Original article

Association of APOB 3´-VNTR alleles with type 2 diabetes, BMI, systolic and diastolic blood pressure

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Abstract

Background: Apolipoprotein B (APOB) is a component of chylomicrons, low-density lipoproteins (LDL), very low-density lipoproteins (VLDL), intermediate density lipoproteins (IDL) and functions as the main protein for transporting cholesterol to peripheral cells. APOB gene has an AT-rich VNTR site at its 3´-untranslated region (3´-UTR). APOB 3´-VNTR alleles with ≥36 repeats have been shown to be strongly associated with increased serum lipid levels, gallstone formation and coronary artery disease. ***Objectives:*** To investigate any possible association of APOB 3´-VNTR alleles with type 2 diabetes, BMI, systolic and diastolic blood pressure. ***Materials and methods:*** APOB 3´-VNTR region in the DNA of non-diabetic controls and type 2 diabetic patients were amplified by polymerase chain reaction (PCR) and the numbers of core repeat in the amplified products were determined. Frequencies of the APOB alleles and genotypes among the controls and the patients were calculated and statistical analyses were performed. ***Results and discussion:*** Here we report for the first time that APOB 3´-VNTR alleles have different distribution frequencies among type 2 diabetic and non-diabetic individuals. We also observed higher body mass index (BMI), systolic and diastolic blood pressures in individuals who had at least one APOB 3´-VNTR allele with ≥35 repeats. ***Conclusion:*** Our study might bridge among the genetic signature of APOB 3´-VNTR, high APOB protein level in blood, diabetes and other co-morbidities.

Keywords: APOB, 3´-VNTR; repeat numbers; Type 2 diabetes.

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Introduction

APOB protein is a component of chylomicrons, low-density lipoproteins (LDL), very low-density lipoproteins (VLDL), intermediate density lipoproteins (IDL) and functions as the main protein for transporting cholesterol to peripheral cells[1-6]. The plasma concentration of APOB protein reflects the total number of potentially atherogenic particles [2,4] and, therefore, is predictive of high risk of coronary heart disease that may not be otherwise detected from routine lipid profile [2,7-10]. High APOB protein level in blood is correlated with cerebrovascular and coronary artery diseases as well as increased risk of diabetes[1,3,11,12].

Variable number of tandem repeats (VNTRs), also known as minisatellites, comprise a significant portion (~3%) of the human genome [13]. These are predominantly localized in the sub-telomeric region of chromosomes and have a core repeat unit of 10 to100 base pairs[6,14]. VNTRs are stably inherited in a Mendelian fashion across generations and

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considered as the most informative markers for genetic characterization [15]. Hyper-variable nature of many VNTR loci have found useful applications in genetic linkage analysis, forensic identification, paternity testing, anthropological research and phylogenetic studies[6,15,16]. One such VNTR is located 73bp upstream of the second polyadenylation signal at the 3′-end of the human Apolipoprotein B (APOB) gene on the short arm of chromosome 2[15,17,18]. The APOB 3′-VNTR region consists of tandem repeats of AT-rich DNA sequences (14-16bp in length) [17].

Longer alleles of APOB 3′-VNTR site has been shown to be associated with different disease conditions. APOB gene alleles with larger 3′-VNTR repeat numbers occur more frequently in gallstone patients [19]. The most common cause of death in diabetes is atherosclerotic cardiovascular disease resulting from dyslipidemia [20]. APOB 3′-VNTR alleles with >37 repeats have been shown to be strongly associated with increased lipid levels and coronary artery disease [18]. In this study, we investigated whether there is any association of the number of repeats at the APOB 3′-VNTR locus with type 2 diabetes, body mass index (BMI), systolic and diastolic blood pressures.

Materials and methods

DNA samples: Blood samples and additional information (e.g., systolic and diastolic blood pressure, body weight and height, other disease conditions, family history, etc) were collected with written consent from 67 unrelated Bangladeshi individuals diagnosed to have type 2 diabetes and 46 non-diabetic controls. Males and females represented 56.31% and 43.69%, respectively, of the studied population. Blood glucose level was measured following overnight fasting and two hours after meal. DNA was extracted from whole blood using Genomic DNA mini kit (AGB100, ATP Biotech, Taiwan). Purity and concentration of extracted DNA samples were measured using Nano Drop UV-Vis Spectrophotometer (Thermo Fisher Scientific Inc.).

Sequence amplification and detection: APOB 3′-VNTR region of the studied samples were amplified by PCR using primers already described by Ruixing *et al.* [21] in a thermal cycler (Gene Atlas G, Astec Co. Ltd.). About 20 to 50 ng of genomic DNA was used for amplification in a final reaction volume of 25 µl containing PCR buffer (EP0712, Thermo Scientific, USA), 1.0 µl of each primer (10 µM), 1 µl dNTP-mix (10 mM) (R0191, Thermo Scientific, USA) and 1U Taq polymerase (EP0712, Thermo Scientific, USA). The cycle condition was as follows: an initial denaturation step at 94°C for 5 minutes, then 33 cycles- each with denaturation at 94°C for 30 seconds, annealing at 58°C temperature for 60 seconds, and elongation at 72°C for 60 seconds followed by a final extension at 72°C for 5 minutes. The amplified products were resolved in 2% agarose gel with 0.5x Tris Borate EDTA (TBE) buffer in a horizontal Agarose gel electrophoresis system along with DNA size markers (300003, GeneON). DNA bands were observed in a gel documentation system (WGD-30, Witeg) following staining with ethidium bromide in 0.5x TBE buffer and photographed with Wise Capture II™ software.

Allele size determination and statistical analysis: Sizes of PCR products were determined using the software "Gel Analyzer" [22] by comparing their migration relative to DNA size markers (300003, Gene ON). PCR products of the patients and the controls were run side by side in gels. The number of core repeats in the amplified products was calculated using Microsoft Excel following the formula described by Ruixing *et al.* [21]. The alleles were designated according to the number of core repeats. Frequencies of the APOB alleles and genotypes of the individuals and other statistical analyses were performed using GenAlEx 6.5 [23] and GraphPad Prism® software.

Ethical approval was received from local ethics committee before data collection was initiated.

Results

All together 24 different alleles were identified in the studied population (Table 1). Despite the less number of non-diabetic individuals than the diabetic patients in this study, the former had more APOB 3′-VNTR alleles (21 alleles) compared to the later (17 alleles). The smallest and the largest alleles had 17 and 47 repeats, respectively. Alleles at both ends of the distribution had relatively low frequencies. Among the non-diabetic individuals, allele with 35 repeats had the highest frequency (0.163) followed by allele with 39 (0.152) repeats. Among the diabetic patients allele with 37 repeats was the most common (0.209) followed by allele with 35 (0.164) repeats. In combined population data (diabetic and non-diabetic together), allele with 37 had the highest frequency (0.173) followed by allele with 35 (0.164) and 39 (0.128) repeats.

Table 1: APOB 3´-VNTR allele frequencies in non-diabetic control (NDC) and type 2 diabetic (T2D) individuals.

APOB 3´ -VNTR Allele	NDC	T2D	Combined
17	0.022	0.000	0.009
18	0.022	0.000	0.009
19	0.022	0.000	0.009
20	0.011	0.000	0.004
28	0.000	0.007	0.004
29	0.000	0.022	0.013
30	0.043	0.000	0.018
31	0.011	0.030	0.022
32	0.033	0.030	0.031
33	0.011	0.037	0.027
34	0.043	0.082	0.066
35	0.163	0.172	0.168
36	0.098	0.090	0.093
37	0.120	0.209	0.173
38	0.109	0.090	0.097
39	0.152	0.104	0.124
40	0.043	0.030	0.035
41	0.011	0.067	0.044
42	0.011	0.007	0.009
43	0.033	0.000	0.013
44	0.000	0.007	0.004
45	0.022	0.007	0.013
46	0.011	0.000	0.004
47	0.011	0.007	0.009

These 24 alleles made up 53 different genotypes among the diabetic and non-diabetic individuals (Table 2). The most frequent genotype among the diabetic patients was 37,37(0.119) followed by 35,35(0.104). In non-diabetic individuals the most frequent genotype was 37,35 and 39,39 (0.109) followed by 35,35 (0.087). In combined population of diabetic and non-diabetic individuals the most frequent genotype was 35,35 (0.097) followed by 37,35 (0.088), and 37,37 (0.080).

Table 2: Genotypes frequencies at APOB 3'-VNTR locus in non-diabetic control (NDC) and type 2 diabetic (T2D) individuals.

NDC		T2D	
Frequency	Genotype	Frequency	Genotype
17,17	0.022	29,29	0.015
18,18	0.022	31,31	0.015
19,19	0.022	32,32	0.015
30,30	0.022	33,33	0.015
34,32	0.022	34,34	0.045
34,34	0.022	35,29	0.015
35,32	0.022	35,32	0.015
35,35	0.087	35,35	0.104
36,30	0.022	36,28	0.015
36,32	0.022	36,31	0.015
36,36	0.043	36,33	0.015
37,34	0.022	36,36	0.045
37,35	0.109	37,31	0.015
37,37	0.022	37,35	0.075
38,20	0.022	37,37	0.119
38,30	0.022	38,33	0.015
38,31	0.022	38,34	0.015
38,33	0.022	38,36	0.015
38,36	0.043	38,38	0.03
38,38	0.043	39,34	0.045
39,35	0.022	39,36	0.015
39,36	0.022	39,37	0.045
39,37	0.043	39,39	0.045
39,39	0.109	40,32	0.015
40,40	0.043	40,36	0.015
41,37	0.022	40,37	0.015
43,43	0.022	40,38	0.015
45,45	0.022	41,35	0.015
46,42	0.022	41,37	0.03
47,43	0.022	41,38	0.045
		41,39	0.015
		41,41	0.015
		42,33	0.015
		44,34	0.015
		45,38	0.015
		47,35	0.015

In our study, APOB 3´-VNTR alleles with 37 repeats showed statistically significant ($p<0.001$) difference in frequencies between the diabetic and non-diabetic individuals (Table 1). Overall distribution of APOB 3´-VNTR allelic frequencies in these two groups

was different. Combined frequencies of alleles with ≥35 repeats as well as genotypes of individuals with at least one allele with ≥35 repeats were more common among the diabetic individuals- although this difference was not statistically significant. We divided the diabetic and the non-diabetic studied population in two groups – one included individuals who had both APOB alleles with <35 repeats and the second group had at least one allele with ≥35 repeats. We did not compare the lipid profiles of diabetic and non-diabetic individuals in this study. But individuals having at least one allele with ≥35 repeats had higher Body mass index (BMI), systolic and diastolic blood pressures (in both males and females) (Figure 1).

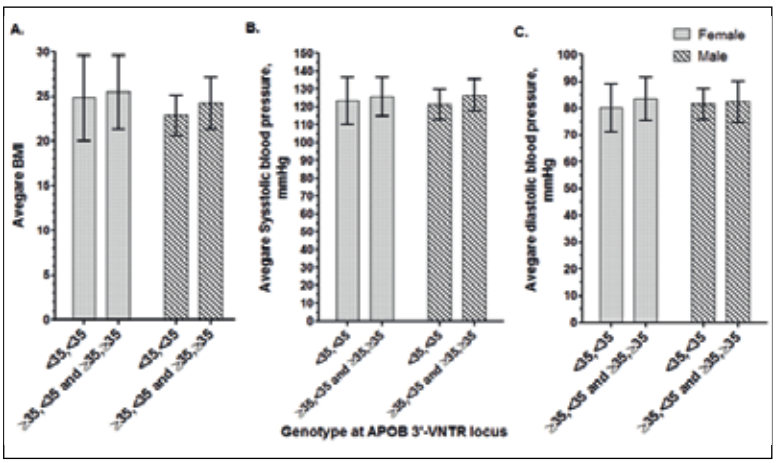

Figure 1: Association of APOB 3′-VNTR alleles with BMI, systolic and diastolic blood pressure. A. Irrespective of the disease condition, individuals with one or both APOB 3′-VNTR alleles with ≥35 repeats had higher BMI values, although none of these differences were statistically significant. **B and C.** In both males and females, individuals with one or both APOB alleles with ≥35 repeats had higher systolic and diastolic blood pressure values, although none of these differences were statistically significant.

Discussion

This is the first study to investigate any possible association of the number of repeats at the APOB 3′-VNTR locus with type 2 diabetes, body mass index (BMI), systolic and diastolic blood pressures. Earlier studies have found alleles with 35, 37 and 39 repeats to be the most common ones in different populations worldwide[16,18,24-32]. In two different Indian sub-populations[18,29], allele with 35 repeats was found to be the most prevalent. In our study, allele with 35 repeats had the highest frequency (0.163) among the non-diabetic individuals, whereas the diabetic patients had the allele with 37 repeats as the most common (0.209).

APOB 3′-VNTR alleles with relatively larger number of repeats have association with a number of disease conditions. Alleles with higher repeats (≥36 repeats) were shown to be strongly correlated with coronary heart disease[18,33-35]. APOB 3′-VNTR alleles with higher repeats are also significantly associated with higher levels of total cholesterol (TC) and low density lipoprotein-cholesterol (LDL-C) [36,37]. Kanani *et al.* detected raised APOB protein in 56.7% of the type 2 diabetics [7]. But no earlier study looked into any possible association of APOB 3′-VNTR alleles with diabetes.

Overall distribution of APOB 3′-VNTR allelic frequencies between the control and diabetic individuals was different. APOB 3′-VNTR alleles with 37 repeats showed statistically significant ($p<0.001$) difference in frequencies between the diabetic and non-diabetic individuals. Combined frequencies of alleles with ≥35 repeats as well as the genotypes of individuals having at least one allele with ≥35 repeats were more common among the diabetic individuals. We also observed that individuals having at least one allele with ≥35 repeats had higher Body mass index (BMI), systolic and diastolic blood pressures (in both males and females). These differences, however, were not statistically significant. In this study we did not measure APOB level in serum to correlate with 3′-VNTR repeat numbers in diabetic and non-diabetic individuals. Earlier studies have reported high APOB protein level in serum of individuals with larger APOB 3′-VNTR alleles [18,36,37]. In Canadian aboriginal people, high level of plasma APOB protein was found to be associated with type 2 diabetes as well as a better predictor of risk compared to LDL or HDL cholesterol [12]. Hashemi *et al.* found that serum APOB protein levels in diabetic children (aged 9–18 years) and healthy children with diabetic parents were significantly higher than the healthy children of similar age with nondiabetic parents [20]. Atherosclerotic coronary artery disease (CAD) is the most common co-morbidity in type 2 diabetes and the risk of death by CAD among diabetic individuals is greater by 3-folds compared to the non-diabetics[38]. CAD can at least partially be attributed to abnormalities in lipid and lipoprotein metabolism [39]. Our observation that the APOB 3′-VNTR alleles with larger repeats are more prevalent in type 2 diabetic individuals might make the bridge among the genetic

signature of APOB 3′-VNTR, high APOB level in blood, diabetes and other co-morbidities.

The mechanism through which 3′-VNTR polymorphism influence serum lipid level is not clearly elucidated yet[18]. Since these VNTR repeats are present in the 3′untranslated region (3′-UTR) of APOB gene, these are not supposed to play roles in the structure and/or function of APOB protein. Alleles with the higher repeat numbers may be in linkage disequilibrium with other genes which may have some effect on the regulation of gene expression[5]. High repeat numbers may also increase stability/half-life of APOB mRNA or enhance translation through interaction with other proteins or factors. For example, APOB 3′-VNTR region is AT-rich [17,40]. Human antigen R (HuR)/ELAV1 protein is a key player in both mRNA turnover and translation regulation processes in eukaryotic cells and binds to sites called AU-rich elements (ARE) [41]. There are different opinions on the actual consensus motif of AREs. Barreau *et al.* [42] suggested that AREs are just sequence elements of 50–150 nucleotides which are rich in adenosine (A) and uridine (U) bases. Proteins like HuR may play role in increased expression of APOB alleles with high number of AT-rich repeats. This and other possibilities may be explored to decipher the role of APOB 3′-VNTR repeat number with its expression level and associated diseases.

Conclusion

APOB 3′-VNTR alleles have different distribution frequencies among type 2 diabetic and non-diabetic individuals. We observed that individuals harboring at least one allele with ≥35 repeats have higher body mass index (BMI), systolic and diastolic blood pressures. Our study might bridge among the genetic signature of APOB 3′-VNTR, high APOB protein level in blood, diabetes and other co-morbidities. The mechanism behind the influence of the 3′-VNTR polymorphism on serum lipids is not known yet. Molecular interaction between APOB 3′-VNTR site and different regulatory proteins may be explored to decipher the role of APOB 3′-VNTR repeat number on its expression level and associated diseases.

Declaration

There is no known conflict of interest.

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