**APO A1-75 G to A substitution associated with severe forms of CAD, lower levels of HDL and apoA-I among Northern Indians**

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**Abstract.** Apolipoprotein A-I (APOA1 gene, apoA-I protein) is the major protein for plasma high density lipoprotein (HDL). The relationship of APOA1-75G/A polymorphism with lipid profile and coronary artery disease (CAD) is unclear. Out of 370 individuals initially recruited, 164 angiographically proven CAD patients (≥ 70% stenosis) and 36 individuals with normal coronaries or insignificant CAD (NCAD, ≤ 50% stenosis) from Delhi and adjoining areas were selected for analysis based on the set criteria. Polymorphism was determined by PCR followed by MspI restriction digestion. Lipid profile was estimated by enzymatic kit and apoA-I levels by immunoturbidimetry. A highly significant increasing trend in ‘A’ allele frequency was observed with the rise in severity of CAD: NCAD (0.097) < SVD (single vessel disease) (0.117) < DVD (double vessel disease) (0.223) < TVD (triple vessel disease) (0.291). In comparison to GG individuals, the OR of ‘A’ allele carriers to develop SVD, DVD, TVD was 1.3, 2.8 and 4.2 respectively (\(p_{\text{trend}} = 0.007\)). Analysis of intergenotypic variations in the lipid profile revealed significantly lower levels of HDL and apoA-I among ‘A’ allele carriers as compared to GG (patients). Our study, first of its kind from India, suggests that ‘A’ allele may contribute to severity of CAD and low levels of HDL & apoA-I. However, an in depth study with a larger set of sample is necessary.

Keywords: APOA1, apolipoprotein, CAD, polymorphism, India

**1. Introduction**

Central to pathogenesis of atherosclerosis is deposition of cholesterol in artery wall and within activated macrophages (foam cells). HDL is involved in the transport of cholesterol away from arterial walls to the liver, a process referred to as reverse cholesterol transport (RCT). In addition, HDL has other important atheroprotective functions including anti-inflammatory, anti-oxidant properties of HDL, though their elucidation is as yet unclear [1].

Apolipoprotein A-I (APOA1 gene, ApoA-I protein) is the major protein of high density lipoprotein (HDL). It is a 243 amino acid long peptide, synthesized mainly in the liver and to some extent in the small intestine. ApoA-I is the *in vivo* activator of Lecithin: cholesterol acyl transferase (LCAT), responsible for the esterification of cholesterol and thus, a major participant in the regulation of RCT [2].

APOA1 gene is present along with APOC3 and APOA4 genes on chromosome 11 (11q23.3-qter) [3]. Variations in the APOA1-C3-A4 genes have been associated with dyslipidemia and coronary artery disease (CAD) [4]. In this context, a common G to A transition, 75 bp upstream from the transcription start site in APOA1 gene, (denoted as −75 bp, −76 bp or −78 bp in different studies) has received most of the attention [5]. Many of the studies showed an association of rare ‘A’
allele of APOA1-75G/A polymorphism with higher levels of HDL and/or apoA-I [6–14]. However, others did not confirm this association [15–21]. Interestingly, an inverse association has also been reported [22]. A meta-analysis showed that rare ‘A’ allele might be associated with only marginally higher (by ∼0.5 g/l) apoA-I concentration [23].

Studies pertaining to the association of ‘A’ allele with CAD have also revealed controversial findings. In an Australian CAD patient study, Wang et al. described a positive relationship between ‘A’ allele and severe forms of CAD [20]. There was another report of higher ‘A’ allele frequency in myocardial infarction (MI) and angina patients as compared to healthy individuals [24]. However, studies on Solvenian [25] and Taiwanese population [26] did not reveal any preferential distribution of ‘A’ allele among the CAD population. Recently, in a Japanese study [21], no association was found between the polymorphism and MI.

APOA1-75G/A polymorphism has been studied in different ethnic groups with the notion that ‘A’ allele may determine an individual’s HDL levels and propensity to develop CAD. India is heading towards a CAD epidemic. Low HDL is one of the predominant cardiovascular risk factor among Indians. Despite all these facts, no data has so far been made available from this part of the world. We are reporting here our findings on the APOA1-75G/A polymorphism and its association with lipid profile and severity of CAD among Northern Indians.

2. Materials and methods

2.1. Study subjects

A total of 370 individuals coming for angiography at the department of cardiology, AIIMS, New Delhi were recruited in the study. Of these, only 200 individuals (36 controls, 164 patients) were selected for the investigation based on the criteria that all of these were 1) residents of Delhi or adjoining areas, sharing fairly similar socioeconomic, cultural pattern and dietary habits, 2) voluntary participants in the study and 3) with no cardiomyopathy or valvular disease. Individuals angiographically proven to have normal coronaries (N = 27) or ≤ 50% stenosis (N = 9) in one of the major coronary artery, formed the NCAD group (control group). The criteria for the control group were taken from the pioneer work of Wang et al. [20]. Subjects having ≥ 70% stenosis in one, two and three major coronary arteries were classified as SVD (single vessel disease, N = 43), DVD (double vessel disease, N = 54) and TVD (triple vessel disease, N = 67) patients respectively. A statistician was consulted for the study design. The study was approved by the ethical committee of All India Institute of Medical Sciences, New Delhi and its guidelines were observed.

2.2. Lipids, lipoproteins and apolipoprotein A-I

Venous blood sample was collected from each individual after at least 12 hours of fasting. Lipid profile was monitored using enzymatic kits (Randox laboratories limited, UK). All the chemicals used in the study were procured from Sigma Chemical Co., USA until or unless the source is specified.

ApoA-I levels were estimated in plasma by immunoturbidimetry using anti-human apoA-I antibody (from rabbit, DAKO) and modifications were made to the method reported by Mount et al. [27].

2.3. APOA1-75G/A polymorphism

DNA was extracted from blood by salting out method [28]. 100–500 ng of DNA was amplified in a thermocycler (PTC-100, MJ Research Inc., USA) using 1 unit of Taq DNA Polymerase (Life Technologies Inc., USA) in a 25 ul reaction mixture containing 10 picomole forward primer: 5’-AGG GAC AGA GCT GAT CCT TGA ACT CTT AAG-3’ and reverse primer: 5’-TTA GGG GAC ACC TAG CCC TCA GGA AGA GAT CCT TGA ACT CTT AAG-3’ (MWG Biotech GmbH, Germany) [8]. DNA was initially denatured for 5 minutes at 95°C, annealed at 57°C for 2 minutes and heated at 70°C for 1 minute. The cycling conditions were set to heat the samples at 95°C for 30 seconds, at 57°C for 45 seconds and at 72°C for 1 minute. The cycle was repeated 40 times followed by final extension at 72°C for 10 minutes.

About 9 ul of the PCR product was digested at 37°C overnight with 10 units of MspI restriction enzyme (New England Biolabs Inc., USA) in the presence of 1 ul of 10 X buffer provided with the restriction enzyme. The digested PCR product was resolved on a 8% polyacrylamide gel using 1XTBE buffer (89 mM Tris Borate, 2 mM EDTA, pH8.3) at 250 V for 2 hours and visualized by silver staining. Substitution from G to A, at −75 bp results in the loss of the MspI site. The presence of 183 bp represents the ‘A’ allele. The genotypes were referred to as GG, GA and AA.
The genotype distribution was in Hardy-Weinberg equilibrium in all the study groups. Prevalence of ‘A’ allele \( (p\text{ trend} = 0.0001) \): NCAD (0.097) < SVD (0.117) < DVD (0.223) < TVD (0.291) increased significantly with the rise in severity of CAD (Table 2). After adjusting for age and sex, the OR of ‘A’ allele carriers to develop SVD, DVD and TVD was 1.3, 2.8 and 4.2 respectively as compared to GG individuals \( (p = 0.007, \text{Table 3}) \). ‘A’ allele carriers were almost two times more prevalent in CAD group \( (37.8\%) \) as compared to NCAD group \( (19.4\%, p = 0.036, \text{Table 2}) \). Higher prevalence of ‘A’ allele was observed in CAD patients \( (0.223) \) as compared to NCAD group \( (0.097, p = 0.012) \).

Age and sex adjusted intergenotypic variations in lipid, lipoprotein and apolipoprotein A-I levels with respect to APOA1-75G/A polymorphism has been summarized in Table 4. Rare ‘A’ allele carriers were associated with lower levels of HDL in patients \( (9.7\% \text{ lower}, p = 0.014) \) and NCAD \( (10.8\% \text{ lower}, p = \text{NS}) \). Likewise, ‘A’ allele carriers had 5% lower levels of apoA-I as compared to GG individuals in the patients group \( (p = 0.0280) \). Similar trend was observed in the SVD, DVD and TVD group. No gradient was observed in the HDL and apoA-I levels with the rise in severity of CAD (data not shown).

### 4. Discussion

Our study, first of its kind from India, brings forth the relationship of APOA1-75G/A polymorphism with lipid profile and severity of CAD. People residing in different regions of India may express variable genotype-phenotype relationship and hence, susceptibility to CAD. The study, therefore, was confined to the residents of close geographical premise of Delhi and surrounding areas visiting the department of Cardiology, AIIMS, New Delhi for angiography. The study subjects might be of heterogeneous ethnicity, but shared fairly common socio-economic cultural background and dietary habits. Wang et al., in a study pertaining to the association of ‘A’ allele with severity of CAD, used NCAD category as the referent [20]. We followed the same study design and criteria for NCAD in this study. The criteria for NCAD was no or \( \leq 50\% \) stenosis in one of the major coronary artery disease and for patients was \( > 50\% \) stenosis [20]. Our patients, however, were those who were angiographically proven to have \( \geq 70\% \) stenosis in one of the major coronary artery. Of 370 individuals who were initially enrolled, only 200
individuals fulfilled the selection criteria and could be included for the analysis. This limited the sample size in different groups.

APOA1-75G/A polymorphism in the promoter region of the gene results in the loss ofMspI site (Rare allele: ‘A’). The genotype distribution was found to be in Hardy-Weinberg equilibrium in all the study groups. The frequencies of various genotypes decreased in the order of GG > GA > AA.

A highly significant increasing trend in the ‘A’ allele frequency with respect to severity is the key finding of our study. Similar trend was obvious in the OR of ‘A’ allele carriers to develop single, double and triple vessel disease as compared to GG in the patients (9.7% lower, p = 0.014; χ² trend = 10.13, df = 1, p trend = 0.0015).

Some observations have been reported in two other Northern Indian studies [29,30]. However, a larger study sample is essential to explain these observations.

In conclusion, base change at −75 bp of APOA1 gene was found to be associated with severe forms of CAD and lower levels of HDL-C & apoA-I in this representative group of Northern Indians. Thus, our findings suggest that individuals harboring −75 base change might be at a higher risk of developing severe forms of CAD and having lower levels of HDL and apo A-I. However, the major limitation of the study has been small sample size, and an exhaustive investigation, with a large sample size, is warranted.

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References

[1] A.S. Wierzbicki, Have we forgotten the pivotal role of high density lipoprotein cholesterol in atherosclerosis prevention, *Curr. Med. Res. Opin.* 21 (2005), 299–306.

[2] C.J. Fielding, V.G. Shore and P.E. Fielding, A protein co-factor of lecithin: cholesterol acyl transferase, *Biochem. Biophys. Res. Commun.* 46 (1972), 1493–1498.

[3] S.K. Karathanasis, Apolipoprotein multigene family: tandem organization of human apolipoprotein AI, CIII, and AIV genes, *Proc. Natl. Acad. Sci. USA* 82 (1985), 6574–6578.

[4] G. Assmann, A. von Eckardstein and H. Funke, High density lipoproteins, reverse transport of cholesterol, and coronary artery disease. Insights from mutations, *Circulation* 87(Suppl III) (1993), III28–III34.

[5] M. Groenendijk, R.M. Cantor, T.W.A. de Bruin and G.M. Dallinga-Thie, The ApoAI-CIII-AIV gene cluster, *Atherosclerosis* 157 (2001), 1–11.

[6] F. Pagani, A. Sidoli, G.A. Giudici, L. Barenghi, C. Vergani and F.E. Baralle, Human apolipoprotein A-I gene promoter polymorphism: association with hyperalphalipoproteinemia, *J. Lipid Res.* 31 (1990), 1371–1377.

[7] Q.H. Meng, P. Pajukanta, L. Valsta, A. Aro, P. Pietinen and M.J. Tikkanen, Influence of apolipoprotein A-I promoter polymorphism on lipid levels and responses to dietary change in Finnish adults, *J. Intern. Med.* 241 (1997), 373–379.

[8] H. Paul-Hayase, M. Roseneau, J.P. Van Bervliet, J.P. Deslypere and S.E. Humphries, Polymorphisms in the apolipoprotein (apo) AI-CIII-AIV gene cluster: detection of genetic variation determining plasma apo AI, apo CIII and apo AIV concentrations, *Hum. Genet.* 88 (1992), 439–446.

[9] C.-F. Xu, F. Angelico, M. Del Ben and S. Humphries, Role of genetic variation at the apo AI-CIII-AIV gene cluster in determining plasma apoAI levels in boys and girls, *Genet. Epidemiol.* 10 (1993), 113–122.

[10] N. Saha, J.S.H. Tay, P.S. Low and S.E. Humphries, Guanidine to adenine (G > A) substitution in the promoter region of the apolipoprotein AI gene is associated with elevated serum apolipoprotein AI levels in Chinese non-smokers, *Genet. Epidemiol.* 11 (1994), 255–264.

[11] P.J. Talmud, S. Ye and S.E. Humphries, Polymorphism in the promoter region of the apolipoprotein AI gene associated with differences in apolipoprotein AI levels: The European Atherosclerosis Research Study, *Genet. Epidemiol.* 11 (1994), 265–280.

[12] G. Sigurdsson, Jr., V. Gudnason, G. Sigurdsdsson and S.E. Humphries, Interaction between a polymorphism of the Apo A-I promoter region and smoking determines plasma levels of HDL and Apo A-I, *Arterioscler. Thromb.* 12 (1992), 1017–1022.

[13] A. Minnich, G. Delangavant, J. Lavigne, G. Roederer, S. Lussier-Cacan and J. Davignon, G → A substitution at position 75 of the Apolipoprotein A-I gene promoter-evidence against a direct effect on HDL cholesterol levels, *Arterioscler. Thromb. Vasc. Biol.* 15 (1995), 1740–1745.

[14] M.I. Kamboh, C.E. Aston, C.M. Nestlerode, A.E. McAllister and R.F. Hamman, haplotype analysis of two APOAI/Msp I polymorphisms in relation to plasma levels of apo A-I and HDL-cholesterol, *Atherosclerosis* 127 (1996), 255–262.

[15] F. Civeira, M. Pocovi, A. Cenarro, C. Garces and J.M. Ordovas, Adenine for guanine substitution −78 base pairs to the apolipoprotein (APO) A-I gene: relation with high-density lipoprotein cholesterol and apoA-I concentrations, *Clin. Genet.* 44 (1993), 307–312.

[16] D.E. Barre, R. Guerra, R. Verstraete, Z. Wang, S.M. Grundy and J.C. Cohen, Genetic analysis of a polymorphism in the human Apolipoprotein A-I gene promoter: effect on plasma HDL-cholesterol levels, *J. Lipid Res.* 36 (1994), 1292–1296.

[17] H. Akita, H. Chiba, M. Tsuji, S.P. Hui, Y. Takahashi, K. Matsuno and K. Kobayashi, Evaluation of G-to-A substitution in the apolipoprotein A-I gene promoter as a determinant of high density lipoprotein cholesterol level in subjects with and without cholesteryl ester transfer protein deficiency, *Hum. Genet.* 96 (1995), 521–526.

Table 4

| Parameter | Groups | GG | GA/AA | p Value |
|-----------|--------|----|-------|---------|
| TC (N = 164) | CAD | 198.77 ± 52.55 | 191.33 ± 52.58 | 0.383 |
| (N = 36) | NCAD | 192.02 ± 46.85 | 159.07 ± 49.37 | 0.108 |
| LDL (N = 102) | CAD | 124.63 ± 44.64 | 120.63 ± 44.67 | 0.580 |
| (N = 29) | NCAD | 119.02 ± 38.12 | 99.91 ± 40.18 | 0.247 |
| HDL (N = 7) | CAD | 39.69 ± 9.58 | 35.83 ± 9.62 | 0.014 |
| (N = 6) | NCAD | 40.01 ± 10.25 | 35.69 ± 10.80 | 0.329 |
| LDL/HDL | CAD | 3.33 ± 1.43 | 3.44 ± 1.44 | 0.636 |
| (N = 39) | NCAD | 3.13 ± 1.21 | 2.85 ± 1.28 | 0.582 |
| TG (N = 36) | CAD | 166.92 ± 74.49 | 170.57 ± 74.72 | 0.762 |
| (N = 29) | NCAD | 162.10 ± 61.92 | 114.31 ± 65.25 | 0.078 |
| ApoA-I (g/l) | CAD | 0.93 ± 0.14 | 0.88 ± 0.14 | 0.028 |
| (N = 39) | NCAD | 0.92 ± 0.14 | 0.86 ± 0.14 | 0.3675 |

All the parameters expressed in mg/dl (except apoA-I).
moter region: a study in hypertriglyceridaemic patients, *Hum. Hered.* **44** (1994), 94–99.

[19] R.E. Peacock, A. Hamsten, J. Johansson, P. Nilsson-Ehle and S.E. Humphries, Associations of genotypes at the apolipoprotein AI-CIII-AIV, apolipoprotein B and lipoprotein lipase gene loci with coronary atherosclerosis and high-density lipoprotein subclasses, *Clin. Genet.* **46** (1994), 273–282.

[20] X.L. Wang, S.X. Liu, R.M. McCredie and D.E.L. Wilcken, Polymorphisms at the 5’-end of the apolipoprotein AI gene and severity of coronary artery disease, *J. Clin. Invest.* **98** (1996), 372–377.

[21] K. Shoji, T. Mannani, Y. Kokubo, Y. Goto, H. Nonogi and N. Iwai, An association analysis between APOA1 polymorphisms and high density lipoprotein (HDL) cholesterol level and myocardial infarction (MI) in Japanese, *J. Hum. Genet.* **49** (2004), 433–439.

[22] A. Matsunaga, J. Sasaki and T. Mori, Apolipoprotein A-I gene promoter polymorphism in patients with coronary artery disease and healthy controls, *Natr. Mediat. Cardiovasc. Dis.* **5** (1995), 269–275.

[23] S.H.H. Juo, D.F. Wyszynski, T.H. Beaty, H.Y. Huang and J.E. Bailey-Wilson, Mild association between the A/G polymorphism in the promoter of the apolipoprotein A-I gene and apolipoprotein A-I levels: a metaanalysis, *Am. J. Med. Genet.* **82** (1999), 235–241.

[24] J.R. Reguero, G.I. Cubero, A. Batalla, V. Alvarez, S. Hevia, A. Cortina and E. Coto, Apolipoprotein A1 gene polymorphisms and risk of early coronary disease, *Cardiology* **90** (1998), 231–235.

[25] D. Petrovic, M. Tiore and B. Peterlin, Effect of apolipoprotein E polymorphism and apolipoprotein A-I gene promoter polymorphism lipid parameters and premature coronary artery disease, *Folia Biol. (Prague)* **46**(5) (2000), 181–185.

[26] J.H. Wu, J.-T. Kao, M.-S. Wen and S.-K. Lo, DNA polymorphisms at the apolipoprotein AI-CIII loci in Taiwanese: correlation of plasma APOCIII with triglyceride level and body mass index, *J. Formos. Med. Assoc.* **99** (2000), 367–374.

[27] J.N. Mount, E.M. Kearney, M. Rosseneu and B.M. Slavin, Immunoturbidimetric assays for serum apolipoprotein A1 and B using Cobas biocentrifugal analyzer, *J. Clin. Pathol.* **41** (1988), 471–474.

[28] S.A. Miller, D.D. Dykes and H.F. Polesky, A salting out procedure for extracting DNA from human nucleated cells, *Nucleic acid Res* **16** (1988), 1215.

[29] V.K. Bahl, M. Vaswani, D. Thatai and H.S. Wasir, Plasma levels of apolipoprotein A-I and B in Indian patients with angiographically defined coronary artery disease, *Int. J. Cardiol.* **46** (1994), 143–149.

[30] R. Gupta, S. Vasisht, V.K. Bahl and H.S. Wasir, Correlation of lipoprotein(a) to angiographically defined coronary artery disease in Indians, *Int. J. Cardiol.* **57** (1996), 265–270.