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Genetic Variation at the Apo B 3'VNTR in Czech General Population and in Czech Diabetes Mellitus Type II Patient Group

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INTRODUCTION

Apolipoprotein B (Apo B) is a major apolipoprotein of the cholesterol and triacylglycerol-rich lipoproteins in plasma (chylomicrons, very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL) and lipoprotein (a) particles). Apo B is essential for the formation and secretion of these lipoproteins. It is involved in the assembly and secretion of both chylomicrons from the small intestine and VLDL from the liver, and thus in the transport of cholesterol and triglycerides in serum. Besides, it functions as a ligand for an LDL-receptor that mediates the cellular uptake of cholesterol. Dyslipidemia and dyslipoproteinemia, which are often observed in DM II, are characterized by elevated cholesterol, triglyceride (TG), VLDL-TG and Apo B serum levels and by the usually decreased HDL-cholesterol level. All these represent primary risk factors of cardiovascular disease and can be found in up to 70% of the DM II patients.

The human Apo B gene has been cloned, mapped and characterized from cDNA and the high genetic variation of the Apo B gene has been determined (Lusis et al., 1988; Berg et al., 1986; Young et al., 1990) in different association studies dealing with the genetic linkage between the Apo B gene variation and different clinical phenotypes, such as hypercholesterolemia (Weisgraber et al., 1988; Soria et al., 1989), hypertriglyceridemia, or coronary artery disease. Several Apo B polymorphisms (esp. Xba I, Eco RI, MspI, ins/del in signal peptide region, 3´VNTR of the gene) have been described but their influence on plasma lipid levels and their association with coronary heart disease remains controversial (Law et al., 1986; Rajput-Williams et al., 1988; Saha et al., 1992; Aalto-Setala et al., 1988; Darnfors et al., 1989; Talmud et al., 1987).

The genetic variation of the Apo B gene is involved in the regulation of lipid and apolipoprotein blood levels, which confirms that this gene may be one of the candidate genes participating in the development of atherosclerosis (Ye et al., 1995).

The Apo B 3´VNTR, located 73 bp downstream from the 3´end of the second polyadenylation signal of the Apo B gene on chromosome 2p 24 (21) (Ludwig et al., 1989), consists of the dimeric AT-rich core repeat sequence of 30 bp. There are at least 15 different alleles in length and about 77% of the population are heterozygous for different alleles at this site. Different alleles vary in length, ranging over about 400 bp as a result of the numbers of two related 15 bp hypervariable elements (*i. e.* 30 bp). It is one of the most highly polymorphic genetic loci in man. The direct DNA sequence determination clearly shows that the exact sequence of the repeat units among alleles of the same size is not necessarily identical. Several recent studies have confirmed that some types of alleles have effect on lipid and lipoprotein levels when present but the results are inconsistent (Turner et al., 1995). DNA variability in the Apo B 3'VNTR has been reported to be associated with myocardial infarction (Hegele et al., 1986; Friedl et al., 1990) and 3'VNTR-L allele (number of repeat units more than 39) was present in patients with coronary heart disease apparently more frequently when compared with the frequency in control subjects (Ye et al., 1995).

The aim of our study was to ascertain the allelic variability at the Apo B 3´VNTR in Czech DM II patients in comparison with Czech general population and to determine the effect of this genetic polymorphism on serum lipid levels.

MATERIALS AND METHODS

Subjects

The Czech general population comprised 200

unrelated individuals (Prague area) of both genders (115 males, 85 females). The clinical data of these subjects were not available, the impaired glucose tolerance and diabetes were not determined in this group and therefore they could not be excluded. The Czech DM II patient group comprised 142 unrelated individuals (Prague area) of both genders (51 males, 91 females).

Methods

The genomic DNA was extracted from leukocytes of peripheral blood using common methods (Maniatis et al 1989). The VNTRs were determined by PCR amplification of this region using the primer sequencies (Boerwinkle et al., 1989).

5´ primer 5' ATGGAAACGGAGAAATTATG 3' and
3´ primer 5' CCTTCTCACTTGGCAAATAC 3' (Amitoff).

In the total volume of 50 µl, each amplification reaction contained (final concentrations): 100 ng of target DNA, 10 mmol/l Tris- HCl pH 8.3, 50 mmol/l $MgCl_2$, 0.01 % gelatine, 2.5 U Taq DNA polymerase (Appligene), 0.5 µmol/l of each primer and 200 µmol/l of each dNTP (USB). The denaturation step of the PCR was carried out at 94° C for 1 minute, annealing at 58°C for 1 minute, and extension at 72° C for 4 minutes in a programmable heat block (Perkin-Elmer Thermal Cycler).

PCR products (20 µl) were electro-phoresed on 2% Metaphor agarose gel (Sigma) at 50 V for 5 hours. The subsequent separation of alleles provided a high resolution, allowing differentiation of up to a single repeat unit. The nomenclature introduced by Boerwinkle (1989) was followed throughout the study. Having measured the relative migration of the PCR products with respect to the DNA size standards (from 50 to 1000 bp, USB), the observed DNA-fragments ranged in size from 510 to 930 bp - each allele differed in length from its neighbouring allele by 30 bp *i.e.* two 15 bp - repeat units.

The statistical analysis was performed by means of the Statistica software package. Allele frequencies of the Apo B 3'VNTR in both groups were determined by direct gene counting. Differences in obtained genotype frequencies in comparison with the expected values for Hardy-Weinberg equilibrium were estimated by the exact test (Guo et al., 1992). We considered the statistical significance to be 0.01. An agreement of the allele distribution in both groups was tested by the t-statistic which was calculated in the following way:

$$t = \sqrt{\frac{N_1 N_2}{N_1 + N_2}} \cdot \frac{p_1 - p_2}{\sqrt{p(1-p)}}$$

$$p = \frac{(p_1 N_1) + (p_2 N_2)}{N_1 + N_2}$$

where, N_1 = total number of alleles in the general population group, N_2 = total number of alleles in the DM II patients, p_1 = allele frequency in the general population group, p_2 = allele frequency in the DM II patients. We considered the statistical significance to be $p<0{,}0034$ ($p = 0.05$/number of comparison between groups, *i. e.* 15).

The influence of Apo B genotypes on serum lipid levels was tested by the non-parametric Kruskal-Wallis test (persons on beta-blockers, diuretics and hypolipidemic drugs were excluded). We considered the statistical significance to be 0.05.

RESULTS AND DISCUSSION

The comparison of the VNTRs allele distributions between the Czech general population and the Czech DM II patient group is illustrated in figure 1.

Fifteen segregating alleles and fifty-one different genotypes were detected in Czech general population (Table 1) and thirteen segregating alleles and forty-eight different genotypes were detected in the Czech DM II patient group (Table 2). The frequency distribution of alleles at the VNTR site was, as expected, bimodal in the Czech general population group. One mode was around 35-VNTR and 37-VNTR allele. The less frequent mode was observed at 47-VNTR and 49-VNTR allele. Similar bimodality in the allele distribution was described by others (Boerwinkle et al 1989; Ludwig et al., 1989). The bimodal distribution was not so apparent

in the DM II patient group (Fig. 1). No deviations from Hardy-Weinberg equilibrium were detected in general population (P = 0.029), but the frequency distributions of genotypes in the DM II patient group were not in Hardy-Weinberg equilibrium (P=0.01). The disequilibrium was caused, as expected, mainly by the occurance of a higher number of 35-/35-VNTR homozygotes.

The most common genotypes of the Apo B 3'VNTR in the DM II patient group and corresponding lipid levels are given in Table 3.

The comparison of allele frequencies between general population and the DM II patient group by the t-statistic indicated a significant difference in the frequency of allele 41-VNTR (Fig. 1). Thus, the influence of allele 41-VNTR in the genotype on plasma lipid levels (cholesterol, HDL-cholesterol and triglyceride) was estimated. It was tested by non-parametric Kruskal-Wallis test. No significant differences among the groups were found (Table 4).

Our data suggest that the polymorphism of the Apo B 3'VNTR does not influence cholesterol, HDL-cholesterol and triglyceride levels in the Czech DM II patients.

Table 1: Genotype and allele frequency distributions at the Apo B 3'VNTR in Czech general population

	Allele *Length of alleles in bp*															*Number of alleles*	*Frequency*
	25 510	27 540	29 570	31 600	33 630	35 660	37 690	39 720	41 750	43 780	45 810	47 840	49 870	51 900	53 930		
25	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	2	0.5
27		-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	0.25
29			-	1	2	3	1	-	-	-	-	1	-	-	-	8	2.0
31				2	4	4	5	-	1	1	1	6	4	-	-	31	7.75
33					3	8	8	3	-	-	3	7	4	2	1	49	12.25
35						17	29	3	2	-	4	3	6	1	-	98	24.5
37							15	7	-	1	1	10	4	3	-	99	24.75
39								5	-	-	1	1	1	1	-	27	6.75
41									-	-	-	-	-	-	-	3	0.75
43										-	-	-	-	-	-	2	0.5
45											1	-	1	-	-	13	3.25
47												3	1	-	-	36	9.0
49													-	1	-	22	5.5
51														-	-	8	2.0
53															-	1	0.25
Total																400	100

Table 2: Genotype and allele frequency distributions at the Apo B 3'VNTR in Czech DM II patients

	Allele *Length of alleles in bp*													*Number of alleles*	*Frequency*
	27 540	29 570	31 600	33 630	35 660	37 690	39 720	41 750	43 780	45 810	47 840	49 870	51 900		
27	-	-	-	-	-	1	-	-	-	-	-	1	-	2	0.7
29		1	-	1	1	1	-	-	-	1	-	-	-	8	2.82
31			2	5	2	6	-	-	-	-	-	-	1	18	6.34
33				4	4	9	2	3	1	3	1	1	-	40	14.08
35					13	9	1	2	1	2	2	1	-	51	17.96
37						16	5	2	1	5	7	3	-	81	28.52
39							-	2	-	1	-	1	-	12	4.23
41								3	-	1	1	1	-	19	6.69
43									1	2	-	2	-	9	3.17
45										-	-	-	-	15	5.28
47											1	2	-	12	4.23
49												-	-	15	5.28
51													-	2	0.7
Total														284	100

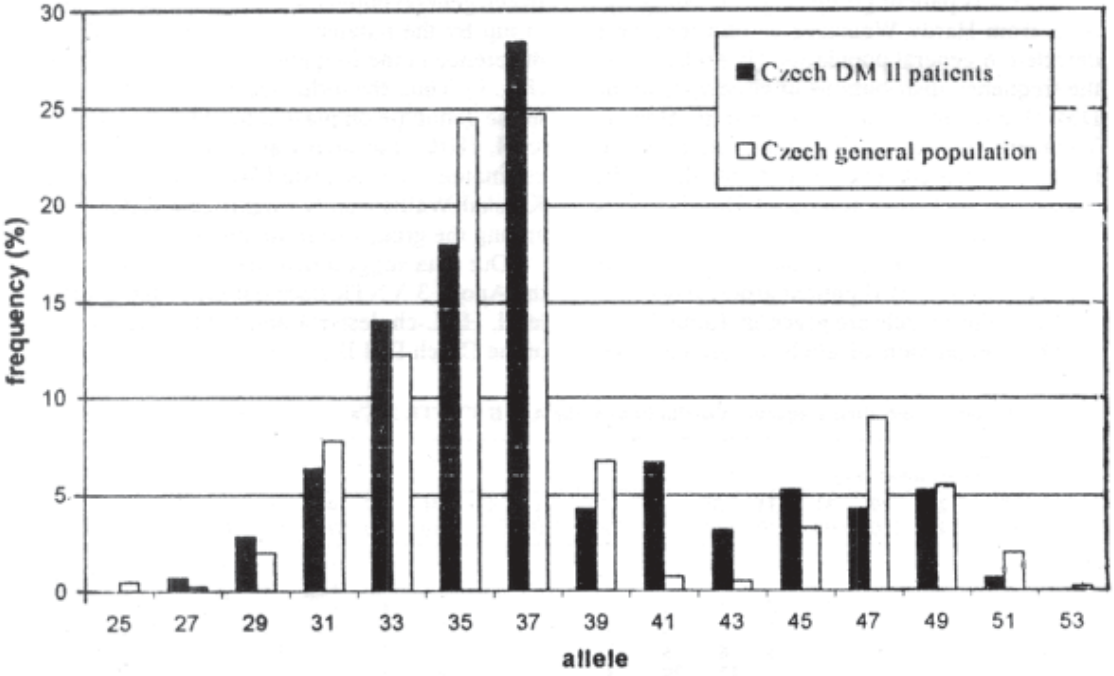

Fig. 1. Frequency distribution of the Apo B 3'VNTR alleles in Czech general population and in Czech DM II patients

allele	25	27	29	31	33	35	37	39	41	43	45	47	49	51	53
t-statistic	0.233	0.376	0.486	0.481	0.482	0.041	0.27	0.161	<0.001	0.006	0.187	0.016	0.901	0.165	0.399

Table 3: The most common genotypes of the Apo B 3'VNTR (frequency higher than 2%) in the DM II patient group and corresponding lipid levels

3'VNTR genotype	*Number of carriers*	*Relative frequency* (%)	*Triglyceride** *(mmol/l)*	*Cholesterol** *(mmol/l)*	*HDL-chol** (mmol/l)
29/33	3	2.1	1.46±0.86	6.2±1.35	1.11±0.4
31/33	5	3.5	1.6±0.51	6.81±1.68	1.31±0.58
31/37	6	4.2	1.53±0.92	5.76±0.33	1.3±0.52
33/33	4	2.8	1.13±0.37	4.94±0.67	1.35±0.15
33/35	4	2.8	5.35±3.24	6.7±1.08	1.04±0.29
33/37	9	6.3	2.36±1.76	6.28±1.13	1.18±0.38
33/41	3	2.1	1.08±0.62	7.53±1.63	1.51±0.22
33/45	3	2.1	1.38±0.91	6.65±1.94	1.98±0.08
35/35	13	9.2	1.97±1.96	6.49±1.31	1.44±0.35
35/37	9	6.3	1.87±1.09	6.34±0.51	1.58±0.47
37/37	16	11.3	2.03±1.21	6.35±1.43	1.34±0.36
37/39	5	3.52	2.09±0.6	6.25±0.76	1.28±0.18
37/45	5	3.52	3.83±3.32	7.26±0.63	1.04±0.34
37/47	7	4.93	2.66±1.58	7.04±1.66	1.3±0.36
37/49	3	2.1	3.07±0.53	7.48±0.79	1.27±0.13
41/41	3	2.1	6.71±8.29	8.57±3.14	1.04±0.26

*(patients on hypolipidemic drugs were excluded

Table 4: The influence of allele 41-VNTR in genotype on plasma lipid levels tested by the Kruskal-Wallis test (KW test)

	Total	*Number of genotypes*				
	number	*41/41*	*41/–*	*–/–*	*KW test*	*P*
Cholesterol	142	13	25	104	0.212	0.8994
HDL-cholesterol	136	13	14	99	2.565	0.2773
Triglyceride	142	13	25	104	2.799	0.2467

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KEY WORDS Diabetes Mellitus Type II. Apo B 3'VNTR. Allele Frequencies. Czech Population

ABSTRACT A sample of 200 unrelated individuals from the Prague area representing the Czech general population was studied for the Apo B 3'VNTR (variable number of tandem repeats) polymorphism. Polymorphisms at the Apo B loci are associated with various manifestations of diseases such as dyslipidemias, dyslipoproteinemias, Diabetes Mellitus Type II (DM II).The influence of the Apo B 3'VNTR alleles polymorphism on plasma lipid levels (cholesterol, HDL-cholesterol, triglycerides) was studied in a sample of 142 unrelated DM II patients. The allele distribution in Czech general population was in Hardy-Weinberg equilibrium, in contrast to the Czech DM II patient group. No apparent association between the Apo B 3'VNTR genotype and plasma lipid levels (cholesterol, HDL-cholesterol, triglycerides) was observed.

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