The contribution of candidate genes to the response of plasma lipids and lipoproteins to dietary challenge

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Abstract

The possible role of four candidate genes in lipid and lipoprotein response to diet was examined in 214 members of two large kibbutz settlements in Israel. Four site polymorphisms (signal peptide insertion/deletion, XbaI, EcoRI and MspI) of the apo B gene, the common apo E genotypes, three common mutations (T-93G, S447stop and N291S) of the LPL gene and the CETP I405V RFLP were determined. The average reduction induced by diet in participants with the absence of the EcoRI restriction site (L4154) of the apo B gene compared with those found to be homozygotes for the restriction site (G/G4154) were: 16.2 and 8.0 mg/dl for total cholesterol (TC) (P < 0.01); and 15.6 and 6.2 mg/dl for LDL-C (P = 0.007), respectively. TC and LDL-C baseline levels were significantly different among the apo-E genotypes, yet there were no significant effects on lipid and lipoprotein dietary response. Triglyceride baseline values were significantly lower (P = 0.007) among subjects with the LPL S447stop mutation and HDL-C was significantly lower (P = 0.008) among subjects found to be heterozygous for the LPL N291S mutation. A heterogeneous response for triglyceride was observed for individuals with the S291 allele as compared to those individuals who were found to be homozygous for the N291 allele. No differences in dietary responsiveness were observed among the apo E and CETP genotypes. In conclusion, our results suggest that sequence variation(s) in the coding region of the apo B gene linked to the EcoRI polymorphism are associated with total cholesterol and LDL-C responsiveness to dietary manipulation. In our study population, LPL mutations had a significant effect on TG and HDL-C baseline levels and on their response to diet. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Lipids; Lipoproteins; Genetics; Diet

1. Introduction

Levels of plasma lipids and lipoproteins can be altered by diet and the magnitude of dietary effects varies between individuals [1–4]. These differences could result from different dietary adherence or may be due to true inter-individual variation in the response [1,5]. Other factors that may influence the response include sex [6], age [7], BMI [8] and the basal level of plasma lipids [9].

It is well established that genetic variation contributes to the basal plasma levels of lipids and lipoproteins. Various studies have shown an association between lipid and lipoprotein levels and the apolipoprotein B (apo-B) RFLPs [10–14], however this has not been consistent [15,16]. In addition, studies have indicated that the apo E polymorphism influences plasma lipid levels [17]. Recent studies have shown that variation at the lipoprotein lipase (LPL) and at the cholesterol ester transfer protein (CETP) loci also contribute to between-individual variation in plasma lipid and lipoprotein levels within the normal population [18–22].

It has been proposed that genetic variation may also contribute to the variability of these levels over time and in response to environmental exposure [23–26].
Recent studies provided evidence that genetic variability at apolipoprotein gene loci may contribute to the variation in lipid and lipoprotein response to dietary manipulations [27–36].

The present study examined the association between genotypes at the apo B, apo E, LPL and CETP loci with the response of plasma lipids and lipoproteins to dietary manipulation. This was carried out in 214 individuals who participated in dietary experiments in which fatty acid composition and cholesterol intake were modified while total energy intake remained constant.

2. Material and methods

The study was performed on healthy members of two large kibbutz settlements. Before the beginning of the study, all eligible subjects were assembled and the purpose of the study, its performance and requirements, on the part of the participants, were explained by the investigators. Signed consent was obtained from all subjects agreeing to participate.

Each subject underwent a medical examination and a routine biochemical screening. Subjects with endocrine or metabolic disturbances, such as diabetes mellitus, hypothyroidism, or those who reported any other cause of secondary dyslipoproteinemia were excluded. Also excluded were subjects who were found to consume more than 20% of their energy intake outside the kibbutz. Prior to the intervention, subjects were asked to record their entire food intake for several days. This enabled the investigators to calculate the energy requirements of each participant and to plan his/her diet accordingly.

Two different diets were administered in a crossover design. The first diet was characterized by a high content of saturated fatty acid and cholesterol (HSC) while the second diet consisted of low saturated fatty acid and low cholesterol content (LSC). The two diets were administered to the randomly allocated groups for a 4-week period (period 1) followed by a wash-out period of 4 weeks consisting of the participants’ regular home diet. Thereafter, subjects were given the other of the two diets for a second 4-week period (period 2). During the last week of each dietary period the participants were asked again to keep a record for several days a record of all foods eaten. The food records were coded by dieticians. Quantities of food were coded by frequencies, with one frequency representing the weight or volume of a standard serving or part of it. For several composite dishes, such as cakes or fillings, standard recipes were used to estimate their composition. A set of coding rules was used to estimate the amount of fat absorbed during cooking or frying and factors were calculated for converting raw materials to cooked and baked foods. The nutrient content of the codes was derived from several sources including local food producers and retailers, and local laboratory analyses for some items. For more general items, local food tables were applied [37]. These records were analyzed and the results are presented in Table 1. Total caloric intake was not significantly different between the two diets, but the SFA content and the amount of cholesterol intake did differ.

At the beginning and at the end of each period, fasting blood was drawn twice within 2–3 days for determination of lipids and lipoproteins. Participants’ weights were measured at several occasions throughout the study and an attempt was made to immediately identify all subjects who showed weight change. Such subjects where then advised by the dieticians how to adjust their energy intake so as to control the weight.

<table>
<thead>
<tr>
<th>Table 1 Composition of the diets during the two periods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal/day)</td>
</tr>
<tr>
<td>Baseline</td>
</tr>
<tr>
<td>LSC&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Period 1</td>
</tr>
<tr>
<td>Period 2</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>HSC&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Period 1</td>
</tr>
<tr>
<td>Period 2</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

<sup>a</sup>SFA, saturated fatty acids.

<sup>b</sup>LSC, low SFA/cholesterol diet; HSC, high SFA/cholesterol diet.
3. Laboratory methods

Plasma cholesterol and triglycerides were determined by an enzymatic procedure (Vitalab, Vital Scientific, Diesen, Netherlands). HDL-cholesterol (HDL-C) concentrations were determined after precipitation of apo-B-containing lipoproteins with phosphotungstic acid. Low-density lipoprotein cholesterol (LDL-C) was estimated by the Friedewald equation [38], which has been validated in our population [39].

The apo B insertion/deletion site in exon 1, and the XbaI, MspI, and EcoRI RFLPs at the 3' end of the apo B gene were studied. The polymorphism at the 5' end of the apo B gene involves the insertion/deletion of 9 bp in exon 1 which results in the addition or deletion of the three amino acids in the signal peptide [40]. The XbaI RFLP involves the third base of threonine codon 2488: the apo B gene involves the insertion of 9 bp in exon 5 near the 5' boundary of exon 6 (5'-GCCAGATAAACTTGGTG-3'). The 3' mismatch PCR primer was located in exon 6 near the Asn291Ser mutation (5'-CTGTTTTGGCTCTGACTGTA-3'). PCR amplification reactions were performed; 20 ml of the PCR product was digested with 10 U of RsaI, and the digested fragments were separated on 2% agarose gel.

Identification of the CETP I405V RFLP was performed as described previously [45]. This RFLP involves an A→G nucleotide substitution in exon 14 which does not result in the creation or disappearance of an enzyme restriction site. Therefore, a mismatch primer that introduced an RsaI restriction site in the presence of the mutation, was used. Target sequences were amplified with a 5'-PCR primer 5'-CTGTTTCCAACTTGACTGAG-3'). The 3' mismatch PCR primer was 5'-CGCCGGCCGGCCGGCCGGCGTCCGGCGCCCGCCGCCCATGGACTGAGAGAAG-3'). The amplification reaction mixture included 10 mmol/l Tris–HCl; pH 9.0, 50 mmol/l KCl, 0.1% (w/v) gelatin, 1.5 mmol/l MgCl2, 1% Triton X-100 and 20 mg/ml BSA containing 0.1–0.5 (µg genomic DNA and final concentrations of 100–200 µmol/l dNTPs and 0.5 µmol/l primers in a total volume of 50 µl. After initial denaturation (10 min, 95°C), 0.3–0.5 U thermostable DNA polymerase was added, followed by 30 amplification cycles at 95°C for 1 min, 60°C for 1 min, and 72°C for 1 min, with a final extension step of 10 min at 72°C. Some 20 µl of the PCR reaction product was digested with the restriction enzyme according to the instructions of the manufacturer in a total volume of 20 µl for 2 h at 72°C. Thereafter, fragments were separated on 1–2% agarose gel (Boehringer Mannheim, FRG) and stained with ethidium bromide. DNA restriction fragments were visualized and analyzed on transilluminator.

4. Statistical methods

Blood lipids and lipoproteins were each adjusted for sex and age effects by multiple linear regression. In addition, the association between change in lipid and lipoprotein variables with BMI, were examined. The hypothesis of effect of the diet, the existence of a period effect, and the possible interaction between dietary effects, and the possible interaction between dietary
Table 2
Baseline values and lipid changes adjusted for sex and age by apo B polymorphic sites

<table>
<thead>
<tr>
<th></th>
<th>T2488 (XhoI)</th>
<th>A3611G (MspI)</th>
<th>G4154L (EcoRI)</th>
<th>Signal peptide (SP)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ +</td>
<td>+ -</td>
<td>- -</td>
<td>SP(27/27)</td>
</tr>
<tr>
<td></td>
<td>(n = 15)</td>
<td>(n = 107)</td>
<td>(n = 88)</td>
<td>(n = 104)</td>
</tr>
<tr>
<td>Baseline</td>
<td>214.8 ± 33.9</td>
<td>215.1 ± 36.4</td>
<td>214.9 ± 34.4</td>
<td>214.1 ± 34.3</td>
</tr>
<tr>
<td>Change</td>
<td>1.1 ± 21.8</td>
<td>11.5 ± 23.3</td>
<td>11.2 ± 22.4</td>
<td>10.6 ± 22.3</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>170.3 ± 33.2</td>
<td>170.6 ± 38.7</td>
<td>172.9 ± 35.2</td>
<td>170.5 ± 35.0</td>
</tr>
<tr>
<td>Change</td>
<td>0.3 ± 23.4</td>
<td>11.2 ± 21.5</td>
<td>9.7 ± 23.0</td>
<td>9.8 ± 21.7</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>38.7 ± 8.6</td>
<td>37.6 ± 7.4</td>
<td>37.2 ± 7.4</td>
<td>37.3 ± 7.5</td>
</tr>
<tr>
<td>Change</td>
<td>1.3 ± 4.7</td>
<td>1.0 ± 5.5</td>
<td>1.5 ± 6.5</td>
<td>0.7 ± 5.3</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>147.0 ± 116.0</td>
<td>146.6 ± 100.0</td>
<td>154.4 ± 66.3</td>
<td>154.0 ± 76.5</td>
</tr>
<tr>
<td>Change</td>
<td>16.7 ± 67.6</td>
<td>-1.4 ± 50.4</td>
<td>4.8 ± 51.2</td>
<td>3.4 ± 53.5</td>
</tr>
</tbody>
</table>

*P ≤ 0.01.
treatment and period in this basic two-period crossover design were assessed by means of \( t \)-tests according to Fleiss [46]. Analysis of variance was performed in order to examine the homogeneity of baseline levels and changes across the genotype groups.

5. Results

Study participants comprised 108 males and 106 females. The mean of age was 46.4 years (range 15–74) for men and 44.0 years (range 14–70) for women. The means of BMI were 26.0 (± 3.1) and 25.7 (± 4.2) for men and women, respectively. Since the variability of the change in lipids and lipoproteins was found to be associated with age and not with BMI, further analyses were performed on sex and age adjusted values. Our results from the crossover study indicate an overall significant effect of the diets on plasma cholesterol, LDL-C and triglyceride concentrations (data not shown). For all variables, no carry over effect was demonstrated, and the order of the given diets had no effect on the response of the lipid phenotypes. Due to these results, the mean values of the two determinations made at the end of the LSC diet compared with that determined at the end of the HSC diet, irrespective of the order of the diets, were used in order to test the null hypothesis that phenotypic change was not associated with the genetic variation at the gene locus under study (Tables 2–5).

The frequency of apoB polymorphisms is similar to those previously described in the Israeli population [14,35]. The frequency distribution of apoE genotype

<table>
<thead>
<tr>
<th>Total cholesterol</th>
<th>LDL cholesterol</th>
<th>HDL cholesterol</th>
<th>Triglyceride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>Baseline</td>
<td>Baseline</td>
<td>Baseline</td>
</tr>
<tr>
<td>Change</td>
<td>Change</td>
<td>Change</td>
<td>Change</td>
</tr>
</tbody>
</table>

Table 3 Baseline values and lipid changes adjusted for sex and age by apo E genotypes

Table 4 Baseline values and lipid changes adjusted for sex and age by LPL polymorphic sites

Table 5 Baseline values and lipid changes adjusted for sex and age by CETP I405 polymorphic site
was in Hardy–Weinberg equilibrium and in a relatively uniform pattern (allele frequencies: $e2 = 0.079$, $e3 = 0.815$, $e4 = 0.105$) similar to that previously described in other populations [47]. All the LPL and CETP polymorphisms were also in Hardy–Weinberg equilibrium.

Table 2 presents the average lipid and lipoprotein levels at baseline and the average changes from the end of LSC diet to the end of the HSC diet for the different apo B genotypes. Subjects homozygous for the apo B X\(\text{ba}1\) restriction site allele (designated as + +) showed a smaller change in plasma cholesterol (1.1 mg/dl) and LDL-C (0.3 mg/dl) levels compared to subjects with the X-allele (11.4 mg/dl) and LDL-C (10.6 mg/dl). However, these differences in change between the two X\(\text{ba}1\) genotype groups were not statistically significant for total cholesterol ($P = 0.098$) and for LDL-C ($P = 0.098$). Subjects heterozygous for the less frequent apo B EcoRI allele (G/L4154) showed a significant change in plasma cholesterol (16.2 mg/dl) and LDL-C (15.6 mg/dl) levels, compared with a smaller change for cholesterol (8.0 mg/dl) and LDL-C (6.2 mg/dl) among subjects homozygous for the apoB EcoRI restriction site allele (G/G4154). These differences in change between the EcoRI genotype groups were statistically significant ($P = 0.01$ for total cholesterol and $P = 0.007$ for LDL-C). The Apo B signal peptide polymorphism appeared to influence the response of HDL-C to the intervention diet. HDL-C change among the SP27/24 (insertion/deletion alleles) heterozygotes was greater than that observed in the SP27/27 genotype; however, this difference was not significant ($P = 0.099$).

Table 3 indicates that although TC and LDL-C baseline levels were significantly different among the apo-E genotypes, there were no significant apo E genotype effects on lipid and lipoprotein changes.

Mean TG and HDL-C on the basal diet and the average response to diet were significantly different among LPL genotypes (Table 4). Triglyceride baseline values were higher ($P = 0.092$) and HDL-C significantly lower ($P = 0.008$) among subjects found to be heterozygous for the LPL N291S mutation. A heterogeneous response for HDL-C was observed for individuals harboring the S291 allele compared to those homozygous for the N291 allele. Baseline triglyceride levels were also found to be significantly affected by the LPL S447stop mutation. Finally, we observed a statistically non-significant ($P = 0.1$) influence of the LPL-93 mutation on the triglyceride response to the dietary change; Triglyceride declined among the T/G heterozygotes compared to a slight increase among those individuals with the T/T genotype.

In the present study, there were no significant effects of the CETP genotypes on lipid and lipoprotein baseline levels and on their response to dietary manipulations (Table 5).

6. Discussion

In this study of 214 Israeli subjects, conducted in a free-living setting, various polymorphisms at five major gene loci were determined. The response to diet was significantly greater in participants heterozygous for the absence of the apo B EcoRI site (G/L4154) as compared to those individuals with the genotype G/G4154. The EcoRI L4154 allele, which occurs at frequency of 0.1–0.2, in most populations has been associated with CHD and with increased plasma cholesterol concentration in some studies [15,48–50], but not in all [16,51]. The EcoRI site of the apo B gene has been also suggested as a ‘variability’ gene affecting the apo B level in the plasma [52,53]. Moreover, in a study of identical twins, the presence of the EcoRI cutting site was found to be associated with a decreased co-twins difference in serum cholesterol [54]. In a study of fifty-one subjects who were classified as diet responsive or non-responsive, the responders more frequently had the EcoRI cutting site absent then the non-responders [29]. In a recent controlled dietary study conducted on 44 healthy middle-aged subjects, the increase of plasma total and LDL cholesterol was greater in apoB G/G4154 subjects compared with those individuals with the other genotypes [55]. Although in other studies this association was not evident [35,56], a meta-analysis of all published dietary trials confirmed the role of EcoRI locus [55].

The mechanism through which variation at the apo B EcoRI locus may affect the response in cholesterol levels to diet is not fully understood. This association could be explained by variation in the synthetic rate or in the catabolism of apo B and apo B containing lipoproteins. The EcoRI RFLP reflects a single base pair change in exon 29 that results in an amino acid change from glutamic acid to lysine [41]. This sequence variation is located in a region presumed to be near the two putative LDL receptor binding sites of apolipoprotein B [57]. Such variation in the receptor-binding site of apo B could lead to different affinity for the LDL receptor and thereby different catabolism. However, there was no effect of the EcoRI polymorphism on the binding of LDL to LDL receptor [58], although this does not exclude other effects in vivo.

Several studies have indicated a significant role of apo E genotypes on cholesterol and LDL-C response to dietary treatment [59]. It has been suggested that the differences in cholesterol absorption [60], in apoB production and cholesterol synthesis [61] and/or in affinity to the B, E receptor [62] in subjects with varying apo E alleles may alter lipids response to diet. However, in other studies, such relationships were not demonstrated [59]. In the present study sample, subjects with the e3e4 genotype had significantly greater TC and LDL-C baseline levels, than in individuals with other apo E geno-
types. However, no apo E effects on the response of lipids and lipoproteins to dietary challenge was observed. Ethnic differences, baseline dietary fat and cholesterol intake and baseline lipid levels are among other reasons why studies on the role of apoE in modulation of dietary response have given inconsistent results. Results from a meta-analysis which included 1015 normo- and hypercholesterolemic participants in 16 studies have suggested that an apoE effect may be more evident when the total amount of dietary fat is changed, than when compositional changes are made [63].

Lipoprotein lipase plays a central role in lipoprotein metabolism and genetic variations in the LPL locus have been associated with lipid levels and CHD risk [18,19]. In our study the N291S mutation was found to be associated both with level and variability in TG and HDL-C response to diet. Data from other studies have shown those with one or more alleles for the LPL S291 mutation had significantly higher TG levels, as well as a greater increase in plasma TG over a 3-year period compared to non-carriers [64]. In a group of young MI survivors and healthy matched controls, plasma TG concentrations following an oral fat load were consistently higher among carriers of the LPL S291 as compared to non-carriers, with the differences being more pronounced in the late postprandial period (8–12 h after the fat load) [65]. In the European Atherosclerosis Research Study (EARS), allelic frequencies for S291 mutation did not differ between subjects with parental history of MI (cases) and those without such a history [66]. Yet, among the cases, carriers of the S291 allele had higher TG levels 6 h post an oral fat tolerance test ($P \leq 0.04$) than non-carriers. In vitro mutagenesis and expression in COS cells demonstrated that the S291 allele resulted in an LPL protein with activity reduced to 30–50% compared to the wild-type protein and a decreased stability [64].

In our study, the Stop447 mutation in the LPL gene was found to be associated with levels of TG but did not alter lipid response to diet. In a study of MI patients and age-matched controls, no significant association was found among the healthy subjects between lipid variables and genotypes of the S447stop mutation [67]. However, in monozygotic twins, the Stop447 mutation was associated with significantly smaller within-pair differences in plasma HDL-C, total cholesterol, and triglyceride levels [68]. This suggests that individuals without this mutation are more susceptible to fluctuations in their lipid and lipoprotein levels in response to environmental exposure. In addition, the HindIII RFLP has also been found to be strongly associated with variability in lipid response to diet manipulations [63,69]. It the ECTIM control populations the S447stop mutation was in nearly complete disequilibrium with the HindIII RFLP ($D/D_{max} = 0.97, \ P < 10^{-4}$) [70]. It has been shown that this mutation leads to a truncation of the two carboxyl-terminal amino acids of LPL and mutations affecting the carboxy region have been reported to alter the LPL-specific activity in vitro [71]. Yet these findings are inconsistent [72] and the mechanism through which this mutation exerts level gene and variability gene effects requires further investigation.

Rare mutations in the CETP gene causing partial or full CETP deficiency, that markedly elevate HDL-C and markedly lower VLDL-C, have been described in various populations. Recently, the ECTIM study found evidence of a gene-environment interaction involving the CETP TaqI B polymorphism, HDL-C, and alcohol consumption, albeit very high levels of alcohol intake [29]. Our study did not find significant effects of the CETP genotypes on lipid and lipoprotein response to dietary manipulations and we are unaware of other studies that have examined the relationship between dietary response and the CETP gene.

In summary, the metabolic response to changes in dietary intake of saturated fatty acids and cholesterol is likely to be under multifactorial control. Our results suggest that sequence variation(s) in the coding region of the apo B gene linked to the EcoRI polymorphism may have a modifiable influence on total cholesterol and LDL-C changes. In our study population, TG and HDL-C response to dietary manipulations were affected by LPL mutations. However, other factors, including genes investigated here, may also be involved and should be re-examined in larger studies in order to uncover their possible role in plasma lipid response to diet, and so contribute to revealing the mechanisms involved in differential responsiveness.

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