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Lack of association of MFN2 (rs1474868) gene polymorphism with essential hypertension in south Indian population

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Abstract

Essential hypertension (EH) is the most common condition which contributes to ~ 95% of cases and has no clear identifiable basis. Although, EH has a strong genetic basis, the identification of genes associated with it has been difficult to achieve because of the complexity of regulation of blood pressure, its multifactorial nature and the presence of multiple susceptibility genes that have profound environmental and gene gene interactions. A case-control association study was conducted to investigate the possible involvement of *MFN2* (*rs1474868)*polymorphism in essential hypertensive patients. A total of 568 cases and 604 controls were recruited for this study. The SNP marker of *MFN2* gene *(rs1474868)* did not show any significant difference between the case and control groups in south Indian population studied. The genotype frequency was almost the same for both the case and control data sets. The result of the present study indicates that *MFN2* polymorphism is not associated with essential hypertension in south Indian population.

Key words : Essential hypertension, *MFN2, HSG*, SNP, PCR-RFLP

INTRODUCTION

Essential hypertension (EH) is defined as a chronic increase in blood pressure (BP) for which there is no known, single underlying cause. BP must be tightly regulated to ensure uninterrupted perfusion to all vital organs. A transient interruption in the blood flow leads to loss of consciousness. Conversely, high BP provides no metabolic gain but might increase damage to blood vessels and vital organs [1]. EH is the most important modifiable risk factor for coronary artery disease, stroke, congestive heart failure, end-stage renal disease and peripheral vascular disease [2]. The renal, neural and endocrine systems regulate blood pressure by an intricate network of physiological pathways involving extracellular fluid volume homeostasis, cardiac contractility and vascular tone. Any derangement in these pathways due to intrinsic (genetic) or extrinsic (environmental) factors or a combination of both can result in low or high blood pressure [3].

Essential hypertension has evolved as a global challenge afflicting one billion people worldwide (~ 4.5% of the world population), accounting for 9.4 million deaths per year [4]. Due to increasing longevity in the past few decades, the prevalence of EH is shown to rise with age, as it affects 25 - 35% of the adult population and about 60 - 70% beyond 70 years [5]. Blood pressures also change patterns, as age advances. Systolic BP increases with age and continues throughout life in contrast to DBP. DBP is a potent cardiovascular risk factor until 50 years of age, whereas SBP is associated with the most common form of HTN above 50 years [6]. The prevalence is also higher in men than women before 60 years of age, but equal after this age. Both men and women who were normotensive at age 55 - 65 and survive upto 80 - 85 years have a life time hypertension risk of 90% [7].

The prevalence of EH has been increasing in India, both in rural and urban areas. Pooled epidemiological data obtained from various parts of India have reported that hypertension has increased by 30 times over a period of 55 years in urban population and 10 times over a period of 36 years in rural population [8]. A community based cross-sectional study in rural Maharashtra including 1297 subjects aged 19 years and above reported a hypertension prevalence of 7.24% (94 subjects). Of the 94 hypertensive subjects examined, the lowest prevalence of HTN was reported in individuals of age group 19 - 28 years and highest prevalence of 31% was recorded among individuals of age group >79 yrs. In males, prevalence of hypertension was less (44.68%) as compared to females (55.22%) [9]. A recent study by Gupta *et al.* (2011) [10], reported an overall HTN prevalence of 38.2% in a rural population of Haryana.

In view of the epidemiological data mentioned above several genetic variants and mutations have been studied for decades globally which indicated both positive and negative associations with EH. The present study was aimed to analyze the association of an SNP marker *(rs1474868)* of *MFN2* gene in south Indian population. Mitochondria are cellular organelles which play a fundamental role in respiration, substrates oxidation, ATP production and apoptosis. In addition, it is also involved in essential biochemical pathways, amino acid synthesis, steroid metabolism, iron metabolism, fatty acid oxidation and calcium homeostasis. Dynamic changes are known to occur in the shape, localization and number of mitochondria even within a single eukaryotic cell. Mitochondrial remodeling is regulated by cell division and fusion in response to physiological and environmental signals [11]. This process is vital to accommodate diverse demands on mitochondrial function in various cell types during growth, differentiation and maintenance [12]. Equilibrium between fusion and fission of mitochondria should be maintained

so as to ensure proper energy metabolism, oxidation, calcium signaling and apoptosis. Proteins which are involved in the control of mitochondrial fusion are mitofusin 1 and 2 found in the outer membrane and OPA1 located in the inner mitochondrial membrane. Mitochondrial fusion is a two-step process which is carried out by combined action of *MFN1* and *MFN2* which brings about outer membrane fusion and OPA1 regulating the inner membrane fusion [13].

The association of *MFN2* with essential hypertension has been recorded in recent years. Mitofusin2, is also known as hyperplasia suppressor gene (*HSG*) and mitochondrial assembly regulatory factor (MARF). It is composed of 757 residues [14] and is highly expressed in heart, but is downregulated in heart in response to hypertrophic stimuli [15]. It belongs to the family of large GTP-binding proteins and located on the short (p) arm of chromosome 1 at position 36.22 [16]. The expression product of *MFN2/HSG* is completely dependent on its GTPaseacivity [17]. It participates in mitochondrial fusion and contributes to the maintenance and operation of the mitochondrial network. Though mitofusin is described as a mitochondrial protein, it is also found in endoplasmic reticulum (ER), controlling ER morphology and its tethering to mitochondria [18]. Dysregulation of mitofusin 2 is shown to trigger vascular proliferative disorder in experimental animal models as well as in essential hypertensive patients [11,19].

Mitofusin 2 and hypertension

Ras signaling is the central pathway for a wide array of cardiovascular diseases such as hypertensive proliferation, injury-associated arterial restenosis, cardiac hypertrophy and failure, angiogenesis and endothelial dysfunction. Checkpoints in Ras pathway have been the major focus of cardiovascular biology and medicine [20]. *MFN2* being an endogenous Ras inhibitor suppress VSMC proliferation by inhibiting Ras-Raf-ERK1/2 pathways [17]. Any deregulation of *MFN2* expression has been linked to vascular proliferative disorders such as hypertension, atherosclerosis and restenosis after vascular injury [21]. Since, EH is a disease characterized by hyperplasia of VSMCs, *MFN2* can be considered as a potential therapeutic target for EH and other cardiovascular disorders. Reduced expression of *MFN2* has also been reported in obese [22] and diabetic subjects [23]. Recently, two studies in Chinese population [24, 25] have identified novel variants exhibiting strong association with essential hypertension.

MATERIALS AND METHODS

All the samples were selected based on the 7th (2003) JNC report and WHOISH guidelines for management of hypertension [26]. The clinical investigations were carried out by qualified physicians and informed consent was obtained from all the patients and controls. Five ml of venous blood was collected from hypertensive patients (n = 568) and controls (n = 604) between the age group of 20-82 years. Patients' samples were collected from four different areas: 1. Govt. Hospital, Headquarters Dindigul, Tamilnadu, 2. K.S. Hospital, Kilpauk, Chennai, Tamilnadu, 3. Government Hospital, Headquarters Chennai, Tamilnadu, India

Table 1: Base-line data of normotensive controls and hypertensive patients

	CONTROLS (N=604)		PATIENTS (N=568)	
Sex (M:F)	1: 1.06		1.08: 1	
Age (Years)				
Males				
Mean + SD	54.4 ± 12.10		54.5 ± 11.27	
Females				
Mean + SD	54.4 ± 12.87		54.5 ± 11.55	
Systolic blood pressure (SBP) mmHg				
Mean + SD	116.8 ± 7.54		154.0 ± 19.93*	
Diastolic blood pressure (DBP) mmHg				
Mean + SD	77.9 ± 4.69		94.7 ± 12.36*	
Body Mass Index (BMI) (kg/m^2)	**N**	**%**	**N**	**%**
	293		**295**	
Males (N)				
Underweight	16	5.46	24	8.14
Normal	177	60.41	143	48.47*
Overweight	87	29.69	103	34.92
Obese	13	4.44	25	8.47*
	N	**%**	**N**	**%**
	311		**273**	
Females (N)				
Underweight	31	9.97	20	7.32
Normal	180	57.88	129	47.25*
Overweight	87	27.97	100	36.64*
Obese	13	4.18	24	8.79*

and 4. Voluntary Health Services, Adyar, Chennai, Tamilnadu, India. Age and sex matched control samples were collected from healthy volunteers and patients who visited outpatient clinics with minor ailments without hypertension in previous records. Patients with the history of diabetes mellitus, hyperlipidaemia, liver or renal disease, myocardial infarction and other causes of secondary hypertension were excluded from the study. All the subjects were recruited based on standard questionnaire and written informed consent was obtained. The study was approved by Institutional Human Ethical Committee.

Genotyping: Genomic DNA was extracted from the buffy coat of EDTA anti-coagulated blood by using Miller *et al.* (1988) [27] salting out method. Genotype analysis for both the SNP marker was based on PCR-RFLP method. PCR was performed in mastercycler gradient (Eppendorf, Hamburg, Germany). PCR was performed in 20 µl volumes using 100ng of genomic DNA, 200µM of dNTP, 5pmol/µl of forward: VP30: 5'-TTCAGGGAGGGCAGAGG -3' and VP31: 5'-ATGCAGCTGCAGGCTGG -3' (Eurofins MWG Operon, Bangalore, India), 2mM $MgCl_2$ and 0.5U of Taq DNA polymerse (Prime Taq DNA polymerase, Korea) and was amplified following the PCR conditions which involves an initial denaturation at 94°C for 4 min, annealing at 63°C for 45 secs, extension at 72°C for 45 secs and a final extension at 72°C for 4 min. 3 µl of PCR product was checked on a 1% agarose gel (Fig.1A). 15 µl of PCR product was digested using restriction enzyme *TaqI* procured from New England Biolabs, England. Digestion was carried out at 37°C for 2 hours. The digested product was visualized on a 2.5% agarose gel and the results were documented (Fig.1B). Sequencing analysis was performed to confirm genotypes and the sequence chromatograms (Fig. 2) were analyzed using CHROMAS 2.31 software (Technelysium, Australia). The comparison of allele frequencies between

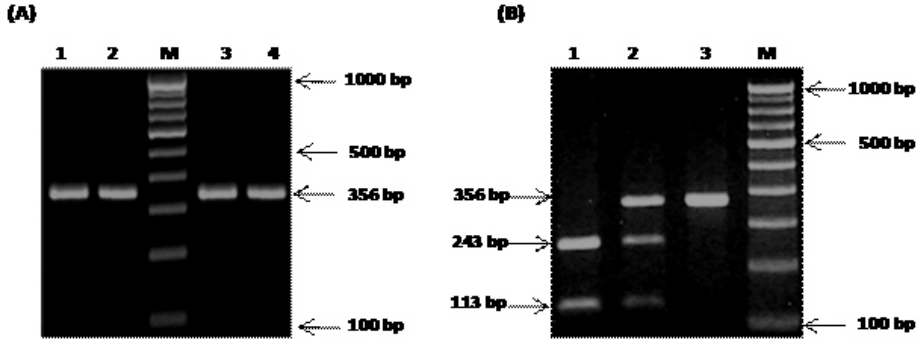

Figure 1: Agarose gel electrophoregram showing (A) Amplicons of size 356 bp fragment run along with 100bp DNA ladder (Lanes 1-4), (B) *TaqI digestion* of PCR amplified products for genotyping (Lanes 1 AA, 2 AG, 3 GG).

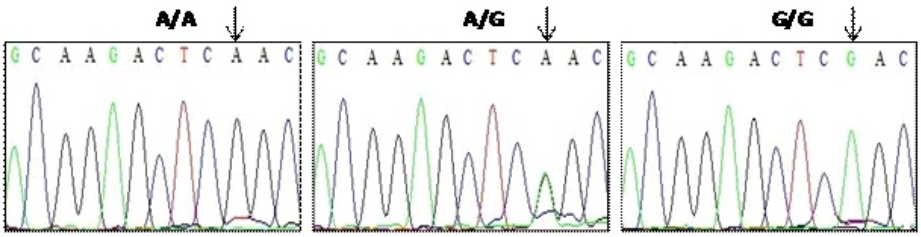

Figure 2: Sequence chromatograms of the genotypes: Homozygous wild-type (AA); Heterozygous (AG); Homozygous variant (GG).

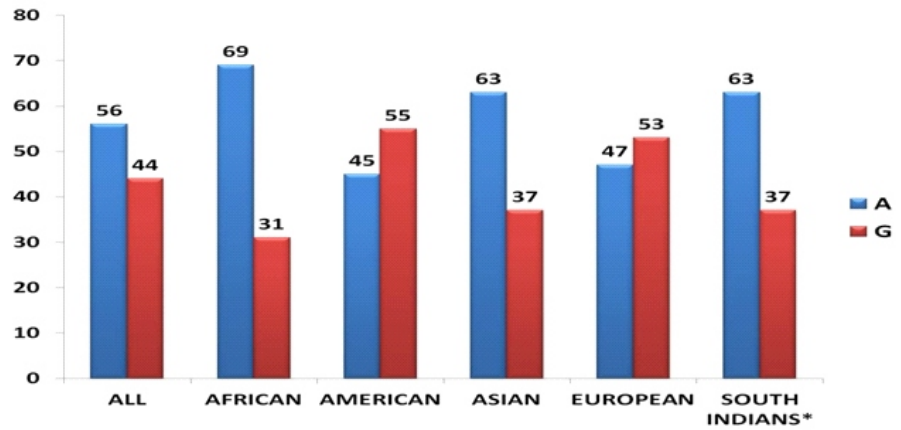

Figure 3: Allele frequency for rs1474868 polymorphism of MFN2 gene in different populations from around the world (http://browser.1000genomes.org/Homo_sapiens/).

Table 2: Genotype frequencies of *MFN2 (rs1474868)* gene polymorphism among the cases and controls.

Genotypes	AA	AG	GG	HWE p value*
Cases N = 568 (%)	232 (40.8)	262 (46.1)	74 (13.0)	0.92
Controls N = 604 (%)	239 (39.6)	281 (46.5)	84 (13.9)	0.99

*For departure from Hardy-Weinberg equilibrium (HWE), chi square with one degree of freedom.
The genotype frequency of cases and controls do not differ significantly χ 2df (P = 0.839)

Table 3: Overall genotype distribution of the *MFN2* gene polymorphism *(rs1474868)* in cases and controls.

	Cases N=568 (%)	Controls N=604 (%)	Unadjusted OR [95% CI]	P-Value	Adjusted OR* [95% CI]	P-value
Dominant						
AA	232 (40.8)	239 (39.6)	1.153 [0.8304 - 1.6008]	0.395	1.044 [0.826 – 1.320]	0.719
AG + GG	336 (59.2)	365 (60.4)				
Recessive						
GG	74 (13.1)	84 (13.9)	1.104 [0.7015 - 1.7376]	0.669	0.925 [0.660 – 1.297]	0.653
AG+AA	494 (86.9)	520 (86.1)				
Additive A	726 (63.9)	759 (62.8)	1.048 [0.8854 - 1.2393]	0.589	-	-
G	410 (36.1)	449 (37.2)				

Table 4: Gender specific distribution of *MFN2 (rs1474868)* gene polymorphism in male subjects

	Cases N=295 (%)	Controls N=293 (%)	Unadjusted OR [95% CI]	P-Value	Adjusted OR* [95% CI]	P-value
Dominant						
AA	128 (43.4)	117 (39.9)	1.153 [0.8304 - 1.6008]	0.395	1.160 [0.834 – 1.614]	0.378
AG + GG	167 (56.6)	176 (60.1)				
Recessive						
GG	46 (15.6)	42 (14.3)	1.104 [0.7015 - 1.7376]	0.669	1.114 [0.707 – 1.758]	0.642
AG+AA	249 (84.4)	251 (85.7)				
Additive A	377 (63.9)	368 (62.8)	1.049 [0.8271 - 1.3292]	0.696	-	-
G	213 (36.1)	218 (37.2)				

different ethnic groups was performed from the data obtained from 1000 genome browser (http://browser.1000genomes.org/) (Fig. 3).

Statistical analysis: All the continuous variables were expressed as mean ± standard deviation. Student's t-test was used for comparison of means of different variables. χ2 analysis was used to test for deviation of genotype distribution from Hardy-Weinberg equilibrium and to determine whether any significant differences in allele or genotype frequencies between cases and controls. The association between genotypes and hypertension risk was analyzed by calculating odds ratio (OR) at 95% confidence interval (95% CI). Statistical tests including logistic regression analysis were performed using the statistical package SPSS 14.0 version (SPSS Inc., Chicago, Illinois, USA). *P* value < 0.05 was considered to be statistically significant.

RESULTS

The allele frequency of the study population (A-63% and G-37%) was found to be the same for other Asian ethic groups as

Table 5: Gender specific distribution of *MFN2 (rs1474868)* gene polymorphism in female subjects

	Cases N=273 (%)	Controls N=311 (%)	Unadjusted OR [95% CI]	P-Value	Adjusted OR* [95% CI]	P-value
Dominant						
AA	104 (38.1)	122 (39.2)	0.953 [0.6827 - 1.3312]	0.779	0.944 [0.674 – 1.321]	0.736
AG + GG	169 (61.9)	189 (60.8)				
Recessive						
GG	28 (10.3)	42 (13.5)	0.732 [0.4402 - 1.2172]	0.229	0.734 [0.440 – 1.225]	0.237
AG+AA	245 (89.7)	269 (86.5)				
Additive						
A	349 (63.9)	391 (62.7)	1.047 [0.8244 - 1.3288]	0.708		
G	197 (36.1)	231 (37.1)				

*Odds ratio according to genotypes were estimated after adjusting the confounding variables for BMI.

observed from the figure 3. The observed and expected genotype frequencies of the control (p = 0.092) and case (p = 0.099) group were in accordance with Hardy-Weinberg equilibrium (Table 2). There was no significant difference between case and control groups at χ^{2df} (p value = 0.839). Overall genotype distribution (Table 3) and gender specific distribution did not show any significant difference in the genotype or allele frequencies between case and control groups of both male (Table 4) and female subjects (Table 5).

DISCUSSION

Mitofusin, belongs to a family of GTP-binding proteins which is an essential component of the mitochondrial machinery. Mitochondrial oxidative stress due to imbalances in mitochondrial fusion and fission contributes to decline in mitochondrial function. This can lead to a wide variety of pathologies including hypertension and atherosclerosis [21]. A recent study in the Chinese population revealed a gender based association between *rs1474868* of *MFN2* gene with EH. The study revealed significant difference between normotensive and hypertensive male population [24]. Another study in Chinese population also revealed an A > G variation of 5'-non coding region viz., -1248 of *MFN2* gene to be associated with hypertension [25]. The SNP marker of *MFN2* gene *(rs1474868)* did not show any significant difference between the case and control groups in south Indian population studied. The genotype frequency was almost the same for both the case and control data sets. Gender-based study did not produce any significant association with EH. Mitofusin, which is highly expressed in heart, could be a vital target in studying the genetic basis of hypertension. Although, this protein has a potential role in several cardiovascular pathologies, the genetic basis has not been investigated to a larger extent. Globally, the genetic association of the polymorphism in mitofusin gene with that of EH has not been well established in other populations/ethnic groups. Hence, a clear conclusion could not be obtained to ascertain the correlation of *rs1474868* polymorphism with hypertension.

CONCLUSION

In conclusion, the present study shows the lack of association of *MFN2* marker *(rs1474868)* with essential hypertension in south Indian population. Inconsistency in the results could be due to genetic and environmental heterogeneity among different ethnic groups. Since a wide variety of physiological systems exhibit pleiotrophic effects and interact in complex fashion to influence BP, the effect of the variants studied may be masked by the effect of unstudied variants. Thus, multiple variants need to be analyzed to decipher the molecular profile of this population. The knowledge acquired from the genetic association studies coupled with further functional studies will aid us to understand the underlying biological mechanisms of the observed associations and develop efficient preventive strategies or therapy for essential hypertension. Further investigations are required to unravel joint effects of several candidate genes to dissect out the complex phenotype and gender specific association of essential hypertension.

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