Influence of ApoB100 3′ hypervariable repeats on acute myocardial infarction

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ABSTRACT

Background and objective The 3′ flanking region of apolipoprotein B (ApoB) 100 gene is known to contain short A+T-rich DNA sequences which are hypervariable in nature and called the variable number of tandem repeats (VNTRs). It results in different alleles of ApoB100. The present study extends the investigation of whether there is a correlation between the presence of these alleles and acute myocardial infarction (MI).

Methods We examined ApoB genotypes in 230 acute MI patients and 300 healthy controls. PCR based genotyping was done for ApoB 3′ VNTRs.

Results We recoded 3′ApoB-VNTR alleles through three- and five-allelic models based on different sizes and found that large repeats (>37) were significantly associated with acute MI ($p<0.0001$). These large repeats (>37) were also significantly associated with higher lipid levels in the MI group.

Conclusion Patients with 3′ApoB-VNTR large repeats (>37) are more susceptible to acute MI development.

INTRODUCTION

Coronary artery disease (CAD) is a major public health problem in developing as well as developed nations. It has emerged as a major killer in India during recent decades. In the last couple of years, several studies have shown the association of genetic factors with the pathogenesis of both CAD and myocardial infarction (MI). The apolipoprotein-B (ApoB) 100 gene is one of the very well characterised genetic variants in this respect. Cloning and sequencing of the ApoB gene has identified a number of genetic polymorphisms, which contribute to the susceptibility of developing CAD. The ApoB gene consists of an AT rich core repeat sequence of 11–16 bp, located about 73 bp downstream from its 3′ end. This minisatellite hypervariable region is designated as the ApoB 3′ variable number of tandem repeats (VNTRs) or ApoB 3′ hyper variable elements (HVE). This locus is highly polymorphic and about 26 alleles have been reported with two basic types of 15-bp repeats (X and Y).[1] The presence of high allelic variability at ApoB 3′VNTR is due to the complex mutational pattern. Earlier stepwise mutational model studies reflected gain or loss of one or a few repeat units during replication; this was responsible for creating high polymorphism at the ApoB 3′VNTR region.[2] ApoB VNTR polymorphism is considered to be a suitable locus to study the relationship between the allele size distribution of these minisatellites and disease origin. Different studies of diverse ethnic set-up have used different alleles of ApoB to investigate the association between high lipid levels and CAD.[3–5] However, their size (HVE21–HVE 64), as well as distribution, varied from one population to other.[6–8] No study been conducted to investigate the occurrence of acute MI with these alleles. We hypothesised that ApoB 3′ HVE allele size variability may be useful to analyse their relation with acute MI. In this study we have attempted to analyse the allele frequency of ApoB100 3′ HVE in a north Indian population.

MATERIALS AND METHODS

Subjects

A total of 230 acute MI patients from Uttar Pradesh, a northern province of India, were selected. All sample and data collection for the patient cohort was done at eight centres in Uttar Pradesh which were mostly secondary or tertiary care teaching institutes/hospitals. A total of 300 sex and ethnically matched normal healthy controls were also genotyped. The collection site of the control samples was Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGIMS), Lucknow. In order to achieve a confidence level of 90% and a significance level of $p<0.05$, at least 188 patients were needed in each group. Sample size was calculated using following formula:

$$\text{Sample size} = (\text{z score})^2 - \text{SD} \times (1 - \text{SD})/(\text{CI})^2$$

z score for 90% confidence level=1.645, SD=0.5, CI=0.06.

All patients taking any lipid lowering drugs in the last 4 weeks or having secondary causes of dyslipidaemia (eg, long standing uncontrolled diabetes, hypothyroidism, liver disease, nephrotic syndrome) were excluded from the study. Blood samples were collected, after obtaining written informed consent, within 24 h of the onset of symptoms of acute MI . The ethics committee of the institute approved the study.

DNA Extraction

DNA was extracted from the blood by the salting out method using phenol-chloroform, as described by Comey *et al*,[9] and was purified by ethanol precipitation.

Analysis of VNTR

ApoB 3′ VNTR amplification was carried out using the protocol described by Boerwinkle *et al*[10] and PCR, using a forward and reverse oligonucleotide primer encompassing the entire ApoB 3′ VNTR sequence. The sequence of the primer used was 5′ ATGCAAACGGAGAAATTATG 3′ and 5′ CCTTCTCACTTGCCAAATAC 3′. PCR was performed in an M.J. Research thermocycler, with 26 cycles of denaturation at 94°C for 1 min, and

annealing and extension at 58°C for 6 min. The amplified product was electrophoresed in 5% polyacrylamide gel, and allele sizing was done using the ApoB3′HVR allelic ladder (AlphaImage 1220 v5.5) and commercial ladder (New England Biolabs) shown in online supplementary figure S1.

Statistical analysis

Allele and genotypic frequency analysis for ApoB 3′ VNTR was done using the POPGENE-16 version. Comparison of the different ApoB genotypes among controls and patients was done by the χ^2 test. Association between variables was tested using stepwise logistic regression analysis. ORs were calculated with a 95% CI limit and $p<0.05$ was considered significant.

RESULTS

Basal demographic, medication history and association of risk factors with acute MI

Demographic data along with risk factors among patients and controls are given in table 1. Of the 230 acute MI patients studied, 130 (56.52%) were on β blockers, 87 (37.82%) were taking calcium channel blockers, 118 (51.30%) were on ACE inhibitors, 125 (54.34%) were taking clopidogrel and 123 (53.47%) were on aspirin. Age, body mass index (BMI) and smoking were significantly associated with acute MI. We also found that higher BMI and smoking habit were significantly associated with acute MI. With respect to biochemical parameters, mean TC (total cholesterol), HDL (high density lipoprotein) and LDL (low density lipoprotein) levels were significantly higher in patients than controls.

Allelic distribution of ApoB-VNTRs in study participants

The relative frequencies of VNTR in acute MI patients and controls are shown in figure 1. A total of 17 different alleles were observed in 530 subjects (HVE21–HVE49). The VNTR allele 35 occurred with the highest frequency, that is, 20.8% and 30% in patient and control groups, respectively. Interestingly, HVE36 was totally absent in patients while HVE29 was absent in controls. We further proposed two different models for ApoB-VNTR alleles coding and analysed them to identify any association with acute MI (table 2).

Table 1 Biochemical parameters of acute MI patients and controls

Demographics		Patients (n=230)	Controls (n=300)
Sex			
Male	n (%)	196 (85.2%)	255 (85.0%)
Female	n (%)	34 (14.8%)	45 (15.0%)
Age (years)	Mean±SD	49.97±11.91*	44.93±13.17
Risk factor profile and clinical characteristics of the patient cohort: n (%)			
BMI		24.11*	22.13
Diabetes		52 (16.9%)	0
Hypertension		75 (24.0%)	0
Family history of CAD		81 (26.2%)	0
Smoking		132 (42.9%)*	99 (33.0%)
Alcohol consumption		97 (31.3%)	92 (30.7%)
Non-vegetarian diet		103 (44.8%)	140 (46.5%)
Biochemical parameters in patient and control group: mean±SD			
Total cholesterol (mg/dL)		173.95±47.84*	135.70±30.34
Triglycerides (mg/dL)		150.60±92.28	141.10±61.99
HDL cholesterol (mg/dL)		41.93±10.95*	35.38±7.01
LDL cholesterol (mg/dL)		102.45±38.30*	81.05±23.97
VLDL cholesterol (mg/dL)		29.30±15.55	29.23±12.73

*p<0.05.
BMI, body mass index; CAD, coronary artery disease; HDL, high density lipoprotein; LDL, low density lipoprotein; MI, myocardial infarction; VLDL, very low density lipoprotein.

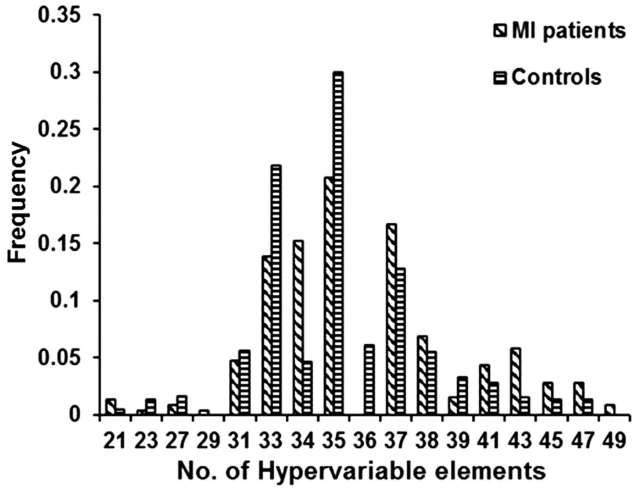

Figure 1 ApoB 3′VNTRs (variable number of tandem repeats) allele frequency distribution among myocardial infarction (MI) patients and controls.

First we categorised ApoB-VNTR alleles into three different groups. The first group (I) contained lower repeats (alleles 21–33), the second group (II) comprised medium repeats (alleles 34–37), while the third group (III) consisted of large repeats (alleles 38–49) (table 2). In patient versus control group comparisons, small repeats were statistically significant and higher in the control group ($p=0.001$), while large repeats were associated with acute MI compared to controls ($p<0.0001$). However, the differences were not statistically significant in groups containing medium repeats. The genotypic analysis was further categorised into five different groups of ApoB VNTRs (table 2). Group I had alleles lower than 33 repeats. Group II contained alleles of 33 repeats and group III had alleles of 34, 35 and 36 repeats. The group containing alleles of 37 repeats was classified as group IV, while the group with alleles higher than 37 repeats was called group V. Our results further demonstrated that higher repeats of variable HVE >37 and acute MI were significantly associated ($p<0.0001$).

We also observed a total of 15 types of genotype on the basis of five different groups of VNTR alleles (see online supplementary table S1). Genotypes 2/5 (group II and group V) and 5/5 (group V only) were significantly higher in patients ($p<0.0001$ and 0.02, respectively), while genotype 2/3 (group II and group III) was significantly higher ($p<0.0001$) in controls.

Predictors of acute MI

We further determined predictors of acute MI by stepwise logistic regression analysis. We found that ApoB-VNTR large repeats (>37) along with age, TC, BMI and HDL cholesterol remained independent factors, which influenced the progression of acute MI. Smoking and ApoB VNTR repeats <37 showed no significant association after multivariate adjustment (table 3).

Correlation of lipid parameters with genotypes of five allelic models

We compared lipid concentration between patients and control group with different genotypes based on five allelic models; for

Table 2 Allelic model of ApoB VNTRs in control and acute MI groups

Group	Allele	MI patients (n=230) Frequency (n)	Controls (n=300) Frequency (n)	χ^2	OR (95% CI)	p Value
Three-allelic model						
I	21–33	0.219 (101)	0.310 (186)	10.33	0.62 (0.47 to 0.82)	0.001
II	34–37	0.528 (243)	0.536 (322)	0.04	0.96 (0.75 to 1.23)	ns
III	38–49	0.252 (116)	0.153 (92)	19.17	1.90 (1.43 to 2.53)	<0.0001
Five-allelic model						
I	<33	0.080 (37)	0.097 (55)	0.28	0.86 (0.56 to 1.34)	ns
II	33	0.139 (64)	0.210 (131)	10.35	0.57 (0.41 to 0.80)	0.001
III	34–36	0.360 (166)	0.430 (245)	2.27	0.81 (0.63 to 1.05)	ns
IV	37	0.167 (77)	0.128 (77)	1.36	1.36 (0.96 to 1.92)	ns
V	>37	0.2520 (116)	0.153 (92)	15.50	1.86 (1.37 to 2.52)	<0.0001

ApoB, apolipoprotein B; MI, myocardial infarction; VNTRs, variable number of tandem repeats.

this analysis we only studied the combinations, which revealed significance for 2/3, 2/5 and 5/5 (table 4).

When genotypic analysis was done, a significant association was observed between higher repeats HVE 38–49/38–49 and lipid levels, except for very low density lipoprotein (VLDL) cholesterol. A higher level of cholesterol was observed in HVE 33/38–39, but the association did not reach statistical significance.

DISCUSSION

ApoB VNTR alleles with higher repeat numbers have been reported to occur more frequently in CAD patients than in the healthy control group,[4] but no data are available for acute MI patients. In this study we showed an allele specific association between ApoB 3′ VNTR variability and acute MI. In our population we found total 17 alleles for ApoB 3′ VNTR. We have seen that VNTR 35 was the most common allele followed by VNTR 33 and VNTR 37 in MI patients. One study found 12 segregating alleles among 319 individuals in a study of allelic frequency distribution at the hypervariable locus 3′ to the ApoB gene.[6] The authors studied five different populations and found that the two most frequent alleles, 37 and 39, were present in all the populations. A unimodal distribution study in an African population showed a peak on allele 35 or 37. In another study, a higher frequency of smaller alleles (≤33) as well as larger alleles (≥37) was found in an African American population in comparison to Caucasians.[11] This differences in the allelic association with CAD patients in different studies conducted by various research groups on different ethnic populations may be due to ethnic variation at this polymorphic site.

For further comparison, we used three- and five-allelic models of ApoB 3′ VNTR in acute MI versus a control group. In the three-allelic model, we recoded VNTR alleles into three classes (small, 21–33 repeats; medium, 34–37 repeats; and large, 38–49 repeats) and found that the frequency of larger repeats was significantly higher in MI patients in comparison to the controls. Similarly, the five-allelic model demonstrated a higher frequency of longer repeats of VNTR alleles (>37) in acute MI cases. This observation indicates that when individual alleles were considered, minor differences were observed at the allele frequency level. These differences became more prominent when we grouped the alleles into three- or five-allelic models; higher repeat alleles were more associated with disease. Stepwise regression analysis revealed that higher repeat (>37) VNTR alleles influence acute MI independently.

As lipids are a major risk factor for atherosclerosis and ApoB is a major component of low density lipoprotein, we further tried to correlate the difference in the lipid profile in both groups with ApoB 3′ HVR. We had earlier shown that lipid levels are significantly higher in younger CAD patients.[12] A low

Table 3 Multivariate risk factor association with acute MI

	OR	95% CI	p Value
Age	1.046	1.028 to 1.064	<0.0001
ApoB VNTRs >37	2.221	1.268 to 3.892	0.0053
BMI	1.344	1.222 to 1.478	<0.0001
Total cholesterol	1.024	1.028 to 1.064	<0.0001
HDL	1.057	1.027 to 1.088	0.0002

ApoB, apolipoprotein B; BMI, body mass index; HDL, high density lipoprotein; MI, myocardial infarction; VNTRs, variable number of tandem repeats.

Table 4 Lipid concentration in patient and control groups in individuals with genotype of five-allelic model

VNTR genotype of five-allelic model	Patients (n=230)	Controls (n=300)	p Value
Total cholesterol			
HVE 33/34–36	152±45 (8)	134±23 (48)	0.01
HVE 33/38–49	154±39 (19)	115±12 (6)	ns
HVE 38–49/38–49	193±55 (20)	151±20 (11)	0.01
Triglycerides			
HVE 33/34–36	151±102 (8)	129±43 (48)	0.01
HVE 33/38–49	162±88 (19)	113±57 (6)	ns
HVE 38–49/38–49	163±82 (20)	125±50 (11)	<0.05
High density lipoprotein			
HVE 33/34–36	40±16 (8)	35±6.6 (48)	<0.001
HVE 33/38–49	38±10 (19)	34±7.6 (6)	ns
HVE 38–49/38–49	48±15 (20)	35±6.7 (11)	0.009
Low density lipoprotein			
HVE 33/34–36	82±15 (8)	76±42 (48)	0.001
HVE 33/38–49	93±30 (19)	61±21 (6)	ns
HVE 38–49/38–49	114±47 (20)	93±18 (11)	0.01
Very low density lipoprotein			
HVE 33/34–36	30±20 (8)	26±8.5 (48)	0.01
HVE 33/38–49	22±161 (19)	35±17 (6)	ns
HVE 38–49/38–49	32±16 (20)	24± 10 (11)	ns

HVE, hyper variable elements; VNTR, variable number of tandem repeat.

level of HDL is a powerful independent risk factor for the future risk of CAD, irrespective of TC level. However, in our study, serum HDL-cholesterol levels were significantly higher in patients than in controls. This reason may be that our study patient population had acute MI; within 24 h of onset of symptoms, plasma HDL has been found to be in the range of 40–45 mg/dL in different studies, which started falling significantly from day 2.[13 14] We further evaluated the correlation between VNTR genotypes and plasma lipid profile. Interestingly, a significant association was observed between the HVE 38–49 alleles and higher levels of TC, triglyceride (TG), LDL and HDL cholesterol, and a non-significant but marked association was observed between the HVE 38–49 alleles and higher levels of VLDL cholesterol. In conclusion, we have shown that genetic variants in the 3′ HVR region of the ApoB gene are associated with changes in cholesterol levels. Because the >37 repeats of the VNTR allele are associated with higher lipid levels, individuals carrying this allele may be at a higher risk of developing acute MI.

However, the genetic architecture of acute MI is complex. An undoubted limitation of our study is the small sample size, which may temper the statistical association. However, these limitations do not detract from the main study conclusions that ApoB3′VNTR is correlated with a risk of acute MI. In conclusion, our study provides new evidence in support of an association of higher repeats of ApoB 3′VNTR and acute MI.

Contributors N Singh performed all experiments and wrote the manuscript. N Sinha and SK provided all clinical data. SA supervised the study design and manuscript writing. CMP helped with the statistical analysis. All authors have read and approved the final manuscript.

Competing interests None.

Ethics approval Ethics Committee of SGPGIMS.

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