Abstract

Familial defective apolipoprotein (apo) B-100 (FDB) is caused by R3500Q mutation of the apo B gene resulting in decreased binding of LDL to the LDL receptor. Two other apo B mutations, R3500W and R3531C, affecting binding are known to date. We screened the apo B gene segment around codon 3500 by heteroduplex analysis and single strand conformation polymorphism (SSCP) analysis in a total of 373 hyperlipidemic individuals. Two single-base mutations were detected and confirmed by DNA sequencing. One mutation, ACA3528 → ACG change, resulted in degenerate codon with no amino acid substitution. The other mutation, CGG3500 → CAG mutation, resulted in an Arg 3500 → Gln substitution (R3500Q). The prevalence of heterozygote in this selected population was 0.3% (95% confidence interval, 0.01–1.5%) for the R3500Q mutation, and 2.4% (95% confidence interval, 1.1–4.5%) for the previously described R3500W mutation. The results suggest that the R3500Q mutation is not a significant factor contributing to moderate hypercholesterolemia in Chinese (P = 0.027). Family studies of the R3500Q carrier revealed a further two individuals heterozygous for the mutation, both of whom were hypercholesterolemic. Analysis of the R3500Q allele using six diallelic markers and the 3'HVR marker revealed a haplotype which was the same as that reported in a Chinese American but differed from that reported in a Chinese Canadian. Our data support limited multiple recurrent origins for R3500Q in Chinese population. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Familial defective apolipoprotein B-100; R3500Q mutation; Haplotype

1. Introduction

Familial defective apolipoprotein (apo) B-100 (FDB) is an autosomal dominant condition resulting in hypercholesterolemia [1,2]. It is caused by the substitution of arginine by glutamine at codon 3500 of the apo B gene (apo B R3500Q) [3]. Apo B-100, encoded by the apo B gene, is the main protein of low density lipoprotein (LDL) and is the ligand through which LDL binds to its receptor in the process of receptor-mediated endocytosis [4]. The mutation affects the function of apo B-100 by decreasing LDL receptor binding affinity [5].

Recently two other mutations have been identified, each caused by a C→T transition, one at nucleotide 10 707 and the other at nucleotide 10 800. The change at nucleotide 10 707 results in the substitution of arginine by tryptophan at codon 3500 (apo B R3500W) [6], and the change at nucleotide 10800 results in the substitution of arginine by cysteine at codon 3531 (apo B R3531C) [7]. In a general population of whites, R3500Q and R3531C are equally common with a frequency of 0.08%, whereas R3500W is mostly likely very rare [8]. R3500W causes hypercholesterolemia and LDL derived from the R3500W heterozygote is dysfunctional in that it allows only poor growth of a LDL cholesterol-dependent cell line [6]. R3531C only reduces binding to LDL receptor by about 30% in heterozygote in vitro [7] and R3531C carriers in the general population are not hypercholesterolemic [8].
Haplotyping analysis of the affected alleles, using markers within and/or flanking of the apo B gene, suggesting that R3500Q mutations in several Western populations were always present on an allele with the same underlying haplotype, designated as haplotype 194 [9–13]. On the other hand, three different haplotypes were found in one German [14] and two Chinese subjects [15,16]. Conversely, the same haplotype was associated with R3500W alleles in 10 unrelated Chinese [17,18]. That different haplotypes in subjects of Asian and Scottish descent indicates R3500W mutations arose independently in different ethnic backgrounds [6]. On different chromosomal background, R3531C alleles were also associated with different haplotypes [7].

In a previous study, we reported the detection and haplotype analysis of nine heterozygous R3500W mutations in 373 unrelated hyperlipidemic Chinese [18]. However, it is apparent that other functional mutations may be present. We have therefore screened this original population for the presence of any mutations by heteroduplex analysis and single strand conformation polymorphism analysis.

2. Materials and methods

2.1. Subjects and lipid measurements

Blood samples were obtained after an overnight fast from 373 hyperlipidemic patients attending the lipid clinic of Veterans General Hospital-Taipei and Wei Gong Memorial Hospital. These patients had cholesterol (TC) concentrations ≥ 5.17 mmol/l (not desirable values according to NCEP guidelines [19]) without tendon xanthomas. No diagnosis of familial hypercholesterolemia (FH) was made in these patients. Of these patients, 258 had triglyceride (TG) values < 2.27 mmol/l, and 115 had concentrations above this value. The mean cholesterol values for Chinese men and women living in Taiwan in 1990 were 5.19 and 5.30 mmol/l, respectively [20]. TC and TG were measured by enzymatic methods [21,22]; HDL and LDL were determined by precipitation with phosphotungstic acid/magnesium chloride and heparin/sodium citrate. The lipid profiles were analysed using reagents supplied by E. Merck (Darmstadt, FRG), according to the CHOD-PAP method.

2.2. DNA preparation and PCR amplification

DNA was isolated from leukocytes, as previously described [18]. A 345-bp fragment flanking nucleotide 10 551–10 895 of the apo B gene [23] was amplified by PCR using primers and conditions described by Tybjærg-Hansen et al. [24].

2.3. Mutation screening

PCR products (3 µl) were mixed with an equal volume of 95% formamide buffer and electrophoresed at 300 V for 24 h on a low cross-linking 28 cm × 16.5 cm × 1 mm polyacrylamide gel (20% polyacrylamide (w/v), 0.2% N,N′-methylenebisacrylamide (w/v), containing 30% (w/v) urea in 0.6× TBE). Gels were stained with ethidium bromide and photographed under ultraviolet light. PCR products were restricted into 141- and 204-bp fragments by EcoRI and subjected to SSCP analysis using GeneGel Excel gels as recommended by the manufacture (Pharmacia Biotech, Uppsala, Sweden). Aberrant products were sequenced by the dideoxynucleotide chain termination method [25] as previously described [18]. Screening for the identified R3500Q mutation was performed using mutagenic PCR [26] that creates a MspI restriction site in the normal allele but not in the mutant allele. The PCR amplified products (nucleotides 10 684–10 895) were digested with MspI and separated on a 10% polyacrylamide gel.

2.4. Haplotype markers

Seven markers were analyzed as described [9] to establish haplotypes at the apo B locus. These markers include a 9-bp insertion/deletion in signal peptide, five biallelic RFLP sites (ApaLI in exon 4; AluI in exon 14; XbaI and MspI sites in exon 26; EcoRI site in exon 29), and a hypervariable repeat region (HVR) downstream of the apo B gene. Alleles were identified as + or −, depending on whether or not the 9-bp insertion or particular restriction site was present. The HVR alleles were identified by comparison with known markers and recorded according to the nomenclature of Ludwig et al. [27].

3. Results

3.1. Heteroduplex and SSCP analyses

A region of 345 bp in the exon 26 of the apo B gene was amplified and screened for mutations. Out of 373 individuals screened, additional slower migrating heteroduplex bands were observed from 11 individuals. Nine of these were shown to be heterozygous for the apo B R3500W mutation from a previous study [18]. The heteroduplex molecules of the remaining two, D163 and A145 (Fig. 1, lanes 2 and 3), had a different electrophoretic mobility compared to that of known R3500W heterozygote (lane 1), indicating different point mutations. Similarly, different aberrant patterns were observed after SSCP analysis (data not shown), indicating different underlying mutations in A145 and D163.
3.2. Mutation identification

The mutations in A145 and D163 were revealed by DNA sequencing. Individual A145 had a mutation in codon 3528 (ACA → ACG), a degeneracy codon change that did not result in substitution of threonine. By dot blotting and allele-specific oligonucleotide hybridization, A145 was shown to be heterozygous for the mutation (data not shown). Individual D163 has mutation in codon 3500 (CGG → CAG), which resulted in substitution of arginine by glutamine (R3500Q). The recurrent R3500Q mutation was confirmed by mutagenic PCR and \( \text{MspI} \) digestion (Fig. 2).

3.3. Family studies

A total of 12 family members of the R3500Q carrier gave consent to be screened for the mutation. As shown in Fig. 2, the proband (II-3) inherited the mutation from his father (I-1) and transmitted the mutation to his son (III-3). None of his siblings (II-2 and II-5) carry the mutation. The clinical and biochemical data for this FDB family are shown in Table 1. All of the FDB subjects in this family were hypercholesterolemic but none of them had evidence for atherosclerotic disease. The abnormal high TG value in the proband is due to his lifestyle as a businessman and inappropriate diet.

3.4. Haplotype analysis on the R3500Q allele

The genotypes at seven polymorphic loci were examined in this FDB family (Table 2). A total of 26 chromosomes yielded three independent haplotypes. The R3500Q allele was associated with the haplotype B: SP + /ApaLI + /AluI − /XbaI − /MspI + /EcoRI + /30 3'HVR repeats in the family. This haplotype was designated as 195 according to the nomenclature of Ludwig and McCarthy [9] and was identical to that reported in association with the R3500Q allele in a Chinese American [15].

4. Discussion

In this report, a total of 373 unrelated hyperlipidemic individuals were screened by heteroduplex analysis and SSCP analysis for the presence of apo B-100 mutations. The heteroduplex method used in this study appeared able to differentiate among R3500W and R3500Q types. Distinct SSCP band patterns were observed only after performing analysis at both 8 and 15°C (data not shown).

Studies of the prevalence of FDB among hypercholesterolemic Caucasian populations range between 1 and 6% [2]. In our study, 10 (one R3500Q and nine R3500W) of the 373 hypercholesterolemic subjects screened were positive. Our data suggest the prevalence of FDB among the Chinese subjects with hypercholesterolemia is 2.7%. With one R3500Q and nine R3500W detected in our selected population, the results suggest that the R3500Q mutation is not as significant a factor contributing to moderate hypercholesterolemia in Chinese as it is in Caucasians.
Table 1
Lipid characteristics of the proband and immediate family

<table>
<thead>
<tr>
<th>Individual</th>
<th>Sex</th>
<th>Age (years)</th>
<th>R3500Q</th>
<th>TC (mmol/l)</th>
<th>LDL (mmol/l)</th>
<th>HDL (mmol/l)</th>
<th>TG (mmol/l)</th>
<th>Weight (kg)</th>
<th>Height (m)</th>
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<tr>
<td>I-1</td>
<td>M</td>
<td>71</td>
<td>+</td>
<td>6.12</td>
<td>3.82</td>
<td>1.58</td>
<td>0.62</td>
<td>60</td>
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<td>I-2</td>
<td>F</td>
<td>62</td>
<td>-</td>
<td>6.87</td>
<td>5.40</td>
<td>1.60</td>
<td>1.26</td>
<td>53</td>
<td>1.51</td>
</tr>
<tr>
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<tr>
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<td>4.70</td>
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<td>1.64</td>
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<tr>
<td>D163b</td>
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<td>+</td>
<td>7.08</td>
<td>3.28</td>
<td>1.09</td>
<td>6.52</td>
<td>88</td>
<td>1.75</td>
</tr>
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<td>1.34</td>
<td>0.95</td>
<td>1.02</td>
<td>50</td>
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</tr>
<tr>
<td>II-6</td>
<td>M</td>
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<td>-</td>
<td>5.30</td>
<td>3.54</td>
<td>1.09</td>
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<td>88</td>
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<tr>
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<td>0.96</td>
<td>0.49</td>
<td>13</td>
<td>0.90</td>
</tr>
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</table>

a TC, total cholesterol; LDL, LDL cholesterol; HDL, HDL cholesterol; TG, triglyceride. No tendon xanthomas was observed in the proband and immediate family.
b The proband (individual D163 and II-3 in pedigree).

In the proband and immediate family, clear cosegregation of hypercholesterolemia with the R3500Q mutation was observed. The cholesterol concentrations of the R3500Q carriers in the family were moderately increased (6.12–7.08 mmol/l), similar to those reported (6.43–7.29 mmol/l) for Chinese R3500Q carriers [15,16]. The serum cholesterol concentration of 6.67 ± 0.50 mmol/l (mean ± S.D.) in the six Chinese het-
erozygous for R3500Q ([15,16] and this study) and 6.77 ± 1.27 mmol/l in the 15 Chinese R3500W carriers [17,18] were not significantly different (P = 0.727). Whereas significantly lower cholesterol concentrations were observed in Chinese R3500Q heterozygotes (6.67 ± 0.50 mmol/l, n = 6) than in the Caucasian R3500Q carriers (8.3 ± 1.9 mmol/l, n = 238; P < 0.001) [28]. The milder phenotype of FDB in Chinese is not due to any effect of this mutation, but may be due to differences in dietary fat consumption since Chinese on average have lower cholesterol levels than Caucasians. A traditional low-fat diet modulating the phenotype of Chinese patients with heterozygous familial hypercholesterolemia was also reported [29].

In the kindred, the R3500Q mutation was unambiguously assigned to the haplotype SP+/ApoLI+/AlsI−/XbaI−/MspI+/EcoRI+/3‘HVR repeats. Comparison of this haplotype with those reported [9,14–16] strongly suggests that the R3500Q mutation in the Chinese individual in the study of Bersot et al. [15] and our index case is identical by descent. As the Chinese individual and proband’s father were both natives of China, it is therefore probable that the R3500Q mutation arose in a common ancestor of Chinese origin many years ago.

Haplotype markers are ancient and predate human racial divergence. Little or no recombination in the 3’ region of the apo B gene was observed [30]. The R3500Q alleles associated with two different haplotypes in the index case of Abdel-Wareth et al. [16] and ours are likely to be recurrent mutation events like that of the factor VIII gene [31]. The hypermutable CpG dinucleotide is frequently associated with point mutations of various genes [32].

In summary, we identified an apo B-100 R3500Q heterozygote in hyperlipidemic Chinese by the heteroduplex method as well as SSCP analysis. Comparison of nine R3500W carriers detected previously suggests that the R3500Q mutation does not appear to be a significant factor contributing to moderate hypercholesterolemia in Chinese. Together with two cases described, the presence of two different haplotypes associated with this mutation among three Chinese individuals supports limited multiple recurrent origins of the R3500Q mutation in the Chinese population.

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References


