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Association of SH2B3 (rs3184504) polymorphism in essential hypertensive patients in south Indian population

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ABSTRACT

Introduction: Essential hypertension (EH) is considered to be the major risk factor associated with cardiovascular, cerebrovascular and renal diseases. Molecular target identification is the key to the development of drug targets. SNP profiling is a basic method which provides insights into targets associated with disease phenotype. The present study is a case control model used to associate EH with one of the promising cell signalling marker *SH2B3* expressed in inflammatory conditions.

Results: The subjects recruited for the study were genotyped for *rs3184504* polymorphism of *SH2B3* gene. Female subjects with CC genotype were 1.4 times more susceptible to EH than male subjects. A significant association was observed for CC genotype on adjusting BMI (p value = 0.030, OR = 1.455, 95% CI = 1.036 – 2.042) in females. Consequently, the C vs T allele comparison in additive model also showed a significant difference (p value = 0.023, OR = 1.455, 95% CI = 1.036 – 2.042).

Conclusion: Although, several drugs have been developed for combating EH, the incident rate of the disease seems to rise over the past few decades. This fact clearly describes the ineffectiveness of current drugs to control BP and lack of awareness among individuals about the available treatment modalities. Personalized medicine designed to match the physiological conditions of the patient based on his genotype could safely and effectively control the disease. In this context, the *SH2B3* (*rs3184504*) polymorphism is considered to be a significant marker associated with EH in south Indian population especially in female subjects.

Keywords: Essential hypertension, *SH2B3*, SNP, ARMS-PCR, Sequencing.

INTRODUCTION

Inflammation and hypertension are linked and this has been proved by the presence of circulating inflammatory molecules such as C-Reactive Protein (CRP) and Interleukin-6 (IL-6) in hypertensive patients (Pauletto and Rattazzi, 2006). Extracellular signals relayed from the plasma membrane to specific intracellular sites are a key step of cellular regulation leading to inflammation. Cellular responses to external intrinsic signals are coordinated through specific protein–protein and protein–phospholipid interactions mediated by ‘‘adaptor proteins’’. Adaptors have multiple functions such as determining the localization of signalling proteins in the cell, coordinating the signals involved in cell activation and bringing together the enzymes and substrates that drive the activation process (Figure 1). Expression of these adaptor proteins is either ubiquitous or restricted to selected cell types, where they play a specialized role by controlling differentiation and function (Devalliere and Charreau, 2011).

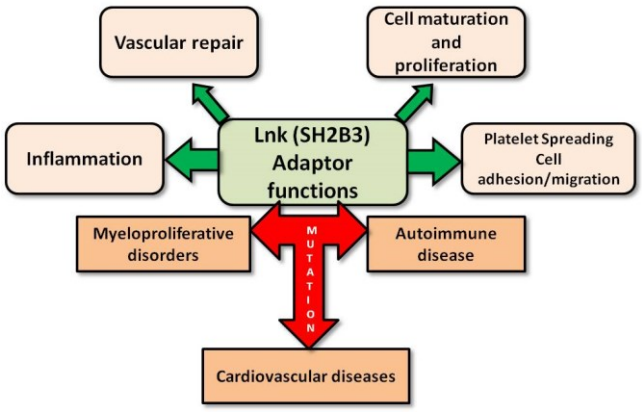

Fig. 1. SH2B3 adaptor functions and outcome of mutation in the gene.

Lnk (*SH2B3*) is a member of the SH2B family of adaptor proteins which are implicated in integration and regulation of multiple signaling events. The SH2-B (Src homology 2-B) protein family contains SH2B1 and SH2B2, originally named SH2-B and APS (adaptor protein with PH and SH2 domains), respectively. Lnk is structurally composed of a number of functional domains: a carboxyl-terminal Src homology 2 (SH2) domain, which is essential for specific binding to phosphotyrosine residue, a pleckstrin homology (PH) domain, which recognize phosphoinositides and control protein translocation to the cell membrane, proline-rich regions, dimerization domain (DD) and several putative tyrosine phosphorylation motifs (Maures *et al.,* 2007). Lnk has been shown to negatively control receptor activation such as stem cell factor (SCF) receptor (Simon *et al.,* 2008), thrombopoietin receptor (MPL) (Seita *et al.,* 2007) erythropoietin receptor (EPOR) (Tong *et al.,* 2005), platelet-derived growth factor receptor (PDGFR) (Gueller *et al.,* 2011) and macrophage colony-stimulating factor receptor (c-Fms) (Gueller *et al.,* 2010).

Lymphocyte-specific adaptor protein and disease associations:

Functional investigation of the effect of the *SH2B3* genotype in response to lipopolysaccharide and muramyl dipeptide revealed that carriers of the *SH2B3 rs3184504* risk allele showed stronger activation of the NOD2 recognition pathway. This suggests that *SH2B3* plays a role in protection against bacterial infection (Zhernakova *et al.,* 2011). Recently, genetic studies reported a role for Lnk gene polymorphism and mutations in various diseases including type 1 diabetes (T1D) (Reddy *et al.,* 2011), hypertension (Levy *et al.,* 2009), myocardial infarction (Gudbjartsson *et al.,* 2009), coeliac disease (Hunt *et al.,* 2008), myeloproliferative diseases (Oh *et al.,* 2010), erythrocytosis (Lasho *et al.,* 2010), systemic lupus erythematous (Gateva *et al.,* 2009), rheumatoid arthritis (Coenen *et al.,* 2009) and multiple sclerosis (Alcina *et al.,* 2010).

Lymphocyte-specific adapter protein and hypertension:

Experimental evidences suggest that there is a link between hypertension and inflammation (Pauletto and Rattazzi, 2006). It involves complex interplay between systemic inflammation, vascular cells activation and structural changes in the arteries. *SH2B3* gene, mapped to the locus 12q24 was recently found to be associated with coronary heart disease and hypertension. The SNP *rs3184504* in *SH2B3* is one of the blood pressure SNPs determining a risk allele for both systolic and diastolic blood pressure (Levy *et al.,* 2009). The SNP *rs3184504*, in exon 3 is a missense variant (R262W; 784 T>C) that introduces an amino acid substitution (arginine to tryptophan) in the PH domain involved in plasma membrane targeting (Li *et al.,* 2000). These genetic variations can affect protein function by altering gene expression and protein levels or by altering the structure of the encoded protein.

The *rs3184504* T allele, associated with increased blood pressure is known to cause increased cytokine production (Zhernakova *et al.,* 2011). The SNP impacts

blood pressure through an action specific to cells outside of the immune system which supports the hypothesis for a role of Lnk in vascular biology and homeostasis. In addition, the involvement of this SNP with a panel of autoimmune diseases may also suggest that immune response pathways may influence blood pressure by mechanisms not yet clearly defined.

METHODOLOGY

All the samples were selected based on the 7th (2003) JNC report and WHOISH guidelines for management of hypertension (Chalmers *et al.,* 1999). 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 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 salting out method (Miller *et al.,* 1988). ARMS PCR was carried out to genotype the SNP of *SH2B3* gene. A total of three primers were used, with two forward and one common reverse primer, where each of the forward primer was specific to a particular allele. Therefore for every DNA sample, two PCR reactions were carried out, each containing one of the allele specific forward primer (F1; F2) and the common reverse primer. The genotypes were directly identified by electrophoresing the products on a 1-1.5 % agarose gel. Amplicons observed with both the primers (F1 and F2) were designated as heterozygous, whereas amplicons with just one set of primer (F1+R/F2+R) is designated as either homozygous wild-type (CC) or homozygous mutant (TT) (Fig. 2a). The primer sequences are as follows: Forward 1:VP9: 5'–ATCCAGGAGGTCCGGC-3', Forward 2:VP10 5'-ATCCAGGAGGTCCGGT- 3', Reverse - VP11: 5'-TGCACTCCGAGAGCTC-3'.

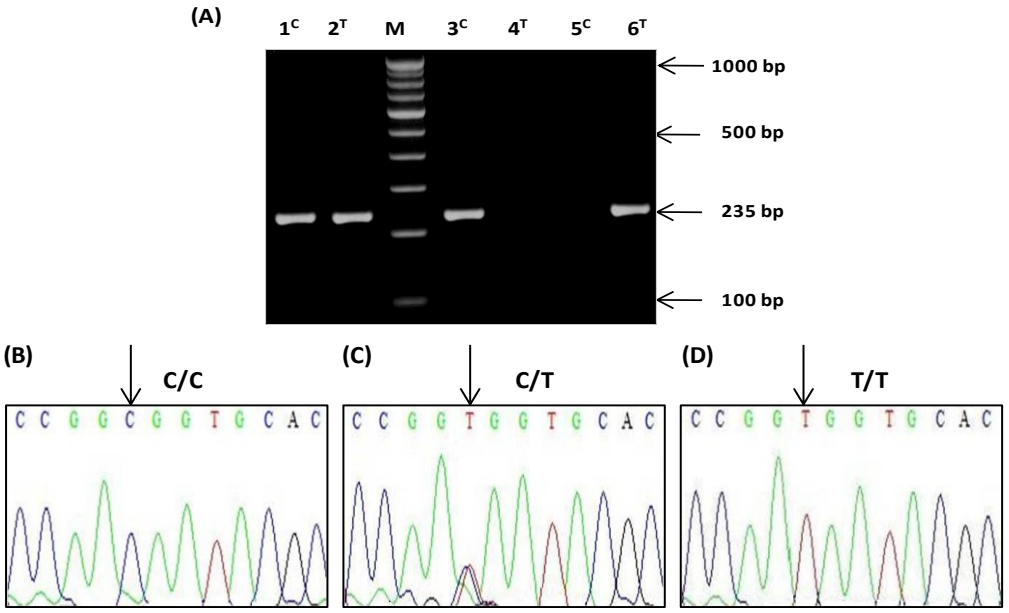

Fig.2. C/T polymorphism of *SH2B3* (*rs3184504*) gene: (A) Allele specific PCR amplification (235 bp) demonstrating the genotypes [M = 100 bp DNA marker] Lane 1 and 2 - CT – same sample amplified by both the sets of primers, hence heterozygote; Lane 3 and 4 - CC – sample amplified with C allele specific primer set, hence homozygote; Lane 5 and 6 – TT sample amplified with T allele specific primer set, hence homozygote. Sequence chromatograms of the genotypes: (B) Homozygous wild-type (CC); (C) Heterozygous (CT); (D) Homozygous variant (TT).

The PCR reaction conditions are as follows: initial denaturation at 94°C for 4 mins, denaturation at 94°C for 45 secs, annealing at 58°C for 45 secs, extension at 72°C for 45 secs, for 35 cycles followed by a final extension at 72°C for 4 mins. Sequencing analysis was performed to confirm genotypes and the sequence chromatograms (Fig. 2b, c, d) were analyzed using CHROMAS 2.31 software (Technelysium, Australia). The comparison of allele frequencies between 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. $\chi 2$ 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 analysed 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 genotype and sequence chromatograms of the *SH2B3* gene polymorphism *(rs3184504)* are shown in figure 2. The comparison of allele frequencies revealed the distribution of C and T alleles of the study population (C-78% and T-22%) matched the frequencies of American population (C-70% and 30%) (Figure 3). The observed and expected genotype frequencies of the control and case group were in good agreement with Hardy-Weinberg equilibrium.

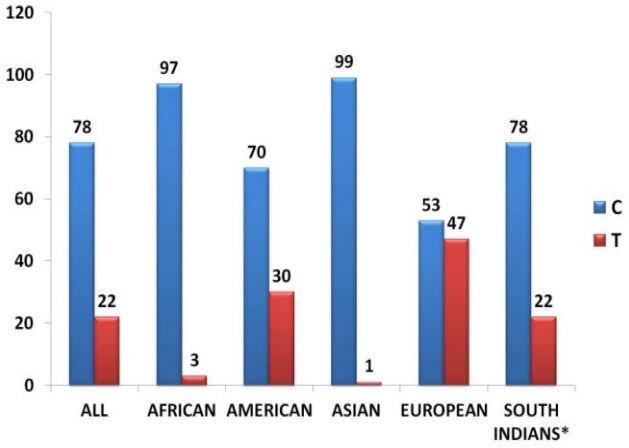

Fig.3. Ethnic distribution of allele frequencies among different populations with the present study group*

Table 1: Base-line data of normotensive controls and hypertensive patients * p value less than 0.01

		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**	**%**
Males (N)		**293**		**295**	
	Underweight				
	Normal	16	5.46	24	8.14
	Overweight	177	60.41	143	48.47*
	Obese	87	29.69	103	34.92
		13	4.44	25	8.47*
Females (N)		**N**	**%**	**N**	**%**
	Underweight	**311**		**273**	
	Normal				
	Overweight	31	9.97	20	7.32
	Obese	180	57.88	129	47.25*
		87	27.97	100	36.64*
		13	4.18	24	8.79*

Table 2: Overall genotype distribution of the *SH2B3* gene polymorphism (*rs3184504)*

10	Cases N=568 (%)	Controls N=604 (%)	Unadjusted OR [95% CI]	P-Value	Adjusted OR* [95% CI]	P-value
Dominant						
CC	357 (62.9)	358 (59.3)	1.163 [0.9190 - 1.4709]	0.209	1.208 [0.952 – 1.532]	0.120
CT + TT	211 (37.1)	246 (40.7)				
Recessive						
TT	22 (3.9)	38 (6.3)	0.600 [0.3504 - 1.0279]	0.063	0.626 [0.365 – 1.073]	0.089
CT + CC	546 (96.1)	566 (93.7)				
Additive						
C	903 (79.5)	924 (76.5)	1.191 [0.9791 - 1.4491]	0.080	-	-
T	233 (20.5)	284 (23.5)				

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

Table 3: Gender specific distribution of *SH2B3* (*rs3184504*) gene polymorphism in male subjects

	Cases N=295 (%)	Controls N=293 (%)	Unadjusted OR [95% CI]	P-Value	Adjusted OR* [95% CI]	P-value
Dominant						
CC	183 (62.0)	183 (62.5)	0.982 [0.7036 - 1.3709]	0.916	1.001 [0.715 – 1.402]	0.994
CT + TT	112 (38.0)	110 (37.5)				
Recessive						
TT	12 (4.1)	15 (5.1)	0.786 [0.3614 - 1.7091]	0.543	0.834 [0.382 – 1.820]	0.648
CT + CC	283 (95.9)	278 (94.9)				
Additive						
C	466 (79.0)	461 (78.7)	1.019 [0.7703 - 1.3480]	0.895	-	-
T	124 (21.0)	125 (21.3)				

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

Table 4: Gender specific distribution of *SH2B3* (*rs3184504*) gene polymorphism in female subjects

	Cases N=273 (%)	Controls N=311 (%)	Unadjusted OR [95% CI]	P-Value	Adjusted OR* [95% CI]	P-value
Dominant						
CC	174 (63.7)	175 (56.3)	1.366 [0.9787 - 1.9062]	0.067	1.455 [1.036 – 2.042]	**0.030**
CT + TT	99 (36.3)	136 (43.7)				
Recessive						
TT	10 (3.7)	23 (7.4)	0.476 [0.2224 - 1.0191]	0.056	0.490 [0.228 – 1.053]	0.067
CT + CC	263 (96.3)	288 (92.6)				
Additive						
C	437 (80.0)	463 (74.4)	1.377 [1.0441 - 1.8155]	**0.023**		
T	109 (20.0)	159 (25.6)				

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

The overall genotypic distribution did not show any significant difference between case and control groups which is evident from a p value of 0.130 at χ^{2df} (Table 2). No significant difference was observed with male subjects (Table 3). However, a marginal significance was (p = 0.067) observed on CC vs CT + TT comparison between cases and control groups of the dominant model in female subjects.

A significant difference was observed for the same genotype model on adjusting BMI (p value = 0.030, OR = 1.455, 95% CI = 1.036 – 2.042). Furthermore, the C vs T allele comparison in additive model shows a significant difference (p value = 0.023, OR = 1.455, 95% CI = 1.036 – 2.042) (Table 4).

DISCUSSION

SH2B3 gene has a wide range of clinical significance. It has been shown to be strongly associated with essential hypertension (Newton-Cheh *et al.,* 2009), celiac disease (Hunt *et al.,* 2008), type I diabetes mellitus (Todd *et al.,* 2007) and other autoimmune diseases (Gudbjartsson *et al.,* 2009). It encodes Lnk, an adaptor protein that mediates the interaction between extra-cellular receptors, such as the T-cell receptor, the thrombopoietin receptor and intracellular signaling pathways. The SNP *rs3184504* is a nonsynonymous SNP in exon 3 of *SH2B3* gene, leading to R262W (arginine to tryptophan) change in the pleckstrin homology domain. Ripatti *et al.* (2010), carried out case-control analysis and prospective cohort study including subjects from Finland and Sweden. The reports suggest that the marker *rs3184504* of *SH2B3* gene was associated with cardiovascular disease (OR = 1.10; P = 0.011) and myocardial infarction (OR = 1.15; P = 0.012).

Meta-analysis by the CHARGE and Global BPgen consortium have attained a genome wide significance for *SH2B3* (*rs3184504*) with systolic blood pressure (P = 4.5 X 10^{-9}) (Levy *et al.,* 2009). Another report on GWAS in African American population also showed association with EH (P = 0.009) (Fox *et al.,* 2011). The T allele of *rs3184504* correlates with high diastolic blood pressure and is common in HapMap CEU (frequency = 0.45), whereas absent in Hapmap YRI, JBT and CHB samples which is an evidence for recent positive selection. Positive selection of the T allele was also observed in four European and Saharawi population. When a genetic variation is under positive selection it increases in prevalence in a population. Climate, diet and pathogen load causes a selective pressure in populations world-wide resulting in global allele frequency variation (Zhernakova *et al.,* 2011).

The minor allele T, leading to a missense mutation results in the loss of *SH2B3* function. This report suggests that the minor allele arose with an intermediate frequency in European-derived populations, conferring selective advantage of immune response to infectious pathogens. Although enhancing *SH2B3* activity might seem attractive to reduce risk for multiple diseases, the evidence for positive selection of an apparent loss-of-function allele and pleiotropic consequences suggest that enhancing *SH2B3* activity could have unintended consequences (Newton-Cheh *et al.,* 2009).

In the present study, the genotype frequency between cases and controls did not differ significantly (P = 0.130). Though there was no association in model based study for the overall genotype analysis, female subjects showed a significant association with essential hypertension. In the dominant model, CC genotype was found to be the risk genotype with an adjusted p value of 0.030 (OR = 1.455). Hence the risk that is estimated is 1.4 times more in individuals with CC genotype when compared to the other two genotypes. However, the unadjusted p value showed only a marginal significance with a p value of 0.067 (OR = 1.366). The additive model for female subjects also showed that the C allele poses a risk on an individual's blood pressure phenotype (p value = 0.023, OR = 1.377). No such association was observed in the male subjects.

Pharmacogenomics Responses of Antihypertensive Responses (PEAR) study was performed to investigate whether the loci/SNP associated with BP/hypertension are also associated with BP response to antihypertensive drugs. The PEAR participants were Caucasian (60%) and African American (40%) hypertensive individuals. Around 37 SNPs were analysed for this purpose. The associations of these markers with BP response to atenolol and hydrochlorothiazide (HCZT) monotherapy were assessed in 768 hypertensive patients. The SNP marker *rs3184504* of *SH2B3* gene was also assessed. This marker showed opposite effect of association in African Americans in comparison to Caucasians. The C allele was linked with better BP reduction in Caucasians treated with HCZT, whereas a slight increase in BP was

observed in African Americans. The variation in the drug response due to ethnic disparity could also be precipitated by other underlying factors involved in the blood pressure regulation (Johnson *et al.,* 2009).

CONCLUSION

Hypertension management is the prime need for prevention of complications due to essential hypertension. The anti-hypertensive drugs such as diuretics, beta-blockers, angiotensin converting enzyme inhibitors, calcium channel blockers and angiotensin receptor blockers, have contributed minimal to control BP in the population, which is quite evident from the prevalence data for EH. Designing an antihypertensive drug likely to be most effective for an individual patient should be the goal of current treatment modalities. Inter individual variation in terms of genetic polymorphisms has been found to underlie pathophysiology of diseases which can also affect the efficacy of therapy. Substantial evidences from GWAS in different populations have immensely contributed to the knowledge about the role of *SH2B3* gene with EH. Being a non-synonymous SNP leading to Arg262Trp change, this marker has attracted attention for functional analysis. Both genetic and functional validation is warranted to reveal the effect of this marker on blood pressure regulation.

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Conflicts of Interest: The author stated that no conflicts of interest.

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