown to be associated with circulating cholesterol concentrations, to have potential functional effects (i.e. located in a missense region), and/or to have higher minor allele frequencies. Influences of the SNPs of interest on responses of cholesterol concentrations to dairy consumption were assessed following the extraction of genomic DNA from white blood cells, by using the DNeasy® Blood & Tissue Kit (QIAGEN, USA), and quantification on the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., USA). DNA samples were genotyped by using TaqMan® assays on StepOnePlus™ Real-

Time PCR System (Applied Biosystems® Inc., USA). For quality control of SNP genotyping, each 96-well plate contained two duplicate samples and a negative control. The concordance rate for genotyping was >99.0%.

Statistical analyses

Endpoint values of the dairy and dairy-free diets were compared by using SAS 9.4 (SAS Inc., USA), with a statistical significance set at $p \leq 0.05$ for all analyses. Effects of the study diets on cholesterol concentrations were assessed by PROC MIXED model for repeated measures, with diet and sex included as fixed factors, and study site and subjects included as random factors. Genotype by diet interactions were analyzed by SAS ANCOVA, with sex included as a fixed factor, followed by Tukey-Kramer adjustments, and are presented as changes from dairy-free in response to dairy treatment (delta values of post-dairy minus post-dairy-free diets). Minor allele homozygotes were merged with the SNP heterozygotes and compared to the major allele homozygotes. Variables with a skewed distribution, as detected by Shapiro-Wilk test, were $\log_{10}$ transformed before analysis. Findings are reported as least-square means $\pm$ SE unless otherwise indicated. Deviations from Hardy-Weinberg equilibrium (HWE) for the genotypes of each SNP were tested by using a web-based calculator by Michael H. Court (Tuft University), where p values > 0.05 were considered consistent with the HWE.

Results

Baseline characteristics

From both study sites 124 individuals completed the study, of which 101 individuals (29 men and 72 women), including $n = 41$ from Winnipeg and $n = 60$ from Quebec City, provided consent for the genotyping tests. Study participants were mainly Caucasians (75%), in

addition to 16% Asians, 3% Africans, 2% Hispanics, and 4% other ethnicities. Baseline characteristics and compliance to the dairy diet have been previously reported (Abdullah et al. 2016). No difference in cholesterol concentrations between genotypes was observed at baseline (**Table S1**).

Responses of serum cholesterol concentrations to dairy consumption

Responsiveness of serum cholesterol concentrations to dairy versus dairy-free diets, with and without interactions with individual genotypes under study, has been previously reported (Abdullah et al. 2016). Genotype distribution for each SNP did not deviate from HWE ($p = 0.265$, 0.367 , and 0.429 for rs6720173, rs3808607, and rs760241, respectively).

When a combinatory approach was applied (**Fig. 1**), where SNPs rs6720173, rs3808607, and rs760241 were assessed in combinations of genotypes, maximal increases ($p = 0.0016$) in serum LDL-cholesterol concentrations ($+0.38 \pm 0.14$ mmol/L, $n = 7$) were observed among homozygotes for the G-allele of rs6720173 who also carried the rs3808607-G allele and rs760241-A allele, in comparison to maximal decreases among carriers of the rs6720173-C allele who were also homozygotes for the T-allele of rs3808607 and G-allele of rs760241 (-0.37 ± 0.12 mmol/L, $n = 9$). Other combinations of genotypes in these SNPs showed varying cholesterolemic responses to dairy consumption, some of which were also statistically significant, as illustrated in Fig. 1. While similar trends were observed for total cholesterol ($p < 0.0001$), there was no between-genotype difference in serum HDL-cholesterol (Fig. 1) or triglycerides concentrations following dairy intake (not shown).

Discussion

We investigated the serum cholesterol responses to dairy consumption in interaction with the combination of three common SNPs; in *ABCG5*, a gene encoding a half-transporter that is responsible for cholesterol excretion from enterocytes into the intestinal lumen and from hepatocytes into the bile (Jakulj et al. 2010), *CYP7A1*, a gene encoding the rate-limiting enzyme for bile acid synthesis in the liver, which is the classical mechanism for cholesterol removal from the body (Chiang 2009), and *DHCR7*, a gene encoding the enzyme that catalyzes the terminal reaction in the cholesterol biosynthesis pathway (Luu et al. 2015). The observations reveal a genetic basis that may contribute to some of the heterogeneity in the response of cholesterol to dairy consumption as reported in the literature (Drouin-Chartier et al. 2016). To our knowledge, this is the first demonstration of such influence of combinations of genetic variants on serum cholesterol responses to a feeding intervention.

The genes and SNPs of interest in the present study are known to play important roles in cholesterol metabolism and have, particularly in the case of rs6720173 and rs3808607, been shown to associate with cholesterol concentrations, with (Abdullah et al. 2016; Wang et al. 2016; Herron et al. 2006) and without (Li et al. 2012; Weggemans et al. 2002) interaction with diet. These SNPs are thus likely to also be linked to diseases affected by hypercholesterolemia, such as CVD. Although, to our knowledge, studies associating such gene by diet interaction impact on hard endpoints are lacking.

The exact mechanism of action of our observations is yet to be defined. However, in light of previous observations by others and findings of our work, it is possible that carriers of the combination of rs6720173-GG, rs3808607-G, and rs760241-A genotypes may show a lower efficiency in the efflux of cholesterol from enterocytes into the intestinal lumen, and less

ability to catabolize cholesterol through the formation of bile acids (De Castro-Oros et al. 2010), while synthesizing less cholesterol through lower activity of 7-dehydrocholesterol reductase (Gylling and Miettinen 1989; Miettinen et al. 1990), leading to higher cholesterol concentrations following dairy intake. Noteworthy, is that following dairy versus dairy-free diets, all carriers of the rs6720173-GG, rs3808607-G, and rs760241-A genotype set showed higher LDL-cholesterol concentrations (average 19%), whereas all carriers of the rs6720173-C, rs3808607-TT, and rs760241-GG genotype set showed lower LDL-cholesterol concentrations (average -10%). The clinical implication of these observations is likely significant given evidence from epidemiologic studies, clinical trials, and meta-analyses that suggests at least 1% reduction in CVD incidence or mortality due to cardiovascular events with each 1% reduction in LDL-cholesterol or total cholesterol concentrations (Mihaylova et al. 2012; Robinson et al. 2005; Gould et al. 1995). More research is needed to confirm such hypothesis, however, on the basis of the fact that dairy products constitute several nutrients that may individually show varying cholesterolemic effects in interactions with different genotypes, and the influence that other lifestyle and genetic factors may play in this context.

The crossover design and use of commercially-available dairy products that are habitually consumed by the general public in Canada are strengths of this study. Limitations include the unevenly distributed frequencies of genetic variants and the population size, which may have been too small for such genotyping investigations. Considering the lack of specific sample size and power calculation for detecting gene by diet interaction, the associations reported herein must be interpreted in the context of the study's sample size, as well as the free-living setting.

In summary, this candidate gene study suggests a combinatory action of common variants of genes along the cholesterol metabolic pathway that may work in conjunction with each other

to amplify individual genotyping impacts on serum cholesterol responses to dairy consumption. Given the complexity of the human genome, replication of the findings in prospective recruitment designs with sufficient power and larger sample sizes, and under fully controlled living conditions, is warranted to ensure better views into the potential clinical implications of the present work. Such further research is expected to contribute to development of predictive commercial tests and may over the long run improve personalized advice specifically tailored to dairy-based food choices.

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Conflict of interest statement

Peter Jones has received funding in the past from Dairy Farmers of Canada and Danone Inc.

Benoît Lamarche and Patrick Couture have received funding from Dairy Farmers of Canada and Dairy Australia. Benoît Lamarche has received funding from Danone Inc. and has acted as the Chair of the Expert Scientific Advisory Committee of Dairy Farmers of Canada.

Mohammad Abdullah and Peter Eck report no conflict of interest.

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Figure Legends

Fig. 1. Responses of serum LDL-cholesterol and HDL-cholesterol concentrations to the 28 day consumption of dairy compared with dairy-free diet in normocholesterolemic individuals stratified by the combinations of *ABCG5* rs6720173, *CYP7A1* rs3808607, and *DHCR7* rs760241 genotypes ($n = 7$ rs6720173-GG + rs3808607-G + rs760241-A, $n = 42$ rs6720173-GG + rs3808607-G + rs760241-GG, $n = 5$ rs6720173-GG + rs3808607-TT + rs760241-A, $n = 17$ rs6720173-GG + rs3808607-TT + rs760241-GG, $n = 6$ rs6720173-C + rs3808607-G + rs760241-A, $n = 11$ rs6720173-C + rs3808607-G + rs760241-GG, $n = 2$ rs6720173-C + rs3808607-TT + rs760241-A, $n = 9$ rs6720173-C + rs3808607-TT + rs760241-GG). Labeled means without a common superscript letter are significantly different, $p \leq 0.05$. *ABCG5*, ATP-binding cassette sub-family G member 5; *CYP7A1*, cholesterol 7 α -hydroxylase; *DHCR7*, 7-dehydrocholesterol reductase; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; Δ cholesterol, change in serum cholesterol concentrations (post-dairy minus post-dairy-free diets).