



Original Research Article

Prevalence of AdipoQ (+276 G/T) gene polymorphism in Punjabi menopausal women with and without family history of cardiovascular disease

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ABSTRACT

Introduction: Cardiovascular disease (CVD) is a serious threat in female population particularly after menopause. The prevalence of CVD with associated risk factors in Punjabi population after menopause is rapidly increasing. Adiponectin polymorphism ADIPOQ +276G/T is located in intron 2 which results from G to T substitution. The variants of adiponectin gene are found to be associated with obesity, metabolic syndrome markers and cardiovascular disease.

Aim: The present study aimed to screen the prevalence of +276 G/T polymorphism of AdipoQ gene in menopausal women of Punjab with and without the family history of CVD

Materials and Methods: Genomic DNA was extracted from intravenous blood for all the participants. Genotyping of +276 G/T polymorphism in the AdipoQ gene was done by polymerase chain reaction (PCR) – based Restriction Fragment Length Polymorphism (RFLP).

Results: Prevalence of TT genotype was higher among cases (22.3%) than among controls (4.4%). Statistical significant difference ($p < 0.05$) was observed in the frequency distribution of TT genotype ($p = 0.020$) in cases with positive family h/o CVD. TT genotype also provided significant risk ~9 folds towards CVD predisposition [$p = 0.001$; OR=9.90(3.77-25.99)] in cases with negative family h/o CVD.

Conclusion: The present study reveals that prevalence of TT genotype was found to be more in cases as compared to controls and also positive family history of CVD alone does not seem to be a good predictor as individual risk for cardiovascular disease in Punjabi menopausal women.

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1. Introduction

Cardiovascular disease (CVD) is one of the leading causes of death in women.¹ Several surveys conducted across the country, have shown a rising prevalence of major risk factors for CVD in urban as well as rural population.² So, the need becomes imperative to investigate the various risk factors associated with the pathogenesis and progression of atherogenic CVD. Moreover, it has already been reported that CVD risk factors become more prevalent after menopause.³ The relationship between genetic variants associated with coronary heart disease (CHD) and measured CHD risk factors is complex, with some genetic markers

associated with multiple risk factors and other markers showing no association with risk factors.⁴ Adiponectin is a polypeptide hormone that is produced and secreted into the blood by mature adipocytes. Adiponectin influences a number of metabolic processes particularly glucose and fatty acid metabolism in the liver and muscles. It plays an important role in anti-inflammatory, antiatherosclerotic and insulin-sensitizing activities.⁵ Several adiponectin gene (ADIPOQ) single nucleotide polymorphism (SNPs) have been shown to influence adiponectin levels and have been associated with risk for obesity, T2D and CVD.⁶

Human adiponectin gene referred as AdipoQ or APM1 is located in chromosome 3q27, has a structural homology with collagen VII and IX, complement factor CIq and TNF family⁷ and has been known to play an important

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role in inflammation, immune system and arteriosclerosis.⁸ The most extensively studied polymorphism within the adiponectin gene is ADIPOQ +276G/T variant located in intron 2 and is a result of a G to T substitution.⁹ The variants of adipo gene were found to be associated with obesity, metabolic syndrome markers and cardiovascular disease.^{10–12} Genetic variations of AdipoQ gene may lead to alterations in gene expression or changes in protein structure subsequently affecting biological functions of AdipoQ, eventually leading to individual's susceptibility to coronary artery disease (CAD).¹³

Adiponectin gene polymorphisms have been shown to differ between ethnicities and interactions may exist in ethnic genetic heterogeneity or genetic and environmental factors or intergenetic interactions.¹⁴ ADIPOQ polymorphism may be linked to each other or can even be linked to other unidentified genes which could also impact individual susceptibility to cardiovascular disease.¹³ There may be transmission of genetic factors that are linked with maladaptive responses to environmental or conventional risk factors.

An important risk factor associated with heart disease is family history of heart disease.^{15,16} The role of genetic factors in excess familial risk of heart disease increases with early onset of heart disease.¹⁵ Family history of premature CAD is defined as myocardial infarction (MI) or sudden death before 55 years of age in father or other male first – degree relative, or before 65 years of age in mother or other female first degree relative.¹⁷ The increased risk of cardiovascular disease characteristic to family history can be caused by shared genetic, environmental and behavioral factors and many common conventional risk factors. Family history can interact with several modifiable risk factors for heart disease.

2. Aims and Objectives

The present study was aimed to screen the prevalence of +276 G/T polymorphism of AdipoQ gene in menopausal women of Punjab with and without the family history of CVD and whether positive family history of CVD plays an individual role as risk factor for CVD in menopausal women of Punjab.

3. Materials and Methods

3.1. Participants

In the present study 265 menopausal women with CVD as cases and 258 menopausal women without CVD as controls were recruited from Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab (India). Menopausal women (Both cases and controls) were further classified according to the family h/o CVD into two groups. One group was with positive (with) family h/o CVD and the other group was with negative (without) family h/o CVD.

3.2. Selection criteria for cases and family h/o CVD

If a participant reported that they had ever been diagnosed with any cardiovascular disease such as, coronary heart disease, cerebrovascular disease, peripheral heart disease, angina or heart attack by a physician, were considered as cases. Participants (Both cases and controls) were asked whether any of their close biological (blood) relatives, including father, mother, sisters or brothers ever had any heart problem. If yes were considered as menopausal women with positive family h/o CVD

3.3. Genotyping

Genomic DNA was extracted from intravenous blood for all the participants by salt precipitation method.¹⁸ Genotyping of +276 G/T polymorphism in the AdipoQ gene was done by polymerase chain reaction (PCR) – based Restriction Fragment Length Polymorphism (RFLP).

Table 1: PCR profile

Initial Denaturation	95°C for 5 min
Denaturation	94°C for 45 sec
Annealing	58°C for 45 sec
Primer Extension	72°C for 45 sec
Final Extension	72°C for 5 min
Hold	4°C

The ADIPOQ gene polymorphism with respect to BsmI recognition sequence is due to guanidine to thymine base pair change which results in loss of BsmI restriction site. The reaction mixture was prepared in PCR tubes. The amplification was carried out in an Eppendorf Mastercycler Gradient thermal cycler. The PCR products were checked on 2% agarose gel. Fragment size of 241 bp was observed devoid of any non specific amplification against 100 bp ladder. The amplicons were then incubated with the restriction endonuclease (RE) enzyme (BsmI) for one and a half hour at 60°C. The reaction mixture of 15 μ l consisted of 4.4 μ l of distilled water, 1.5 μ l of NEB buffer, 1 unit of BsmI enzyme (0.1 μ l) and 9.0 μ l of amplicon.

The digested product along with positive and a negative (undigested amplicons) control were then electrophoresed on 2% agarose gel stained with ethidium bromide to detect the presence/absence of mutation. In case of the presence of BsmI cutting site, the amplified DNA fragment was digested into two fragments of 148 bp and 93 bp which represented homozygous wild (GG) type, uncut single band of 241 bp as homozygous mutant (TT) type and three bands of 241 bp, 148 bp and 93 bp as heterozygous (GT) type (Figure 1).

3.4. Statistical analysis

Frequencies of genotype and allele in cases and controls were compared using Chi-square (χ^2) test. Odds ratio (OR) at 95% confidence interval (CI) were calculated to estimate

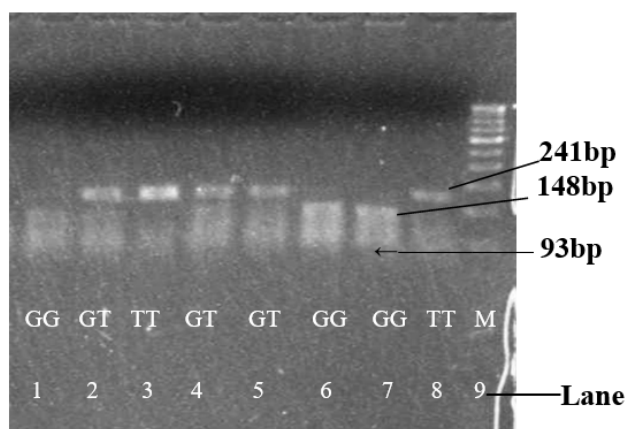


Fig. 1: Photograph of RFLP PCR showing allele specific PCR amplified products for ADIPOQ G>T polymorphism
Lane 9 - M - Marker (100bp) Lane 1, 6, 7- GG (148, 93 bp)
Lane 2, 4, 5 - GT (241+148/93bp)
Lane 3, 8 - TT (241bp)

the risk/protection of AdipoQ gene for disease etiology. The statistical analysis was performed using Statistical Package for Social Science program (version 16.0; SPSS Inc., Chicago, IL).

4. Results

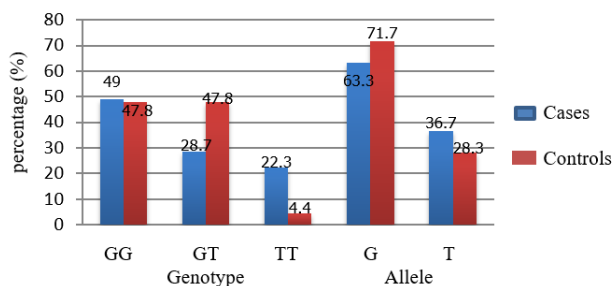


Fig. 2: Distribution of genotype and allele frequencies of +276 G/T of AdipoQ gene in menopausal women with positive family h/o CVD

As shown in Table 2 and Figure 2 the frequency of GG genotype with positive family h/o of CVD observed in cases was a little more (49%) than in controls (47.8%). The frequency of GT genotype was found to be more in controls (47.8%) than in cases (28.7%). However, distribution of TT genotype was higher among cases (22.3%) than among controls (4.4%). Statistical significant difference ($p < 0.05$) was observed in the frequency distribution of genotype ($p = 0.020$). The frequency of wild type allele (G) was higher among controls (71.7%) than among cases (63.3%) while, frequency of risk allele (T) was found to be higher in cases (36.7%) than in controls (28.3%). No significant difference

($p = 0.205$) was found on comparing the two groups.

Table 3 demonstrates model analysis. Under recessive model (TT vs GT+GG) analysis TT genotype provided significant risk ~6 folds towards CVD predisposition [$p = 0.014$; OR=6.32(1.41-28.31)]. Dominant model (GT+TT vs GG) [$p = 0.071$, OR=0.52 (0.27-1.00)] revealed no significant difference ($p > 0.05$) in the distribution while, co-dominant model (GT vs GG+TT) [$p = 0.041$; OR=0.43(0.21-0.91)] provided protection for CVD on comparing the two groups with positive family h/o CVD.

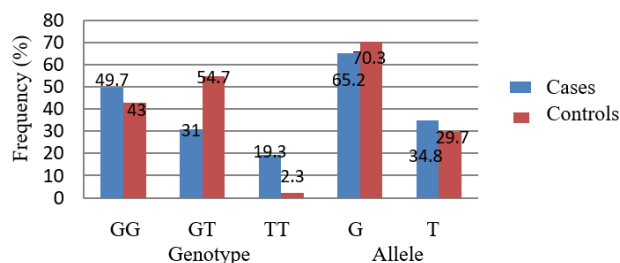


Fig. 3: Distribution of genotype and allele frequencies of +276 G/T of AdipoQ gene in menopausal women with negative family h/o CVD

Similarly, in Table 4 and Figure 3 the frequency of GG genotype observed in menopausal women with negative family h/o of CVD was more (49.7%) in cases than in controls (43%). The frequency of GT genotype was found to be more in controls (54.7%) without family h/o of CVD than in cases (31%). However, distribution of TT genotype was higher among cases (19.3%) than among controls (2.3%). Statistical significant difference ($p < 0.05$) was observed in the frequency distribution of genotype ($p = 0.001$). The frequency of wild type allele (G) was higher among controls (70.3%) than among cases (65.2%) while, frequency of risk allele (T) was found to be higher in cases (34.8%) than in controls (29.7%) but, no significant difference ($p = 0.156$) was observed on comparing the two groups.

Model Analysis is shown in Table 5. Under recessive model (TT vs GT+GG) analysis TT genotype provided significant risk ~9 folds towards CVD predisposition [$p = 0.001$; OR=9.90(3.77-25.99)]. Dominant model (GT+TT vs GG) [$p = 0.222$, OR=0.76 (0.50-1.14)] revealed no significant difference ($p > 0.05$) in the distribution while, co-dominant model (GT vs GG+TT) [$p = 0.001$; OR=0.37(0.24-0.56)] provided protection for CVD on comparing menopausal women without family h/o CVD.

5. Discussion

Several factors or mechanisms could interfere in the association between ADIPOQ SNPs, adiponectin levels and cardiometabolic diseases. Prevalence of family history varies depending upon the age at which it is assessed.

Table 2: Prevalence of +276 G/T of AdipoQ gene in menopausal women with positive family h/o CVD

Genotype/Allele	Menopausal women with positive family h/o CVD		p value OR (95% CI)
	Cases (n=94) n (%)	Controls (n=46) n (%)	
Genotype GG	46 (49)	22 (47.8)	0.020*
GT	27 (28.7)	22 (47.8)	
TT	21 (22.3)	02 (4.4)	
Allele G	119 (63.3)	66 (71.7)	0.205 1.47 (0.85-2.53)
T	69 (36.7)	26 (28.3)	

*p<0.05 was considered statistically significant; OR- odds ratio; CI-confidence interval

Table 3: Model analysis for prevalence of SNP +276 G/T of AdipoQ gene in menopausal women with positive family h/o CVD

Model	Menopausal women with positive family h/o CVD (n=140)	
	p value	OR (95% CI)
Dominant (GT+TT vs GG)	0.070	0.52 (0.27-1.00)
Recessive (TT vs GT+GG)	0.014*	6.32 (1.41-28.31)
Co-dominant (GT vs GG+TT)	0.041*	0.43 (0.21-0.91)

*p<0.05 was considered statistically significant; OR- odds ratio; CI-confidence interval

Table 4: Prevalence of SNP +276 G/T of AdipoQ gene in menopausal women with negative family h/o CVD

Genotype/Allele	Menopausal women with negative family h/o CVD		p value OR (95% CI)
	Cases (n=171) n (%)	Controls (n=212) n (%)	
Genotype GG	85 (49.7)	91 (43)	0.0001**
GT	53 (31)	116 (54.7)	
TT	33 (19.3)	05 (2.3)	
Allele G	223 (65.2)	298 (70.3)	0.156 1.26 (0.93-1.71)
T	119 (34.8)	126 (29.7)	

*p<0.05 was considered statistically significant; OR- odds ratio; CI-confidence interval

**p<0.01 highly significant

Table 5: Model analysis for prevalence of SNP +276 G/T of AdipoQ gene in menopausal women with negative family h/o CVD

Model	Menopausal women with negative family h/o CVD (n=383)	
	p value	OR (95% CI)
Dominant (GT+TT vs GG)	0.222	0.76 (0.50-1.14)
Recessive (TT vs GT+GG)	0.001**	9.90 (3.77-25.99)
Co-dominant (GT vs GG+TT)	0.001**	0.37 (0.24-0.56)

*p<0.05 was considered statistically significant; OR- odds ratio; CI-confidence interval

**p<0.01 highly significant

Parental history of premature heart attack has been shown to increase the risk in women by ~70%.¹⁹ Sibling history of CVD has been shown to increase the odds of CVD in women by 45%. In a recent international study of individuals with premature ACS more women (28%) than men (20%) had a family h/o of CAD.²⁰ Women with a family history had a higher prevalence of each traditional risk factor (hypertension, obesity, dyslipidemia, DM) as compared to women without a family history.²¹

Familial aggregation of CVD may be related to aggregation of specific risk factors as mentioned above or specific behaviors such as smoking and alcohol use that may have genetic and environmental influences. The effect size of any contributor to risk may be small or may have an enhanced risk when an environmental contributor is present, or may be large but may affect only a small population.

In the light of above mentioned risk factors our study highlighted on common risk factor that is family h/o heart disease.

In the present study TT genotype of recessive model of inheritance of cases with positive family h/o of CVD conferred ~6 fold high risk for CVD as compared to controls. Interestingly, TT genotype of women with CVD that is cases with negative family h/o CVD conferred more risk that is ~9 folds than control subjects with negative family h/o CVD. It means family history of CVD seems not be that predictive to foresee CVD risk in the subjects. Moreover, number of women suffering from heart disease with negative family h/o CVD (n=171) was also observed to be more than women with positive family h/o CVD (n=94). It means the mechanism underlying the genotype-phenotype association is not directly related to

+276 T SNP. Another possible reason could be due to interaction of susceptibility genes, environmental factors and conventional risk factors such as physical activity, dyslipidemia, hypertension and oxidative stress^{22,23} which contribute significantly to the pathogenesis of CVD. In another study it was observed that aggregation among siblings suggests that early life familial environmental exposures may increase heart failure risk, because siblings share the family environment at similarly young ages, as opposed to children and parents whose ages generally differ by two to four decades.²⁴ According to Moonesinghe et al. in their study family history interacts with modifiable risk factors for heart disease.¹⁵ The recognition of these factors mentioned above should have impact on future studies seeking to identify genetic and environmental risk factors of cardiovascular disease.

6. Conclusion

The present study reveals that prevalence of TT genotype was found to be more in cases as compared to controls both in women with and without family h/o CVD. Moreover, positive family history of CVD in Punjabi menopausal women does not seem to be a good predictor as individual risk factor for CVD as such because, the prevalence of TT genotype was also found to be significantly high in cases with negative family history of CVD. It is not essential that positive family history of CVD may identify a high risk subpopulation. Interaction of susceptibility genes, environmental factors along with conventional risk factors also contribute drastically to the pathogenesis of CVD. Modifiable risk factors should be planned to reduce the overall risk of heart disease. Therefore, regular screening of these factors for all subjects should be followed.

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8. Source of Funding

None.

9. Conflict of Interest

None.

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