Gene 532 (2013) 211–215

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Methods paper

Effect of a *GFOD2* variant on responses in total and LDL cholesterol in Mexican subjects with hypercholesterolemia after soy protein and soluble fiber supplementation

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ARTICLE INFO

Article history:
Accepted 15 September 2013
Available online 21 September 2013

Keywords:
Hypercholesterolemia
Soy protein
Soluble fiber
Low saturated fat diet
GFOD2

ABSTRACT

Background: Although dietary treatments can successfully reduce blood lipids in hypercholesterolemic subjects, individual variation in that response has on occasion been linked to allelic differences. SNP rs12449157 has shown association with HDL-C concentrations in GWAS and falls in the glucose-fructose oxidoreductase domain containing 2 (*GFOD2*) locus. Of interest, previous data suggest that this SNP may be under environmentally driven selection. Thus, the aim of this study was to assess if rs12449157 may mediate the response of lipid traits to a dietary supplementation (DS) with soy protein and soluble fiber in a Mexican population with hypercholesterolemia.

Methods: Forty-one subjects with hypercholesterolemia were given a low saturated fat diet (LSFD) for 1 month, followed by a LSFD + DS that included 25 g of soy protein and 15 g of soluble fiber (S/SF) daily for 2 months. Anthropometric, clinical, biochemical and dietary variables were determined. We analyzed the gene–diet interaction between the *GFOD2* genotype, with the minor allele frequency of 0.24, and the DS on total cholesterol (TC) and LDL-C concentrations.

Results: Hypercholesterolemic subjects with *GFOD2* rs12449157 G allele had higher serum TC and LDL-C at the baseline and showed a greater response to the LSCD + S/SF (−83.9 and −57.5 mg/dl, respectively) than those with *GFOD2* AA genotype (−40.1 and −21.8 mg/dl, respectively) ($P = 0.006$ for TC, 0.025 for LDL-C, respectively).

Conclusion: The observed differences in allele-driven, diet-induced changes in blood lipids may be the result of a recent environmentally driven selection on the rs12449157 minor allele. Variation in the *GFOD2* gene contributes to the genetic basis for a differential response to a cholesterol- or lipid-lowering diet.

1. Introduction

Hypercholesterolemia is a major health problem associated with an increased risk of cardiovascular disease (NCEP, 2001). Several dietary strategies have been used as a first step to reduce blood lipids as recommended by the National Cholesterol Education Program Expert Panel. Previous work has demonstrated the hypocholesterolemic effect of a dietary supplementation (DS) consisting of a combination of soy protein and soluble fiber (S/SF) integrated in a low saturated fat diet (LSFD) in a Mexican group with hypercholesterolemia (Torres et al., 2009). This dietary supplementation is defined as the combination of two or more functional foods and is designed to reduce the biochemical abnormalities of risk factors for specific diseases. We have shown that a significant decrease occurs in total cholesterol (TC), LDL cholesterol (LDL-C) and triglycerides (TG) in response to the dietary treatment but without any significant association between LDL-C levels and genetic variants in genes relevant to LDL-C homeostasis, including *ABCG5*, *ABCG8*, *APOE*, *APOA1* and *ABCA1* (Torres et al., 2009). This suggests that other genetic variants may contribute to the diverse responses to specific dietary treatments (Bouchard and Ordovas, 2012).

Genome-wide association studies (GWAS) have identified scores of genetic variants that appear to contribute to human cardiovascular disease risk (Ordovas and Guevara-Cruz, 2013). The GFOD2 protein, encoding glucose-fructose oxidoreductase domain containing 2, catalyzes the oxidation–reduction of glucose and fructose to gluconolactone and sorbitol. *GFOD2* SNP rs12449157 previously was demonstrated to be associated with blood lipid levels (Clark et al., 2003; Richardson et al., 2011a). Of note, rs12449157 also was shown to have an unusually high

Abbreviations: ATP III, National Cholesterol Education Program Adult Treatment Panel III; DS, dietary supplementation; *GFOD2*, glucose-fructose oxidoreductase domain containing 2; GWAS, genome-wide association studies; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LSCD, a low-saturated fat and low-cholesterol diet; miRNA, microRNA; S/SF, soy/soluble fiber; SNP, single nucleotide polymorphism; TC, total cholesterol; TG, triglycerides; F_{ST}, fixation index.

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http://dx.doi.org/10.1016/j.gene.2013.09.055

F_{ST} value (Cheng et al., 2009) with allele frequencies varying widely among HapMap Phase 3 populations (Table 1). As the F_{ST} statistic measures population differentiation where higher F_{ST} values suggest local positive adaptation, it is plausible that these variants have been driven to higher frequencies by environmental factors, and one such factor could be the diet or a change in the physical environment that exerts an impact on cultivated foodstuffs (Lai, 2012).

As Europeans and Native Americans are ancestors of Mexicans and as both European and Mexican populations showed important differences in allele frequencies at rs12449157, it is anticipated that this variant could have effects on lipids in the Mexican population similar to those seen in other studies (Clark et al., 2003; Richardson et al., 2011a). Alternatively, the effect of this SNP could be uncovered by an analysis of gene diet interactions. Therefore, the aim of this study was to assess the association between rs12449157 and the combined effect on lipid concentrations of rs12449157 and a dietary supplementation with soy protein and soluble fiber in a group of Mexican subjects with hypercholesterolemia.

2. Subjects and methods

2.1. Study population and study design

We included 41 subjects with hypercholesterolemia from a previously described cohort and all of these subjects did complete the dietary intervention (Torres et al., 2009). The participants were Mexican mestizos who were informed of the protocol, and written informed consent was obtained from all participants. This study was approved by the Committee for Human Research at the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán. The study was a prospective cohort design. The subjects were instructed to consume an isocaloric LSCD (Table 2), based on the National Cholesterol Education Program Adult Treatment Panel III (ATP III) for 4 weeks — period 1 (2001). After 4 weeks, all participants were instructed to consume the isocaloric LSCD including a DS based on 25 g of soy protein and 15 g of soluble fiber (S/SF) for a period of 2 months (Anderson et al., 1995; Zhan and Ho, 2005). Body weight was measured monthly, and blood samples were obtained at 1-month intervals after a 12-hour overnight fast. The participants had no history of cardiovascular, renal or liver disease, diabetes, or hypertension, had no history of smoking and were not taking hypolipidemic agents. The subjects were also asked to maintain their normal physical activity level throughout the study.

Table 1
F_{ST} values for rs12449157 among HapMap Phase III data.

Population	F_{ST}
ASN	0.0059
EUR	0.0040
AFR	0.0841
AEA	0.8399

F_{ST}: differentiation among populations.
ASN: samples of Asian, CHB, CHD and JPT.
CHB: Han Chinese in Beijing, China.
CHD: Chinese in Metropolitan Denver, Colorado.
JPT: Japanese in Tokyo, Japan.
EUR: samples of European, CEU and TSI.
CEU: Utah residents with Northern and Western European ancestry.
TSI: Toscans in Italy.
AFR: samples of African, YRI, ASW, LWK, and MKK.
YRI: Yoruba in Ibadan, Nigeria.
ASW: African ancestry in Southwest USA.
LWK: Luhya in Webuye, Kenya.
MKK: Maasai in Kinyawa, Kenya.
AEA: ASN, EUR and AFR.

Table 2
Composition of the low-saturated, low-cholesterol diet (LSCD) used during the study.

Percentages refer to total daily energy intake	LSCD diet
Carbohydrates, %	60
Protein, %	15
Total fats, %	25
Polyunsaturated fatty acids, %	7
Monounsaturated fatty acids,%	12
Saturated fatty acids, %	6
Fiber, g/d	22
Cholesterol, mg	60

2.2. Anthropometric and biochemical determinations

Anthropometric variables, including height, weight and waist circumference, were measured in subjects using standard techniques (Lohman et al., 1988) During each visit, a 5-ml blood sample was obtained after 12 h of fasting. The blood was centrifuged at 400 ×*g*, and the serum was separated and stored at −20 °C until analysis. The serum was analyzed for TC, TG, and HDL-C. TC and TG were determined enzymatically (SERA-PAK Plus, Bayer de México, Mexico City) (Allain et al., 1974; Bucolo and David, 1973; Werner et al., 1981). Serum HDL-C was determined using an immunoassay method (Nauck et al., 1998) (DiaSys Diagnostics Systems GmbH, Holzheim, Germany), and LDL-C was calculated using the method described by Friedewald et al. (1972).

2.3. Genotyping

During the first visit, an additional 5-ml blood sample was withdrawn, and DNA was extracted from the leukocytes (Miller et al., 1988). Genotypes of the *GFOD2* SNP rs12449157 were determined using a polymerase chain reaction (PCR)-based TaqMan allele discrimination assay (ABI Prism 7900HT Sequence Detection System; Applied Biosystems, Foster City, CA).

2.4. Statistical analyses

The results are expressed as the mean ± SEM. Variables were assessed using the Kolmogorov–Smirnov *Z*-test to examine the distribution type. If the outcome variable did not exhibit a normal distribution, a logarithmic transformation was undertaken prior to analysis. To examine the differences between genotypes in clinical and biochemical characteristics and in percentage change after treatment, comparisons were tested with the Student *t*-test. Differences between the basal and final biochemical parameters were evaluated by one-way ANOVA. When the main effects were identified by the initial analysis, we conducted a post-hoc analysis using Bonferroni correction. To determine genotypic effects on blood lipids in response to the diet, we used a repeated-measure ANOVA adjusted for age, sex, and baseline weight. The genotype frequencies were examined using a goodness-of-fit test to determine whether the observed values differed from Hardy–Weinberg equilibrium. $P < 0.05$ was considered significant for biochemical parameters. The data were analyzed using SPSS for Windows (Version 10.00; SPSS Inc., Chicago, Ill).

3. Results

Distribution of the three genotypes of the *GFOD2* variant rs12449157 (AA, AG, and GG) were 23, 16 and 2, respectively. These genotypes were distributed according to Hardy–Weinberg equilibrium ($P > 0.05$). The G allele frequency was 0.24 in these hypercholesterolemic subjects. Considering the moderate to low frequencies of both the G allele and the GG genotype, subjects with AG and GG genotypes were combined into a single genotype group – G allele carriers – for further analysis.