Taq1B CETP polymorphism, plasma CETP, lipoproteins, apolipoproteins and sex differences in a Jewish population sample characterized by low HDL-cholesterol

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Abstract

Mean high-density lipoprotein cholesterol (HDL-C) concentrations are low in the Jewish population of Israel. With this in mind we assessed the association of the Taq1B CETP polymorphism, plasma CETP mass and plasma lipid, lipoprotein and apolipoprotein concentrations in a sample of 884 Jerusalem residents aged 28–32. The allele frequency (0.435 ± 0.017(S.E.)) is similar to that reported elsewhere. There was a strong (apparently codominant) association of the Taq1 B allele with plasma CETP in both sexes, and an inverse association with HDL-C and apo A-1, significant in women and undiminished upon adjustment for plasma CETP. There was evidence in this population for an admixture of two plasma CETP distributions, with 9% belonging to a distribution with the higher mean, pointing to a possible major gene effect. Mean plasma CETP was higher in women than men. Plasma CETP was inversely associated with HDL-C in men but not in women (P < 0.05 for the sex difference, multivariate analysis), inversely related to the HDL-C/apo A-1 ratio in men and positively related in women (P < 0.005 for the sex difference), and was positively associated with total cholesterol (TC) and low-density lipoprotein cholesterol in both sexes, and with the TC/HDL-C ratio and apo B in men alone. The sex differences may reflect dissimilarities in the regulatory function of CETP in lipid exchange. The absence of an unusual allele frequency of the Taq1B CETP polymorphism and its relatively modest association with HDL-C argue against an important role for this or strongly linked sites in determining the low population levels of HDL-C in Israel. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Apolipoproteins; CETP; Genetic polymorphisms; HDL cholesterol; Jews; LDL cholesterol; Sex differences; Taq1B

1. Introduction

Plasma high-density lipoprotein cholesterol (HDL-C) concentrations are low in the Jewish population of Israel in both men and women compared with North American and European populations [1,2]. The consistent inverse association between HDL-C and coronary heart disease (CHD) evident elsewhere [3,4] is apparent also in the Israeli population [5–9]. In light of this, elucidation of the environmental and genetic determinants of HDL-C may be especially informative in the Jewish population of Israel. Among the environmental attributes in this population relevant to low HDL-C levels are its dietary characteristics including a high polyunsaturated fatty acid and relatively low saturated fatty acid intake [10], and an extraordinarily small alcohol consumption [11].

Regulation of HDL-C concentration has a substantial genetic component with an estimated genetic heritability of 0.55 in the Jewish population of Jerusalem, similar to that in the US [12]. Segregation analysis pointed to a major recessive gene for depressed HDL-C with an allele frequency of 0.06 and a polygenic component of 0.45 [13]. A recently reported association of
heterozygous Gaucher mutations, and consequent elevated levels of glucocerebrosidase, with depressed HDL-C concentrations is a step in identifying major gene involvement [14]. The allele frequency, although relatively high in Ashkenazi Jews [15,16], is far too low to explain the low HDL-C levels in Israeli Jews of European origin, and cannot explain the low levels evident also among the other ethnic origin groups comprising the Jewish population. Given that some of the ethnic groups had been separated geographically in the diaspora for 2000–2500 years, any influential mutations affecting HDL-C are likely to be of biblical or pre-biblical vintage.

The function of HDL in reverse transport of cholesterol from the periphery to the liver is believed to play a key role in its protective effect [17]. Cholesterol ester transfer protein (CETP) partakes in the process of reverse transport, by transferring esterified cholesterol from the HDL molecule to triglyceride-rich lipoproteins, in exchange for triglycerides [18,19]. However, the implications of the action of CETP are not well understood. There are apparently conflicting reports as to the association of CETP mass or activity with the risk of coronary heart disease [20–24].

Some studies [25–27] have shown an inverse association of plasma CETP with HDL-C, but this is not consistent [22,28]. There is some evidence that HDL-C regulation by CETP is dependent on plasma triglyceride (TG) levels [29,30]. Polymorphisms of the CETP gene, in particular a frequent Taq1B RFLP [22,25,27,31–36], but also others such as the Msp 1 and Rsa 1 RFLPs [27,37], are associated with HDL-C, but this too is not consistent [34,38,39]. These genetic polymorphisms are associated in some studies [22,25–27], but not all [33], with altered plasma CETP activity or mass, which may mediate the relation with HDL-C.

We studied the interrelationship of the Taq1B RFLP, plasma CETP levels, plasma HDL-C and low density lipoprotein cholesterol (LDL-C) and the A-1 and B apolipoproteins in a population sample of young Jewish adults of differing ethnic origin in Jerusalem.

2. Methods

2.1. Sample

The Jerusalem Lipid Research Clinic (LRC) initially examined 8646 17-year-olds between 1976–1979 and a sample comprising 6950 of their parents. The youngsters were sequentially sampled at their induction medical examinations for military service which is obligatory in Israel for the Jewish population except for female religious objectors. The study design, response rates and characteristics have been described previously [40].

For the offspring follow-up study we drew a sex-stratified random sample of 1093 men and 753 women from the original 8646 participants. Non-Jerusalem residents according to the national population registry, women who were pregnant or up to 3 months after giving birth or were breastfeeding up to 6 months after birth, and institutionalized people or those unable to provide informed consent were excluded. A total of 786 men (71.9%) and 469 women (62.3%) were eligible for the study; 9.2% of eligible men and 9.4% of women could not be located, 17.8% of men and 23.0% of women refused to participate, and 570 men (72.5%) and 314 women (67.0%) were examined in 1989–1991 at ages 28–32 years.

Ethical approval for the study was granted by the Hadassah University Hospital Helsinki Committee.

2.2. Data collection

Blood was drawn with minimal venous constriction after an overnight fast. Information on sociodemographic characteristics, personal and family medical history, reproductive history, and health-relevant behaviors including smoking, alcohol intake, exercise and diet was obtained by a standardized interview. Trained technicians made standardized measurements of blood pressure, heart rate and anthropometric variables (weight, height, waist, hip and arm circumference, and subscapular, triceps and supra-iliac skinfold thickness). Red blood cells were separated for erythrocyte membrane fatty acid analysis by gas chromatography and theuffy coat was separated for DNA preparation. Serum and EDTA plasma samples were aliquotted into cryotubes and stored at −70 to −80°C until examination.

2.3. Laboratory methods

Genomic DNA was prepared from separated buffy coat by a salting out procedure. Determination of the Taq1B polymorphism located on intron 1, using PCR, was performed as previously described [22] for 819 individuals (527 men and 292 women), comprising 93% of the total sample examined.

Since plasma CETP concentration and CETP activity are well correlated, \( r \sim 0.75–0.85 \) [41,42], it is frequently assumed that measurement of CETP mass provides information, albeit imperfect, on CETP activity. Plasma CETP mass was measured from frozen samples by a two-site immunoenzymatic assay [43] in the first 714 subjects sequentially examined (81% of the study population). This method is associated with an intra- and inter-assay coefficient of variation of 3.6 and 8.4%, respectively. Both genotype and plasma CETP measures were available for 662 individuals (75% of the sample).
Plasma total cholesterol, HDL-C, and TG concentrations were determined by an enzymatic method on a Cobas bio autoanalyzer. HDL-C was measured after precipitation of apo B containing lipoproteins by phosphotungstic acid and magnesium chloride, and LDL-C was calculated by the Friedewald method. Apo A-I and apo B were determined by an immunoturbidimetric assay read on a Cobas Bio autoanalyzer. Correction was made for laboratory drift in the measurement of the apolipoproteins over the duration of the study.

2.4. Statistical methods

The allele frequency was determined by direct counting. Conformity with Hardy–Weinberg equilibrium was checked. Plasma CETP was log transformed to reduce skewness in its distribution and geometric means and their approximate S.D. or 95% confidence intervals (CI) are presented. Plasma TG was also log transformed.

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3. Results

3.1. Population characteristics (Table 1)

A third of the women and almost half the men smoked regularly. Alcohol intake was extremely low in both sexes (as has been noted among Jewish populations), more so in women. Men were more overweight than women at this age. Only 22% of the men and 15% of the women reported vigorous leisure-time exercise at least once weekly. Of the women 21% were using an oral contraceptive.

Mean plasma total cholesterol (TC), LDL-C and TG levels were significantly higher in men than women, apo B levels were similar, and as expected HDL-C was higher in women. A salient characteristic of this study population is the low level of plasma HDL-C in both sexes, irrespective of European (‘Ashkenazi’), North African or Asian origin. This is reflected also in the relatively high ratio of TC to HDL-C, notable in men. Mean apo A-1 levels were low, consistent with the low HDL-C levels, and were as expected significantly higher in women than in men. The ratio of HDL-C to apo A-1 was higher in women than in men, indicating a higher cholesterol content per molecule of protein in HDL among women.

Mean plasma CETP was significantly lower in men (geometric mean 1580 µg/l (95% CI 1521–1639 µg/l)) than women (1735 µg/l (1646–1829 µg/l), (P = .004)).

3.2. Taq1B polymorphism

3.2.1. Allele frequency

The frequency of the B2 allele, representing the absence of the cutting site, was 0.435 ± 0.017(S.E.).
3.2.2. Association with plasma CETP

In this population, the Taq1B genotype was associated with plasma CETP levels in an apparently codominant mode with the B2 homozygote showing the lowest levels and the B1B2 heterozygote intermediate ($P \leq 0.0002$ for trend) (Table 2). The geometric mean CETP of the B1B1 compared with the B2B2 homozygotes was 22% higher in men and 42% higher in women, there being no sex difference in CETP levels among B2B2 homozygotes and an increasing female–male difference through B1B2 heterozygotes to B1B1 homozygotes. However, although the effect appeared to be larger in women, a formal test for a sex–genotype interaction was not statistically significant ($P = 0.14$).

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>Geometric mean</td>
<td>S.D.</td>
<td>n</td>
<td>%</td>
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<td>B2B2</td>
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<td>22.4</td>
<td>1390</td>
<td>578</td>
<td>42</td>
<td>18.4</td>
</tr>
<tr>
<td>B1B2</td>
<td>192</td>
<td>44.2</td>
<td>1572</td>
<td>616</td>
<td>105</td>
<td>46.1</td>
</tr>
<tr>
<td>B1B1</td>
<td>145</td>
<td>33.4</td>
<td>1693</td>
<td>660</td>
<td>81</td>
<td>35.5</td>
</tr>
<tr>
<td>Totala</td>
<td>434</td>
<td>100.0</td>
<td></td>
<td></td>
<td>228</td>
<td>100.0</td>
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<td>$P$ ANOVA 2 d.f.</td>
<td>0.001</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>$P$ linear trend</td>
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<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
<td></td>
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</tbody>
</table>

*a CETP genotype and phenotype available for 434 men and 228 women.

### Table 3

Association of Taq1B CETP gene polymorphism with adjusted lipids, lipoproteins and apolipoproteins

<table>
<thead>
<tr>
<th></th>
<th>HDL-C</th>
<th>TC/HDL-C</th>
<th>Apo A-I</th>
<th>HDL-C/Apo A-I</th>
<th>LDL-C</th>
<th>Apo B</th>
<th>TGb</th>
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<tbody>
<tr>
<td>Men</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B2B2</td>
<td>37.5</td>
<td>4.89</td>
<td>94</td>
<td>0.41</td>
<td>108</td>
<td>69</td>
<td>107</td>
</tr>
<tr>
<td>B1B2</td>
<td>36.6</td>
<td>4.97</td>
<td>92</td>
<td>0.40</td>
<td>105</td>
<td>71</td>
<td>120</td>
</tr>
<tr>
<td>B1B1</td>
<td>35.5</td>
<td>5.22</td>
<td>92</td>
<td>0.39</td>
<td>111</td>
<td>70</td>
<td>114</td>
</tr>
<tr>
<td>$P$ ANOVA (2 d.f.)</td>
<td>0.203</td>
<td>0.16</td>
<td>0.48</td>
<td>0.37</td>
<td>0.088</td>
<td>0.59</td>
<td>0.073</td>
</tr>
<tr>
<td>$P$ for trend (1 d.f.)</td>
<td>0.074</td>
<td>0.069</td>
<td>0.29</td>
<td>0.18</td>
<td>0.22</td>
<td>0.89</td>
<td>0.31</td>
</tr>
<tr>
<td>n</td>
<td>526</td>
<td>526</td>
<td>526</td>
<td>526</td>
<td>503</td>
<td>488</td>
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</table>

<table>
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<th>Women</th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>B2B2</td>
<td>49.1</td>
<td>3.58</td>
<td>105</td>
<td>0.46</td>
<td>100</td>
<td>71</td>
<td>84</td>
</tr>
<tr>
<td>B1B2</td>
<td>46.2</td>
<td>3.69</td>
<td>102</td>
<td>0.45</td>
<td>97</td>
<td>68</td>
<td>78</td>
</tr>
<tr>
<td>B1B1</td>
<td>45.1</td>
<td>3.72</td>
<td>100</td>
<td>0.44</td>
<td>96</td>
<td>69</td>
<td>87</td>
</tr>
<tr>
<td>$P$ ANOVA (2 d.f.)</td>
<td>0.11</td>
<td>0.70</td>
<td>0.10</td>
<td>0.58</td>
<td>0.70</td>
<td>0.35</td>
<td>0.14</td>
</tr>
<tr>
<td>$P$ for trend (1 d.f.)</td>
<td>0.042</td>
<td>0.44</td>
<td>0.036</td>
<td>0.40</td>
<td>0.34</td>
<td>0.33</td>
<td>0.61</td>
</tr>
<tr>
<td>n</td>
<td>292</td>
<td>292</td>
<td>292</td>
<td>292</td>
<td>291</td>
<td>276</td>
<td>292</td>
</tr>
</tbody>
</table>

*a* Adjusted for BMI, smoking, alcohol intake, exercise and oral contraceptive use.

*b* Geometric mean.

(There was a modest deviation from Hardy–Weinberg equilibrium with a relative deficiency of B1B2 heterozygotes $\chi^2 = 4.71, P = 0.03$). The allele frequencies by country of origin groups were 0.40 (0.033) for Israel, 0.388 (0.039) for Europe and countries of emigration from Europe, 0.441 (0.031) for Asia and 0.508 (0.036) for North Africa ($P = 0.08$ for the ethnic difference).

#### 3.2.3. Association with lipoproteins and apolipoproteins

The Taq1B RFLP was associated with multivariable adjusted plasma HDL-C and apo A-I levels (Table 3). The B1 allele was associated with lower HDL-C and with lower apo A-I, both statistically significant only in women. The associations in women were undiminished upon control for plasma CETP, indicating that they are not mediated by CETP. The ratios of HDL-C/apo A-I and TC/HDL-C, and the levels of LDL-C, TG or apo B did not differ significantly between the genotypes.

We assessed the association of HDL-C with TG within genotype and sex categories (Table 4). In men the correlation in the B2B2 and B1B2 groups was high compared with the B1B1 homozygotes ($\chi^2 = 9.82, 2$
Table 4
Pearson correlations of triglycerides (ln transformed) and HDL-C* within CETP Taq1B genotypes

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Correlation</th>
<th>( \chi^2 ) For heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B2B2</td>
<td>116</td>
<td>-0.45</td>
<td></td>
</tr>
<tr>
<td>B1B2</td>
<td>232</td>
<td>-0.51</td>
<td>( \chi^2 = 9.82, \text{ d.f.} = 2 )</td>
</tr>
<tr>
<td>B1B1</td>
<td>179</td>
<td>-0.25</td>
<td>( P = 0.007 )</td>
</tr>
<tr>
<td>Allb</td>
<td>527</td>
<td>-0.41</td>
<td></td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B2B2</td>
<td>54</td>
<td>-0.20</td>
<td></td>
</tr>
<tr>
<td>B1B2</td>
<td>140</td>
<td>-0.37</td>
<td>( \chi^2 = 1.50, \text{ d.f.} = 2 )</td>
</tr>
<tr>
<td>B1B1</td>
<td>98</td>
<td>-0.28</td>
<td>( P = 0.47 )</td>
</tr>
<tr>
<td>Allb</td>
<td>292</td>
<td>-0.31</td>
<td></td>
</tr>
</tbody>
</table>

* Both unadjusted for covariates.

b \( \chi^2 \) test for heterogeneity between the sexes = 2.49, 1 d.f., \( P = 0.12 \).

d.f., \( P = 0.007 \), who also have the highest plasma CETP levels. In women there was no genotype effect; the correlations were non-significantly lower overall than in men and were similar to that in B1B1 men.

No interaction was found between genotype and either alcohol intake, BMI, or smoking in determining HDL-C levels.

3.3. Plasma CETP

3.3.1. Association with behavioral risk factors

Cigarette smoking was associated with significantly lower plasma CETP levels in women (geometric mean 1571 vs. 1845 \( \mu g/l \) in nonsmokers, \( P = 0.0035 \)). Male smokers had non-significantly lower plasma CETP than nonsmokers (1534 vs. 1612 \( \mu g/l \), \( P = 0.19 \)), with the association evident only in the B2B2 genotype (nominal borderline significance). Consequently the sex difference in plasma CETP was smaller in smokers.

Following Hannuksela et al. [26], we determined whether smoking modifies the association of the Taq1B polymorphism with plasma CETP. In both sex-specific and sex-pooled analyses there was no evidence for a smoking–genotype interaction. There was no association of plasma CETP with BMI, alcohol intake or exercise in either sex, nor was there evidence for a genotype–BMI interaction on CETP levels.

3.3.2. Association with lipoproteins and apolipoproteins

In unadjusted correlation analysis, plasma CETP was significantly related to lipoprotein and apolipoprotein levels (Table 5). In men plasma CETP was inversely correlated with HDL cholesterol (\( r = -0.13, \ P = 0.006 \)), in contrast with women in whom the association was non-significantly positive (\( r = 0.10, \ P = 0.10 \)). The sex difference in association was significant (\( P = 0.003 \)). There was no significant correlation with apo A-I in either sex. Consequently, the ratio of HDL-C to apo A-I was inversely related to plasma CETP (\( r = -0.18 \)) in men and positively related in women (\( r = 0.15 \) \( P < 0.0001 \) for sex difference). For TC and LDL-C the correlations were significantly positive in both sexes, and for apo B only in men (\( P = 0.05 \) for sex difference). For the ratio of TC to HDL-C the correlation was correspondingly positive in men (\( r = 0.18 \)) and almost zero in women (\( P = 0.054 \) for sex difference). There was no association with TG. To assess whether the sex difference in the CETP–HDL-C association might be determined in part by male versus female patterns of fat distribution, we tested for an interaction with waist to hip ratio in each sex. There was no evidence for effect modification.

We further explored these associations in an analysis of quintiles of plasma CETP, adjusting for BMI, smoking, alcohol intake, exercise and oral contraceptive use (Table 6). In men HDL-C was highest in the bottom...
Table 6
Plasma lipids, lipoproteins and apolipoproteins by CETP quintiles and by sex

<table>
<thead>
<tr>
<th>CETP quintilesb</th>
<th>N</th>
<th>TC</th>
<th>HDL-C</th>
<th>TC/HDL-C</th>
<th>Apo A-1</th>
<th>HDL-C/Apo A-1</th>
<th>LDL-C</th>
<th>Apo B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men (n = 470)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1 (313.0–1192.0)</td>
<td>104</td>
<td>161.2</td>
<td>38.7</td>
<td>4.43</td>
<td>92.0</td>
<td>0.42</td>
<td>98.0</td>
<td>67.1</td>
</tr>
<tr>
<td>2 (1192.1–1460.0)</td>
<td>97</td>
<td>172.4</td>
<td>35.6</td>
<td>5.23</td>
<td>91.0</td>
<td>0.39</td>
<td>110.5</td>
<td>70.6</td>
</tr>
<tr>
<td>3 (1460.1–1811.0)</td>
<td>96</td>
<td>167.4</td>
<td>34.9</td>
<td>5.05</td>
<td>89.5</td>
<td>0.39</td>
<td>107.1</td>
<td>69.9</td>
</tr>
<tr>
<td>4 (1811.1–2261.0)</td>
<td>95</td>
<td>174.1</td>
<td>36.9</td>
<td>5.23</td>
<td>94.1</td>
<td>0.39</td>
<td>109.3</td>
<td>71.7</td>
</tr>
<tr>
<td>5 (2261.1–8408.0)</td>
<td>78</td>
<td>175.9</td>
<td>34.4</td>
<td>5.44</td>
<td>93.9</td>
<td>0.36</td>
<td>114.4</td>
<td>73.4</td>
</tr>
<tr>
<td>P ANOVA (4 d.f.)</td>
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<td>0.005</td>
<td>0.011</td>
<td>0.0003</td>
<td>0.081</td>
<td>0.001</td>
<td>0.001</td>
<td>0.11</td>
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<td>P for trendc</td>
<td></td>
<td>0.0015</td>
<td>0.016</td>
<td>0.0001</td>
<td>0.14</td>
<td>0.0001</td>
<td>0.0003</td>
<td>0.01</td>
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<td><strong>Women (n = 244)</strong></td>
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<td>1 (313.0–1192.0)</td>
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<td>146.5</td>
<td>45.8</td>
<td>3.32</td>
<td>85.5</td>
<td>0.45</td>
<td>85.2</td>
<td>68.2</td>
</tr>
<tr>
<td>2 (1192.1–1460.0)</td>
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<td>3.88</td>
<td>88.1</td>
<td>0.43</td>
<td>95.1</td>
<td>71.0</td>
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<tr>
<td>3 (1460.1–1811.0)</td>
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<td>158.3</td>
<td>49.1</td>
<td>3.40</td>
<td>95.0</td>
<td>0.47</td>
<td>92.9</td>
<td>65.9</td>
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<tr>
<td>4 (1811.1–2261.0)</td>
<td>50</td>
<td>163.6</td>
<td>46.3</td>
<td>3.74</td>
<td>93.1</td>
<td>0.45</td>
<td>99.8</td>
<td>70.2</td>
</tr>
<tr>
<td>5 (2261.1–8408.0)</td>
<td>62</td>
<td>164.6</td>
<td>46.4</td>
<td>3.69</td>
<td>94.1</td>
<td>0.49</td>
<td>99.2</td>
<td>67.6</td>
</tr>
<tr>
<td>P ANOVA (4 d.f.)</td>
<td></td>
<td>0.037</td>
<td>0.113</td>
<td>0.039</td>
<td>0.050</td>
<td>0.48</td>
<td>0.072</td>
<td>0.39</td>
</tr>
<tr>
<td>P for trendc</td>
<td></td>
<td>0.003</td>
<td>0.36</td>
<td>0.26</td>
<td>0.011</td>
<td>0.19</td>
<td>0.011</td>
<td>0.70</td>
</tr>
</tbody>
</table>

a All lipids variables adjusted for BMI, smoking, alcohol intake, exercise and oral contraceptive use.
b Sex-pooled.
c With quintiles coded as 1–5 in a linear regression.

3.3.3. Admixture (commingling) of the plasma CETP distribution

Admixture was assessed for men and women with low HDL-C levels (below 35 and 45 mg/dl, respectively), and for the whole population without regard for HDL-C levels. There was no evidence for more than one distribution fitting the low HDL-C population. However, in the total study population two distributions fitted significantly better than one \( P < 0.001 \). Of the population 91% were included in a lower distribution and 9% in an upper distribution with a mean 2.3 S.D. above the major mode.

3.3.4. Effect modification by plasma TG

We tested a hypothesis \([29,30]\) that regulation of plasma HDL-C levels by CETP is dependent on TG levels (i.e. an inverse association is present only if TG is elevated), using ANOVA with tertile cutoff points for CETP and TG. There was no significant interaction. In men, the inverse association with HDL-C was slightly attenuated \( P = 0.062 \) upon adjustment for TG, and the direct relation with TC/HDL-C and with HDL-C/Apo A-1 remained strong and independent of TG, and was not limited to the upper quintile of TG \( (\geq 178 \text{ mg/dl}) \). In women the positive CETP–HDL-C relationship was enhanced \( P = 0.014 \) and was not dependent on TG level (however, the upper quintile cut-off point was only 118 mg/dl). The absence of effect modification was reexamined treating ln TG as a continuous variable; again there was no support for the hypothesis. Similarly, the association of CETP with HDL-C was also independent of BMI levels.

4. Discussion

The low mean HDL-C observed in this sample confirms previous reports \([1,2]\) and is characteristic of the Jewish population of Israel. Apo A-1 levels were correspondingly low. The TC/HDL-C ratio was high in men. The low alcohol intake, low level of exercise and relatively high proportion of smokers evident in our sample all serve to depress the HDL-C levels. In a comparison of Jerusalem, Czech and Bavarian men \([2]\), using the same standardized laboratory, there were profound dif-
ference in HDL-C, averaging 9–10 mg/dl lower in Jerusalem, and in parallel the TC/HDL-C ratio was higher. Prominent environmental differences between these populations, other than those associated with geographic location, are the exceedingly low alcohol intake in Israel [11], confirmed in our study, and the unusual dietary fatty acid intake. In the EURAMIC study monounsaturated fatty acids, predominantly linoleic acid, comprised 25% of adipose tissue in a Jerusalem population sample, a considerably larger proportion than in eight European countries including Germany [46]. It is in this context that we assessed the associations of the Taq1B CETP polymorphism and plasma CETP concentration with regulation of HDL-C levels.

4.1. Taq1B allele frequencies

The allele frequencies in the ethnic groups comprising the Jewish population were relatively homogenous and differed little from those reported in European populations with higher mean HDL-C levels. For example, in the ECTIM study that included samples from Northern Ireland and France [22], and in a Dutch sample [27], the B2 allele frequency was 0.40–0.41, in a Scottish study 0.46 [33], and in Italian and Greek migrants to Australia 0.43 and 0.48, respectively [34]. In Sinhalese Sri Lankans the frequency was 0.49 [32]. The similarity across populations suggests that this polymorphic marker is ancient.

4.2. Taq1B association with HDL-C

Our finding of an association of the B1 allele with lower HDL-C accords with studies in Dutch [27], Norwegian (also with A-1) [31], Scottish (no association with apo A-1) [25,33], Sinhalese Sri Lankan [32] and in Greek migrant samples [34], but differs from studies in Italian migrants [34] and Finns [39] which suggests differing linkage disequilibrium in different ethnic groups, differing gene–environment interactions, or the effects of chance or selection. Sib-pair linkage studies were inconsistent, one showing an association of the CETP gene locus with HDL-C [35] and one not [38]. We did not confirm the sex interaction reported by Kauma et al. [36], who showed that the association of the B1 allele with lower HDL-C was restricted to women in a Finnish population sample, although in our data and those reported by Mitchell et al. [34] the relationship appeared to be stronger in women, and in our study was significant only in women. Sex modification with the Taq1A RFLP was observed in a French-Canadian population where the association was restricted to females [47].

Our findings differ somewhat from the ECTIM study [22] where no relationship was observed in men who drank < 25 g/day alcohol (which would include almost our entire study population), whereas among heavier drinkers a strong positive association with HDL-C was evident in B2B2 homozygous men. We were unable to adequately assess effect modification by alcohol, although alcohol intake even at the low levels prevalent in the Israeli population is positively associated with HDL-C and inversely related to CHD (Kark, J.D., unpublished data).

A stronger effect of the Taq1B RFLP on plasma HDL-C or apo A-1 and on CETP in nonsmokers reported in several studies [26,31,33] was not confirmed by our study, the ECTIM study [22] or a Finnish study [39].

The Taq1B RFLP is unlikely to be functional, does not affect coding, and is probably a marker for functional variants [22]. Kuivenhoven et al. [27] infer that the B1 allele serves as a marker for specific CETP haplotypes, such as Taq1B-Msp1-Rsa1, that may affect HDL metabolism. In that study the M1 allele (Msp1 RFLP) was also strongly associated with low HDL-C. However, these variants do not affect the CETP structure in contrast with the Rsa 1 RFLP (Ile405val) shown by Funke et al. [37] to be associated with increased HDL-C. This was confirmed by Kuivenhoven et al. [27] who showed, however, that this locus was responsible for only 3.6% of the variance in CETP concentration in their population. In our population as in ECTIM [22] there was little effect of the Taq1B polymorphism on the apo B-containing lipoproteins.

Therefore, taking account of both the absence of an unusual allele frequency of the Taq1B RFLP in the Jerusalem population and its weak association with HDL-C in men and albeit stronger association in women, it is unlikely that this site, either functionally or by linkage disequilibrium with functional sites, is an important determinant of the low HDL-C levels in our population. Consequently, other gene loci must exert a strong influence, or strong and highly prevalent environmental determinants, such as common dietary practices, should play a dominant role. Gene–environment interactions are likely to be important.

We further explored the association of the Taq1B CETP polymorphism with plasma CETP, and the relationships of CETP with plasma lipoproteins not so much as in a quest to explain the low HDL-C levels in Israel, but rather to assess the relationships and functions of CETP within this context.

4.3. Taq1B and plasma CETP concentration

Our data are consistent with a number of studies showing an association of the Taq1B RFLP with plasma CETP levels or activity [22,25–27], but not all [33]. The association was strongly evident in both sexes among these 30-year-olds. We did not confirm studies
suggesting that the association of Taq1B with plasma CETP activity is affected by smoking [26]. Furthermore, there was no evidence for an effect modification by BMI, another important determinant of HDL-C. Although the Taq1B polymorphism explained only 3.1% (men) and 7.6% (women) of the variance in plasma ln CETP in our population, it has been estimated that at least 20% of the between-individual variation in CETP is attributable to the CETP gene; this leaves a large proportion of variance due to other factors [28]. These may include other regulatory enzymes such as LCAT [41].

4.4. Admixture analysis

Tato et al. [21] reported a bimodal distribution for CETP among a sample with low HDL-C, suggesting heterogeneous determination of low HDL-C levels. This was not confirmed by Kuivenhoven et al. [27] or in our study. We did observe, however, an admixture of two plasma CETP distributions in the overall population, pointing to the likelihood of a major gene effect on CETP in this population, with about 10% belonging to a distribution characterized by a high mean CETP; nonetheless, an environmental factor could produce this result.

4.5. Plasma CETP and plasma lipid variables

It is believed that elevated CETP activity may be an important determinant of low HDL-C [21]. However, the functional relationship between CETP activity and HDL-C is not well understood. An inverse relationship between plasma CETP and HDL-C has been described in some studies [21, 26, 27] but not in others [22, 28, 33, 48]. Freeman et al. [33] postulated that differences in CETP activity may be seen only in extreme conditions such as hyperalphalipoproteinemia (which is not consistent with our findings or those of others [21, 27]) and that, in the general population, gene effects on CETP activity are not responsible for the relation between the CETP gene polymorphism and HDL-C. This latter interpretation is supported by our findings and by McPherson et al. [28] and Fumeron et al. [22] who concluded that the effects of the CETP gene on plasma CETP and HDL-C are independent, but not by the inference of Kuivenhoven et al. [27].

A notable finding in our study was a significant effect modification by sex of the association of plasma CETP with HDL-C, in men the association being inverse whereas in women it tended to be positive. Although the apo A-1 containing lipoproteins are the main carrier of plasma CETP, a positive association in the multivariate-adjusted analysis was evident only in women. These findings are consistent with a different effect of CETP in men and women on cholesterol ester–TG exchange. This could alter the cholesterol content of HDL as reflected by the significantly declining ratio of HDL-C to apo A-1 with increasing CETP evident in men, but not in women. This interpretation is consistent also with an apparent unequal correlation of TG with HDL-C between the sexes in those with the B2 allele, which although suggestive was not statistically significant (Table 4).

Several studies [29, 30] indicate that regulation of HDL-C by CETP depends on TG levels, with an inverse relationship with HDL-C and apo A-1 evident only in the presence of high TG. In men with normal TG, CETP was positively associated with apo A-1 and HDL-C [29, 48] (in contrast to our findings for HDL-C in men), but adjustment for LPL, which is strongly correlated with CETP, eliminated the association [29]. We did not confirm findings of differing CETP–HDL-C relationships by level of TG in this population sample of young Jewish adults in Jerusalem in which the proportion with elevated TG (> 200 mg/dl) was small. The associations were largely independent of TG levels.

Some authors have concluded that the association of plasma CETP with lipoproteins is stronger for the apo B-containing lipoproteins than for HDL cholesterol [18], as seen in ECTIM [22] and a Japanese study [41], where an association was reported with LDL-C, but not with HDL-C. In our data there was a consistent association of CETP with LDL-C in both sexes, in contrast with a significant interaction of sex with CETP on HDL-C.

4.6. Sex differences in association

We summarize a number of differences observed between the sexes: (1) Plasma CETP levels were significantly higher among women, consistent for example with studies in Japanese [41]. (2) The association of the Taq1B1 allele with plasma CETP and the inverse B1 allele associations with HDL-C and apo A-1 appeared to be stronger in women than men, but these differences were not statistically significant. (3) A significant inverse association of smoking with plasma CETP was restricted to women; the female excess in plasma CETP was consequently reduced in smokers. (4) There were statistically significant sex differences in the association of plasma CETP with HDL-C and with the ratio of HDL-C to apo A-1, but not with TC or LDL-C. These differences suggest different mechanisms regulating the content of cholesterol in HDL in women and men. A significant multivariate-adjusted positive association of CETP with apo A-1 was apparent only in women, consistent with an inference of a sex difference in the affinity of CETP for apo A-1 [49].

In conclusion, while we detected findings of interest relating to the determinants of plasma CETP and lipoprotein relationships with CETP, there is little evidence...
that the CETP Taq1B polymorphism or strongly linked loci play an important role in setting the low HDL-C levels in the Jewish population of Israel. Low HDL-C is almost certainly a heterogeneous combination of conditions with different metabolic determinants, some associated with increased risk of CHD and some not [50,51]. Currently there are no simple available laboratory measures to readily define and quantify these components of low HDL-C at the population level. Individuals with low plasma HDL-C and normal TG often have an increased fractional catabolic rate for apo A-1, reflecting abnormal HDL metabolism [52,53], and which may explain the low apo A-1 levels in our population. Recently, hepatic triglyceride lipase activity was found to be elevated in Turkish men and women, who represented another population characterized by low HDL-C levels [54]. Comparative studies of apo A-1 metabolism and of hepatic lipase activity in Jewish and non-Jewish populations are warranted. A continued search for major genes and major environmental effects regulating HDL-C in this population and for gene–environment interactions should be rewarding.

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References


