Apolipoprotein B (XbaI) allele frequencies in an Egyptian Population: impact on blood lipids

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Background: Apolipoprotein B (ApoB) is the major protein component of chylomicrons, low-density lipoproteins (LDL), very low density lipoproteins (VLDL) and intermediate-density lipoproteins (IDL) and is the ligand for the LDL receptor. ApoB plays an important role in the maintenance of cholesterol homeostasis and lipoprotein metabolism. Numerous restriction fragment length polymorphisms of the ApoB gene have been reported to be associated with variation in lipid levels, obesity and/or coronary artery disease. Purpose: This study assesses the effect of Apo B restriction fragment length polymorphism (XbaI) on lipid and lipoprotein concentrations in an Egyptian population. Methods: Blood samples from 355 unrelated individuals aged 19 – 70 years were analysed and genotyping was performed using the XbaI restriction enzyme after polymerase chain reaction (PCR) amplification. Results: Allele frequencies obtained for X- and X+ were 69 and 31 %, respectively. Presence of the X+ allele was associated with increased levels of total cholesterol, ApoB, and LDL. X+/X+ homozygous individuals in our sample displayed the lowest mean levels and homozygous X-/X- the highest, with heterozygous individuals displaying intermediate levels. Conclusion: These findings confirm the impact of ApoB genetic variation on some lipid related factors levels in an Egyptian population.

ABSTRACT

Introduction

Atherosclerosis and coronary artery disease (CAD) are growing health problems worldwide, affecting an increasingly young population [1, 2]. Established risk factors for CAD include age, hypertension, smoking, diabetes, elevated total cholesterol, and decreased high-density lipoprotein (HDL) cholesterol [3,4]. The variability of serum levels of lipoproteins is believed to be important [5]. A number of candidate genes has been implicated in the pathogenesis of CAD and atherosclerosis [6,7]. One of the genetic variants is located in the apolipoprotein B (ApoB) gene. It has been reported that ApoB impacts total atherogenic particle number in plasma, and is superior to LDL cholesterol as an index of the lipid-related risk of vascular disease and as a guide to the adequacy of LDL-lowering therapy [8]. However, for historical reasons, cholesterol, and more specifically LDL-cholesterol, remain the accepted indicators for atherosclerosis risk. We suggest that ApoB measurement should be included in guidelines as an indicator of cardiovascular risk. This has become the position of the three major Canadian guideline groups [9-11]. The latest European guidelines acknowledge the value of ApoB, but highlight the current problem of restricted laboratory availability [12].

ApoB, the largest of all human apolipoproteins, is a major constituent of low-density lipoproteins and of the triglyceride-rich lipoproteins. ApoB has a fundamental role in the transport and metabolism of plasma cholesterol serving as a ligand for the receptor-mediated uptake of low-density lipoproteins by various cells [13 -15]. It occurs in plasma as two major immunologically distinct isoforms, ApoB-100 and ApoB-48. ApoB-100 is synthesized in the liver and plays a role in the packaging of very low-density lipoprotein particles, whereas ApoB-48 is synthesized in the intestine and plays a role in the formation of chylomicrons [13 -17]. The two isoforms share a common N-terminal sequence.

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The shorter ApoB-48 protein is produced after RNA editing of the ApoB-100 transcript at residue 2180 (CAA->UAA), resulting in the creation of a stop codon, and early translation termination. The ApoB gene is located on the short (p) arm of chromosome 2 between positions 24 and 23. Human ApoB-100 cDNA is 14 kilobases in length and encodes a 4563-amino acid precursor protein. This gene comprises 29 exons and 28 introns. The distribution of introns is extremely asymmetrical, most of them appearing in the 5' terminal one-third of the gene. Although most of the exons fall within the normal size limits for mammalian genes, two are unusually long: 1906 and 7572 base pairs. The latter exon is by far the longest reported for a vertebrate gene. ApoB is the sole protein component of LDL and VLDL [18-20]. The interaction of ApoB-100 with LDL receptors mediates the uptake of LDL from liver and peripheral cells; hence, it serves the function of solubilizing cholesterol within LDL [21].

Sequencing of human ApoB revealed a number of polymorphisms that affect lipid metabolism and cardiovascular disease (CVD) [22]. Because of the central role of apolipoprotein in lipid metabolism, even small changes in the protein structure or function may be of physiological importance [23]. Mice overexpressing mApoB have increased levels of LDL "bad cholesterol" and decreased levels of HDL "good cholesterol" [24]. Mice containing only one functional copy of the mApoB gene show the opposite effect, being resistant to hypercholesterolemia. Mice containing no functional copies of the gene are not viable [25]. A polymorphism on codon 2488 of exon 26 in the human ApoB gene has been shown to influence the triglyceride (TG), LDL-C, and ApoB levels that are associated with atherosclerosis and CVD [26-28]. This polymorphism arises from a single nucleotide change. A cytosine base in the "wild type" allele becomes a thymine base in the "rare" allele [28,29]. This base change creates a cutting site for the restriction endonuclease XbaI and the polymorphism is known as ApoB XbaI polymorphism, which is silent. This means that it does not change the amino acid sequence of ApoB [27]. The aim of this study was to determine the allele frequencies of the XbaI polymorphism of ApoB in an Egyptian population. We then investigated possible correlations with pathologies associated with LDL-C, Chol, and ApoB levels and the risk of hyperlipidemia associated with this gene.

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Materials and methods

Subjects

Three hundred and fifty five unrelated subjects (215 men and 140 women), aged 19 - 70 years were randomly recruited in Cairo. At study entry, all subjects completed a questionnaire covering data on demographics, family status, education level, smoking habits, history of cardiovascular disease, blood pressure, medications, age, weight, height and sex. Written informed consent was obtained from each subject. Body mass index (BMI; the body mass in kilogram divided by the square of the individual's height in meters) was calculated. Obesity was defined by a body mass index (BMI) > 30 kg/m2. Hypertension was defined as the existence of a systolic blood pressure > 140 mmHg or blood pressure diastolic > 90 mmHg (or receiving antihypertensive treatment according to WHO recommendations) [30]. The presence of diabetes was determined by fasting glucose > 125 mg/dl. Dyslipidemia was defined as total cholesterol (TC) > 250 mg/dl and/or triglycerides (TG) > 87.5 mg/dl and a value of LDL-cholesterol (LDL-C) > 160 mg/dl.
Biochemical analysis of blood

Blood samples were taken in EDTA tubes, in the morning after a 12-hour fast. Plasma was immediately separated from the cell pellet after centrifugation at 250 g for 15 minutes at 4°C. Total cholesterol (TC) and triglycerides (TG) were measured by enzymatic colorimetric tests [31,32]. High-density lipoprotein cholesterol (HDL-C) concentrations were determined by enzymatic assay after phosphotungstic acid and magnesium precipitation [33]. Low-density lipoprotein cholesterol (LDL-C) was calculated according to Friedewald’s formula and very-low-density lipoprotein cholesterol (VLDLC) was calculated using the formula TG/5 [34]. Fasting blood sugar was assayed by glucose oxidase-peroxidase method [35]. Apolipoproteins A1 and B (ApoA1 and ApoB) were determined by immunoturbidimetric methods. Coefficients of variation (CV) for total cholesterol, HDL-C, and triglyceride measurements were below 5%.

DNA isolation and genotyping

Genomic DNA was isolated from peripheral blood. The isolated DNA was of good quality (absorbance 260 nm/280 nm, ratio > 1.75). PCR was used to amplify a 230 bp fragment in the ApoB gene using the oligonucleotide primers F: 5’-AAATAACCTTAA TCATCAATTGT-3’ as upstream primer and R: 5’- GGTTCCTTAG CAGCAAGAGTC-3’ as downstream primer (36). Each amplification was performed using 100 ng of total genomic DNA in a final volume of 25 μL containing 40 pmol of each oligonucleotide, 0.2 mmol/L of each dNTP, 1.5 mmol/L MgCl2, 10 mmol/L Tris (pH 8.4), and 0.25 units of Taq polymerase (Fermentase Co. Canada). Hybridization was carried out in a DNA thermal cycler (Bio-Rad Laboratories; Foster City, California, USA) in which DNA templates were denatured at 95°C for 5 min, amplification consisting of 35 cycles in 95°C for 45s, 60°C for 1 min, and 72°C. Products were subjected to restriction enzyme analysis by digestion at 37°C for 2 h with 10 U/ml of XbaI restriction endonuclease in each 10 μL of PCR sample in the buffer recommended by the manufacturer of the endonuclease (Roche Co. Mannheim, Germany). The fragments separated by electrophoresis on 2% agarose gels. After electrophoresis, gels were stained with 1 mg/dl ethidium bromide solution for 10 min, and DNA fragments were visualized by gel documentation (Bio-Rad Laboratories; Foster City, California, USA). DNA samples were quantified by absorbance measurement and this allowing samples concentration to be normalised to produce consistent results. The genotypes for all samples were reassessed twice to confirm the results and ensure reducibility. Some suspected genotypes were validated by purifying the PCR products using GeneJET gel extraction and DNA cleanup kit (Thermo Fisher Scientific Inc. USA) and were directly sequenced in an ABI Prism 310 Automated Sequencer, using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems).

ApoB gene XbaI polymorphism status

The following nomenclature has been used to specify the genotypes at ApoB XbaI:
- X-/X- samples contain 230 bp fragments (wild-type, CC, or homozygous absence of restriction site)
- X-/X+ samples contain 230, 160, 70 bp fragments (CT, heterozygous absence of restriction site deletion)
- X+/X+ samples contain 160 and 70 bp fragments: (rare-type, TT, or homozygous presence of restriction site).

Statistical analysis

All statistical calculations for the biochemical data were performed using Statistical Package for the Social Sciences (SPSS) version 11.5 (IBM Corporation). Data were expressed as mean ± SD, whereas categorical variables were summarized as frequencies and percentages. Data was evaluated by t test and one-way analysis of variance. Allele and genotypic frequencies for ApoB XbaI were calculated with the gene counting method. For Xba polymorphism, deviation from Hardy–Weinberg equilibrium and allele frequency were tested by using power marker software [38]. For all tests, a two-sided p value less than 0.05 was used as the level of significance.

Result

A summary of the clinical, biochemical characteristics and genotype frequencies of the 355 studied individuals is presented:

Table 1 shows that our sample of Egyptian women has a significantly increased prevalence of obesity, levels of total cholesterol, HDL cholesterol and ApoA1 compared to men. Smoking levels were much higher in men than women.

Table 2 shows allele frequencies obtained for X- and X+ were 72.7 and 27.3%, respectively; the X-/X- genotype presented the highest frequency (54.6%), X+/X- (36.2%) and the X+/X+ genotype had the lowest frequency (9.2%). The genotype frequencies did not deviate significantly from the Hardy-Weinberg equilibrium.

The mean values of BMI and average concentrations of lipid parameters by genotype are shown in Table 3. A non-significant effect is observed on the BMI (p = 0.069).

Biochemical changes in serum lipids were compared with the genotype status. A small but significant increase in cholesterol levels is associated with the X+ allele (X+/X+: 186 ± 17.27 vs., X-/X-: 175 ± 15.21 mg/dl p 0.041), LDL-C (X+/X+: 115 ± 32.43 vs., X-/X-: 110 ± 31.39 mg/dl p 0.021), and ApoB (X+/X+: 119 ± 11.47 vs., X-/X-: 107 ± 11.35 mg/dl p 0.019) levels. The ApoB concentration in the presence of the X+/X+ genotype is elevated by comparison to X-/X+ and X-/X-.
Table 1: Clinical characteristics and biochemical parameters of the sample population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Population (n=355)</th>
<th>Males (n=215)</th>
<th>Females (n=140)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>49.46 ± 9.34</td>
<td>51.11 ± 9.48</td>
<td>48.36 ± 10.17</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index (BMI) (kg/m2)</td>
<td>27.01 ± 6.21</td>
<td>29.67 ± 6.43</td>
<td>25.52 ± 4.51</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cigarette smokers (%)</td>
<td>8.9</td>
<td>18.6</td>
<td>0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetestype 2(%)</td>
<td>17.5</td>
<td>15.8</td>
<td>10.7</td>
<td>NS</td>
</tr>
<tr>
<td>Dyslipidemia (%)</td>
<td>17.8</td>
<td>15.7</td>
<td>21.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Obesity (%)</td>
<td>51.5</td>
<td>39.5</td>
<td>69.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Blood Glucose (mmol/L)</td>
<td>6.04 ± 3.55</td>
<td>6.04 ± 2.49</td>
<td>6.04 ± 4.77</td>
<td>NS</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>192.1 ± 23.4</td>
<td>178.2 ± 32.5</td>
<td>193 ± 24.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>46.1 ± 11.5</td>
<td>42.2 ± 19.40</td>
<td>48.9 ± 11.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>114 ± 26.3</td>
<td>114 ± 21.7</td>
<td>113 ± 29.2</td>
<td>NS</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>191 ± 29.3</td>
<td>189 ± 36.5</td>
<td>192 ± 22.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apolipoprotein A1 (mg/dl)</td>
<td>141 ± 21.6</td>
<td>121 ± 25.9</td>
<td>149 ± 28.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apolipoprotein B (mg/dl)</td>
<td>115 ± 19.5</td>
<td>117 ± 12.5</td>
<td>113 ± 13.5</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 2: Numbers and frequency of subjects with the ApoB Xbal gene polymorphism X-/X- (CC), X-/X+ (CT) and X+/X+ (TT) genotype and X- (C) and X+ (T) alleles of the sample population

<table>
<thead>
<tr>
<th>ApoB</th>
<th>Genotype</th>
<th>Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
<td>CT</td>
</tr>
<tr>
<td>Males</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>(n=215)</td>
<td>(54.4)</td>
<td>(36.3)</td>
</tr>
<tr>
<td>Females</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>(n=140)</td>
<td>(45.7)</td>
<td>(35.7)</td>
</tr>
<tr>
<td>Total</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>Population</td>
<td>(51)</td>
<td>(36.1)</td>
</tr>
</tbody>
</table>
Table 3: Body mass index and lipid profile variables according to genotypes in the total population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bodymass index (kg/m²)</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>Apo A1 (mg/dl)</th>
<th>Apo B (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-/X- (CC)</td>
<td>27.2±5.6</td>
<td>175±15.21</td>
<td>123±18.65</td>
<td>42.6±21.26</td>
<td>104±33.39</td>
<td>131±11.25</td>
<td>105±10.35</td>
</tr>
<tr>
<td>(n=181)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>X-/X+ (CT)</td>
<td>28.5±4.5</td>
<td>179±16.23</td>
<td>112±19.65</td>
<td>44.3±22.29</td>
<td>110±31.39</td>
<td>131±13.23</td>
<td>107±11.37</td>
</tr>
<tr>
<td>(n=128)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>X+/X+ (TT)</td>
<td>29.3±3.6</td>
<td>186±17.27</td>
<td>134±12.63</td>
<td>43.5±23.25</td>
<td>115±32.43</td>
<td>132±15.29</td>
<td>119±11.47</td>
</tr>
<tr>
<td>(n=46)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>P value (ANOVA)</td>
<td>0.069</td>
<td>0.041</td>
<td>0.131</td>
<td>0.167</td>
<td>0.021</td>
<td>0.91</td>
<td>0.019</td>
</tr>
</tbody>
</table>

TC: Total cholesterol; HDL-C: High density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; TG: Triglyceride. NS: not significant. ANOVA: analysis of variance statistical test

Discussion

This study is the first to examine the relationship between the ApoB XbaI polymorphisms and plasma lipid parameters in the Egyptian population. We have shown that ApoB polymorphisms are linked in a statistically significant manner to cholesterol, ApoB protein, and LDL-C levels. The genotype frequencies observed in this study were in Hardy-Weinberg equilibrium and the allele frequencies found were similar to those described in other populations [39-41]. The frequency of the rare allele X+ XbaI polymorphism observed in our population (0.45) is similar to that observed in Finland (0.38), Italy (0.37) and Brazil (0.40) [42-44]. The X+ frequency is lower than that reported in France (0.47 to 0.46), Denmark (0.49), Switzerland (0.55) and England (0.57) [45-49], and very much greater than that reported in Nigeria (0.15), China (0.01) and in Japan (0.05) [8, 50-52]. BMI is the parameter most often used to evaluate the degree of obesity. In our study, the increase in BMI was not significant (p = 0.09) in subjects with X+/X+ and this is in agreement with the results obtained in a Caucasian and Indian population [53-55].

Cholesterol has been the most discussed lipoprotein-related proatherogenic risk variable. However, risk appears more directly related to the number of circulating atherogenic particles that contact and enter the arterial wall than to the measured concentration of cholesterol in these lipoprotein fractions. The atherogenic lipoprotein particles contain a single molecule of ApoB, so the concentration of ApoB provides a direct measure of the number of circulating atherogenic lipoproteins. The plasma levels of ApoB are also associated with CVD. Evidence is accumulating that ApoB might be a better risk indicator for cardiovascular events than LDL-C [55] and the monitoring of ApoB gene polymorphisms could be helpful for disease prediction. Numerous studies have attempted to relate the presence of a XbaI restriction polymorphism of the ApoG gene for ApoB to lipid abnormalities and atherosclerosis. The results, so far, are inconsistent with studies on Caucasian populations [44, 56-58] or Asian [51, 59, 60] finding no association between lipid parameters and X+ allele. Other studies on healthy subjects [61-63] and on patients with non-insulin dependent diabetes [64] concluded the presence of an association. Zaman et al. in a Japanese population showed that XbaI polymorphism was not significantly associated with total cholesterol and HDL-C levels [65]. The allele X+ has also been associated with elevated triglycerides [54, 66, 67]. A previous study on 2166 Caucasians showed similar effects of ApoB XbaI polymorphism on LDL-C and cholesterol levels [68]. A study in a North Indian population showed that the mean value of total serum cholesterol and ApoB was significantly higher in CVD patients carrying homozygous X+ genotypes as compared to controls [39].

The mechanisms explaining the association of XbaI polymorphism to lipid disturbances are unknown. Since ApoB is an important component of each LDL-C particle, genetic variations that affect either the level or the structure of ApoB are most likely to act in a co-dominant fashion [69]. Our results of different XbaI genotypes that were associated with total cholesterol, LDL-C, and ApoB levels are in agreement with this suggestion. However, it is noticeable that the XbaI polymorphism in ApoB results from a mutation in exon 26 at the third base of the triplet (ACC → ACT) encoding the threonine located at position 2488 of the apolipoprotein B without changing the sequence of amino acid. Therefore, the effect of this polymorphism on lipid concentrations is probably not due to the ApoG gene product itself. Many authors hypothesize a linkage disequilibrium between the XbaI polymorphism and a causal mutation not yet identified and responsible for these variations. In other words the observed associations probably result from co-segregation with one or more
functional variants in the ApoB gene or a gene located nearby [70,71]. Confirmation of this hypothesis could partly explain the inconsistency of results observed between different ethnic or geographical populations. Furthermore, Demant et al. [72] suggest that ApoB produced by the homozygous X+X+ may have a slightly modified structure that would reduce its ability to interact with the LDL receptor. The causal mutation in linkage disequilibrium with the Xbal locus could be located in the region of the gene encoding the ApoB binding site of the LDL receptor. Altering the rate of clearance of LDL can cause disturbances to lipoprotein metabolism, thus explaining the variations observed, not only at the level of LDL-cholesterol and ApoB but also, total cholesterol, triglycerides, ApoA1 concentrations and deposition of triglycerides in adipose tissue from the lipoprotein particles containing ApoB (VLDL). CVD, ApoA1 concentrations and deposition of triglycerides in adipose tissue, thus explaining the variations observed, not only at the level of LDL-cholesterol and ApoB but also, total cholesterol, triglycerides, ApoA1 concentrations and deposition of triglycerides in adipose tissue from the lipoprotein particles containing ApoB (VLDL). CVD, a leading cause of death in most industrialized countries, is a multifactorial disease [72] and in future, more studies on the association of apolipoprotein polymorphisms with lipid factors is suggested. It may help to identify high-risk groups for CVD and may be of use in genetic screening for CVD patients of different genetic/ethnic background.

References


