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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.

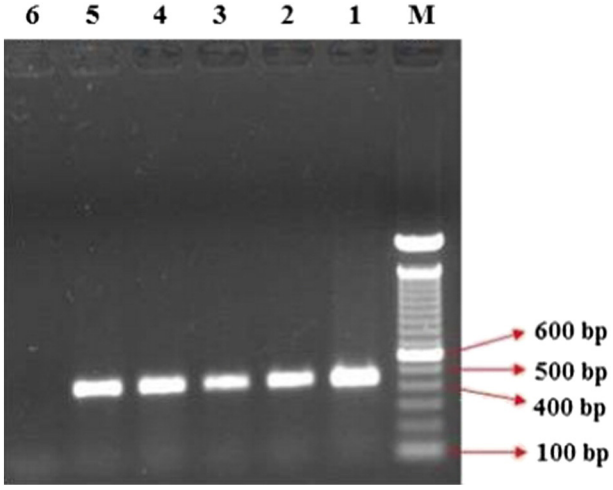

Fig. 1. Amplified PCR product in 1% agarose gel. Lanes 1 to 5: PCR product (435 bp), lane 6: negative control, lane M: 100 bp DNA ladder (Invitrogen, USA).

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

Table 2
Baseline characteristics of the sample population.

Parameter	Values
Subjects, n (%)	
Male	95 (66.33%)
Female	55 (36.67%)
Age (years)	42.87 ± 11.86
Serum apo A-I (mg/dL)	120.96 ± 28.01

2.4. Biochemical analysis

Concentration of apolipoprotein A-I (apo A-I) in serum was determined by immunoturbidometry in Vitros 5600 Integrated System autoanalyser using commercially available reagents and kits (VITROS Chemistry Products, Ortho-Clinical Diagnostics Inc. USA). Quality control measures were undertaken using control material (ApoA1 Performance Verifier 1) to ensure precision and accuracy of the test results.

2.5. Statistical analysis

All statistical analyses were performed using SPSS version 11.5 (SPSS, Chicago, USA). For continuous variables, the results were reported as means with standard deviation (SD). The Kolmogorov–Smirnov test was performed to ensure normal distribution of the data. For the qualitative variables, proportions were summarized.

The allelic and genotype frequencies of the two SNPs and the linkage disequilibrium between them were determined using POPGENE 2.0 software (Yeh and Boyle, 1997). Conformity to Hardy Weinberg Equilibrium (HWE) was assessed by goodness of fit Chi-square test (χ^2) and G-square test. The gender-wise frequencies of the polymorphisms were also calculated. Besides, diplotype frequency of the SNPs was ascertained. Allelic frequencies obtained in our population were compared to those reported by other studies using χ^2 test.

Comparison of continuous variables across genotypes was carried out by unpaired *t*-test or one way analysis of variance (ANOVA), whereas, categorical data were compared by χ^2 test (with Yates correction wherever required). A *P* value of less than 0.05 was considered statistically significant and that less than 0.01 was considered highly significant.

Table 3
Frequency distribution of the genotypes and alleles of the G-75A and C+83T polymorphisms.

		Genotype frequency			Allelic frequency	
G-75A	Sample size (n)	GG	GA	AA	G	A
Total	150	91 (60.7%)	51 (34%)	8 (5.3%)	0.78	0.22
Male	95	54 (56.8%)	33 (34.7%)	8 (8.4%)	0.74	0.26
Female	55	37 (67.3%)	18 (32.7%)	0	0.84	0.16
	$\chi^2 = 5.29$ ($df = 2$, $P = 0.07$)				$\chi^2 = 3.05$ ($df = 1$, $P = 0.08$)	
		Genotype frequency			Allelic frequency	
C+83T	Sample size (n)	CC	CT	TT	C	T
Total	150	131 (87.3%)	19 (12.7%)	0	0.94	0.06
Male	95	83 (87.4)	12 (12.6%)	0	0.94	0.06
Female	55	48 (87.3)	7 (12.7%)	0	0.94	0.06
	$\chi^2 = 0.0003$ ($df = 1$, $P = 0.99$)				$\chi^2 = 0.00$ ($df = 1$, $P = 1$)	

The numbers outside the parentheses indicate the counts. Numbers within parentheses indicate the proportions. Differences in genotype frequency and allelic frequency between males and females were analysed by Chi-square (χ^2) test. *df* = degree of freedom, a *P* value less than 0.05 was considered statistically significant, and that less than 0.01 as highly significant.

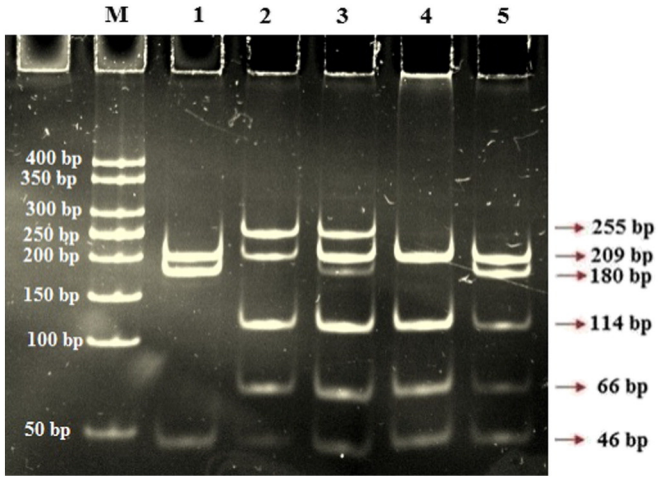

Fig. 2. Genotyping of the G-75A and C+83T sites of the *APOA1* gene in 12% polyacrylamide gel following digestion of the PCR product by *Msp*I restriction enzyme. Lane M: 50 bp DNA ladder (ThermoFisher Scientific, USA), lane 1: AA/CC (46, 180 and 209 bp), lane 2: GG/CT (46, 66, 114, 209 and 255 bp), lane 3: GA/CT (46, 66, 114, 180, 209, 255 bp), lane 4: GG/CC (46, 66, 114 and 209 bp), lane 5: GA/CC (46, 66, 114, 180 and 209 bp).

3. Results

The baseline characteristics of the sample population are shown in Table 2. It comprised 63.33% male and 36.67% female subjects. The ages ranged from 21 to 65 years.

We found both the G-75A and the C+83T loci to be polymorphic in the current population. The genotype and the allelic frequencies for the two polymorphisms are presented in Table 3. The frequency for the rare A allele of the G-75A locus was 0.22. All the three possible genotypes, namely, GG, GA and AA were detected. The homozygous GG variant was the most prevalent (60.7%). The AA genotype was not found in females. On the other hand, for the C+83T locus, the rare T allele frequency was 0.06. We detected only the homozygous CC and heterozygous CT genotypes. The CC genotype was predominant (87.3%). The homozygous TT variant was not encountered in any of the study subjects. The distributions of G-75A and C+83T polymorphisms were consistent with a population at Hardy–Weinberg equilibrium. The genotype and allelic frequencies for the G-75A site with respect to the A allele was apparently lower in females than that in males, but, it was not statistically significant ($P > 0.05$). However, for the C+83T polymorphism, the frequencies were very similar in the two sexes.

We encountered 5 combinations of the two polymorphisms in the studied population: GG/CC, GG/CT, GA/CC, GA/CT and AA/CC (Fig. 2). The diplotype frequencies are shown in Table 4. Overall, the GG/CC double homozygous variant was the most prevalent combination, while the double heterozygous GA/CT variant was the least common. The diplotype frequencies did not differ significantly between

Table 4
Combined distribution of the G-75A and C+83T polymorphisms.

	Sample size (n)	Diplotype frequency				
		GG/CC	GA/CC	AA/CC	GG/CT	GA/CT
Total	150	77 (51.3%)	46 (30.7%)	8 (5.3%)	14 (9.3%)	5 (3.3%)
Male	95	45 (47.4%)	30 (31.6%)	8 (8.4%)	9 (9.5%)	3 (3.1%)
Female	55	32 (58.2%)	16 (29.1%)	0	5 (9.1%)	2 (3.6%)
$\chi^2 = 5.53$ ($df = 4$, $P = 0.24$)						

The numbers outside the parentheses indicate the counts. Numbers within parentheses indicate the proportions. Differences in diplotype frequency between males and females were analysed by Chi-square (χ^2) test. *df* = degree of freedom. A *P* value less than 0.05 was considered statistically significant, and that less than 0.01 as highly significant.

Table 5
Comparison of the allelic frequencies of the G-75A and C+83T polymorphisms found in the study with other studies.

Reference	Population/place of study	Sample size (n)	Sample characteristics	Allelic frequency G-75A		Chi square value (χ^2)	*P* value	Allelic frequency C+83T		Chi square value (χ^2)	*P* value
				G allele	A allele			C allele	T allele		
Indian populations											
Current study	Assam, northeast India	150	Healthy volunteers	0.78	0.22	–	–	0.94	0.06	–	–
Chhabra et al. (2003)	North Indians, New Delhi, Delhi	142	Healthy controls (from a case–control study)	0.82	0.18	1.24	0.26	NA	NA		
Dixit et al. (2007)	North Indians, Lucknow, India	322	Healthy controls (from a case–control study)	0.741	0.259	1.49	0.22	0.9465	0.0536	0.04	0.84
Rai et al. (2008)	Punjabi, Chandigarh, India	100	Controls without evidence of coronary artery disease (from a case–control study)	0.705	0.295	3.2	0.07	0.92	0.08	0.47	0.49
Shanker et al. (2008)	Mumbai & Bangalore, India	274	Healthy relatives of CAD and stroke patients	0.81	0.19	0.92	0.33	0.85	0.15	14.12	<**0.01**
Padmaja et al. (2009)	Tamil, Pondicherry, India	185	Healthy volunteers	0.789	0.211	0.04	0.85	0.957	0.043	0.65	0.42
Dawar et al. (2010)	North Indians, New Delhi, India	50	Controls without history of coronary artery disease (from a case–control study)	0.58	0.42	14.22	<**0.01**	0.68	0.32	44.01	<**0.01**
Khan et al. (2012)	Jammu & Kashmir, India	200	Controls free of coronary artery disease (from a case–control study)	0.63	0.37	18.03	<**0.01**	1	0		
Biswas et al. (2013)	West Bengal, India	150	Controls without cardiovascular disease (from a case–control study)	0.71	0.29	3.51	0.06	0.92	0.08	0.64	0.42
Other Asian populations											
Bai et al. (1996)	Japan	119	Control subjects (from case–control study)	0.865	0.135	5.96	<**0.05**	NA	NA		
Yang et al. (2010)	Korea	179	Healthy controls (from a case–control study)	0.788	0.212	0.02	0.88	NA	NA		
Heng et al. (2001)	Malay, Singapore	283	Newborns	0.69	0.31	7.3	<**0.01**	0.97	0.03	3.8	0.051
Heng et al. (2001)	Indian, Singapore	326	Newborns	0.79	0.21	0.067	0.7	0.98	0.02	9.23	<**0.01**
Heng et al. (2001)	Chinese, Singapore	467	Newborns	0.7	0.3	6.77	<**0.01**	0.96	0.04	1.76	0.184
Li et al. (2008)	Hei Yi Zhuang ethnic group, Guangxi, China	474	Healthy subjects	0.70	0.30	6.42	<**0.05**	NA	NA		
Li et al. (2008)	Han ethnic group, Guangxi, China	564	Healthy subjects	0.66	0.34	15.37	<**0.01**	NA	NA		
Jia et al. (2005)	Sichuan, China	307	Healthy subjects	0.752	0.248	0.69	0.4	0.936	0.064	0.004	0.95
Chien et al. (2008)	Taiwan	281	Normal controls only (from a case–control study)	0.684	0.316	8.54	<**0.01**	NA	NA		
Al-Yahyaee et al. (2004)	Oman	150	Healthy subjects	0.783	0.217	0.009	0.92	0.933	0.067	0.03	0.87
Daneshpour et al. (2012)	Iran	823	Participants of Tehran Lipid and Glucose Study (TLGS)	0.862	0.138	12.74	<**0.01**	0.946	0.054	0.07	0.78
Al-Bustan et al. (2013)	Kuwait	549	Healthy volunteers	0.807	0.193	0.91	0.34	0.964	0.036	2.73	0.09
Africa											
Smach et al. (2011)	Tunisian Arab descendants, Tunisia	150	Elderly controls (age 71 ± 1.78 years)	0.8	0.2	0.25	0.62	NA	NA		
Kamboh et al. (1999)	African, Nigeria	786	Civil servants	0.899	0.101	32.5	<**0.01**	0.598	0.402	128.5	<**0.01**
Australia											
Wang et al. (1996)	Caucasian, Australia	243	Healthy subjects	0.779	0.221	2.9	0.99	0.959	0.041	1.05	0.305
Europe											
Talmud et al. (1994)	Europe (5 geographical regions, 12 countries)	1078	Controls (from a case control study)	0.858	0.142	11.89	<**0.01**	NA	NA		
Jeenah et al. (1990)	Bristol, UK	96	Healthy men	0.89	0.11	9.098	<**0.01**	NA	NA		
Sigurdsson et al. (1992)	Iceland	315	Subjects enrolled in Icelandic National Diet Survey	0.88	0.12	13.96	<**0.01**	NA	NA		
Dallinga-Thie et al. (1996)	The Netherlands	177	Spouses of familial hypercholesterolemia	0.86	0.14	6.37	<**0.05**	NA	NA		
Reguero et al. (1998)	Spain	200	Controls without cardiovascular disease (from a case–control study)	0.76	0.24	0.28	0.59	0.93	0.07	0.14	0.71
Miroshnikova et al. (2011)	St. Petersburg, Russia	229	Healthy controls without cardiovascular disease	0.79	0.21	0.06	0.8	0.935	0.065	0.023	0.88
North America											
Kamboh et al.	Non-Hispanic Whites,	534	Non-diabetic, normolipidemic subjects	0.817	0.183	1.89	0.16	0.967	0.033	3.96	<**0.05**

(continued on next page)

Table 5 (*continued*)

Reference	Population/place of study	Sample size (n)	Sample characteristics	Allelic frequency G-75A		Chi square value (χ^2)	*P* value	Allelic frequency C+83T		Chi square value (χ^2)	*P* value
				G allele	A allele			C allele	T allele		
(1996)	USA										
Ordovas et al. (2002)	Boston, USA	1577	Participants of Framingham Offspring Study	0.835	0.165	5.53	<**0.05**	NA	NA		
Larson et al. (2002)	Pritikin Longevity Centre, USA	734	Subjects attending lifestyle changing programme of Pritikin Longevity Centre	0.845	0.155	7.06	<**0.01**	0.955	0.045	0.93	0.33
South America											
de Franca et al. (2005)	Admixed population, Brazil	414	Children (ages 5–15 years)	0.819	0.181	1.9	0.17	0.889	0.111	5.97	<**0.05**
Chen et al. (2009)	Sao Paolo, Brazil	334	Elderly population of different ethnicities (age more than 66 years)	0.898	0.102	23.27	<**0.01**	0.79	0.21	32.83	<**0.01**

males and females. The two polymorphic sites were not in linkage disequilibrium.

Table 5 compares the allelic frequencies of the two SNPs obtained in our study with those reported by other workers.

To assess the effect of the two polymorphisms on apo A-I concentrations in serum (Table 6), we analysed the data separately in males and females. This was done to take into account the gender-specific effects. For the G-75A SNP, no significant differences ($P > 0.05$) in serum apo A-I values were noted across the GG, GA and AA genotypes. The findings were consistent in both male and female subjects. Likewise, serum apo A-I levels were comparable between the CC and the CT variants of the C+83T locus too. We further examined the combined influence of the two polymorphic sites by analysing the variation in serum apo A-I levels in a diplotype-specific manner. However, the apo A-I values did not differ significantly across the 5 diplotypes ($P > 0.05$).

4. Discussion

The current study was undertaken to investigate the G-75A and C+83T polymorphic sites of the *APOA1* gene in a sample population from Assam, northeast India from where data was hitherto not available. Both the polymorphisms were found to be in Hardy–Weinberg equilibrium, indicating that they are normally distributed in the study population. For the −75 bp site, the GG genotype was the most common in the study subjects, followed by GA and AA variants respectively. We found that the AA genotype was present only in males. However, it is unlikely that the AA variant is distributed in a sex-specific manner, and we attribute its absence in the female subjects of our sample to chance. As all the AA homozygotes were men, the A allele frequency appeared to be higher in males (0.26) than in females (0.16); though statistically the difference was not significant. For the +83 bp locus, only the CC and the CT variants were detected. The rare T allele frequency was similar in males and females. The homozygous TT genotype was not detected in any of the study subjects. The rarity of the TT genotype seems to be a universal phenomenon as it was not detected in a number of other populations too (Kamboh et al., 1996; Ma et al., 2003; Jia et al., 2005; Dawar et al., 2010; Biswas et al., 2013; Padmaja et al., 2009). When detected, the frequency of this variant was mostly found to be less than 1% (Sigurdsson et al., 1992; Heng et al., 2001; Larson et al., 2002; Miroshnikova et al., 2011; Hamrita et al., 2011). A review of existing literature reveals that only certain samples from northern India have exhibited a comparatively higher prevalence for this genotype (Dixit et al., 2007; Rai et al., 2008; Khan et al., 2012).

The A allele frequency for the G-75A locus in our sample was found to occupy an intermediate position with respect to the other populations of the world. Nigerians had the lowest values (~0.1) (Kamboh et al., 1999), while Chinese had the highest values (~0.3) (Heng et al., 2001; Li et al., 2008) in the spectrum. Our values were considerably higher compared to those of certain Caucasian populations from Europe (Jeenah et al., 1990; Sigurdsson et al., 1992; Talmud et al., 1994; Dallinga-Thie et al., 1996), North America (Larson et al., 2002; Ordovas et al., 2002) and Brazil (Chen et al., 2009). When compared with populations from other Asian countries in East Asia and Southeast Asia regions, the Malays (Heng et al., 2001) and Taiwanese (Chien et al., 2008) were found to have a significantly higher frequency, whereas, the Japanese (Bai et al., 1996) had a significantly lower frequency of this allele. Koreans on the other hand had comparable values (Yang et al., 2010). Amongst the Indian studies, the A allele frequency was seen to be higher in North Indians from New Delhi (Dawar et al., 2010) and Kashmir (Khan et al., 2012) as compared to our sample. Control subjects from West Bengal (Biswas et al., 2013) and Chandigarh (Rai et al., 2008) were also found to have relatively higher values, though this was not statistically significant. In contrast to the G-75A polymorphism, comparatively fewer reports are available on the C+83T polymorphism. The frequency of the rare T allele in our study was considerably lower than in Nigerians (Kamboh et al., 1999) and Brazilians (de Franca et al., 2005; Chen et al., 2009), and higher than in non-Hispanic Whites (Kamboh et al., 1996). It was also different from that reported in other Indian populations from New Delhi (Dawar et al, 2010), Kashmir (Khan et al., 2012), Singapore (Heng et al., 2001), and Mumbai and Bangalore (Shanker et al., 2008).

The similarities in the allelic frequencies of the G-75A and the C+83T polymorphisms seen amongst some populations may be due to ancestral homology. Likewise, the differences in the allelic frequencies observed across some other nationalities of the world are perhaps attributable to the diverse genetic background. This holds true for the different values reported from various parts of India as well, since the Indian population is heterogeneous. The northeastern region of India is sandwiched between two major subcontinental regions — the Indian subcontinent in the west and the East/Southeast Asia region in the east. Owing to the geographical proximity and strategic location, there have been multiple waves of migration to Assam and the other northeastern states from the Indian mainland and the neighbouring Asian countries in the past. The region has thus acted as a 'melting pot' for people of different stocks from the adjoining regions since the ancient times. By and large, the present day population of Assam has an obvious affinity to the other Indian populations, but with discernible Mongoloid elements (Flatz et al., 1972). The region represents a sort of ethnological transition zone between India and neighbouring China, Myanmar and Thailand (Ali and Das, 2003). The rare allelic frequencies of the two *APOA1* polymorphisms in the current population being recorded in the intermediate range between other Indian (Chhabra et al., 2003; Shanker et al., 2008; Padmaja et al., 2009) and Southeast Asian populations (Jia et al., 2005; Heng et al.,

Table 6
Influence of G-75A and C+83T polymorphisms on serum apo A-I levels.

	G-75A				C+83T		
	GG	GA	AA	*P* value	CC	CT	*P* value
Total	121.3 ± 29.5	119.8 ± 26	125.3 ± 25.6	0.87	121.1 ± 28.2	119.9 ± 27.6	0.86
Male	121.8 ± 30.8	114.9 ± 25.1	125.3 ± 25.6	0.47	119.9 ± 28.8	117.9 ± 27.3	0.82
Female	120.4 ± 27.8	128.7 ± 25.9	–	0.39	123.1 ± 27.2	123.3 ± 29.9	0.98

	Diplotypes					
	GG/CC	GA/CC	AA/CC	GG/CT	GA/CT	*P* value
Total	121.6 ± 29.6	119.7 ± 26.3	125.3 ± 25.6	119.6 ± 30	120.8 ± 21.9	0.99
Male	123 ± 31.2	113.9 ± 25.6	125.3 ± 25.6	115.8 ± 29.9	–	0.53
Female	119.5 ± 27.5	130.4 ± 25.9	–	126.5 ± 32.4		0.42

The values are expressed as mean ± standard deviation. Differences between two groups were analysed by unpaired *t*-test and differences between more than two groups were analysed by one-way analysis of variance (ANOVA). A *P* value less than 0.05 was considered statistically significant, and that less than 0.01 as highly significant.

2001; Li et al., 2008; Chien et al., 2008) are perhaps a reflection of the above fact. Indians in general have exhibited a lower frequency for the rare A allele as compared to their Southeast Asian counterparts. A few Indian studies have reported otherwise as an exception to this trend. This may be an outcome of different sampling techniques used in these studies. For instance, a very high frequency of the A allele was observed in the control subjects by Dawar et al. (2010) and Khan et al. (2012). But, this might not be truly representative because these control subjects were selected on the basis of absence of cardiovascular diseases, and hence may not necessarily represent the healthy population.

The direct gene product of the *APOA1* gene is apo A-I. Some studies have suggested that the G-75A polymorphism influences the apo A-I levels in serum. The A allele has been found to be associated with significantly higher values of apo A-I in certain populations (Jeenah et al., 1990; Talmud et al., 1994; Sigurdsson et al., 1992; Rai et al., 2008; Dawar et al., 2010; Kamboh et al., 1996). However, in the current population, the serum apo A-I levels were comparable across the GG, GA and AA genotypes in males as well as in females. Our findings are in agreement with those observed in other populations where no effect of the A allele was noted on serum apo A-I levels (Dallinga-Thie et al., 1996; Heng et al., 2001; Jia et al., 2005). In contrast, in individuals belonging to the Hei Yi Zhuang ethnic group in China, the GG homozygotes had higher serum apo A-I levels than the A allele carrying subjects (Li et al., 2008).

The C+83T SNP, which is situated in the first intron of *APOA1* has been relatively less investigated as opposed to the G-75A SNP. Although few population studies have shown this polymorphism to influence serum apo A-I concentrations (Kamboh et al., 1996; Dawar et al., 2010; Larson et al., 2002), we observed no such effect. The differences in serum apo A-I between the CC and CT genotypes were not significant in either sex of the current population. Our results were in agreement with previous studies (Heng et al., 2001; de Franca et al., 2005; Jia et al., 2005; Padmaja et al., 2009; Chen et al., 2009).

The proposed mechanism by which G-75A SNP alters apo A-I expression is that the G to A substitution alters the transcription efficiency of the gene and the apo A-I production rate (Tuteja et al., 1992; Smith et al., 1992). This site is located in a GC rich sequence of the promoter which is subject to transcription regulation by binding of a 90 kDa binding factor. It is believed that the G to A interchange at the −75 bp site alters the binding affinity of this binding factor to the GC rich region and consequently influences the rate of transcription (Angotti et al., 1994). As for the C+83T SNP which is located in the first intron of *APOA1*, it is a part of the CpG dinucleotide (Shemer et al., 1990). The +83 bp site and the 5′-region of *APOA1* are differentially expressed in tissues. They are methylated in non-expressing tissues, but hypo-methylated in tissues that express the gene (liver). The cytosine to thymine transition at the +83 bp site possibly results in further demethylation, and thus facilitates increased expression of *APOA1* in the expressing tissues (Shemer et al., 1990; Wang et al., 1996). Due to the contradictory findings reported by different studies, it is increasingly believed that the G-75A and the C+83T polymorphic sites are probably in linkage disequilibrium with other regulatory elements that actually control the production of apo A-I. For instance, strong linkage disequilibrium has been described between the G-75A polymorphism and the *Xmn*I polymorphism located in the 5′-flanking region of the *APOA1* gene (Paul-Hayase et al., 1992). The X2-allele of the *Xmn*I polymorphism was found at a higher frequency in hyperlipidemia than in normolipidemia (Kessling et al., 1985). It may also be that the effects of these SNPs on the apo A-I values are seen only in certain ethnic groups. Or the effects attributed to these SNPs in some of the earlier studies were actually due to gene–environment interactions, and not a result of direct influence. Another possibility is that they influence apo A-I production only in specific subgroups like females (Pagani et al., 1990; Minnich et al., 1995). Considering the possibility that these two polymorphisms may influence the apo A-I concentrations in a synergistic manner, we further examined the variation in apo A-I values across the 5 diplotypes encountered in our study population. Still, none of the combinations seemed to be associated with higher apo A-I levels. Thus, irrespective of the gender and irrespective of the combined effect of the G-75A and the C+83T SNPs, these two SNPs did not influence the apo A-I levels in the current population.

5. Conclusion

The G-75A and the C+83T sites of the human *APOA1* gene are polymorphic. We described the distribution of these two polymorphisms in a healthy sample from Assam, in northeast India for the first time. We documented the differences and similarities in the allelic frequencies in the study population with respect to the populations in the adjoining regions and the other parts of the world. We also observed that these polymorphisms do not directly affect the serum apo A-I levels in the studied population. However, with the recent spurt in interest on the role of these two polymorphisms in a plethora of clinical conditions, it remains to be seen how these allelic variants influence various clinical outcomes in this population. We hope our data will be useful for future studies investigating the role of these two polymorphisms in the implicated disorders in the northeast Indian population.

Conflict of interest

The authors declare that they have no conflicts of interest, financial or otherwise associated with the manuscript.

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