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Lack of an Association Between Apolipoprotein B XbaI Polymorphism and Blood Lipid Parameters in Childhood

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Summary

The frequencies of the alleles of XbaI polymorphism in the apolipoprotein B gene were determined in two groups of children, 82 with high (HCG) and 86 with low (LCG) cholesterol levels. A slightly higher incidence of the X2X2 genotype in HCG was found, but the differences were not statistically significant. No relations were found between the XbaI polymorphic site and the levels of serum lipids and lipoproteins. Common XbaI polymorphism in the apolipoprotein B gene does not determine significantly the plasma cholesterol levels in childhood.

Key words

Apolipoprotein B – Children – Gene polymorphism – Hypercholesterolaemia – XbaI

Introduction

Apolipoprotein B (apo B) is the main protein component of VLDL (Very Low Density Lipoprotein), IDL (Intermediate Density Lipoprotein), and LDL (Low Density Lipoprotein) particles, and plays two important roles in lipoprotein metabolism. It is necessary for the production and secretion of VLDL, and it serves as a ligand for the LDL receptor. As a result, it helps in the removal of LDL particles from the plasma and in the transport of their cholesterol to peripheral cells and back to the liver. Mutations of the apo B gene thus could affect lipoprotein metabolism at two key sites.

Apo B consists of 4536 amino acids and about 20 polymorphism sites have been described in the apo B gene (e.g. Lusis 1988). One of them, the XbaI polymorphism, distinguishes the substitution of cytosine (7673) by thymine (Carlsson *et al.* 1986). This substitution does not change the amino acid (threonine 2488) sequence.

The first papers addressing this topic (Law *et al.* 1986, Aalto-Setälä *et al.* 1988) referred to the X2 allele as a disadvantageous one. All the above authors reported an association between the X2 allele and increased levels of triacylglycerols (TG), total cholesterol (TC), and apo B. Another study (Talmud *et al.* 1987) demonstrated, in a randomly selected population sample, an association between the X1X1 genotype and low TC levels. In a study conducted by Tybjaerg-Hansen *et al.* (1991), the highest levels of LDL-cholesterol were shown in X2X2 homozygotes with coronary artery disease (CAD). In hyperlipidaemic patients (Jenner *et al.* 1988), X2X2 homozygotes were found with increased levels of fasting TG.

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However, the subsequent studies produced opposite results. A group of X1X1 homozygotes showed the highest levels of TC (Tybjaerg-Hansen *et al.* 1991), or LDL-cholesterol (Peacock *et al.* 1992). Hansen *et al.* (1993) found the highest levels of TC and apo B in X1X1 homozygotes whose body mass index (BMI) was <25. A dietary study (Friedlander *et al.* 1993) indicated a more marked increase in blood lipid concentrations in individuals with X1X1 receiving a high-cholesterol diet.

The relationship between the XbaI polymorphism of the apo B gene and serum lipid levels was not documented in the Japanese (Aburatani *et al.* 1987) and Chinese (Saha *et al.* 1992) population. This may be due to the much lower cholesterol concentration and a lower incidence of the X2 allele compared to European populations.

An effect of this polymorphism on the incidence of myocardial infarction (MI) has also been described. A significantly higher frequency of the X1 allele was reported in a group of MI patients compared with the controls (Hegele *et al.* 1986). Monsalve *et al.* (1988) also demonstrated an increased frequency of the X1 allele in all groups of patients with CAD. In a study by Myant *et al.* (1989), normocholesterolaemic patients with CAD exhibited increased allele X1 frequencies compared to healthy controls. However, no significant differences regarding the polymorphism and its possible relation to CAD, MI or lipid parameters were reported by Darnfors *et al.* (1989), Xu *et al.* (1990), Paulweber *et al.* (1990), Nieminen *et al.* (1992) and De Lorenzo *et al.* (1993).

The present study was designed to monitor the distribution of the alleles of the XbaI polymorphism in the apo B gene in two groups of children with different levels of total cholesterol selected from 2000 Prague children. Pre-pubertal children are the most suitable group for identifying the genetic factors determining the levels of plasma lipids and lipoproteins, since children are not yet affected by alcohol, smoking and medication. Moreover, environmental factors (diet, physical activity) have affected children for a shorter period of time than adults.

Methods

Two groups of children (low- and high-cholesterolaemic, LCG and HCG) were chosen from those at the opposite ends (LCG – from 5 % to 10 % and HCG from 95 % to 100 %) of the distribution curve of cholesterolaemia obtained from 2000 children aged from 10 to 11 years as described previously (Pistulková *et al.* 1991, Hubáček *et al.* 1994, Poledne *et al.* 1994). The groups differed considerably in their lipoprotein parameters (Table 1). No differences were found in their diet composition, BMI, insulinaemia and thyroxinaemia. Triacyglycerol and cholesterol (in VLDL, LDL and HDL [High Density Lipoprotein] fractions isolated by ultracentrifugation) levels were measured enzymatically, apo B levels using nephelometry in a WHO Regional Reference Laboratory controlled by CDC, Atlanta, USA.

DNA was isolated by the salting out method (Miller *et al.* 1988) from previously diluted and frozen blood. The polymorphism in the gene for apo B was identified using the Southern blotting technique. The allele with the presence of the XbaI restriction site is referred to as X2, and that without as X1.

ANOVA and the chi-square test were used to analyze the statistical significance of differences.

Table 1. Lipid parameters in low-cholesterolaemic (LCG) and high-cholesterolaemic groups (HCG)(levels in mmol/l, with apo B in g/l)

	LCG	HCG	
Total Cholesterol	3.52±0.57	5.49±0.67	p<0.001
Triacylglycerols	0.96±0.59	1.01±0.44	
Apo B	0.81±0.35	1.07±0.38	p<0.001
VLDL-Cholesterol	0.13±0.09	0.20±0.11	p<0.001
LDL-Cholesterol	2.28±0.56	4.02±0.66	p<0.001
HDL-Cholesterol	1.25±0.28	1.44±0.33	p<0.001

Results

The frequency of both alleles and all three genotypes of the apo B XbaI polymorphism in HCG and LCG are shown in Table 2. The X1 allele and X1X1 genotype were found more often in LCG, whereas the X2 allele and X2X2 genotype were found more often in HCG. The differences in the frequency of both alleles were just below the limit of statistical significance.

Analysis of the effect of genotypes on the levels of plasma lipids and lipoproteins did not reveal any significant differences (Table 3). An upward tendency was detected in the levels of TC, LDL-C and apo B from X1X1 *via* X1X2 to X2X2 genotypes inside LCG.

Table 2. Distribution of genotypes and alleles of XbaI polymorphism of the apo B gene in low-cholesterolaemic (LCG) and high-cholesterolaemic groups (HCG)

	LCG		HCG	
Genotype	n	%	n	%
1/1	22	26.2	15	18.3
2/1	42	50.0	40	48.8
2/2	20	23.8	27	32.9
Allele				
1	86	51.2	70	42.7
2	82	48.8	94	57.3

Table 3. Lipid parameters and XbaI polymorphism in the apo B gene in low-cholesterolaemic (LCG) and high-cholesterolaemic groups (HCG) groups (levels in mmol/l, with apo B in g/l)

Genotype	HCG		
	TC	TG	Apo B
1/1	5.60±0.73	1.00±0.51	1.14±0.37
1/2	5.39±0.66	1.07±0.47	1.03±0.39
2/2	5.60±0.64	0.86±0.25	1.05±0.39
	VLDL-C	LDL-C	HDL-C
1/1	0.19±0.15	4.11±0.76	1.43±0.28
1/2	0.22±0.11	3.89±0.58	1.45±0.34
2/2	0.16±0.07	4.15±0.70	1.45±0.36
Genotype	LCG		
	TC	TG	Apo B
1/1	3.38±0.50	0.99±0.57	0.73±0.29
1/2	3.49±0.52	0.85±0.35	0.83±0.39
2/2	3.67±0.67	1.21±0.94	0.90±0.34
	VLDL-C	LDL-C	HDL-C
1/1	0.15±0.15	2.11±0.48	1.25±0.24
1/2	0.13±0.07	2.25±0.52	1.27±0.28
2/2	0.15±0.07	2.45±0.62	1.17±0.32

Discussion

The genetic determination of cholesterolaemia has been intensively investigated since the late 1980s. Although monogenous mutations in the LDL receptor gene causing pathological disorders of cholesterol metabolism (familial hypercholesterolaemia) has been described in detail, it should be noted that most types of hyperlipoproteinaemias are of polygenous origin. Current knowledge suggests at least 20 genes that may exert an effect on lipid metabolism (Lusis 1988).

One of the most closely monitored genes which may affect lipid metabolism is the gene for apo B and its XbaI polymorphism. Various aspects have been investigated, namely the relations of apo B gene to lipid parameters and allele frequencies in differently defined groups of patients and controls.

The differences in the incidence of genotypes between HCG and LCG (Table 2) did not attain statistical significance. No significant differences were found in the relation between lipid parameters and the X1 and X2 alleles within the two compared groups.

A noteworthy finding concerns the upward tendency of TC, LDL-C, and apo B in the LCG. Compared with X1X1 homozygotes, X2X2 homozygotes were found to have the average TC and LDL-C levels higher by 10 % and 20 %, respectively. By contrast, no such tendency was evident in HCG. As a result, this polymorphism may thus have a mild effect on cholesterolaemia in individuals otherwise genetically predisposed to low levels of lipid parameters without a chance to modulate substantially lipid metabolism.

Considering the number of studies describing apo B XbaI polymorphism, and the fact that the majority of described differences corresponded to low levels of statistical significance (usually only $p<0.05$), it is not likely that variations in the results could be explained by the variability of the investigated groups, by the methods of selection, ethnic origin, number of individuals or by the methods employed for data analysis.

Furthermore, in our study with strictly selected high- and low-cholesterolaemic children, no significant differences were found. These results fully correspond to the lack of consistency of the studies described above.

It appears that XbaI polymorphism in the apo B gene does not play an important role in determining the levels of lipid parameters and MI; any positive results must be restricted to a specific population and they are not generally applicable. A further study of this polymorphism alone in relation to MI does not seem to be of any further significance.

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