Distribution of ApoE Variants in Four Northeast Indian Populations

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ABSTRACT

Polymorphisms in the human apolipoprotein E gene (ApoE) have been extensively studied for associations with various complex diseases. Numerous population genetic studies have reported the distribution of ApoE alleles in global populations. In this study, we present new data on the distribution of ApoE polymorphisms among four Northeast Indian populations, namely, Kachari, Rabha, Ahom, and an Indo-European caste group. Allele frequencies, Hardy-Weinberg equilibrium, genotype and phenotype probabilities were based on the two SNPs, rs7412 and rs429358. Of the APOE genotypes, derived from rs429358 and rs7412 SNPs, 3/3 and 3/4 genotypes were the most frequent in all groups and only Rabha showed higher frequency of 2/3 genotype. The Kachari population showed the highest frequency of CT genotype for SNP rs429358 and the highest frequency of allele *E4 in the Indian subcontinent, which was much higher than other study populations of eastern India.

Keywords: apolipoproteins E, apolipoprotein E2, apolipoprotein E3, apolipoprotein E4, Single Nucleotide Polymorphism, Northeast India, population genetics

Introduction

Polymorphisms in the human apolipoprotein E gene (ApoE) are among the most extensively studied genetic variants in the context of associations with numerous complex diseases. Notably, ApoE variants have been associated with Alzheimer's disease[1–3], cardiovascular and metabolic diseases[4–6], and neurological disorders[7]. ApoE gene is located on chromosome 19q 13.2 and consists of four exons and three introns with 3,597 nucleotides producing a 299 amino acid polypeptide[8, 9]. The 34kDa glycoprotein of this gene (OMIM #107741, NCBI GENE ID 348) is involved in lipid metabolism and has been associated with plasma proteins such as chylomicrons, chylomicron remnants, low-density lipoproteins (LDLs), and high-density lipoproteins (HDL)[4, 9]. Three common allele variants ($\varepsilon 2$, $\varepsilon 3$, $\varepsilon 4$) code for three isoforms (E2, E3, E4) of the protein with amino acid substitutions at residues 130 and 176 (E2: Cys at both positions; E3: Cys at 130 and Arg at 176; E4: Arg at both positions). These variations were first analyzed by isoelectric focusing; however, with discoveries of single nucleotide polymorphisms (SNPs), the alleles can be determined by genotypes at two SNPs in the respective positions, rs429358 (T/C) and rs7412 (T/C). The $\varepsilon 4$ allele has cytosine at both SNPs; $\varepsilon 2$ has thymine at both SNPs; $\varepsilon 3$ has thymine at rs429358 and cytosine at rs7412. Allelic combinations result in six phenotypes, three homozygotes (E2/2, E3/3, E4/4) and three heterozygotes (E2/3, E2/4, E3/4). While disease associations are primarily reported in the genetic literature, there are numerous population genetic investigations based on the distribution of the ApoE alleles in global populations, including Indian populations[10, 11]. Northeast Indian populations, however, have not yet been studied for variation at the ApoE locus. In this report, we present new data on the level and extent of APOE genetic polymorphism among four population groups from Northeast India.

Materials and Methods

Subjects

Northeast India is the eastern-most region of India surrounded by the nations of China, Myanmar, Bhutan and Bangladesh. It is connected to the rest of India via a

narrow corridor that passes between Bangladesh and Bhutan. The population of Northeast India is ethnically and linguistically diverse. Linguistically, the populations can be divided into three groups: Tibeto-burmans, Austroasiatics and Indo-europeans[12]. In our study, we analyzed 189 samples belonging to two Tibeto-burman speaking groups, Kachari (n = 50) and Rabha (n = 44), who are the ancestral populations of the region and ethnically of Mongoloid origins; an originally Thai speaking group, Ahom (n = 48), who migrated to Northeast India in the 13th century AD from Myanmar; and an Indo-european speaking caste population (n = 47), who migrated to the region before the Christian era.

Genotyping

DNA was isolated from whole blood using standard phenol-chloroform extraction method. All samples were quantified using Nanodrop 1000®. DNA concentration was normalized for each sample in order to have 25ng/µl DNA in 20µl. Normalized DNA (2µl) was used for genotyping.

Genotyping was performed by PCR using TaqMan assay. For a 5µl PCR, a PCR reaction mix was made using: 2.5µl TaqMan Genotyping Master Mix, 0.125µl Primer/Probe (40X) of SNPs rs7412 and rs429358 obtained from Applied Biosystems, and 0.375µl H_2O. The 3µl PCR mix and 2µl DNA were well mixed on the working plate and put on the thermocycler. Thermocycler specifications were: 50^0C for 2min, initial denature at 95^0C for 10min, and then 40 cycles of denature at 92^0C for 15sec and combined anneal and extension step at 60^0C for 1 min. End-point products were read using Applied Biosystems 7900HT SDS System to assign the genotypes.

Data analysis

Allele frequencies, Hardy-Weinberg equilibrium, genotype and phenotype probabilities from the two SNPs, rs7412 and rs429358, were calculated using Haploview; compared amongst themselves and with Hap-Map and AB allele frequencies of Caucasians (CEU) and Han Chinese (CHB) populations. APOE types were assigned based on the genotypes at the two SNPs (rs429358 and rs7412) (Table 1). The double heterozygotes had the rs429358-

Table 1
APOE GENOTYPE DISTRIBUTION BASED ON SNPS RS429358 AND RS7412

rs429358	rs7412	APOE
T/T	C/C	3/3
T/C	C/C	3/4
C/C	C/C	4/4
T/T	C/T	2/3
T/C	C/T	2/4
T/T	T/T	2/2

rs7412 diplotype T-T/C-C or 2/4 and not C-T/T-C or 1/3 since the C-T haplotype (APOE*1) is rare.

Distribution of ApoE alleles was compared to other Indian and selected world populations. Collated data on APOE allele frequencies was used for correspondence analysis to assess genetic affinities of studied populations.

Results and Discussion

Table 2 and 3 gives the distribution of genotypes and alleles of the two analysed SNPs. It is clear that at rs429358, Kachari population has highest frequency of CT genotype, which is 12–16% higher than the other three populations. At rs7412, there were no homozygous TT genotype observed in any population. There were no significant differences between populations at both loci and Hardy Weinberg equilibrium was maintained for both loci.

Table 4 lists the APOE genotypes derived from rs429358 and rs7412 SNPs. In all groups 3/3 and 3/4 genotype were most frequent and only Rabha showed higher frequency of 2/3 genotype. Both Rabha and Kachari showed significantly lower frequency of 3/3 genotype compared to Ahom and Caste populations. This difference is also reflected in allele frequency distribution where *E3 allele is highest in Ahom (0.865) and lowest in Kachari (0.720). Kachari documented highest frequency of *E4 allele (0.23) while Rabha showed highest frequency of allele *E2 (0.068). Standard errors of these allele frequencies are high due to small sample size. Pair-wise comparisons between populations using chi-

TABLE 2
DISTRIBUTION OF GENOTYPES

Population (N)	rs429358			rs7412		
	CC*	CT*	TT*	CC*	CT*	TT*
Kachari (50)	2 (4%)	19 (38%)	29 (58%)	45 (90%)	5 (10%)	0
Rabha (44)	1 (2.3%)	11 (25%)	35 (74.5%)	38 (86.4%)	6 (13.6%)	0
Ahom (48)	0	11 (22.9%)	37 (77.1%)	46 (95.8%)	2 (4.2%)	0
Caste (47)	0	12 (25.5%)	32 (72.7%)	45 (95.7%)	2 (4.3%)	0

*All genotypes are in Hardy Weinberg equilibrium (HWE)

TABLE 3

SNP ALLELE FREQUENCY DISTRIBUTIONS

Population	rs429358		rs7412	
	C	T	C	T
Kachari	0.23±0.04	0.77±0.04	0.95±0.02	0.05±0.02
Rabha	0.14±0.03	0.85±0.03	0.93±0.02	0.07±0.02
Ahom	0.11±0.03	0.89±0.03	0.98±0.02	0.02±0.02
Caste	0.13±0.04	0.87±0.04	0.98±0.03	0.02±0.02

square did not reveal any statistically significant differences when controlled for Bonferroni correction (all p values > 0.05). Overall chi-square for these populations was also non-significant (Chi-square = 9.48, DF = 9, p = 0.148).

We collated APOE allele frequency data from representative global populations to evaluate the spectrum of allelic variation observed in our study populations (Table 5). A quick glance of this table clearly shows that Kachari population has highest frequency of allele *E4 in the In-

dian subcontinent which is much higher than other study populations and tribal and non-tribal populations of the eastern India (Koch, Angami Naga, Lotha Naga, and Manipur Muslims)[11, 13, 14]. Both Rabha and Kachari also have the lowest frequencies for *E3 allele for both Indian and other Mongoloid populations of the region. The data from this table was used for correspondence analysis to evaluate evolutionary relationship of the study populations.

As noted before, we performed a corresponding analysis of APOE allele frequencies of selected populations (Figure 1). In this analysis, all Indian and Asian populations were considered individually, but average allele frequencies of other populations (European, African, North American, and South American) were used. It is clear from the plot that the four Northeastern Indian populations (Kachari, Rabha, Ahom and Assam Caste) are clearly differentiated from each other and other main Indian and Asian populations. Higher frequencies of *E4 allele among Assam populations should be investigated further as these could have implications for cardiovascular, bone and Alzheimer/dementia related diseases.
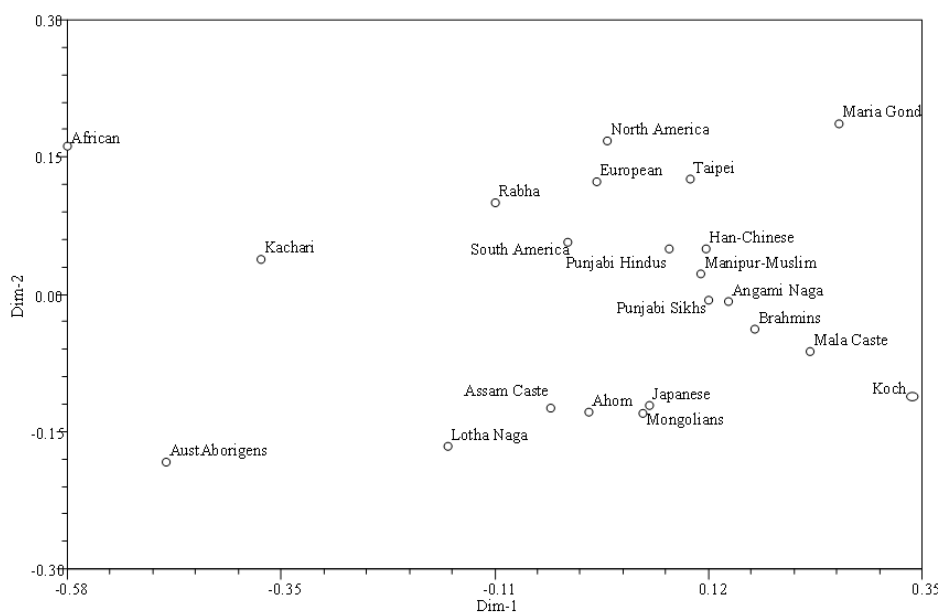

Fig 1. Correspondence Analysis of APOE allele frequencies in selected populations.

TABLE 4

DISTRIBUTION OF APOE GENOTYPES AND ALLELES.

Population	APOE genotypes						Allele Frequencies			HWE P-value
	2.2 (TT/TT)	2.3 (TT/CT)	2.4 (TC/CT)	3.3 (TT/CC)	3.4 (TC/CC)	4.4 (CC/CC)	*E2	*E3	*E4	
Kachari	0	3 (6.0%)	2 (4.0%)	26 (52.0%)	17 (34.0%)	2 (4.0%)	0.50±0.022	0.720±0.044	0.230±0.042	0.79
Rabha	0	6 (13.6%)	0	26 (59.1%)	11 (25.0%)	1 (2.3%)	0.068±0.015	0.784±0.044	0.148±0.038	0.31
Ahom	0	1 (2.1%)	1 (2.1%)	36 (75%)	19 (20.8%)	0	0.021±0.015	0.865±0.035	0.115±0.032	0.70
Caste	0	2 (4.3%)	0	33 (70.2%)	12 (25.5%)	0	0.021±0.015	0.851±0.037	0.128±0.034	0.67

*Based on allelic combinations of rs429358 and rs7412.

TABLE 5
DISTRIBUTION OF APOE ALLELES IN GLOBAL POPULATION

Population		Apo E*2	Apo E*3	Apo E*4	Reference
European					
France	Ile de France	0.068	0.820	0.112	Schachter et al. 1994
Germany	Germans	0.078	0.783	0.139	Menzel et al. 1983
Greece	Greeks	0.053	0.882	0.065	Sklavounou et al. 1997
Italy	Central Italy	0.066	0.851	0.083	Corbo et al. 1995
Netherlands	Amsterdam	0.082	0.751	0.167	Smit et al. 1988
Spain	Andalusia	0.104	0.785	0.111	Fiol et al. 1991
African					
Central African Republic	Pygmies	0.057	0.536	0.407	Zekraoui et al. 1997
Morocco	Moroccans	0.065	0.850	0.085	Valveny et al. 1997
Kenya	Kenyans	0.090	0.590	0.320	Kalaria et al. 1997
South Africa	Khoi-san	0.077	0.553	0.37	Sandholzer et al. 1995
Asian					
China	Han Chinese	0.062	0.863	0.075	Zhang et al. 1999
	Taipei	0.078	0.841	0.081	Liu et al. 2000
Japan	Japanese	0.024	0.882	0.094	Nakai et al. 1998
Mongolia	Mongolians	0.022	0.882	0.096	Chen 1990
India	Punjabi Sikhs	0.050	0.876	0.074	Singh et al. 2006
	Punjabi Hindus	0.061	0.851	0.088	Singh et al. 2006
	Brahmins	0.044	0.898	0.058	Singh et al. 2002
	Maria Gond	0.094	0.876	0.030	Singh et al. 2006
	Koch	0.032	0.968	0.000	Singh et al. 2006
	Angami Naga	0.050	0.883	0.067	Murry et al.2011
	Lotha Naga	0.01	0.827	0.163	Murry et al.2011
	Manipur-Muslim	0.056	0.867	0.077	Asghar et al 2013
	Assam Kachari	0.05	0.727	0.23	present study
	Assam Caste	0.021	0.851	0.128	present study
	Ahom	0.021	0.865	0.115	present study
	Rabha	0.068	0.784	0.148	present study
	Mala caste	0.040	0.921	0.039	Venkatramana et al. 2001
Australia	Aborigens	0.000	0.740	0.260	Kamboh et al. 1991
North America					
USA	Texas	0.090	0.840	0.070	Boerwinkle et al. 1991
	Pennsylvania	0.070	0.820	0.110	Ganguli et al. 2000
	Washington DC	0.095	0.756	0.149	Ghiselli et al. 1982
Greenland	Inuit	0.015	0.776	0.209	Boudreau et al. 1999
South America					
Brazil	Yanomani	0.000	0.850	0.150	Crews et al. 1993
Chile	Santiago	0.243	0.674	0.083	Rollan et al. 1994
Colombia	Bogota	0.080	0.852	0.068	Jacquier et al. 2001
Ecuador	Cayapan Indians	0.000	0.720	0.280	Scacchi et al. 1997
Central America					
Mexico	Mayans	0.000	0.911	0.089	Kamboh et al. 1991
Costa Rica	Costa Ricans	0.030	0.910	0.060	Campos et al. 2001
Caribbean					
Cuba	Cubans	0.070	0.800	0.130	Hardwood et al. 1999

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DISTRIBUCIJA APOE VARIJANATA U ČETIRI SJEVEROISTOČNE INDIJSKE POPULACIJE

SAŽETAK

Polimorfizmi u humanom apolipoproteinu E gena (ApoE) su opsežno proučavani zbog veza s drugim kompleksnim bolestima. Brojne studije populacijske genetika prikazuju distribuciju ApoE alela u svjetskim populacijama. U ovom istraživanju, predstavljamo nove podatke o raspodjeli ApoE polimorfizama među četiri sjeveroistočne populacije u Indiji: Kachari, Rabha, Ahom i Indo-europska kasta. Učestalost alela, Hardy-Weinberg ravnoteža te vjerojatnost genotipa i fenotipa se temelje na dva SNP-ova, rs7412 i rs429358. Od svih APOE genotipova, koji su izvedeni iz rs429358 i rs7412 SNP, 3/3 i 3/4 genotipovi bili su najčešći u svim skupinama, a samo je skupina Rabha pokazala veću učestalost 2/3 genotipa. Stanovništvo Kacharija je pokazala najveću učestalost CT genotipa za SNP rs429358 i najveću učestalost alela * E4 u indijskom potkontinentu, koja je mnogo veća od drugih studija populacija istočne Indije.