Evaluation of Lp[a] and other independent risk factors for CHD in Asian Indians and their USA counterparts

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Abstract **Conventional risk factors for coronary heart disease (CHD) do not completely account for the observed increase in premature CHD in people from the Indian subcontinent or for Asian Indians who have immigrated to the USA. The objective of this study was to determine the effect of immigration to the USA on plasma levels of lipoprotein [a] (Lp[a]) and other independent risk factors for CHD in Asian Indians. Three subject groups were studied: group 1, 57 subjects living in India and diagnosed with CHD (CHD patients); group 2, 46 subjects living in India and showing no symptoms of CHD (control subjects); group 3, 206 Asian Indians living in the USA. Fasting blood samples were drawn to determine plasma levels of triglyceride (TG), total cholesterol (TC), low density lipoprotein [LDL cholesterol (LDL-Chol)], high density lipoprotein [HDL cholesterol (HDL-Chol)], apolipoprotein B-100 (apoB-100), and Lp[a]. Apolipoprotein [a] (apo[a]) size polymorphism was determined by immunoblotting. Plasma TG, apoB-100, and Lp[a] concentrations were higher in CHD patients than in control and USA groups. CHD patients had higher levels of TC and LDL-Chol and lower HDL-Chol than control subjects. However, the USA population had higher levels of TC, LDL-Chol, and apoB-100 and lower HDL-Chol than control subjects. Plasma Lp[a] levels were inversely correlated with the relative molecular weight of the more abundant of each subject's two apo[a] isoforms (MAI), and CHD patients showed higher frequencies of lower relative molecular weights among MAI. Our observed changes in lipid profiles suggest that immigrating to the USA may place Asian Indians at increased risk for CHD. This study suggests that elevated plasma Lp[a] confers genetic predisposition to CHD in Asian Indians, and nutritional and environmental factors further increase the risk of CHD. This is the first report implicating MAI size as a predictor for development of premature CHD in Asian Indians. Including plasma Lp[a] concentration and apo[a] phenotype in screening procedures may permit early detection and preventive treatment of CHD in this population.**—Hoogeveen, R. C., J. K. Gambhir, D. S. Gambhir, K. T. Kimball, K. Ghazzaly, J. W. Gaubatz, M. Vaduganathan, R. S. Rao, M. Koschinsky, and J. D. Morrisett. **Evaluation of Lp[a] and other independent risk factors for CHD in Asian Indians and their USA counterparts.** ***J. Lipid Res.*** **2001.** 42: **631–638.**

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A high incidence of coronary heart disease (CHD) has been observed among Asian Indians immigrating to the USA (1–3) and among native people remaining within the Indian subcontinent (4–6). The mortality rate for CHD in Asian Indians from Singapore is 4 times higher than in Chinese residents from Singapore, and 20 times higher than in blacks from South Africa (7). Moreover, CHD in Asian Indians occurs prematurely and is often more severe than in Europeans (8, 9). The prevalence of CHD within India is significantly higher in southern compared with northern areas (10), and studies have shown that the incidence of CHD in India is higher in urban regions than in rural parts (6, 11). Prevalence of CHD in urban regions of Kerala is as high as 14%, whereas rural Kerala has a prevalence of 7% (10, 12). Traditional risk factors for CHD such as obesity, insulin-dependent diabetes mellitus, smoking, hypertension, and elevated plasma Chol or low density lipoprotein cholesterol (LDL-Chol) levels do not explain the observed increase in CHD incidence among Asian Indians. Earlier reports have shown that serum levels of total Chol (TC) and other blood lipids are normal to low in Asian Indians, and that the incidence of diabetes does not explain the excess cardiac mortality observed in this population compared with subjects of European origin (2, 13). Several studies have identified additional CHD risk factors in Asian Indians, including

Abbreviations: apo[a], apolipoprotein [a]; CHD, coronary heart disease; Chol, cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; Lp[a], lipoprotein [a]; NIDDM, non-insulin-dependent diabetes mellitus; TC, total cholesterol.

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high prevalence of upper body obesity with an increase in waist-to-hip ratio, low levels of high density lipoprotein cholesterol (HDL-Chol), hyperinsulinemia, increased insulin resistance, and non-insulin-dependent diabetes mellitus (NIDDM), especially when subjects are exposed to urban life styles (14–16). Immigration has been selective for urban professionals: 2.5% of Asian Indians in the USA are physicians, and most of the other immigrants are engineers, computer specialists, and businessmen before arrival. Other ethnic groups also tend to develop metabolic abnormalities after migrating to urban environments, but they do not appear to develop premature CHD at rates as high as those of Asian Indians (1).

Lipoprotein [a] (Lp[a]) is a cholesterol ester-rich lipoprotein composed of an LDL particle and a large hydrophilic glycoprotein, apolipoprotein [a] (apo[a]) (17, 18). Elevated plasma levels of Lp[a] confer an increased risk of CHD and studies clearly demonstrate that Lp[a] is an independent risk factor for this disease (19, 20). Because of the association between high plasma Lp[a] concentrations and CHD, the factors controlling plasma Lp[a] levels have been the subject of active study. Apo[a] is highly variable in size because of length polymorphism in the encoding *LPA* gene (21, 22). *LPA* alleles differ in the number of their tandemly repeated nucleotide sequences, each of which encodes a protein motif resembling kringle-IV of plasminogen. The ability of apo[a] to inhibit tissue plasminogen activator-mediated plasminogen activation, and thus to suppress fibrinolysis, is likely a function of the homology between apo[a] and plasminogen (23, 24). Generally it has been observed that the size of an apo[a] polymorph is inversely related to the plasma concentration of Lp[a] (25). From sib-pair analyses, it has been estimated that >90% of the interindividual variation in plasma Lp[a] levels can be attributed to sequence differences at, or closely linked to, the *APO[a]* locus (26). In contrast, 42% of the variability in plasma Lp[a] levels between unrelated individuals has been attributed to the size polymorphism in the apo[a] protein (27). Distribution of plasma Lp[a] levels is known to be influenced by ethnic origin and studies indicate that south Asians living in North America have elevated plasma levels of Lp[a] compared with North American whites (28). Moreover, a study has demonstrated that the serum Lp[a] level is an independent risk factor for CHD in Asian Indians diagnosed with NIDDM (29). This study showed that Lp[a] levels were significantly higher in NIDDM patients with CHD compared with NIDDM patients without CHD and control subjects. Because plasma Lp[a] levels are highly correlated with apo[a] polymorph size distribution, it is possible that this distribution may account, in part, for the tendency of Asian Indians to develop premature CHD.

There are few published studies on plasma Lp[a] levels in Asian Indians and there have been no reports on apo[a] polymorph size distribution in Asian Indian CHD patients. The present study was designed to examine the role of several risk factors for CHD, especially plasma Lp[a] levels and apo[a] polymorph size distribution, in Asian Indian CHD patients versus control subjects, as well as in Asian Indians immigrating to the USA. This is the first report on the contribution of apo[a] length polymorphism to the variability in plasma Lp[a] levels in Asian Indian CHD patients and control subjects, as well as first-generation Asian Indian immigrants to the USA.

MATERIALS AND METHODS

Study subjects

A total of 309 Asian Indians participated in this study. Group 1 consisted of 57 subjects living in the northern regions of India and who were diagnosed with CHD on the basis of a positive coronary angiogram. Group 2 was composed of 46 healthy nondiabetic control subjects who showed no apparent symptoms of cardiovascular disease and were residents of north India. Group 3 was composed of 206 Asian Indians living in the USA and who were recruited at health fairs in the area of Houston, Texas. On the basis of their place of birth within the Indian subcontinent and for purposes of analysis, the USA Asian Indian subjects of group 3 were divided into four subgroups: *1*) north (n = 41) (Pakistan, Punjab, Rajastan, New Delhi, or Uttar Pradesh); *2*) south (n = 81) (Andhra Pradesh, Karnataka, Kerala, Sri Lanka, or Tamil Nadu); *3*) east (n = 38) (Bengal or Orissa); or *4*) west (n = 46) (Maharashtra or Gujarat). The procedures followed were in accordance with the ethical standards of the institutional committee on human experimentation and with the Helsinki Declaration of 1975 as revised in 1983. All participants in the USA group signed an informed consent form before completing a cardiovascular assessment consisting of a health survey, dietary evaluation, and provision of a fasting blood sample. Because not all blood lipid parameters were measured in each subject, the number of samples from which data were derived will be indicated.

Laboratory measurements

Venous blood samples were collected in Vacutainer™ tubes (Becton Dickinson, Franklin Lakes, NJ) containing disodium ethylenediaminetetraacetic acid. Plasma was isolated by low speed centrifugation at 2,000 *g* for 15 min at 4°C, divided into aliquots, and stored at −20°C. Plasma fractions were analyzed for TC (30), HDL-Chol (31), and triglycerides (TG) (32) by the Atherosclerosis Clinical Laboratory (Methodist Hospital, Houston, TX). LDL-Chol was calculated by the Friedewald, Levy, and Fredrickson (33) formula and then corrected for the contribution of Lp[a] cholesterol (Lp[a]-Chol) (34), which is 1.05 × Lp[a] protein. Plasma Lp[a] protein levels were measured by enzyme-linked immunosorbent assay (ELISA) (35), and converted to total Lp[a] mass by using a conversion factor of 3.3. Plasma apoB-100 levels were determined by ELISA, using a monoclonal antibody obtained from Intracel (Rockville, MD).

Apo[a] isoform determination

The relative molecular weights of apo[a] isoforms were determined by a modification of an immunoblotting method previously described (36, 37). This modified method uses high resolution 15 × 25 cm agarose gels, which are capable of separating apo[a] isoforms that differ by only one kringle-IV repeat in length. Apo[a] isoforms were classified according to the total number of kringle-IV repeats in their sequence as determined by comigration with apo[a] molecular weight standards containing 16, 18, 22, 26, 28, 32, and 35 kringle-IV repeats obtained from Intracel. The two isoforms of each subject were classified as "more abundant" (MAI) or "less abundant" (LAI) if the former was ⩾1.5 times more abundant than the latter as judged by densitometry and/or inspection (two observers).

TABLE 1. Demographics of three Asian Indian study groups

	CHD (n = 57)		Control (n = 46)		USA (n = 206)	
	N	%	N	%	N	%
Male	52	91	29	63	134	65
Female	5	9	17	37	72	35
Cigarette smokers	15	26	10	22	5	2
Hypertension (DBP >90)	29	51	2	4	9	4
Diabetes mellitus[a]	8	14	0	0	12	6
Obesity[b]	7	12	3	7	ND	ND
CHD	57	100	0	0	14	7
Family history of CHD	7	12	2	4	62	30
Age, years	52 ± 9		40 ± 11		43 ± 14	

CHD, a group of 57 patients with documented coronary heart disease (CHD) living in northern India; Control, a group of 46 healthy subjects without CHD living in northern India; USA, a group of 206 Asian Indian subjects living primarily in the greater Houston area. ND, not determined; DBP, diastolic blood pressure.

[a] Hb A1c >7%, fasting glucose >126 mg/dl.

[b] More than 20% above ideal body weight.

Statistical analyses

Plasma Lp[a] and apo[a] isoforms displayed nonnormal distribution. Thus, Lp[a] values were subjected to square root transformation and apo[a] isoform distributions were analyzed by nonparametric tests. All normally distributed variables and transformed variables were analyzed with parametric tests. The influences of clinical risk factors on the prevalence of CHD, within each population, were estimated by logistic regression. Two-way comparisons of nonparametric data were performed by Spearman's test. Statistical analyses were performed with Stata software (release 6.0; StataCorp, College Station, TX) and the PRISM package (version 2.0; GraphPad Software, San Diego, CA).

RESULTS

The demographics of the three study populations are presented in **Table 1**. The Asian Indian subjects in the CHD group were predominantly men, who were older and had higher incidences of diabetes and hypertension compared with the Asian Indian control group and USA group. The USA group had significantly fewer smokers but a higher incidence of reported family history of cardiovascular disease than the Asian Indian CHD group and control group.

The plasma lipid profiles of the three study populations are shown in **Table 2**. CHD patients had significantly higher plasma TG levels compared with subjects of the control and USA Asian Indian groups. Mean values for plasma TG levels in all three study populations were below 250 mg/dl. TC and LDL-Chol levels were significantly higher for the CHD and USA groups versus the control group. Control subjects had higher plasma levels of HDL-Chol (mean, 47.4 mg/dl) compared with CHD patients and USA Asian Indians (mean values of 42.3 and 43.4 mg/dl, respectively). Plasma apoB-100 levels were significantly higher for CHD patients versus USA Asian Indian and control groups (mean value of 107.5 vs. 89.2 and 80.2 mg/dl, respectively). Plasma levels of Lp[a] were highly skewed toward the lower concentrations in all three study groups (**Fig. 1**) and ranged from 0.78 to 47.1 mg/dl (mean, 12.7 mg/dl), from 0.99 to 39.1 mg/dl (mean, 9.2 mg/dl), and from 0.15 to 40.7 mg/dl (mean, 8.7 mg/dl) in the CHD, control, and USA groups, respectively. The CHD patients had higher mean plasma levels of Lp[a]

TABLE 2. Plasma lipid parameters of three Asian Indian study groups

			USA Asian Indians				
Analyte	CHD	Control	North India	South India	East India	West India	Total
TG	186.0 ± 101.3[a,b]	139.9 ± 65.5	139.7 ± 95.4	161.4 ± 113.3	137.3 ± 92.7	137.1 ± 63.9	151.3 ± 102.6
TC	193.6 ± 47.3[a]	162.0 ± 25.6	177.3 ± 29.3	193.0 ± 31.9	188.1 ± 38.0	201.8 ± 39.8	191.2 ± 35.3[a]
HDL-Chol	42.3 ± 8.8[a]	47.4 ± 12.9[b]	46.4 ± 11.4	41.5 ± 10.4	40.9 ± 11.1	46.1 ± 12.3	43.4 ± 11.8[a]
LDL-Chol	110.7 ± 40.1[a]	84.2 ± 25.4	104.9 ± 26.7	120.3 ± 27.9	116.3 ± 28.1	126.7 ± 38.6	117.8 ± 32.2[a]
Lp[a]-Chol[c]	4.43 ± 3.29[b]	3.20 ± 2.57	3.24 ± 2.76	3.32 ± 2.86	1.95 ± 2.18[d]	3.31 ± 3.55	3.03 ± 2.88
Apo B-100	107.5 ± 30.9[a,b]	80.2 ± 29.6	82.7 ± 35.1	102.6 ± 30.6	69.9 ± 20.3[d]	88.9 ± 32.2	89.2 ± 32.4
Lp[a]	12.65 ± 9.40[b]	9.15 ± 7.33	9.27 ± 7.89	9.48 ± 8.17	5.57 ± 6.24[d]	9.45 ± 10.15	8.67 ± 8.24

CHD, a group of 57 patients with documented coronary heart disease (CHD) living in northern India; Control, a group of 46 healthy subjects without CHD living in northern India; USA, a group of 206 Asian Indian subjects living primarily in the greater Houston area. Data represent mean values ± SD (mg/dl).

[a] Significantly different from control group ($P < 0.05$).

[b] Significantly higher than total USA Asian Indian group ($P < 0.05$).

[c] Lp[a]-Chol was not measured independently, but rather was calculated from the total Lp[a] value. It is included here to emphasize the small but sometimes significant contribution it can make to the total plasma Chol value.

[d] Significantly different from north, south, and west India subgroups within the USA Asian Indian group ($P < 0.05$).

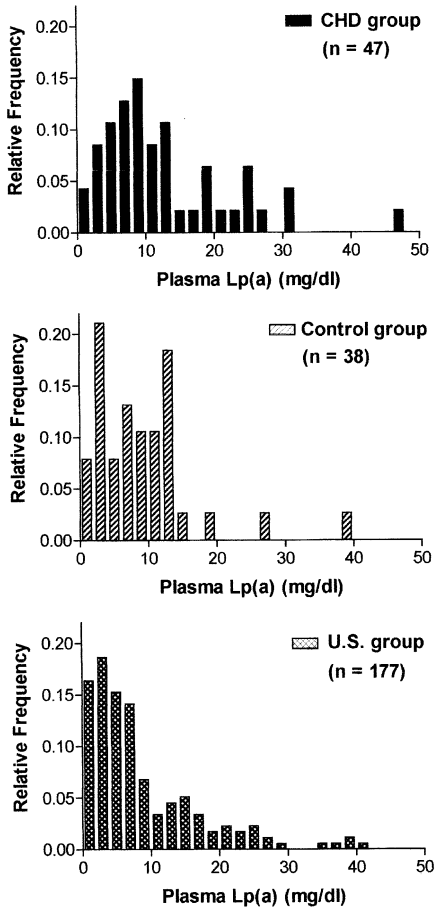

Fig. 1. Frequency distribution of plasma lipoprotein [a] (Lp[a]) concentrations in three Asian Indian study groups.

and Lp[a]-Chol compared with the USA and control study populations, although differences in Lp[a] and Lp[a]-Chol levels between CHD patients and control subjects did not reach significance at the $P < 0.05$ level ($P = 0.068$). The proportion of subjects with plasma Lp[a] levels exceeding 19 mg/dl (upper quartile) was greater in the CHD group versus control and USA Asian Indian groups (25.0% vs. 7.9% and 11.3%, respectively). When plasma lipid data from the USA study population were analyzed on the basis of place of birth, no significant differences were found in plasma levels of TG, TC, HDL-Chol, or LDL-Chol. However, plasma levels of apoB-100, Lp[a], and Lp[a]-Chol were significantly lower in USA Asian Indian subjects born in the eastern regions of the Indian subcontinent.

To identify significant predictors of CHD, we used logistic regression analyses to examine the influence of several clinical risk factors on the prevalence of CHD among Asian Indians living in India versus their USA counterparts (**Table 3**). In the India group, the odds of CHD increased an average of 3.1 times for every 10 years of age ($P < 0.0005$) and were 5.2 times greater in males than females ($P = 0.004$). For every 10-mg/dl increase in TC and LDL-Chol concentration, the risk for CHD increased by 31% ($P = 0.001$) and 35% ($P = 0.001$), respectively, in this study population. Furthermore, the risk for CHD increased by 11% ($P = 0.042$) for every 1-unit increase in the square root of the plasma Lp[a] concentration. Because all diabetics and hypertensive subjects in the India group had CHD, probabilities for these risk factors could not be calculated, and thus diabetes and hypertension were removed from the model. In the USA group, only diabetes was found to be a significant predictor of CHD at the $P < 0.05$ level and the risk for CHD was 6.6 times greater in diabetics than in nondiabetics ($P = 0.013$). None of the hypertensive subjects in this study population had CHD and, therefore, a probability could not be calculated for this risk factor.

The apparent molecular weights of apo[a] isoforms were determined in 281 individuals and the proportions of subjects expressing either one or two isoforms were 24% and 74% of CHD patients, 24% and 76% of control subjects, and 28% and 70% of the USA Asian Indian study group. No apo[a] isoform could be detected in 2%, 0%, and 2% of the subjects in the CHD, control, and USA Asian Indian groups, respectively. The detection limit of a single apo[a] isoform was approximately 2.5 ng and the 2% sodium dodecyl sulfate (SDS)-agarose gel electrophoresis system we used was able to resolve 34 different apo[a] isoforms ranging in length from 7 to 40 kringle-IV repeats (**Fig. 2**).

The apo[a] isoform size frequency distributions of the three study populations are shown in **Fig. 3**. Distributions of each subject's two isoforms, the more abundant isoform (MAI) and less abundant isoform (LAI), are shown in Fig. 3A–C, and the distributions of MAI alone are shown in Fig. 3D–F. When these distributions were compared in terms of quartiles, the 25th percentile of MAI + LAI was 23.0 kringle-IV repeats in the CHD group compared with 25.5 in the corresponding control group (Fig. 3A and B). Similarly, the 50th and 75th percentiles for the CHD group were 29.0 and 33.0, compared with 31.0 and 34.0 for the control group. These results suggest that CHD patients had a higher proportion of apo[a] isoforms with fewer kringle-IV repeats compared with the control group. This hypothesis was further tested by comparing MAI alone among the CHD and control groups (Fig. 3D and E). In the CHD group, the 25th percentile of MAI was 20.0 kringle-IV repeats, substantially lower than the 26.5 kringle-IV repeats in the control group. Logistic regression analysis indicated an odds ratio of 0.73 for increments of 5 kringle-IV units of MAI, although this analysis did not reach significance ($P = 0.101$). Smaller differences were observed for the 50th and 75th percentiles. The larger USA group had MAI + LAI and MAI alone distributions that more closely resembled those of the control subjects than the CHD group. When subjects of the USA Asian Indian study population were grouped according to place of birth, natives from the eastern regions of India had the highest median apo[a] kringle-IV repeats (32 repeats) compared with subjects born in north, south, or west India (29, 31, and 30 kringle-IV repeats, respectively). This higher prevalence of high molecular weight apo[a] isoforms in the subjects born in east India corre-

TABLE 3. Influence of clinical risk factors on the prevalence of CHD in Asian Indians

	India (n = 83)			USA (n = 179)		
Parameter[a]	OR	99% CI	*P*	OR	99% CI	*P*
Effect of age (10-year increment)	3.10	1.56–6 .07	<0.0005[b]	1.55	0.83–2.91	0.072
Gender, male	5.20	1.17–23.18	0.004[b]	1.53	0.25–9.12	0.547
Diabetes mellitus	ND	ND	ND	6.63	0.94–46.77	0.013[b]
Hypertension (DBP >90)	ND	ND	ND	ND	ND	ND
Smoking	1.36	0.36–5.23	0.552	4.10	0.20–82.32	0.226
CHD history	3.32	0.39–28.48	0.151	0.47	0.06–3.67	0.344
TG (10 mg/dl)	1.07	0.98–1.16	0.054	0.98	0.87–1.10	0.057
TC (10 mg/dl)	1.31	1.06–1.62	0.001[b]	0.84	0.66–1.07	0.057
LDL-Chol (10 mg/dl)	1.35	1.07–1.70	0.001[b]	0.84	0.64–1.10	0.096
HDL-Chol (10 mg/dl)	0.96	0.90–1.02	0.082	0.98	0.91–1.06	0.486
Lp[a] (sqare root = 1)[c]	1.14	0.97–1.35	0.042[b]	1.12	0.93–1.34	0.111

India group, Asian Indian subjects living in northern India. USA group, Asian Indian subjects living primarily in the greater Houston area. OR, odds Ratio; 99% CI, 99% confidence interval; ND, not determined; DBP, diastolic blood pressure.

[a] Qualitative and quantitative changes indicated in parentheses.

[b] Significant ($P < 0.05$).

[c] Increments of 1 unit in square root of Lp[a].

sponded to the lowest plasma Lp[a] concentrations in this subgroup (Table 2).

We also investigated the relative contribution of individual apo[a] isoforms to the control of plasma Lp[a] levels. **Figure 4** shows the correlation between apo[a] isoform size and plasma Lp[a] concentration. A significant inverse correlation was found between the more abundant apo[a] isoform size and plasma Lp[a] concentration (Spearman's $r = -0.489$, $P < 0.0001$). On the basis of these data, the calculated contribution of MAI size polymorphism to the variability in plasma Lp[a] concentration was 24%. Although there appeared to be an inverse correlation of less abundant apo[a] isoform sizes with plasma levels of Lp[a], this correlation was weak and only 2% of the variance in plasma Lp[a] levels could be explained by variance in LAI size polymorphism (Spearman's $r = -0.125$, $P = 0.098$).

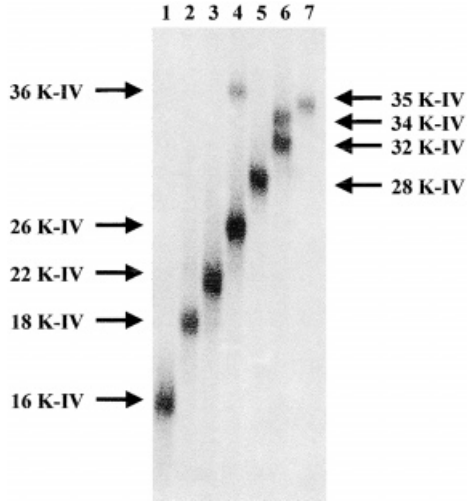

Fig. 2. Immunoblot analysis of apolipoprotein [a] (apo[a]) isoforms after 2% sodium dodecyl sulfate (SDS)-agarose gel electrophoresis. Samples in lanes 1, 2, 3, 5, and 7 each contain 1 apo[a] isoform with 16, 18, 22, 28, and 35 kringle-IV (K-IV) repeats, respectively. Lane 4 contains 2 apo[a] isoforms with 26 and 36 K-IV repeats. Lane 6 contains 2 apo[a] isoforms with 32 and 34 K-IV repeats.

DISCUSSION

The incidence of premature CHD in Asian Indians is among the highest reported for any major ethnic group (38). In contrast to other ethnic populations, it remains higher in immigrant Asian Indians compared with the native people of the country of immigration (1–3). These findings seem to indicate that in addition to nutritional and environmental factors (e.g., increased consumption of calories and fat, higher stress levels, sedentary life style), genetic factors may also contribute to the increased prevalence of CHD in the Asian Indian population.

The plasma lipid profile of the CHD patient population showed significantly higher levels of TG, TC, LDL-Chol, and apoB-100, as well as a lower plasma HDL-Chol level than the control group. These findings are not surprising, because elevated plasma levels of TC and LDL-Chol and low HDL-Chol levels are all known to be highly correlated with an increased risk for CHD. It is surprising, however, that the plasma lipid profile of the USA Asian Indian study group showed a trend similar to that of the CHD group in comparison with that of the Asian Indian control subjects. Asian Indians living in the USA had higher plasma levels of TG, TC, and LDL-Chol, as well as lower HDL-Chol levels than the subjects in the control group. Mean values for plasma TG, TC, and LDL-Chol were within the normal range in both these study groups. This observation is in agreement with previous studies that have reported plasma levels of Chol and other blood lipids to be in the low to normal range in immigrant Asian Indians (2, 28) as well as residents of India (39). However, 8.6% of the subjects in the USA Asian Indian study population had high plasma levels of TC (>239 mg/dl) and 7.4% had plasma LDL-Chol levels >160 mg/dl. The prevalence of high plasma levels of TC and LDL-Chol in the

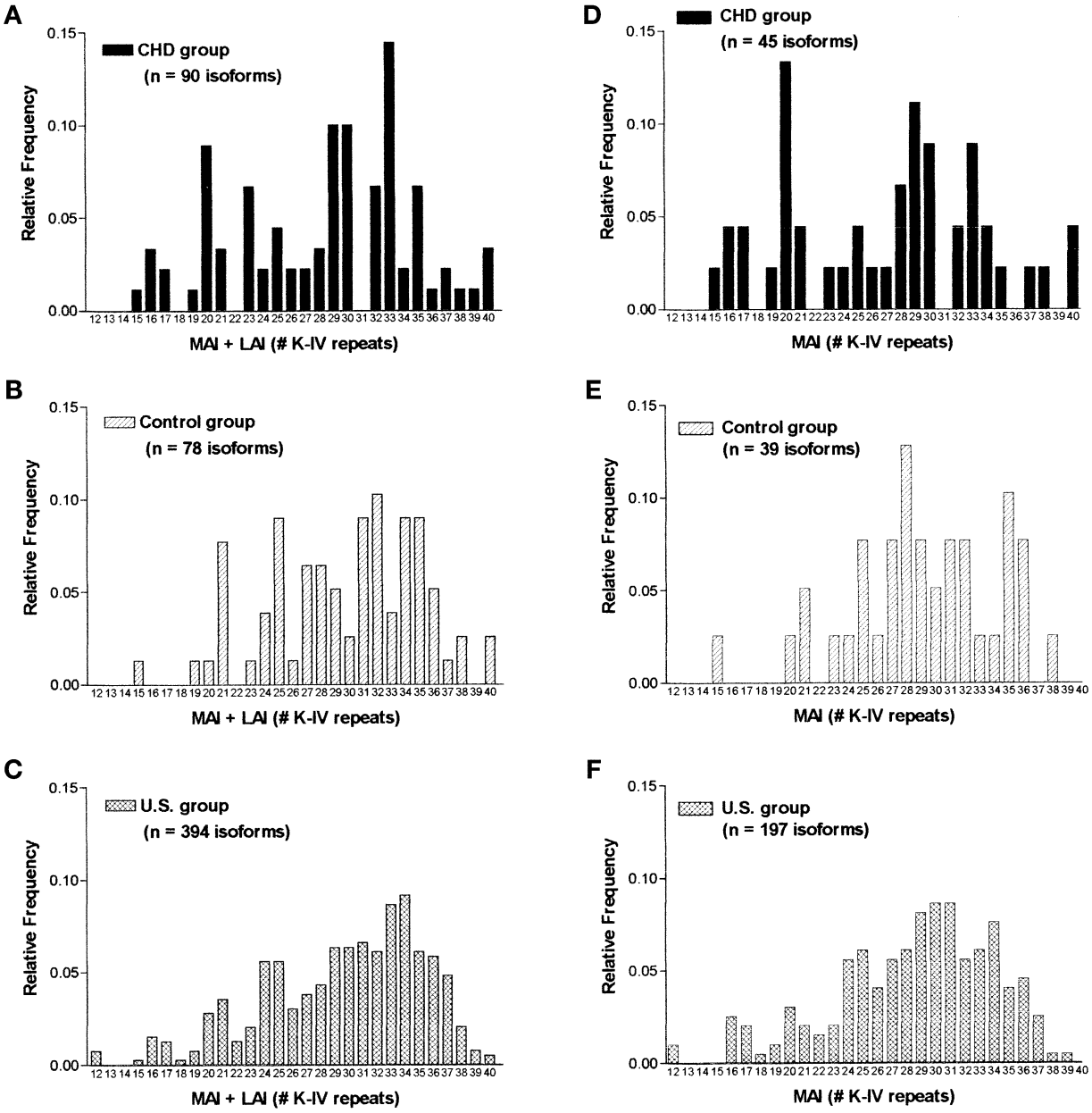

Fig. 3. Frequency distribution of apo[a] isoform sizes in the coronary heart disease (CHD) group, control group, and USA Asian Indian group.

USA Asian Indian group was similar to that of the CHD group (12.3% and 7%, respectively) but was higher than that of the control group (2.2% and 0%, respectively). These data indicate that Asian Indians living in the USA may be at increased risk for CHD.

Plasma Lp[a] levels were highly skewed toward the lower concentrations in all three study populations (Fig. 1), and the mean value was higher in the CHD patient population versus the USA Asian Indian and control groups. These findings are in agreement with previous reported values for plasma Lp[a] levels in Asian Indians (28, 29), but are lower than values reported by this and another laboratory for Caucasian populations (40, 41).

Logistic regression analyses were used to identify significant predictors of CHD among Asian Indians living in India versus their USA counterparts. Age, gender, TC, LDL-Chol, and plasma Lp[a] were significant risk factors for CHD among Asian Indians living in India (Table 3). Because all diabetics and hypertensive subjects in this study population had CHD, probabilities for these risk factors could not be calculated, and diabetes and hypertension had to be removed from the model. In the USA group, only diabetes was found to be a significant predictor of CHD ($P = 0.013$), with a 6.6-times greater chance for incidence of CHD in diabetics than in nondiabetics ($P = 0.013$). However, it should be noted that only 14 subjects, or 7% of the USA study population, had reported CHD. It is quite likely that logistic regression analyses failed to identify significant predictors of CHD because of the low incidence of CHD in the USA group. Furthermore, none of the hypertensive subjects in this study population had CHD and so a probability could not be calculated for this risk factor.

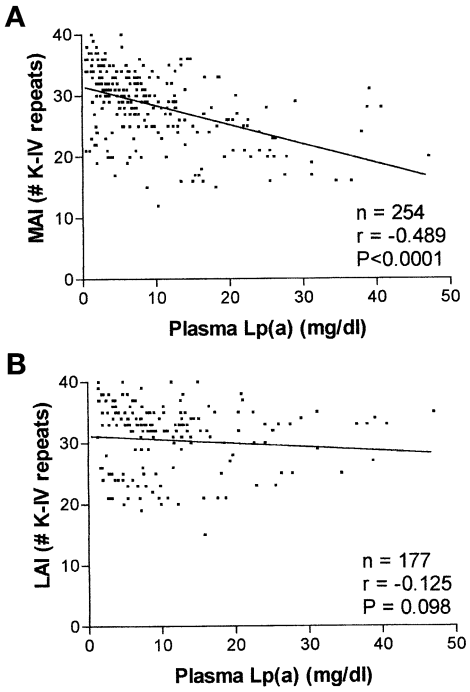

Fig. 4. Correlation of the (A) more abundant (MAI) and (B) less abundant (LAI) apo[a] isoform size with the plasma concentration of Lp[a] in Asian Indians of the three study groups.

Apo[a] isoform size frequency distributions were nonnormal in all three study groups (Fig. 3). Bimodal frequency distributions of apo[a] isoforms have been reported in Caucasian populations from the USA and Scotland (21, 42). A significantly higher proportion of the CHD patients expressed lower molecular weight apo[a] isoforms compared with the USA Asian Indian and control groups. USA Asian Indians who were born in east India expressed the highest percentage of high molecular weight apo[a] isoforms compared with those born in north, south or west India. The high plasma Lp[a] levels found in the CHD patients (mean, 12.65 mg/dl) and the low plasma Lp[a] levels in USA Asian Indians born in east India (mean, 5.57 mg/dl) are indicative of an inverse correlation between apo[a] isoform size and plasma Lp[a] concentration in these Asian Indian populations. It is generally believed that apo[a] polymorph size is inversely correlated with plasma Lp[a] level, and it has been previously reported that 42% of the variability in plasma Lp[a] levels can be attributed to apo[a] size polymorphism (27). However, a study has shown that the contribution of apo[a] isoform size to the control of plasma Lp[a] concentration was considerably lower than previously reported, because the variability in plasma Lp[a] concentration was not uniform across the apo[a] isoform size spectrum (42). These findings led us to a more detailed examination of the association of apo[a] isoform size with plasma Lp[a] concentration in our study populations. We therefore classified each subject's two apo[a] isoforms as either more or less abundant apo[a] isoforms (MAI or LAI, respectively) and examined the relative contribution of individual apo[a] isoforms to the control of plasma Lp[a] levels in all three study groups. A highly significant inverse correlation was found between MAI and plasma Lp[a] levels (Fig. 4A). In contrast, the correlation between LAI and plasma Lp[a] was much weaker (Fig. 4B). Calculated contributions of MAI and LAI size polymorphism were 24% and 2%, respectively. These values are significantly lower than those previously reported by Boerwinkle and colleagues (27). Our values, however, were derived from R^2 whereas Boerwinkle and Sing (43) calculated their values by using a formula-derived estimator. Our data show that MAI size polymorphism is a strong predictor of plasma Lp[a] levels in Asian Indians.

In conclusion, the data from our study suggest that elevated plasma levels of Lp[a] confer a genetic predisposition to CHD in Asian Indians. The generally accepted cutpoint for CHD risk of >30 mg/dl Lp[a] may underestimate that risk among Asian Indians. A more appropriate cutpoint for this population may be >19 mg/dl Lp[a]. The synergistic effect of nutritional and environmental factors, combined with a genetic predisposition, may put Asian Indians living in the USA at increased risk for premature CHD. Apo[a] phenotyping and determination of plasma Lp[a] concentration, as part of a routine screening procedure of independent risk factors for CHD, may aid in the identification of individuals who are genetically predisposed to CHD. Asian Indians appear to be a population well suited for studying the relationship of Lp[a] and premature CHD.

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REFERENCES

1. Enas, E. A., A. Garg, M. A. Davidson, V. M. Nair, B. A. Huet, and S. Ysuf. 1996. Coronary heart disease and its risk factors in first-generation immigrant Asian Indians to the United States of America. *Indian Heart J.* **48:** 343–353.
2. McKeigue, P. M., G. J. Miller, and M. G. Marmot. 1989. Coronary artery disease in south Asians overseas: a review. *J. Clin. Epidemiol.* **41:** 597–598.
3. Balarajan, R. 1991. Ethnic difference in mortality from ischaemic heart disease in England and Wales. *Br. Med. J.* **302:** 560–564.
4. Chadha, S., S. Radhakrishnan, K. Ramachandran, U. Kaul, and N. Gopinath. 1990. Epidemiological study of coronary heart disease in urban population of Delhi. *Indian J. Med. Res.* **92:** 424–430.
5. Gupta, R., H. Prakash, S. Majumdar, S. Sharma, and V. P. Gupta. 1995. Prevalence of coronary heart disease and coronary risk factors in an urban population of Rajasthan. *Indian Heart J.* **47:** 331–338.
6. Bhatia, M. L. 1995. Prevalence of coronary heart disease in India: a contemporary view. *Indian Heart J.* **47:** 339–342.
7. Enas, E. A., J. Dhawan, and S. Petkar. 1997. Coronary artery disease in Asian Indians: lessons learnt and the role of lipoprotein [a]. *Indian Heart J.* **49:** 25–34.
8. Hughes, L. O., U. Raval, and E. B. Raftery. 1989. First myocardial infarction in Asian and white men. *Br. Med. J.* **298:** 1345–1350.
9. Lawrence, R. E., and W. A. Littler. 1985. Acute myocardial infarction in Asians and whites in Birmingham. *Br. Med. J.* **290:** 1472.
10. Begom, R., and R. B. Singh. 1995. Prevalence of coronary artery

disease and risk factors in urban population of south and north India. *Acta Cardiol.* **3:** 227–240.
11. Reddy, K. S. 1993. Cardiovascular disease in India. *World Health Stat. Q.* **46:** 101–107.
12. Raman Kutty, V., K. G. Balakrishnan, A. K. Jayashree, and J. Thomas. 1993. Prevalence of coronary heart disease in the rural population of Trivandrum, Kerala, India. *Int. J. Cardiol.* **39:** 59–70.
13. Forouchi, N., and P. M. McKeigue. 1997. How far can risk factors account for excess coronary mortality in south Asians? *Can. J. Cardiol.* **13:** 47B.
14. Laws, A., J. L. Jeppesen, P. C. Maheux, P. Schaaf, Y. D. Chen, and G. M. Reaven. 1994. Resistance to insulin stimulated glucose uptake and dyslipidemia in Asian Indians. *Arterioscler. Thromb.* **14:** 917–922.
15. McKeigue, P. M., B. Shah, and M. G. Marmot. 1991. Relation of central obesity insulin resistance with high diabetes prevalence and cardiovascular risk in south Asia. *Lancet.* **336:** 383–386.
16. Mohan, V., P. S. Sharp, H. R. Cloke, J. M. Burrin, B. Schemer, and E. M. Kohner. 1987. Serum immunoreactive insulin responses to a glucose load in Asian Indian and European type 2 (non insulin dependent) diabetic patients and control subjects. *Diabetologia.* **29:** 235–237.
17. Utermann, G. 1995. Lipoprotein[a]. *In* The Metabolic Basis of Inherited Disease. C. R. Scriver, A. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill, New York. 1887–1912.
18. Berg, K. 1990. Lp[a] lipoprotein: an overview. *In* Lipoprotein [a]. A. M. Scanu, editor. Academic Press, San Diego, CA. 1–23.
19. Bostom, A. G., L. A. Cupples, J. L. Jenner, J. M. Ordovas, L. J. Seman, P. W. Wilson, E. J. Schaefer, and W. P. Castelli. 1996. Elevated plasma lipoprotein [a] and coronary heart disease in men aged 55 years and younger: a prospective study. *J. Am. Med. Assoc.* **276:** 544–548.
20. Sandholzer, C., E. Boerwinkle, N. Saha, M. C. Tong, and G. Utermann. 1992. Apolipoprotein[a] phenotypes, Lp[a] concentration and plasma lipid levels in relation to coronary heart disease in a Chinese population; evidence for the role of the apo[a] gene in coronary heart disease. *J. Clin. Invest.* **89:** 1040–1046.
21. Lackner, C., E. Boerwinkle, C. C. Leffert, T. Rahmig, and H. H. Hobbs. 1991. Molecular basis of apolipoprotein[a] isoform size heterogeneity as revealed by pulsed-field gel electrophoresis. *J. Clin. Invest.* **87:** 2077–2086.
22. Berg, K., S. Djurovic, and D. L. Rainwater. 2000. Gene encoding the apo(a) protein in LPA. *Atherosclerosis.* **148:** 203.
23. Hajjar, K. A., D. Gavish, J. L. Breslow, and R. L. Nachman. 1989. Lipoprotein[a] modulation of endothelial cell surface fibrinolysis and its potential role in atherosclerosis [letter]. *Nature.* **339:** 303.
24. Edelberg, J. M., M. Gonzalez-Gronow, and S. V. Pizzo. 1989. Lipoprotein[a] inhibits streptokinase-mediated activation of human plasminogen. *Biochemistry.* **28:** 2370–2374.
25. Utermann, G., H. J. Menzel, H. G. Kraft, H. C. Duba, H. G. Kemmler, and C. Seitz. 1987. Lp[a] glycoprotein phenotypes: inheritance in relation to Lp[a]-lipoprotein concentrations in plasma. *J. Clin. Invest.* **80:** 458–465.
26. Boerwinkle, E., C. C. Leffert, J. Lin, C. Lackner, G. Chiesa, and H. H. Hobbs. 1992. Apolipoprotein[a] gene accounts for greater than 90% of variation in plasma lipoprotein[a] concentrations. *J. Clin. Invest.* **90:** 52–60.
27. Boerwinkle, E., H. G. Menzel, H. G. Kraft, and G. Utermann. 1989. Genetics of the quantitative Lp[a] lipoprotein trait. III. Contribution of Lp[a] glycoprotein phenotypes to normal lipid variation. *Hum. Genet.* **82:** 73–78.
28. Anand, S. S., E. A. Enas, J. Pogue, S. Haffner, T. Pearson, and S. Yusuf. 1998. Elevated lipoprotein[a] levels in south Asians in North America. *Metabolism.* **47:** 182–184.
29. Mohan, V., R. Deepa, S. P. Haranath, G. Premalatha, M. Rema, N. G. Sastry, and E. A. Enas. 1998. Lipoprotein[a] is an independent risk factor for coronary artery disease in NIDDM patients in south India. *Diabetes Care.* **21:** 1819–1823.
30. Siedel, J., E. Hagele, J. Ziegenhorn, and A. W. Wahlenfeld. 1983. Reagent for the enzymatic determination of serum total cholesterol with improved lipolytic efficiency. *Clin. Chem.* **29:** 1075–1080.
31. Warnick, G. R., J. Benderson, and J. J. Albers. 1982. Dextran sulfate-Mg^{2+} precipitation procedure for quantification of high-density lipoprotein cholesterol. *Clin. Chem.* **28:** 1379–1388.
32. Nagele, U., E. Hagele, G. Sauer, E. Wiedermann, P. Lehmann, A. W. Wahlefeld, and W. Gruber. 1984. Reagent for the enzymatic determination of serum total triglyceride with improved lipolytic efficiency. *J. Clin. Chem. Clin. Biochem.* **22:** 165–174.
33. Friedewald, W. T., R. I. Levy, and D. Fredrickson. 1972. Estimation of the concentration of low-density lipoprotein in plasma, without the use of the preparative ultracentrifuge. *Clin. Chem.* **18:** 499–502.
34. Li, K. M., D. E. Wilcken, and N. P. Dudman. 1994. Effect of serum lipoprotein[a] on estimation of low-density lipoprotein cholesterol by the Friedewald formula. *Clin. Chem.* **40:** 571–573.
35. Gaubatz, J. W., G. L. Cushing, and J. D. Morrisett. 1986. Quantitation, isolation and characterization of human lipoprotein[a]. *Methods Enzymol.* **129:** 167–185.
36. Marcovina, S. M., Z. H. Zhang, V. P. Gaur, and J. J. Albers. 1993. Identification of 34 apolipoprotein[a] isoforms: differential expression of apolipoprotein[a] alleles between American blacks and whites. *Biochem. Biophys. Res. Commun.* **191:** 1192–1196.
37. Gaubatz, J. W., K. I. Ghanem, J. Guevara, Jr., M. L. Nava, W. Patsch, and J. D. Morrisett. 1990. Polymorphic forms of human apolipoprotein[a]: inheritance and relationship of their molecular weights to plasma levels of lipoprotein[a]. *J. Lipid Res.* **31:** 603–613.
38. Enas, E. A., S. Ysuf, and J. Mehta. 1996. Meeting of the international working group on coronary artery disease in south Asians. *Indian Heart J.* **48:** 727–732.
39. Hughes, K., T. C. Aw, P. Kuperan, and M. Choo. 1997. Central obesity, insulin resistance, syndrome X, lipoprotein[a], and cardiovascular risk in Indians, Malays, and Chinese in Singapore. *J. Epidemiol. Community Health.* **51:** 394–399.
40. Guyton, J. R., G. H. Dahlen, W. Patsch, J. A. Kautz, and A. M. Gotto, Jr. 1985. Relationship of plasma lipoprotein Lp(a) levels to race and to apolipoprotein B. *Arteriosclerosis.* **5:** 265–272.
41. Sandholzer, C., N. Saha, J. D. Kark, A. Rees, W. Jaross, H. Dieplinger, F. Hoppichler, E. Boerwinkle, and G. Utermann. 1992. Apo(a) isoforms predict risk for coronary heart disease: a study in six populations. *Arterioscler. Thromb.* **12:** 1214–1226.
42. Gaw, A., E. A. Brown, and I. Ford. 1998. Impact of apo[a] length polymorphism and the control of plasma Lp[a] concentrations: evidence for a threshold effect. *Arterioscler. Thromb. Vasc. Biol.* **18:** 1870–1876.
43. Boerwinkle, E., and C. F. Sing. 1986. Bias of the contribution of single-locus effects to the variance of a quantitative trait. *Am. J. Hum. Genet.* **39:** 137–144.