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Single nucleotide polymorphisms of *APOA1* gene and their relationship with serum apolipoprotein A-I concentrations in the native population of Assam

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ABSTRACT

Background: There is a growing interest in the role of allelic variants of the *APOA1* gene in relation to a number of disorders. We described two common polymorphisms of the *APOA1* gene, G-75A and C+83T and investigated their potential influence on the serum apolipoprotein A-I (apo A-I) levels in the native population of Assam — a region that is ethnically distinct and from where no information is hitherto available.
Methods: Blood samples were collected from 150 healthy volunteers. Apo A-I levels were estimated by immunoturbidometry. Genotyping was done by a PCR-RFLP method that involved DNA extraction from whole blood, followed by polymerase chain reaction and digestion of the PCR product by *Msp*I restriction enzyme, and analysis of fragment sizes in 12% polyacrylamide gel.
Results: The GG variant at G-75A locus and CC variant at C+83T locus were the most prevalent. GG/CC was the most common combination. Homozygous TT genotype was not detected in any of the subjects. The rare allele frequencies for the G-75A and C+83T sites were found to be 0.22 and 0.06 respectively, which significantly differed from those reported in some other populations in neighbouring regions. Serum apo A-I concentrations did not vary significantly across the detected genotypes. These findings were consistent in both sexes.
Conclusion: We described the distribution of the G-75A and C+83T polymorphisms of the *APOA1* gene in the population of Assam for the first time. These polymorphisms were not found to directly influence apo A-I concentrations in this population either individually or synergistically.

1. Introduction

The human apolipoprotein A-I gene (*APOA1*) belongs to the *APOA1-CIII-AIV* gene cluster, located in chromosome 11q23. This cluster consists of evolutionarily related genes that regulate serum lipid and lipoprotein levels (Karathanasis, 1985; Elshourbagy et al., 1986). The nucleotide sequence of *APOA1* is interspersed by three introns (Karathanasis et al., 1983). *APOA1* codes for apolipoprotein A-I (apo A-I). Since apo A-I is the major protein component of high density lipoprotein (HDL) particles which offer protection against atherosclerosis, therefore, much of the scientific work on apo A-I has traditionally focussed on its atheroprotective role. But, recent studies have implicated derangements in serum apo A-I concentration in some other pathological conditions that are not conspicuously related to atherosclerotic disorders. Thus, genetic variations of *APOA1* that influence serum apo A-I levels are of considerable interest.

The G-75A and C+83T single nucleotide polymorphisms (SNPs) are two common variations of the *APOA1* gene. The G-75A polymorphism, located in the promoter region 75 base pairs (bp) upstream from the transcription start site of *APOA1*, is due to a guanine to adenine interchange (Pagani et al., 1990). The position of this SNP has been variously described as −75 bp, −76 bp and −78 bp. This difference in representation is due to three different transcription start sites being described for *APOA1* by different studies (Higuchi et al., 1988; Sastry et al., 1988). The C+83T polymorphism is located in the first intron of *APOA1*. It is caused by a cytosine/thymine substitution in the position 83 bp downstream from the transcription initiation site (Wang et al., 1995). Each of these SNPs alters the recognition site for the restriction endonuclease *Msp*I, which facilitates their detection by a restriction fragment length polymorphism (RFLP) based technique.

Historically, the G-75A and the C+83T polymorphisms have been investigated in relation to coronary artery disease (CAD) and cardiovascular risk factors (Reguero et al., 1998; Jeenah et al., 1990;

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Sigurdsson, et al., 1992; Talmud et al., 1994; Wang et al., 1996; Bai et al., 1996; Chhabra et al., 2003; Shanker et al., 2008; Dawar et al., 2010; Miroshnikova et al., 2011; Biswas et al., 2013). However, it is increasingly believed that these polymorphisms may have important roles to play in a host of other disorders such as Alzheimer's disease (Vollbach et al., 2005; Smach et al., 2011), multiple sclerosis (Koutsis et al., 2009), schizophrenia (Yang et al., 2010), breast cancer (Hamrita et al., 2011), Parkinson's disease (Swanson et al., 2014), cholelithiasis (Dixit et al., 2007), gout (Cardona et al., 2005), diabetes mellitus (Ma et al., 2003) and acute lung injury following cardio-pulmonary bypass surgery (Tu et al., 2013).

The G-75A and C+83T polymorphisms have been studied globally in different populations. However, no information is available on these polymorphisms from the north-eastern region of India. The distribution of these polymorphisms varies with the ethnicity of the studied population and geography as previous studies have indicated. Geographically, the north-eastern region of India is a unique corridor that links the Indian subcontinent to East Asia and Southeast Asia. Assam is the most populous and second largest (in terms of land area) of the eight north-eastern states. Data about the distribution of a polymorphism in the healthy subjects are a prerequisite to investigating the associations of the polymorphism with the implicated disease. With a renewed interest in G-75A and C+83T polymorphisms worldwide and a variety of diseases being described in relation to them, we attempted to describe these polymorphisms in a sample population from Assam, northeast India for the first time.

Our aim was to study the distributions of G-75A and C+83T polymorphisms of the *APOA1* gene in a cross-section of healthy subjects from Assam, and further compare their frequencies with those observed in other populations from India and elsewhere. Additionally, we assessed the influence of these polymorphisms on the phenotype by determining the variation in fasting serum apo A-I concentrations across the detected genotypes.

2. Materials and methods

2.1. Study subjects

A total of 150 unrelated and apparently healthy individuals of either sex were recruited for the study. All the individuals were inhabitants of Assam and identified themselves as natives of the state, with no history of migration from elsewhere at least in the past four generations. The study was approved by the Institutional Ethics Committee of Gauhati Medical College and Hospital, Assam. All the subjects voluntarily provided informed written consent to participate in the study prior to enrolment.

2.2. Blood sampling

Blood samples (after at least 12 h of overnight fast) were drawn from the median cubital vein in two sets from each subject under sterile conditions. One set consisted of 3 mL of blood anticoagulated in EDTA vials for isolation of DNA and genotyping. The second set included 3 mL of blood collected in plain vials and the serum separated by centrifugation after clot formation for apo A-I estimation.

2.3. DNA isolation and genotyping

Genomic DNA was isolated from peripheral blood leucocytes (obtained from EDTA admixed whole blood) following a rapid DNA isolation protocol (Sambrook and Russell, 2001). The concentration and purity of the genomic DNA was ascertained by measuring the optical density (OD) values at 260 nm and 280 nm. The samples had acceptable purity with the OD ratio (260 nm/280 nm) in the range of 1.7 to 1.9. The integrity was checked by running the extracted DNA samples on 1% agarose gels.

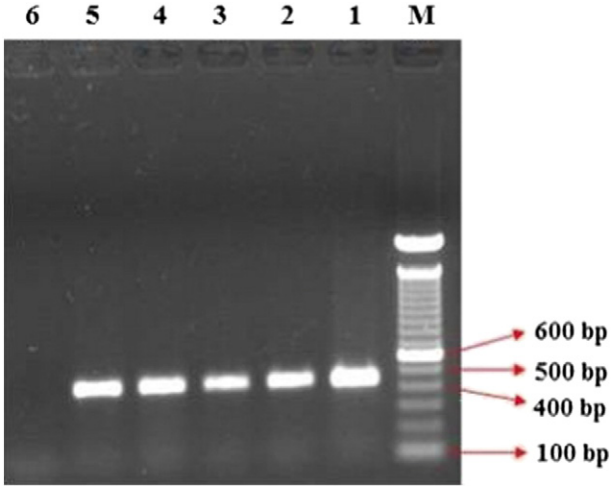

Fig. 1. Amplified PCR product in 1% agarose gel. Lanes 1 to 5: PCR product (435 bp), lane 6: negative control, lane M: 100 bp DNA ladder (Invitrogen, USA).

A 435 bp fragment at the 5′ end of APOA1 gene spanning the promoter region and the first intron was amplified by polymerase chain reaction (PCR) using the following primer pairs as described previously (Larson et al., 2002): forward: 5′-AGG GAC AGA GCT GAT CCT TGA ACT CTT AAG-3′, reverse: 5′-TTA GGG GAC ACC TAC CCG TCA GGA AGA GCA-3′ (Metabion International AG, Germany). The PCR reaction was performed with a 25 μL reaction mixture (1 μL DNA template, 0.5 μL each of forward and reverse primers, 12.5 μL DreamTaq PCR master mix from ThermoFisher Scientific, 10.5 μL nuclease-free water) in a thermal cycler (Bio-Rad Model S1000™, USA) using the following cycling conditions: initial denaturation at 95 °C for 5 min, denaturation at 94 °C for 30 s, annealing at 62 °C for 1 min, extension at 72 °C for 30 s and final extension at 72 °C for 10 min. Steps 2 to 4 were repeated 30 times. The amplified products were run on 1% agarose gels along with 100 bp DNA ladder (Invitrogen, USA), and subsequently photographed under UV light using Gel Doc ™ XR+ system (Bio-Rad, USA) (Fig. 1).

The presence of G-75A and C+83T polymorphisms was ascertained using an RFLP method by digesting the PCR product with *Msp*I restriction enzyme. For this, 10 μL of the PCR product was digested overnight at 37 °C with *Msp*I under conditions specified by the supplier (New England Biolabs Inc., USA). The digestion products were analysed by running them on 12% polyacrylamide gels simultaneously with 50 bp DNA ladder (ThermoFisher Scientific, USA).

As the 435 bp PCR product contains three *Msp*I cutting sites at '−75', '+37' and '+83' loci, its complete digestion would produce 4 fragments of sizes 66 bp, 114 bp, 46 bp and 209 bp. The '−75' and '+83' loci are polymorphic and coincide with the G-75A and C+83T SNPs respectively. The loss of these cutting sites due to polymorphism would produce different fragment sizes. Molecular sizes of the restriction fragments according to the genotype are shown in Table 1. This information was used to determine the genotypes.

Table 1
Molecular sizes of the restriction fragments.

Polymorphism	Molecular sizes of restriction fragments (bp)		
	Wild homozygote	Heterozygote	Mutant homozygote
G-75A	66, 114	66, 114, 180	180
C+83T	46, 209	46, 209, 255	255