Apo lipoprotein A1 gene polymorphisms predict cardio-metabolic risk in South Asian immigrants

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Abstract. *Objectives:* Coronary artery disease (CAD) is a leading cause of death globally with increasing burden in South Asians in the US. Specific genetic variants that influence CAD have not been fully assessed in South Asian Immigrants. The goal is to identify Apo lipoprotein A1 (*APOA1*) gene polymorphisms and their association with CAD risk factors, metabolic syndrome and dysfunctional HDL (Dys-HDL).

Methods: A community-based study on South Asians aged 35–65 years without CAD was conducted. *APOA1* gene sequencing was performed and genotypes compared with cardiovascular findings.

Results: The prevalence of metabolic syndrome and dysfunctional-HDL was 29.7% and 26%, respectively. Six novel *APOA1* gene single nucleotide polymorphisms (SNPs) were analyzed. Three of the six SNPs (G2, G3, and G5) were found to be associated with metabolic syndrome; G2 (T655C) ($p = 0.044$), G3 (T756C) ($p = 0.037$) and G5 (T1001C) ($p = 0.037$). *APOA1* gene SNP G1 (T319C) was highly correlated with low HDL levels ($p = 0.001$). In our study, both associations of *APOA1* SNPs with metabolic syndrome and low HDL remained after age-adjustment.

Conclusion: Discovery of novel gene polymorphisms will help to understand further the causes of excess CAD risk in South Asians so that preventative strategies targeted to high-risk group can be developed.

Keywords: Coronary artery disease, risk factors, South Asian immigrants, polymorphisms, Apo Lipoprotein A1, dysfunctional high density lipoprotein

1. Introduction

Aggressive clinical and public health interventions have resulted in significant reduction in cardiovascular disease (CVDs) and mortality. However, CVD in general and coronary artery disease (CAD) in particular continue to be the leading causes of mortality and morbidity in the US, accounting for more than 40% of all deaths [1]. Moreover, there is troubling evi-

dence that the prevalence of CAD and its risk factors are on the rise in immigrant populations that constitute more than 11% of the US population. Event rates in South Asians (people with ancestors from the Indian subcontinent, i.e., India, Pakistan, Bangladesh, Nepal, Bhutan, and Sri Lanka) have doubled in the past two decades, with the prevalence of CAD in South Asian immigrants (SAIs) as 9.4% [2–4]. South Asian immigrants exhibit a higher prevalence of CAD and risk factors compared with Caucasians [2–5]. These findings are not limited to the US [4–9]. Although South Asians represent the second largest and fastest growing Asian immigrant population in the US, little is known regarding their increased risk for CAD [7]. Not uniform

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groups, SAIs include ethnic subgroups with different cultures and practices, but as a whole, SAIs, compared to other populations, have much higher prevalence of type 2 diabetes (T2D), metabolic syndrome (MS), insulin resistance, central obesity, dyslipidemias [lower HDL cholesterol, increased lipoprotein a-Lp(a), higher triglycerides (TGs) and increased thrombotic tendency], and lower levels of physical activity [3–9]. SAIs as a whole have the highest prevalence of CAD (21.5% in men and 15.9% in women) and a higher mortality due to CAD compared to other immigrant groups and Caucasians [10]. Further, CAD risk factors are present at a younger age in South Asians compared to other populations resulting in CAD at a younger age than in other populations [2]. Conventional risk factors, insulin resistance, or MS (although important in predicting CAD risk), may not fully account for the increased risk in SAIs [9,10]. Approximately one-third of CV events are not readily attributed to traditional CV risk factors [11], often referred to as the *detection gap*, suggesting that other non-traditional or “novel” conditions contribute to CV disease. Thus, a search for additional markers is warranted to promote early detection and prevention of CAD, especially in high-risk groups such as SAIs. Given the high prevalence of CAD and its risk factors in SAIs, a major challenge associated with primary prevention of CAD involves detection of early risk factors as well as early diagnosis of subclinical CAD in those individuals at a high CAD risk but are asymptomatic.

Among the numerous genetic and lifestyle factors associated with CAD, dyslipidemias are one of the most important. HDL cholesterol plays a protective role with low HDL as an independent risk factor for CAD [12]. This protective effect of HDL is related to its role as an anti-atherogenic, anti-oxidant, and anti-inflammatory agent preventing low-density lipoprotein (LDL) oxidation, primarily through Apo lipoprotein A-I (Apo A-I), the main protein component of HDL. Apo A-I is strongly correlated with low HDL levels and function [13]. However, several recent studies in patients with CAD have shown that HDL is not only *ineffective* as an anti-oxidant but, paradoxically, is a pro-oxidant assessed by its lipid peroxide content [13,14].

South Asians in general and SAIs in particular are commonly known to have low HDL levels [5,6]. HDL is likely to be less effective at reverse cholesterol transport (RCT) along with an increased prevalence of metabolic syndrome (MS), T2D and CAD in this population [13–16]. However, presence of *APOA1* gene polymorphisms and associations with low HDL levels,

MS and sub-clinical CAD in this population has not been fully examined [16,17]. In addition, understanding of HDL functionality is important with respect to low HDL levels in SAIs. We predict that SAIs have specific *APOA1* gene polymorphisms that may relate to low HDL levels and the increased CAD risk. The objective of the current study is to identify *APOA1* gene polymorphisms and their association with low HDL levels and other risk factors for CAD in SAIs in the United States. Further, to explore novel non-conventional risk markers for CAD, we determined the association of pro-inflammatory or dysfunction HDL (Dys-HDL) with novel *APOA1* polymorphisms

1.1. Apo lipoprotein A-I

Structure and Function: Apo A-I (*APOA1* gene codes for Apo A-I protein) is the major protein component of HDL, consisting of 243 amino acids. It is synthesized mainly in the liver and to some extent in the small intestine. The inverse relationship between HDL and CAD has been attributed to the role of HDL and its major constituent, Apo A-I, involved in reverse cholesterol transport (RCT) [18,19]. HDL can be separated into two components, HDL₂ and HDL₃, with ultracentrifugation. HDL can also be separated into particles containing Apo A-I without Apo A-II and particles that contain both Apo A-I and Apo A-II [18,20].

The *APOA1* gene is present along with the *APOC3* and *APOA4* genes on chromosome 11 (at 11q23.3-qter). It has been shown previously that one of the gene allele variants (A allele) of the *APOA1* gene contributes to the severity of CAD and low levels of HDL among Northern Indians [21].

2. Methods

The study received Institutional Review Board approval from both the University of Kansas Medical Center and Medical College of Georgia (now called Georgia Health Sciences University). Detailed study methods have been provided previously [15]. Briefly, a prospective cross-sectional study design was used with SAIs belonging to an Indian origin between the ages of 35–65 years and recruited from the main Hindu temples in the States of Georgia, Kansas and Missouri. This age range was chosen because CAD and risk factors occur at younger ages in SAIs compared to other populations [2]. The SAI population in the US is most readily accessed through their temples of

worship. This approach was used, because no national level census or data are available on South Asians for providing an estimate of the total population within the US. Therefore, this study may not be generalizable, however, most SAIs who visit temples on weekends represent several ethnic groups. Information was obtained on socio-demographic status, ethnicity (based on spoken language), personal lifestyle characteristics, and both traditional and non-traditional risk factors for CAD. Twelve-hour fasting blood samples were collected for measurements of high sensitivity C-reactive protein (hsCRP), total lipid testing including total cholesterol, triglycerides (TGs), high density lipoprotein (HDL), low density lipoprotein (LDL), and lipoprotein a (Lp[a]). Insulin, fibrinogen, homocysteine and Apo A-I serum levels were also measured. The diagnosis of MS was made using the International Diabetes Federation (IDF) definition [22].

3. Carotid ultrasound doppler for common carotid intima-media thickness (CCA-IMT)

Assessment of common carotid artery intima-media thickness (CCA-IMT) is a well-recognized method in diagnosing atherosclerosis [23]. Furthermore, CCA-IMT has been used recently, not only as a surrogate end point for atherosclerosis of CAD, but as an accepted indicator of the presence and extent of CAD [24]. Details on carotid CCA-IMT has been published previously [25]. Briefly, B-mode ultrasound scanning of bilateral carotid arteries was performed by a trained non-invasive vascular ultrasound technician using a SonoCalcTM IMT machine (SonoSite, Inc Bothell, WA) with a 10.0 MHz linear array transducer. In order to maintain consistency, the same trained ultrasound technicians were selected at each site to obtain the CCA-IMT scanning results. Both common carotid arteries were scanned with the subject in the supine position. A total of four images were obtained on each side, 1 cm proximal to the carotid bulb using an anterior approach. Images were obtained at an angle to show good lumen-intima and media-adventitia demarcation and were recorded and stored on a disk for off-line analysis. ECG leads were placed to obtain end-diastolic measurements. The CCA-IMT approach for IMT measurements was preferred because of reproducibility [26] and predictability for future cardiovascular events [27]. Any focal thickening of the intima-media complex or carotid plaque was not included in the analysis. Cardiologists were blinded to the partic-

ipants' clinical information and analyzed IMT results using automated edge detection technology software (Sono CalcTM IMT). Measurements of the far wall of the carotid artery were done as they are more indicative of the true thickness of the arterial wall [27]. A CCA-IMT cut-off of ≥ 0.80 mm was chosen and analyzed as positive with IMT measurements based on the available evidence supporting the presence of sub-clinical CAD [26,27].

3.1. Assessment of dysfunctional HDL (Dys-HDL)

According to National Cholesterol Education Program (NCEP) ATP III guidelines, an HDL level < 40 mg/dl is defined as an independent risk factor for CAD and low HDL is often present in high-risk patients with CAD [27]. Current data indicates that a 1% increase in HDL serum concentration can decrease CV risk by 2–3%, independent of LDL levels [27]. However, HDL can have this protective effect only if it is functional. We have shown previously the presence of Dys-HDL in 50% of SAIs without CAD and found an association with lipoprotein [a], low HDL and CCA-IMT (age-adjusted) [15,16].

The diagnosis of Dys-HDL has historically been made with a cell-based assay that requires endothelial cells, smooth muscle cells, and monocytes. However, the use of a cell-based assay is not practical for large-scale studies. Hence, a cell-free assay has been developed to detect dysfunctional HDL [28,29]. The details on Dys-HDL assessment with cell-free assays and the HDL inflammatory index have been published previously [15,16,30].

3.2. DNA isolation and APOA1 gene sequencing

Each whole blood sample (4 mls) was assigned a unique DNA identification code. Genomic DNA was extracted from whole blood (2 mls) using the Qiagen FlexiGene DNA Kit (Qiagen®, Valencia, CA, USA) which yields 4–12 μ g of DNA. An aliquot of DNA was diluted and the absorbance at λ 260 nm and λ 280 nm measured using an Eppendorf Biophotometer for verification of quality and concentration. DNA samples were diluted to 50 ng/ μ l and stored at -80°C .

PCR amplification was performed on genomic DNA to amplify a 1683bp fragment of the *APOA1* gene encompassing the SNPs to be analyzed using forward 5' CGGCAGAGACTATGTGTCCAG 3' and reverse 5' CCAGATCCTTGCTCATCTCC 3' PCR primers. The PCR fragment was purified using the QIA

quick PCR purification kit (Qiagen, Valencia, CA). Sequencing was performed commercially by ACGT Inc. (Wheeling, IL) and results analyzed using Ridom Trace Edit software (Ridom Bioinformatics, Würzburg, Germany) and NCBI BLAST sequence analysis. The reference sequence used was NCBI RefSeq NC_000011.9, derived from the Genome Reference Consortium Human Build 37 (GRCh37), Primary Assembly (<http://www.ncbi.nlm.nih.gov/gene>).

3.3. Data analysis and power calculation

We enrolled 129 first generation SAIs from different ethnic backgrounds. Our pilot study consisted of a fixed sample of 130 participants to assess the association of a novel *APOA1* gene polymorphism with Dys-HDL, MS, low HDL and sub-clinical CAD using CCA-IMT as a surrogate marker for atherosclerosis. *APOA1* gene analysis studies were performed on 94 subjects. The power calculation for assessing Dys-HDL (primary outcome) was based on a chi-square contingency table analysis [Dys-HDL (Yes/No) vs. CCA-IMT (Yes/No)], and from available data on CCA-IMT in South Asians [25]. Assuming that 20% of 130 individuals have positive CCA-IMT, we calculated a 90% power at 5% alpha level and 85% power at 1% alpha level to detect the Dys- HDL difference in two groups.

Baseline socio-demographic characteristics and CAD risk factors were summarized by frequency distributions and percentages for qualitative measures and means and standard deviations for quantitative measures. Maximum likelihood estimates and asymptotic 95% confidence intervals were calculated for the prevalence of disease/diagnosis outcome measures. Bivariate tests of association and odds ratios were performed by simple logistic regression. Multiple logistic regression models were used to assess the relative importance of variables found to be significantly associated with the outcome from the bivariate assessments. All statistical tests were two-sided and performed at the 0.05 level of significance.

4. Results

Of the total sample, complete information was obtained on 129 subjects, constituting our study sample (Table 1). Partial *APOA1* gene sequencing was completed in 94 subjects. The mean age was 51 ± 9.23 years with almost an equal number of males and females (Table 1). The study group presented a ho-

Table 1
Socio-demographics of study sample ($n = 129$)

Variable	n (%)
Age (years)	$51.30 \pm 9.23^*$
Gender	
Male	$51.04 \pm 9.64^*$
Female	$51.68 \pm 8.70^*$
Ethnicity	
South Indian	33 (25.6)
Gujarati	23 (17.8)
Hindi	23 (17.8)
Bengali	10 (7.8)
Punjabi	8 (6.2)
Other	32 (26.2)
Work Type	
Employee full time	91 (70.5)
Homeworker	14 (10.9)
Employee part time	9 (7.0)
Unemployed	6 (4.7)
Other	8 (6.2)
Education	
Postgraduate	67 (51.9)
Graduate	30 (23.3)
Undergraduate	24 (18.6)
Other	1 (0.8)

*Means $\pm$ SD (Standard Deviation).

mogenous mixture of various ethnicities including Hindi speaking (18%), Gujaratis (18%), and South Indians (26%). More than 50% received up to post-graduate level education. The prevalence of CAD risk factors was (a) hypertension- 45% (b) high cholesterol ≥ 200 mg/dl- 41.6%, (c) HDL < 40 mg/dl- 26.4% (d) LDL ≥ 150 mg/dl-16.9%, (e) Lp[a]-35.7% (f) hsCRP (≥ 5)-48.74%, (g) BMI ≥ 23 -78.4% (h) obesity (BMI ≥ 30)-18.2%; and (i) family history of CAD and T2D was 34.4% and 48.4%, respectively (Table 2). About 80% were physically active, based on questionnaire survey data (not shown). Sub-clinical CAD using CCA-IMT ≥ 0.8 mm, as a surrogate marker for atherosclerosis, was seen in 38.5% of subjects. Obesity was also reflected as an increase in waist circumferences in both genders (Table 2). Based on the International Diabetes Federation (IDF) criteria, MS was seen in 29.7% of SAIs without CAD.

4.1. Association of *APOA1* gene SNPs with MS, CCA-IMT and intermediate phenotypes

DNA was analyzed for six *APOA1* gene polymorphisms or SNPs in the SAIs without known CAD (Tables 3 and 4). When individual SNPs were tested directly against MS and CCA-IMT as outcome variables, three of the six SNPs (G2, G3, and G5) were found to

Table 2
Coronary artery diseases (CAD) risk factors and markers ($n = 129$)

Variables	n (%)	Mean $\pm$ Std
BMI		26.37 $\pm$ 5.08
Normal (< 23)	27 (21.62)	21.84 $\pm$ 1.68
Overweight (23–30)	76 (60.14)	25.82 $\pm$ 2.05
Obese ($\geq$ 30)	22 (18.24)	34.07 $\pm$ 6.45
Total LDL		117.63 $\pm$ 35.61
Normal (< 150mg/dl)	103 (83.06)	106.08 $\pm$ 24.73
Abnormal ($\geq$ 150mg/dl)	21 (16.94)	174.95 $\pm$ 24.44
Total HDL		48.38 $\pm$ 10.99
Normal (> 40 mg/dl)	92 (73.6)	52.95 $\pm$ 8.98
Abnormal ($\leq$ 40 mg/dl)	33 (26.4)	35.79 $\pm$ 4.31
Dys-HDL		0.83 $\pm$ 0.74
Normal (< 1.0)	88 (73.95)	0.53 $\pm$ 0.17
Dysfunctional ($\geq$ 1.0)	31 (26.05)	1.71 $\pm$ 1.02
Total Cholesterol		193.17 $\pm$ 38.97
Normal (< 200 mg/dl)	73(58.4)	167.74 $\pm$ 22.34
Abnormal ($\geq$ 200 mg/dl)	52 (41.6)	229.31 $\pm$ 27.37
Triglycerides		160.44 $\pm$ 114.56
Normal (< 150 mg/dl)	73 (58.4)	99.23 $\pm$ 26.88
Abnormal ($\geq$ 150 mg/dl)	52 (41.6)	246.90 $\pm$ 134.70
Lipoprotein [a]		13.61 $\pm$ 18.99
Normal (< 10 mg/dl)	79 (64.23)	4.59 $\pm$ 1.79
Abnormal ($\geq$ 10 mg/dl)	44 (35.77)	30.02 $\pm$ 24.46
Apo lipoprotein A-I		150.36 $\pm$ 31.94
Normal (94–176 mg/dl)	95 (76.0)	142.06 $\pm$ 22.19
Abnormal (else)	30 (24.0)	178.13 $\pm$ 41.30
hsCRP		3.32 $\pm$ 2.56
Normal (< 5 mg/L)	63 (51.22)	1.24 $\pm$ 1.09
Abnormal ($\geq$ 5 mg/L)	60 (48.78)	5.55 $\pm$ 1.60
Homocysteine		10.34 $\pm$ 7.71
Normal (< 12 umol/L)	74 (77.89)	7.96 $\pm$ 2.06
Abnormal ($\geq$ 12 umol/L)	21 (22.11)	18.79 $\pm$ 13.08
CCA-IMT		0.73 $\pm$ 0.16
Normal (< 0.8 mm)	71 (68.54)	0.649 $\pm$ 0.094
Abnormal ($\geq$ 0.8 mm)	32 (31.46)	0.916 $\pm$ 0.15
Waist Circumference (cm)		93.72 $\pm$ 14.08
Male	61 (48.66)	95.53 $\pm$ 12.74
Female	46 (27.98)	91.47 $\pm$ 15.58
Physical Activity		
No	20 (15.50)	
Yes	109 (84.50)	
Smoking		
No	121 (93.80)	
Yes	8 (6.20)	
Type 2 Diabetes (T2D)		
No	89 (69.53)	
Yes	39 (30.47)	
Hypertension [§]		
No	70 (54.69)	
Yes	58 (45.31)	
Family History of T2D		
No	48 (43.24)	
Yes	63 (56.76)	
Family History of CAD		
No	67 (60.36)	
Yes	44 (39.64)	
[†] MS Prevalence		
No	68(53.1)	
Yes	38(29.7)	

[†]MS defined by International Diabetes Federation (IDF); Dys-HDL = Dysfunctional HDL measured by HDL Inflammatory index; hsCRP = high sensitivity C reactive protein; CCA-IMT = Common carotid artery intima media thickness; [§]History/examination and/or blood test.

Table 3
Association of Apo A1 Gene Polymorphisms (un-adjusted) with HDL, Dys-HDL, IMT, and MS ($n = 94$)

Apo A1 SNPs		Dys-HDL		CCA-IMT		HDL		MS	
		< 1	≥ 1	No	Yes	≥ 40 mg/dl	< 40 mg/dl	No	yes
G1-P319 (T319C)	WT	40 (48.2)*	8 (9.64)	31 (45.6)	7 (10.3)	31 (36.5)	18 (21.2)	36 (42.4)	13 (15.3)
	Heterozygous	23 (27.7)	6 (7.23)	20 (29.4)	5 (7.35)	29 (34.1)	0 (0.0)	25 (29.4)	4 (4.71)
	Mutant	5 (6.02)	1 (1.20)	4 (5.88)	1 (1.47)	5 (5.88)	2 (2.35)	3 (3.53)	4 (4.71)
	P-Value [§]	0.902		0.987		0.001		0.053	
G2-P655(T655C)	WT	10 (11.9)	2 (2.38)	11 (15.9)	1 (1.45)	9 (10.6)	3 (3.53)	12 (14.1)	0 (0.0)
	Heterozygous	20 (23.8)	8 (9.52)	15 (21.7)	5 (7.25)	21 (24.7)	8 (9.41)	22 (25.9)	7 (8.24)
	Mutant	39 (46.4)	5 (5.95)	30 (43.5)	7 (10.1)	34 (40.0)	10 (11.8)	29 (34.1)	15 (17.7)
	P-Value	0.187		0.589		0.895		0.044	
G3-P756(T756C)	WT	10 (11.8)	2 (2.35)	11 (15.7)	1 (1.43)	9 (10.5)	3 (3.49)	12 (14.0)	0 (0.0)
	Heterozygous	19 (22.4)	8 (9.41)	14 (20.0)	5 (7.14)	20 (23.3)	8 (9.3)	21 (24.4)	7 (8.14)
	Mutant	41 (48.2)	5 (5.88)	31 (44.3)	8 (11.4)	35 (40.7)	11 (12.8)	30 (34.9)	16 (18.7)
	P-Value	0.127		0.601		0.904		0.037	
G4-P938(C938T)	WT	52 (61.2)	12 (14.1)	42 (60.0)	12 (17.1)	51 (59.3)	15 (17.44)	51 (59.3)	15 (17.4)
	Heterozygous	17 (20.0)	3 (3.53)	13 (18.6)	2 (2.86)	13 (15.1)	6 (6.98)	12 (14.0)	7 (8.14)
	Mutant	1 (1.18)	0 (0)	1 (1.43)	0 (0)	0 (0)	1 (1.16)	0 (0)	1 (1.16)
	P-Value	0.833		0.775		0.169		0.093	
G5-P1001 (T1001C)	WT	10 (11.4)	2 (2.27)	11 (15.1)	1 (1.37)	9 (10.1)	3 (3.4)	12 (13.5)	0 (0.0)
	Heterozygous	28 (31.8)	9 (10.2)	22 (30.1)	6 (8.22)	28 (31.5)	10 (11.2)	27 (30.3)	11 (12.4)
	Mutant	35 (39.8)	4 (4.55)	25 (34.25)	8 (11.0)	29 (32.6)	10 (11.2)	25 (28.1)	14 (15.7)
	P-Value	0.289		0.679		0.995		0.037	
G6-P1149(C1149T)	WT	67 (76.1)	14 (15.9)	52 (71.2)	15 (20.6)	60 (67.4)	22 (24.7)	59 (66.3)	23 (25.8)
	Heterozygous	6 (6.82)	1 (1.14)	6 (8.22)	0 (0)	6 (6.74)	1 (1.12)	5 (5.62)	2 (2.25)
	Mutant	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	P-Value	0.4103		0.238		0.416		0.638	

Dys-HDL, Dysfunctional HDL measure by HDL Inflammatory index, (LDL+HDL)/LDL; CCA-IMT, Common carotid artery intima media thickness; MS, Metabolic Syndrome, Central obesity and two or more of fasting glucose (≥ 100 mg/dl or T2D), obesity, HDL (≥ 40mg/dl), Blood pressure (≥130/≥ 85 mmHg); and Triglycerides (≥ 150 mg/d). *n(%) [§]Fisher – Exact test.

Table 4
Apo A-I Gene Polymorphisms (Age-adjusted) with HDL, Dys-HDL, IMT, and MS ($n = 94$)

	G1-P319 (T319C)		G2-P655 (T655C)		G3-P756 (T756C)		G4-P938 (C938T)		G5-P1001 (T1001C)		G6-P1149 (C1149T)	
	X ²	P	X ²	P	X ²	P	X ²	P	X ²	P	X ²	P
↓HDL	15.917	< 0.0001	0.921	0.337	0.695	0.405	3.028	0.082	0.059	0.808	1.900	0.169
Dys-HDL	0.253	0.615	2.243	0.134	2.824	0.093	0.466	0.495	2.054	0.152	0.056	0.813
MS	0.008	0.929	7.091	0.008	7.245	0.007	5.417	0.020	6.988	0.008	0.125	0.724
CCA-IMT	0.077	0.781	0.163	0.686	0.285	0.093	1.274	0.259	1.576	0.209	0.0001	0.994

[§]P-Values from the Logistical regression Dys-HDL = Dysfunctional HDL measure by HDL Inflammatory index using cell free assay; CCA-IMT = Common carotid artery intima media thickness; MS = Metabolic Syndrome defined by International Diabetes Federation definition [Central obesity and two or more of fasting glucose (≥100 mg/dl or T2D), obesity, HDL (≥ 40 mg/dl), Blood pressure (≥ 130/≥ 85 mmHg)].

be associated with MS: G2 (T655C) ($p = 0.044$), G3 (T756C) ($p = 0.037$) and G5 (T1001C) ($p = 0.037$). The *APOA1* gene SNP G1 (T319C) was highly correlated with low HDL levels ($p = 0.001$). Both associations of *APOA1* SNPs with MS and low HDL remained after age-adjustment (Table 4). Further, odds ratio analysis revealed the importance of *APOA1* SNP association with Dys-HDL. The odds of the presence of two of the SNPs, i.e., G4 (C938T) and G5 (T1001C), with Dys-HDL was 2.516 (1.16, 5.47) and 2.497 (1.27, 4.92), respectively as compared to absence of Dys-HDL. Also, the odds of having SNP G2 (T655C) and G3 (T756C) with MS was 2.791 (1.31, 5.94) and 2.799 (1.32, 5.92),

respectively as compared to those without MS. However, *APOA1* SNP association with CCA-IMT did not reach statistical significance and may be attributed to a small sample size in our study.

5. Discussion

The present study is unique and the first of its kind to focus on SAIs with dyslipidemias and MS and in identifying a significant association with *APOA1* gene polymorphisms with CAD intermediate outcomes. Several recent studies including, the INTERHEART case-

Table 5
Odds Ratio of Apo A1 gene polymorphisms with HDL, Dys-HDL, CCA-IMT, and MS ($n = 94$)

	G1-P319(T319C)	G2-P655(T655C)	G3-P756(T756C)	G4-P938(C938T)	G5-P1001(T1001C)	G6-P1149(C1149T)
↓HDL	0.088 (0.03, 0.29)	0.711 (0.36, 1.43)	0.747 (0.38, 1.48)	2.021 (0.92, 4.46)	0.920 (0.47, 1.79)	0.233 (0.03, 1.85)
Dys-HDL	1.224 (0.56, 2.69)	0.560 (0.26, 1.20)	0.522 (0.24, 1.11)	2.516 (1.16, 5.47)	2.497 (1.27, 4.92)	0.828 (0.17, 3.95)
MS	1.033 (0.51, 2.11)	2.791 (1.31, 5.94)	2.799 (1.32, 5.92)	0.515 (0.16, 1.63)	1.659 (0.75, 3.66)	0.786 (0.21, 3.00)
CCA-IMT	0.885 (0.37, 2.10)	1.190 (0.51, 2.77)	1.254 (0.55, 2.88)	0.710 (0.27, 1.90)	0.574 (0.27, 1.23)	< 0.001 (< 0.001, > 999)

Dys-HDL = Dysfunctional HDL measure by HDL Inflammatory index, (LDL+HDL)/LDL; CCA-IMT = Common carotid artery intima media thickness; MS = Metabolic Syndrome defined by International Diabetes Federation (IDF) definition i.e. Central obesity and two or more of fasting glucose (≥ 100 mg/dl or T2D), obesity, HDL (≥ 40 mg/dl), Blood Pressure ($\geq 130/\geq 85$ mmHg); and Triglycerides (≥ 150 mg/d).

control study showed that nine risk factors (dyslipidemia, diabetes, hypertension, abdominal obesity, tobacco exposure, physical inactivity, psychosocial stressors, low fruit and vegetable intake, and no alcohol consumption) contributed to the risk of myocardial infarction (MI) globally [31–33]. The ratio of Apo lipoprotein (Apo) B/A-I is considered the strongest MI risk factor and accounts for 54% of the total population attributable risk for MI [31,34]. Apo A-I is the main protein of HDL which is responsible for RCT. Although various factors such as genetic variation, diet, exercise, alcohol, smoking, hormones, and certain drugs can significantly influence the levels of HDL and Apo A-I [35], family and twin studies have demonstrated a strong genetic heritability accounting for up to 66% of the variability of HDL and Apo A-I levels [18,36]. Furthermore, 40–60% of the inter-individual variation in HDL concentrations is controlled at the gene level [37]. The strong positive correlation between plasma levels of Apo A-I and HDL suggests that *APOA1* gene polymorphisms may be linked to the variability in HDL levels as well as to its dysfunction [36,37].

More than 40 *APOA1* gene polymorphisms have been identified in several ethnic populations, including South Asians [21,30,37–44]. Each polymorphism may correlate with differing HDL activity and levels. However, not all *APOA1* polymorphisms are associated with CAD. Further, several point mutations have been identified, and one in particular is associated with low levels of HDL but not with an increased CAD risk and is, instead, associated with a reduction in CAD risk [40]. One such *APOA1* mutation encodes the Apo A-I Milano (Apo A-I_{Arg173Cys}) protein.

Qualitative differences in HDL particles, mediated through genetic variability in *APOA1* and other genes, may be as important as quantitative differences in plasma HDL level in determining CAD risk. Therefore, the identified nine risk factors are themselves potentially influenced by genetic variants, which, could act on their own, or in combination with other genetic or lifestyle factors. Genetic variants that influence these risk fac-

tors may also be associated with CAD [45,46]. However, recent studies identifying genetic associations with MI and/or CAD risk factors have largely been conducted among whites [45]. However, whether these findings can be extended to other ethnic groups remain to be demonstrated. In fact, the recent association between chromosome 9 variants and CHD, observed in Caucasians, was not replicated in African-Americans [47]. In addition, SNPs in several lipid-related genes seem to be strongly associated with plasma lipids [47] but inconsistently with CAD. However, recent genome-wide association studies have produced several robust genetic associations with lipid levels and CAD [47,48].

Our results are consistent with the results of these recent genome-wide association studies of blood lipid levels and MS and we identified to be significantly associated with MS and low HDL in SAIs, a group not well represented in research studies. Our collective findings, raise the question of whether HDL levels as measured by current assays, is a marker or mediator of coronary artery disease, now knowing the role of non-functional HDL in sub-clinical CAD measurable by CCA-IMT. Certain single-gene conditions characterized by low or very low HDL levels have premature coronary artery disease as one of their manifestations (for example, Tangier disease, which results from mutations in the *ABCA1* gene), whereas others leading to low HDL levels do not (e.g., Apo A-I-Milano protein). In fact, recent data have shown that HDL particles containing Apo A-I-Milano is more effective than HDL containing normal Apo A-I levels at maintaining endothelial cell homeostasis under stress, due to up-regulation of endothelial nitric oxide synthase expression and down-regulation of vascular cell adhesion molecule expression [48]. Thus, qualitative differences in the HDL particles, mediated through genetic variability in *APOA1* and other genes, may be as important as quantitative and qualitative differences in plasma HDL level in determining CAD risk.

People affected by MS are at an increased risk of CAD and T2D, which are large and rapidly-increasing

causes of illness and death globally. South Asians in general and SAIs in particular have a high prevalence of the MS compared with Europeans, and MS traits are highly heritable in this group [49]. Therefore, MS and its components are a major health concern, particularly in SAIs. The genome-wide association approach has met with some success in dyslipidemia, T2D and obesity phenotypes, with most studies to date being conducted in Europeans [49,50]. Our study has further confirmed a number of previously reported associations, in some cases for the first time in SAIs, and identified novel suggestive associations of *APOA1* gene polymorphisms with MS, requiring further confirmation.

The MS consists of a number of phenotypes that tend to co-occur, raising the question of whether or not they have common genetic mechanisms [50–52]. A number of definitions for the MS have been developed over the years, including those proposed by IDF, National Cholesterol Education Program Adult Treatment Panel III (NCEP ATPIII), or the World Health Organization (WHO) [53]. We chose the IDF definition, which is the most recent and incorporates ethnicity by providing different criteria for MS in different ethnic groups [53]. Most published associations for MS are only with individual component phenotypes, or in some cases with multiple phenotypes but not matching any of the above definitions. Joy et al. [54] reviewed a large number of genetic associations and linkage studies for MS using all definitions, and concluded that these studies have not provided confirmed associations. Our results from SAIs also found no evidence for common genetic mechanisms underlying MS, despite its high prevalence in this population. More work is needed to further understand MS components interactions with genotypes using genome-wide association studies as suggested by other authors [52].

Increased CCA-IMT has been associated with the extent and severity of CAD, and it is also an independent predictor of future MI [24]. A recent review summarizes the current knowledge of the effects of *APOA1* polymorphisms on CCA-IMT [55]. Genetic polymorphisms of *APOA1*/CIII/AIV, APOE and APOB have been suggested to modulate plasma lipid levels as well as the risk of CAD in Caucasians [55]. However, the effects of the polymorphisms in *APOA1* on CCA-IMT especially in SAIs are poorly known. Though we could not find association between novel *APOA1* polymorphisms and CCA-IMT; however, our study brings attention towards an increased need for large-scale studies.

This study has provided novel preliminary results; however, several limitations of this study must be con-

sidered including the cross-sectional design, which precludes any discussion on cause and effect. A longitudinal study would be needed to establish the temporal relationship. A smaller sample size in our study due to budget limitations and generalized results to all South Asian ethnic groups are also potential limitations. Further research is warranted to evaluate the diagnostic and prognostic utility of Apo A-I interactions with phenotypes related to CAD risk in this high risk group.

6. Conclusion

Apo A-I is the major protein component of HDL and known to be associated with HDL levels and its function. Epidemiologic studies have shown that HDL and Apo A-I levels are inversely correlated with the risk of developing CAD. Elevated plasma levels of total LDL, as well as lowered levels of HDL, are proven risk factors for atherosclerosis [9,10]. Functional distinctions between the plasma lipoproteins are mostly explained by specific combinations of Apo lipoproteins. Therefore, many Apo lipoproteins are crucial in the homeostasis and physiological control of lipid metabolism. Polymorphisms of several of these Apo lipoprotein genes have been studied extensively and shown to be associated with variation in plasma total, LDL and HDL levels. Furthermore, the associations between these gene polymorphisms and the clinical manifestations of atherosclerosis, especially CAD, have been investigated to a considerable extent. However, not much research has been done in SAIs, the group with excess CAD risk. Discovery of novel polymorphisms will help to understand further the causes of excess CAD risk in South Asians so that preventative strategies targeted especially to this high-risk group can be developed.

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