APOLIPOPROTEIN E4 POLYMORPHISM AS RISK FACTOR FOR CORONARY HEART DISEASE AMONG INDIAN SUBJECTS

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ABSTRACT :

Apolipoprotein E genotypes and lipid and lipoprotein levels were determined in hypercholesterolemic and angiographically verified CHD subjects and compared against 90 normolipidemic controls. The $\epsilon 4$ allele was significantly prevalent in the hypercholesterolemic and CHD subjects. Significant increase in total cholesterol levels in apo $\epsilon 4$ containing subjects were observed in the hypercholesterolemic and CHD group. The study suggests that the $\epsilon 4$ allele by influencing the lipid levels could act as a risk factor for CHD.

KEYWORDS :

Apolipoprotein E genotypes, alleles.

INTRODUCTION

Coronary heart disease (CHD) is the largest killer in developed countries and is rapidly assuming a similar role in developing countries (1). It has been predicted that cardiovascular disease (CVD) will be the most important cause of mortality in India by the year 2015 (2). Previous clinical and epidemiological studies have recognised plasma lipid and lipoprotein levels as important risk factors for CHD (3, 4, 5). In recent times newer markers like apolipoprotein (apo) E have been found to be associated with CHD and are being studied extensively.

The structural gene locus for apo E on chromosome 19q13.2 is polymorphic (6). Three common alleles $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$ code at a single locus and determine the 6 phenotypes E2/2, E3/3, E4/4, E2/3, E2/4, and E3/4 (7). There exists a wide variation in the distribution of apo E alleles, and the magnitude of the effects of apo E polymorphism on lipid and apolipoprotein levels differ considerably among populations (8). While in Japan, the low

frequency of $\epsilon 4$ allele is associated with a low occurrence of CHD (9), in unrelated Finnish individuals the high frequency of the $\epsilon 4$ allele was found to be in accordance with the higher serum cholesterol levels in the population (10). In subjects with angiographically assessed CHD, the apo E polymorphism was found to influence lipid and lipoprotein levels, thereby predisposing an individual to CHD (11). Sufficient data on prevalence of apo E isoforms, its effect on lipid levels and association with CHD among Indians is lacking. So far only one study on apo E polymorphism has been reported from India (12). The study was conducted on tribal communities from Southern parts of rural India and suggested that the observed low frequency of $\epsilon 4$ allele was a contributing factor to low incidence of CHD.

The present study was undertaken to determine the frequency of apo E alleles and influence of the apo $\epsilon 4$ allele on serum lipid and lipoprotein concentrations and its possible association with the occurrence of CHD.

MATERIALS AND METHODS

The study group selected were not of a specific race or ethnicity. Unrelated heterogeneous group of subjects in the Indian population were selected for this study and comprised of those visiting the Health checkup programme at our

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hospital P.D. Hinduja National Hospital and Medical Research Centre. The subjects were categorized into - those with elevated cholesterol levels [>240 mg/dl, $n=50$] (13), and those with angiographically verified CHD and having normal cholesterol levels ($n=50$). The patient groups were compared against 90 apparently healthy normolipidemic controls (total cholesterol ≤ 220 mg/dl, triglyceride ≤ 200 mg/dl, HDL ≥ 35 mg/dl, and LDL cholesterol ≤ 140 mg/dl as per the National Cholesterol Education Program (NCEP) guidelines (13).

The control subjects had no past or family history of diabetes, hypertension (BP $< 140/90$) and CHD. Of the 90 controls, there were 2 who were smokers and 2 whose smoking status was not known.

Blood was collected in plain and EDTA tubes after an overnight fast of minimum 12 hours. Serum total cholesterol and triglyceride concentrations were measured on Synchron Cx7 Beckman autoanalyzer with standard enzymatic methods (Beckman, USA). HDL cholesterol levels were determined in the supernatant after the serum was subjected to precipitation with $MgCl_2$ and phosphotungstic acid (Beckman, USA). LDL cholesterol was calculated using Friedwald's formula [total cholesterol - HDL cholesterol - (triglyceride/5)] (14). Apo A1 and apo B levels were measured on Beckman Array using rate nephelometry (Beckman, USA).

APO E POLYMORPH IDENTIFICATION

DNA was extracted by salting-out procedure using EDTA blood (15). Apo E genotyping was performed by the method of Hixson and Vernier with slight modifications (16). A 244-bp sequence of the apo E gene containing the polymorphic sites coding for amino acid residues 112 and 158 was amplified by polymerase chain reaction (PCR) in a thermal cycler (model 480 Perkin Elmer Cetus). The 244-bp amplified products were digested with 5 U of Hha I, size fractionated on an 8% nondenaturing polyacrylamide gel, stained with ethidium bromide, and visualized under UV.

STATISTICAL ANALYSIS

The lipid and lipoprotein levels in the 3 groups were expressed as mean $\pm$ SE and the

levels of these biochemical parameters in the patient groups were compared against the controls using the two-tailed, unpaired Students *t* test. Allele frequencies were deduced from genotype frequencies and differences between groups were tested by chi square (χ^2) test. Value of $p < 0.05$ was considered to be significant. One way analysis of variance (ANOVA) was used to test the difference on lipids and lipoproteins according to apo E genotypes. Since very few subjects had the apo E2/2 or E4/4 genotypes, subjects were classified into E2 carriers (E2/2 and E2/3), E3/3 carriers, and E4 carriers (E3/4 and E4/4). Genotype E2/4 was excluded from the analysis.

RESULTS

As shown in Table 1, in both the patient groups serum triglycerides, total Chol / HDL ratio, and apo B were significantly elevated ($p < 0.001$) when compared with the controls. While HDL cholesterol levels in the hypercholesterolemic group did not vary significantly as compared to controls, however their levels in the CHD group were significantly low. Though the angiographically verified CHD subjects and controls had normal total cholesterol levels, the levels were significantly low in the CHD group ($p < 0.05$).

The apo E genotypes were identified on the basis of the digested apo E fragment sizes: apo E2 - 91, 83, 38, and 19 bp; apo E3 - 91, 48, 38, 35, and 19 bp; and apo E4 - 72, 48, 38, 35, and 19 bp (Figure 1). The distribution of the apo E genotypes in the different groups is shown in Table 2. The occurrence of E2/2, E2/4, and E4/4 genotypes was rare in all the groups. While the distribution of the apo E genotypes was significantly different in the CHD group ($\chi^2 = 11.14$, $df = 5$, $p < 0.05$) as compared to controls, it did not vary significantly in the hypercholesterolemic subjects ($\chi^2 = 9.21$, $df = 4$, NS). A significantly increased prevalence of the E4 genotype (E3/4 and E4/4) was observed in the hypercholesterolemic subjects (20.40%, ($\chi^2 = 6.19$, $p < 0.025$), and in the CHD subjects (20.93%, ($\chi^2 = 6.23$, $p < 0.025$) as compared to controls (6.09%) [table 2]. In the controls the frequencies were 0.040, 0.920, and 0.040 for $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$ alleles respectively. The $\epsilon 3$ allele frequency occurred with the highest frequency in the controls followed by the hypercholesterolemic group and then the

angiographically verified CHD group. The $\epsilon 4$ allele frequency was also higher in both these groups as compared to controls (Table 2).

Serum lipid, lipoprotein and apo A1 and apo B levels in relation to apo E genotype are shown in Table 3. On comparing with the E4 controls it was observed that while triglyceride levels were significantly elevated in E4 containing hypercholesterolemic subjects ($p < 0.02$), the HDL cholesterol levels were significantly lowered in E4 CHD subjects ($p < 0.02$). The apo B levels were observed to be significantly elevated in E4 carriers of both hypercholesterolemic ($p < 0.001$) and CHD subjects ($p < 0.03$). Within the patient groups, on comparing with the E3/3 subjects it was observed that total cholesterol levels were significantly elevated in the apo E4 containing subjects of the hypercholesterolemic group by 7.4% and in the CHD group by 16.6%.

DISCUSSION

Apo E plays an important role in lipoprotein metabolism, so the possible association of the different apo E genotypes with a number of plasma lipid and lipoprotein parameters and CHD was explored in the study. Apo E polymorphism studies in several populations have demonstrated the presence of three common apo E alleles- $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$ - that code for the genetic isoforms and determine the six different genotypes (10, 17, 18). Though all the six genotypes were observed in our study it was the apo E3/3 genotype that was predominant in our study population (Table 2). The distribution of the allele frequencies in our normolipidemic control population was 0.920 for $\epsilon 3$, and 0.040 for the $\epsilon 2$ and the $\epsilon 4$ alleles. Similar findings have been reported in the Mala community from rural part of Southern India (12) [Table 4]. In Sardinia the $\epsilon 3$ allele frequency was observed to be 0.897 and could be among the highest reported for any Caucasian population (19). Among Asians, while in the Japanese the $\epsilon 3$ allele frequency was 0.853 (9) in the Chinese it was slightly higher at 0.875 (20). The $\epsilon 4$ allele is generally the next most common allele, exception being the Chinese in Taiwan in whom it occurred less frequently than the $\epsilon 2$ allele (20). While the highest frequency of $\epsilon 4$ allele (0.227) has been observed in the Finns (10), the lowest prevalence of the $\epsilon 4$ allele (0.063) has been from Sardinia (19). Among Asians while

Eto *et al.*, reported a $\epsilon 4$ allele frequency of 0.109 in the Japanese (9), Kao *et al.*, reported a $\epsilon 4$ allele frequency of 0.049 in the Chinese (20). Next is the $\epsilon 2$ allele, which occurs less frequently in the general population. While in the population from Sardinia it was 0.040 (19), in the Finnish the frequency was reported to be 0.041 (10). The $\epsilon 2$ allele frequency in the Japanese was reported to be 0.038 (9) whereas in the Chinese it was comparatively higher at 0.06 (21).

Thus, while the apo $\epsilon 3$ allele frequency in our population is probably among the highest reported for any population, $\epsilon 4$ allele is lower than the Chinese and the Caucasians and the $\epsilon 2$ allele almost similar to the Finnish, Sardinian and Japanese populations. Such differences in the relative frequencies of apo E alleles among different populations can be attributed to mixed range of ethnic, cultural, and geographical differences (8). In spite of these differences the $\epsilon 3$ allele seems to be the most predominant allele in every population.

In the hypercholesterolemic group, in contrast to the normolipidemic control population in whom the $\epsilon 2$ and $\epsilon 4$ alleles occurred with similar frequencies, the $\epsilon 4$ allele occurred more frequently as compared to the $\epsilon 2$ allele (0.110 versus 0.010) [Table 2]. These findings are in accordance with a study conducted on 50 unrelated heterozygous subjects with familial hypercholesterolemia (FH) [0.160 versus 0.010] (22). In our study, the $\epsilon 4$ allele (apo E2/4 was excluded from the analysis) was significantly prevalent in hypercholesterolemic subjects as compared to controls (11.2% v/s 3.7%, $\chi^2 = 5.79$, $df = 1$, $p < 0.025$). Increased prevalence of the $\epsilon 4$ allele has been observed in Germans where E4/4 homozygosity was observed in about 5% of the hypercholesterolemic subjects as opposed to just 2.2% in controls (23).

On comparing the E3/3 hypercholesterolemic subjects with E3/3 normolipidemic controls, as expected, total cholesterol, LDL cholesterol and apo B levels were significantly elevated in the hypercholesterolemic group (Table 3). While triglyceride levels were also significantly elevated, the levels of apo A and HDL between these 2 groups did not vary significantly. Similar results were obtained with the apo E4 containing subjects.

Within the hypercholesterolemic group, it was observed that total cholesterol, triglyceride, LDL cholesterol, and apo B levels were low in E2 carriers, intermediate in E3/3 subjects, and high in E4 carriers (Table 3). Though these differences were observed in the levels of lipid and lipoprotein levels, however they did not vary significantly among the different genotypes with the exception of total cholesterol. (As there was only one E2 carrier in this group, therefore statistical analysis for this group was not conducted). The total cholesterol levels were found to be significantly elevated in E4 carriers by 7.4% as compared to E3/3 genotype (Table 4). The significance remained ($p < 0.02$) even when these subjects were further grouped as E4 present (E3/4 and E4/4) and E4 absent (E2/3 and E3/3) subjects (304.5 ± 11.96 mg/dl v/s 283 ± 3.58 mg/dl). However, when subjects were further categorized into males and females with respect to apo E3/3 and E4 carriers the total cholesterol levels did not vary significantly, indicating the absence of gender effect. This could probably be due to a decrease in the number of subjects within each genotype.

A study by Eto *et al* on FH subjects showed that in apo E4 subjects plasma total cholesterol levels tended to be significantly higher as compared to apo E4 absent group (22). The association of the increased prevalence of $\epsilon 4$ allele (0.227) with elevated cholesterol levels was also observed in the Finnish population (10). These studies suggest that the $\epsilon 4$ allele is associated with modulating the plasma cholesterol levels.

The association of the $\epsilon 4$ allele with plasma cholesterol levels could be due to any of the following reasons. First, the increased binding affinity of the $\epsilon 4$ allele for the receptor may lead to increased plasma cholesterol levels (7, 8). Second reason could be modification of the effects of apo E isoforms on plasma cholesterol by changes in dietary fat and cholesterol intake. This hypothesis receives support from a study by Weintraub *et al.* (24) that hepatic clearance of dietary fat was more rapid in apo $\epsilon 4$ allele carriers than in apo E3/3 subjects and was much slower in apo E3/2 subjects. In addition it was observed by Kesaniemi *et al.* (25) that the intestinal cholesterol absorption was more in apo E4 subjects than in others. The enhanced cholesterol absorption may explain the

increased serum cholesterol response to dietary cholesterol observed in apo E4 carriers as compared to the apo E2 carriers (26).

In angiographically verified CHD subjects (with normal total cholesterol levels) the $\epsilon 4$ allele (calculated from E3/4 and E4/4 genotypes) was significantly prevalent as compared to controls (10.47% v/s 3.7%, $X^2 = 4.63$, $df=1$, $p < 0.05$) [Table 3]. Increased frequency of the $\epsilon 4$ allele (0.324) has been observed in a study on 91 consecutive Finnish men with angiographically confirmed CHD. The study suggested that the high $\epsilon 4$ allele frequency in the Finnish population might be one factor contributing to Finns susceptibility to CHD (27). In Australian men referred for angioplasty and younger than 40 years of age a 16-fold greater prevalence of the apo E4/4 isoform was observed (28). Inheritance of the $\epsilon 4$ allele seemed to confer risk of premature IHD in these males, heterozygotes being especially at risk at a younger age.

In the CHD subjects, total cholesterol, HDL cholesterol, and apo A levels were significantly low as compared to controls, whereas apo B and triglyceride levels were significantly elevated (Table 1). Similar findings were obtained when comparisons were carried out on E3/3 CHD subjects and E3/3 controls (Table 3). Apo A and HDL cholesterol levels were also found to be significantly low in E2 carriers of CHD subjects as compared to E2 controls. Apo B was found to be significantly elevated ($p < 0.03$) in the CHD E4 carriers. In case of total cholesterol an elevation of 8.4% was observed in the E4 carriers of the CHD subjects (189.2 ± 8.16 mg/dl) as compared to E4 controls (179.8 ± 7.72 mg/dl).

Within the CHD group, though the total cholesterol, triglyceride, LDL cholesterol, and apo B were elevated in the apo E4 subjects as compared to E2 and E3/3 carriers, significance was observed only for total cholesterol (Table 3). Total cholesterol levels in the $\epsilon 4$ subjects (189.2 ± 8.16 mg/dl) were elevated by 16.6% ($p < 0.02$) than in the apo E3/3 subjects (162.32 ± 5.48 mg/dl). These observations suggest an indirect influence of $\epsilon 4$ allele on CHD, by its effect on total cholesterol levels. Significance to the value of $p < 0.03$ was also observed when subjects were further grouped as E4 present (189.2 ± 8.16

mg/dl) and E4 absent (164.9 ± 4.89 mg/dl). The subjects were further categorized into males and females and checked for significance between apo E3/3 and E4 carriers. The subjects were age matched and though the total cholesterol levels did not vary significantly between the females, the levels were significantly elevated in E4 males. This could have contributed to the overall effect of E4 carriers on total cholesterol in the CHD group.

These observations suggest that the association of the $\epsilon 4$ allele with CHD is due to its influence on lipids. In Japan a study on IHD subjects (29) showed that E4 carrying IHD patients were characterized by type IIa hyperlipoproteinemia (28.6%) and type IIb hyperlipoproteinemia (42.8%). In Finnish CHD subjects average total and LDL cholesterol levels were found to be significantly elevated in apo E4 carrying subjects than in the other phenotypes (30). The study suggested that the $\epsilon 4$ allele by affecting plasma cholesterol and LDL cholesterol levels might play a role in the development of CHD. In another study involving angiographically verified CHD subjects from Netherlands the apo E phenotypes were found to influence the lipid levels and possibly in that way, indirectly also the risk for CHD (11).

In the normolipidemic control population, which we selected for our study, we did not find any significant influence of apo E polymorph on lipid and lipoprotein levels. While the total and LDL cholesterol levels were low in apo E2 containing subjects, they were elevated in E4 containing subjects as compared to E3/3 carriers but were not statistically significant. Thus the reciprocal effects of the $\epsilon 2$ and $\epsilon 4$ alleles on the lipid and lipoprotein levels [$\epsilon 2$ associated with hypertriglyceridemic and hypocholesterolemic effect and $\epsilon 4$ with hypercholesterolemic and hypotriglyceridemic effect] as observed in the normolipidemic Japanese population (9) was not observed in our study. There was only one subject with apo E4/4 genotype in our normolipidemic group. The total cholesterol, LDL cholesterol, and triglyceride levels in this subject was observed to be more than the average total cholesterol, LDL cholesterol and triglyceride levels in the other

genotypes. Similar findings have been reported in the Indian study conducted on unrelated individuals from 2 different communities (12), wherein apo E4/4 and apo E3/4 genotypes were associated with increased total cholesterol levels as compared to other genotypes. The study also suggested that the low frequency of the $\epsilon 4$ allele could be correlated to the low incidence of cardiovascular disease in the rural population.

One explanation for the lack of association between apo E polymorphism and lipid and lipoprotein levels in our control population could be that as the phenotypic expression is dependent on the interaction between genetic and environmental factors, accordingly the dietary habits among these individuals could be one of the factors. The possible explanation for the absence of the reciprocal effect of the $\epsilon 2$ and the $\epsilon 4$ alleles on the lipid and lipoprotein levels could be due to the low prevalence of $\epsilon 2$ and $\epsilon 4$ alleles in our study population, which can be further, evaluated using a larger population.

Thus in our study an increased prevalence of the $\epsilon 4$ allele in both the hypercholesterolemic and CHD subjects was observed and it was associated with an increase in the serum total cholesterol levels, which is a proven risk factor for CHD. Due to the extreme severity and prematurity of CHD in Asian Indians, an intensive programme of early identification of individuals at high risk as a result of genetic predisposition, primary prevention, and alteration of all modifiable risk factors is urgently required. Apo E polymorphism study could provide an insight into the metabolic fate of lipid and lipoproteins in these individuals. The individuals at risk can be subjected to diet modification or therapeutic intervention, and the development of CHD in these individuals could be either delayed or avoided. A study on larger cohorts can only strengthen the role of apo E polymorphism as predisposing factor for CHD.

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Figure 1

Identification of apo E allele genotyping: analysis of PCR-amplified apo E, digested with the enzyme Hha1, on 8% polyacrylamide gel.

Lane 1 - molecular weight marker pBR 322 DNA/HaeIII digest

Lane 2 - non digested PCR product. The arrows indicate the primer-dimers seen in all the lanes.

Lane 3 & 10 - Apo E2/3 genotype

Lane 4, 5, 6 & 8 - Apo E3/3 genotype

Lane 7 - Apo E3/4 genotype

Lane 9 - Apo E4/4 genotype

Table 1

Biochemical Results in the 3 groups

Parameters N	Control 90	Hyperchol. 50	CHD 50
(M / F)	(45 / 45)	(25 / 25)	(38 / 12)
Age [years]	44.3 ± 9.74	50.7 ± 11.67	56.4 ± 11.8
T. Chol [mg/dl]	176.9 ± 20.4	287.3 ± 27.3 ***	167.2 ± 31.2 **
Trig [mg/dl]	93.8 ± 31.6	187.2 ± 70.3 ***	147.9 ± 70.59 ***
HDL-C [mg/dl]	44.9 ± 9.39	47.5 ± 8.24 *	29.7 ± 7.18 ***
Chol / HDL	4.06 ± 0.77	6.2 ± 1.06 ***	5.94 ± 1.66 ***
LDL-C [mg/dl]	113.5 ± 18.36	202.2 ± 29.9 ***	110 ± 28.57 *
Apo A1 [g/l]	1.15 ± 0.14	1.19 ± 0.18 *	0.94 ± 0.22 ***
Apo B [g/l]	0.91 ± 0.12	1.52 ± 0.24 ***	1.11 ± 0.32 ***

Key : * NS, ** p < 0.05, *** p < 0.001 (significance calculated using two-tailed, unpaired Students test)

T. Chol : Total Cholesterol, Trig : Triglycerides, HDL-c : HDL - cholesterol,

Chol / HDL : Cholesterol / HDL-c Ratio, LDL-c : LDL - cholesterol.

Table 2

Apo E genotypes in the 3 groups and their percentages

Apo E genotype	Control		Hyperchol.		CHD	
	No.	%	No.	%	No.	%
E2 / E2	-	-	-	-	1/50	2
E2 / E3	7/90	7.8	1/50	2	6/50	12
E3 / E3	77/90	85.6	39/50	78	34/50	68
E2 / E4	1/90	1.1	-	-	-	-
E3 / E4	4/90	4.4	9/50	18	9/50	18
E4 / E4	1/90	1.1	1/50	2	-	-
			χ^2 =9.21, NS		χ^2 =11.14, p<0.05	
Allele frequencies :						
Apo E allele	Control		Hyperchol.		CHD	
ε 2	0.040		0.010		0.080	
ε 3	0.920		0.880		0.830	
ε 4	0.040		0.110		0.090	
			χ^2 =7.51, p<0.05		χ^2 = 4.91, NS	

Table 3

Comparison of the biochemical parameters within the 3 groups as compared to E3/3 genotype

Overall		E2(E2/2+E2/3)	E3/3	E4(E3/4+E4/4)
Tcholes	Control	170.57 ± 5.68 (n=7)	177.44 ± 2.46 (n=77)	179.8 ± 7.72 (n=5)
	Hyper	264 (n=1)	283.49 ± 3.65 (n=39)	304.5 ± 11.96(n=10) **
	CHD	177.42 ± 10.41 (n=7)	162.32 ± 5.48 (n=34)	189.2 ± 8.16 (n=9) *
TG	Control	93.29 ± 8.13	92.19 ± 3.75	115.4 ± 7.51
	Hyper	125	180.89 ± 10.64	217.9 ± 25.77
	CHD	149.57 ± 24.82	142.26 ± 11.35	167.89 ± 31.3
HDL	Control	43.83 ± 1.08	45.13 ± 1.12	44.6 ± 3.52
	Hyper	42	47.89 ± 1.29	46.6 ± 3.01
	CHD	32.86 ± 1.89	28.32 ± 1.23	32.22 ± 2.62
LDL	Control	110 ± 4.41	114.19 ± 2.19	110.8 ± 5.66
	Hyper	197	199.08 ± 4.35	214.3 ± 12.54
	CHD	114.57 ± 7.79	105.5 ± 5.2	123.56 ± 8.07
Apo A	Control	1.19 ± 0.06	1.14 ± 0.01	1.22 ± 0.08
	Hyper	1.01	1.21 ± 0.03	1.14 ± 0.04
	CHD	0.86 ± 0.06	0.95 ± 0.04	0.99 ± 0.08
Apo B	Control	0.89 ± 0.05	0.92 ± 0.01	0.83 ± 0.04
	Hyper	1.02	1.5 ± 0.03	1.65 ± 0.09
	CHD	1.01 ± 0.08	1.12 ± 0.06	1.19 ± 0.11

One way ANOVA used for comparison. Key - * - p < 0.02, ** - p < 0.03

Table 4

Relative frequencies of the apo E alleles in the various populations

Population Studied (Reference)	No.	Relative frequencies		
		ε2	ε3	ε4
Munster, W. Germany (23)	1557	0.082	0.782	0.136
Helsinki, Finland (10)	615	0.041	0.733	0.227
Sardinia (19)	633	0.040	0.897	0.063
Asahikawa, Japan (9)	92	0.038	0.853	0.109
Taiwan, China (20)	546	0.076	0.875	0.049
Shanghai, China (21)	71	0.060	0.860	0.070
CHD subjects, Finland (27)	91	0.044	0.632	0.324
CHD subjects, Finland (30)	309	0.023	0.790	0.187
FH subjects, Japan (22)	50	0.010	0.830	0.160
IHD subjects, Japan (29)	109	0.082	0.748	0.170
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Andhra Pradesh, India (12)				
Kshatriya	182	0.052	0.851	0.096
Mala	38	0.039	0.921	0.039
Pooled	220	0.05	0.863	0.086

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