ORIGINAL ARTICLE

Serene Threonine Kinase 39 gene Single Nucleotide A-G Polymorphism rs35929607 is weakly associated with Essential Hypertension in population of Tharparkar, Pakistan

Loung Vasandas Umedani,[1] Bushra Chaudhry,[2] Vikram Mehraj,[3] Muhammad Ishaq[4]

Abstract

Objective: To study the prevalence of Serine Threonine Kinase-39 A?G polymorphism rs3592960 in rural population.

Methods: The random, cross-sectional study was carried out from 2009 to 2010 on 528 subjects in Tharparkar, Pakistan. Data was recorded excluding cases of secondary hypertension. Normotensives were used as the controls and hypertensives as the cases. Genotyping was carried out by tetra-primer amplification refraction mutation system-polymerase chain reaction method and the data was analysed statistically with SPSS-14.

Results: The association of Serine Threonine Kinase-39 rs35929607 with essential hypertension was as 3.07 (95% confidence interval 2.10-4.49) units/mmHg per G allele (p= 0.001). Raised systolic BP >140mmHg showed 0.76 (95% CI, 0.47-1.23) (p= 0.235) and raised diastolic BP >90mmHg showed 0.93 (95% CI, 0.61-1.44) units/mmHg per G allele (p= 0.735). Frequency of the risk allele G was less (33.3%) than that of allele-A (66.7%), (p= 0.0001). The effect size of genetic factors was non-significant, β= 0.062 (p= 0.153) for GG homozygotes and β= -0.013 (p= 0.772) for AG heterozygotes. Effect size of risk factors (age>50 years, diabetes and body mass index >23) was found significantly associated with essential hypertension, β= 0.747 (p= 0.000). The risk factors increased the effect by 12.04 fold in GG genotype and further 3 fold influence of risk factors is required with single allele-G in case of AG heterozygotes.

Conclusion: Essential hypertension risk conferred by this polymorphism in the study population is different from the previously reported European population, suggesting that the variant G allele remains less associated in the absence of environmental risk factors.

Keywords: Serine-threonine kinase-39 (STK39) gene, Single nucleotide polymorphism (SNP) STK39 rs35929607, Essential hypertension, Cardiovascular diseases, STK39 Genotypes, Effect size of Risk Factors, Pakistani population. (JPMA 63: 199; 2013)

Introduction

Essential hypertension (EHTN) and other cardiovascular diseases (CVD) are associated with progressively increasing morbidity and mortality across the globe.[1] Hypertension affected 26.4% of the adult population in the world in 2000.[2] It is expected to increase to about 60% from the present figure (from 972 million to 1.56 billion) by 2025.[2] About 7.1 million deaths occur per year globally due to hypertension.[3] Etio-pathogenesis of EHTN encompasses combined effects of genetic factors and many environmental risk factors.[4] Wang et al in December 2008 showed that SNP rs35929607 in Serine Threonine Kinase 39 (STK 39) gene is associated with raised blood pressure.[5] In this European Genome-wide association (GWA) study, functional single nucleotide polymorphism (SNP) rs35929607 provided a more significant association with hypertension than the other SNPs of this gene (i.e. p= 0.04 for rs35929607, p= 0.09 for rs3754777, and p= 0.12 for rs6749447). The SNP rs35929607 is located in the intronic region of gene. The SNP consistent with rs35929607 was regarded as being either a better surrogate for the functional variant or it is the functional variant by itself.[5] Therefore, we studied the prevalence of SNP rs35929607, genotypes and allele frequencies and performed comparative analysis of the effect size of risk factors in a Pakistani population.

STK39 contains 18 exons and covers a 293.65 kilo base region i.e. from 168812426 to 168518776 on the reverse strand of the gene as reported in National Centre for Biotechnology Information (NCBI) 36. Chromosomal location of STK39 is at 2q24.3. Variants of STK39 human gene produce proteins which influence blood pressure by increasing STK39 expression and consequently alter renal sodium excretion. On Affymetrix 100K platform, several SNPs are found located within 5 kb of STK39.[5] This gene encodes a STK in response to renal tubular cell hypotonicity or low sodium state (i.e. cellular-stress). This leads to phosphorylation of cation chloride coupled co-

[1,2]Department of Biological and Biomedical Sciences, Aga Khan University, Karachi, Pakistan, [3]Unité Mixte de Recherche 6236, Centre National de la Recherche Scientifique, France, [4]Department of Biochemistry, United Medical and Dental College, Ibrahim Hydri, Korangi Creek, Karachi, Pakistan.

Correspondence: Loung Vasandas Umedani. E-mail: loung.umedani@aku.edu

transporters producing activation of p38 Mitogenactivated protein (MAP) kinase pathway. These mechanisms result in the absorption of sodium and water to increase intravascular volume and blood pressure.[6-9]

The presence of the G allele of rs35929607 may increase the activity of downstream sodium potassium chloride co-transporter (NKCC2) and sodium chloride cotransporter (NCC), which would promote Na+ re-absorption, thereby increasing intravascular volume and blood pressure.[5]

Among the various genes attributed to EHTN, some are more important for the overall blood pressure regulation mechanism. For example, angiotensin converting enzyme (ACE) gene, renin (RENIN) gene, angiotensin II receptor 1 (AGTR1) gene and genes involved in the feedback loops of lipid regulation e.g. apolipoprotein A1 (APOA1) etc. What is important for the future is to look at definite number of genetic polymorphisms contributing to genetic variation depending on both genetic and environmental effects.[4] Therefore, SNPs in various genes reflecting the gene interactions will constitute the most important tools to clarify the feedback loops that operate to raise the blood pressure.

Complex disorders like hypertension, type-2 diabetes mellitus and atherosclerosis are known to have a polygenic basis.[10] Hypertension and dyslipidaemia cluster more often than any other common cardiovascular diseases.[11] South Asians and US populations exhibit a higher prevalence of metabolic syndrome.[12-15] The studies of genetic polymorphisms and allelic alterations explore the genetic attributes of the disease. Present strategies towards decreasing hypertension and cardiovascular diseases emphasise awareness and practice of non-pharmacological interventions, especially physical activity and exercise.[16]

Material and Methods

The study was undertaken after approval from the Ethics Review Committee (ERC) of the Aga Khan University (AKU). The research area was located at a site far removed from urban Karachi. The consent form containing information about potential risks and benefits of participating in the study was administered and the signature was obtained from the subjects. In the absence of a signature, a thumb impression of the participant was obtained. In case of children, a parent or guardian's signature or thumb impression was also obtained. AKU ERC approved this procedure of taking the consent. We employed qualified physicians who could assess the clinical condition and co-morbidities and could take good history. We carried out preliminary visits to research area followed by multiple trips to get accurate phenotypic data.

The random cross-sectional study was carried out from 2009 to 2010 on 528 subjects in Tharparkar, Sindh, Pakistan, which is located at 24° 42' 0" North and 70° 11' 0" East coordinates. We recruited the subjects by random selection. The sample size was calculated statistically with confidence level of 95% by assuming 20% prevalence of hypertension among the study population; and we also incorporated 10% design effect in the sample size. We genotyped 528 subjects compared to the calculated sample size of 405 subjects to get satisfactory outcome. The prevalence of genotypes and frequencies of the allele A and G were calculated. The effect size of genetic factors was comparatively analysed with the effect size of risk factors i.e. advanced age (>50 years), presence of comorbid (diabetes) and raised body mass index (BMI) >23 adjusted to raise the blood pressure. BMI of Asian criteria was used due to the subjects being of Dravidian race.[17]

All subjects with a known history of hypertension, newly diagnosed cases and subjects taking anti-hypertensive medication were included. All subjects below 7 years were excluded. The younger population is more exposed to a sedentary life pattern due to current usage of computer games and electronic media so hypertension can occur in paediatric age group as well. We therefore recruited children above 7 years in the study.

Subjects with poor compliance for recruitment and non-compliance in donating blood samples were excluded. A careful past history was obtained by qualified physicians to exclude the secondary causes of hypertension e.g. renal, endocrinological, cortisone-induced and subjects with diabetes as a predisposing cause of hypertension. Hypertensive subjects were also assessed for presence of its complications and other associated comorbids (i.e. stroke, ischaemic heart disease and development of diabetes mellitus after the onset of hypertension etc.)

Anthropometric and demographic data were recorded for each subject. Three blood pressure measurements were taken from the left arm at 15-minute intervals in the resting state from each individual. Any individual with systolic blood pressure >140mmHg and diastolic blood pressure >90mmHg on all the three occasions was diagnosed as hypertensive. Hypertensive subjects were designated as the cases, while normotensives as the controls.

Blood samples were collected in tubes containing ethylenediaminetetra acetic acid (EDTA) and stored at 4°C immediately.

Deoxyribonucleic acid (DNA) was extracted from the

whole blood by phenol-chloroform-isoamyl alcohol method (Centre for Cell and Gene Therapy, Vector Development Lab., Texas, USA). FASTA sequence showing point mutation in STK39 gene at SNP rs35929607 was obtained from the NCBI website.[18] Primers were designed for tetra primers amplification refraction mutation system-polymerase chain reaction (ARMS-PCR) method by using the software http://cedar.genetics.soton.ac.uk/public_html/primer1.html.[19] (viz. inner forward CTC ATG GAA TTA AAG GAT TAT TAG GAT AAC G Mer31, Outer forward AAC ACT CTC ACA AGA AGA GAT CCC AGT G Mer28, Inner reverse CAC ATT TTG GCA GTG TTT GGA CAG CT Mer26, Outer reverse CTC CCA GGT CGT TTT CAA ACA AAA ATA A Mer28).

All subjects were genotyped with 100% quality controls and we got accurate results. In order to detect allele A, we used tetra primer ARMS-PCR reaction. The detection of the allele G was carried out by using ARMS PCR reaction. PCR was carried out using an Eppendorf Gradient Thermocycler. The reaction mix was incubated and the following programme was used: 95°C for 7 minutes (initial denaturation); 95°C for 45 seconds (denaturation, 44 cycles); 66°C for 45 seconds (annealing, 44 cycles); 72°C for 45 seconds (extension, 44 cycles); 72°C for 7 minutes (Final extension) and 4°C (hold phase).

The PCR products were separated by 2.5% agarose gel electrophoresis run at 120V for 30 to 40 minutes, and the gel was transferred to Gel Doc (BioRad) for visualisation under ultraviolet light. Bands for the required product sizes were obtained (i.e. outer primers 349bp, allele-A 175bp and allele-G 231bp).

Data was analysed statistically for prevalence of individual homozygous i.e. AA and GG genotypes, and heterozygous AG genotypes, frequencies of alleles A and G and comparative analysis of effect size of genetic versus risk factors. Assessment for concordance with Hardy Weinberg Equilibrium (HWE) was performed. Chi-square and odds ratio with 95% confidence limitation for frequencies of allele A and allele G was calculated. Statistical softwares used for these analyses were SPSS 14, Microsoft Excel and Epi-Info 1.1.

Results

Of the total, 273 (52%) were males, and 255 (49%) females. All subjects were genotyped for STK39 SNP. The mean age of the subjects was 37.08±16.42 years.

Genotype frequencies were found to be 191 (36.2%) for AA; 322 (61.0%) for AG; and 15 (2.8%) for GG. Frequency of reference allele (A) was 704 (66.7%) and of rare allele (G) was 352 (33.3%) (χ^2 = 73.1 and P= 0.0001) (Table-1). A sanity check indicated that the population did not fall in concordance with HWE and it deviated from the right population structure and appeared to be stratified, signifying the fact that there were hypertensive phenotypes present in it. The controls were also tested for HWE which also showed a similar pattern.

Significant differences in the distribution of three genotypes according to clinical phenotype were observed (Table-2). Occurrence of AG genotype was found higher in the majority of subjects (59% [n=44] in hypertensive subjects; 64% [n=27] in subjects with raised systolic BP; 59% [n=33] in raised diastolic BP; 59% [n=162] in males and 63% [n=160] in females). Subjects who became diabetic had 57% (n=19) occurrence of AG genotype. Phenotypes which differed and did not fall in HWE were the presence of hypertension (p= 0.008), asthma (p= 0.01), systolic BP >140mmHg (p= 0.02) and diastolic BP >90mmHg (p= 0.02). Such deviations are to be expected when there is a marked association with the clinical phenotype, especially in homogenous populations. The diabetic subjects showed a borderline value for falling in HWE (p= 0.06).

The odds ratio of STK39 rs35929607 with essential hypertension was 3.07 (95% confidence interval 2.10-4.49) units/mmHg per G allele (p= 0.000) (Table-3). Association of STK39 rs35929607 polymorphism with raised systolic BP >140 was 0.76 (95% CI 0.47-1.23) units/mmHg per G allele (p= 0.235). Association of STK39 rs35929607 polymorphism with raised diastolic BP >90mmHg was 0.93 (95% CI 0.61-1.44) units/mmHg per G allele (p= 0.735). Frequency of allele G was less (i.e. 33.3%) than that of allele A (66.7%), (p= 0.0000). Frequency of allele G was also non-significantly associated with hypertensive subjects which later on became diabetic [Odds ratio 1.08; 95% CI, 0.61-1.90, (p= 0.787) units per G allele].

Table-1: Statistical genotype frequencies, allele frequencies and Hardy Weinberg Equilibrium analysis of the studied population.*

Genotype	**Observed number**	**Genotype frequency**
11	191	0.362
12	322	0.610
22	15	0.028
Total Genotyped	528	
Allele	**Observed number**	**Allele frequency**
1	704	0.667
2	352	0.333
Total	1056	
$\chi 2$	73.129	
p-value*		$\leq$ 0.0001

* Sanity check indicating the subjects of selected population (n = 528) deviated from HWE.
1: allele-A, 2: allele-G.

The effect size of genetic factors was tested and compared with the effect size of the risk factors (i.e. advanced age >50 years, presence of diabetes and BMI >23) adjusted to raise blood pressure. The blood pressure raising effect due to risk factors was found to be highly significant in GG homozygotes i.e. β= 0.747 (p= 0.001). This effect is 1.6 fold higher than the risk factor effect of single allele (i.e. allele G in AG heterozygotes, β= 0.469 (p= 0.000). In contrast, the effect size due to genetic factors alone in these patients was found to be non-significant i.e. β= 0.062 (p= 0.153) for GG homozygotes and β= -0.013 (p= 0.772) for AG heterozygotes (Table-4). Similar findings were obtained in case of analyses carried out for isolated raised systolic (BP>140) and raised diastolic blood pressure (BP>90). The risk factors increased the effect by 12.04 fold in GG genotype and the risk factors need to be 3 times higher to create significant effect in the presence of single G allele in case of AG genotype.

The effect size of the risk factors was further looked for the

Table-2: Genotypes, allele frequencies and χ^2 to show significance of variables with Hardy Weinberg Equilibrium (HWE).

Basic Characteristics	Genotype Frequencies			Alleles Frequencies		χ^2	p-value
	AA, n (%)	AG, n (%)	GG, n (%)	A, n (%)	G, n (%)		
Males ¤	102 (37)	162 (59)	9 (3.3)	366 (67)	180 (33)	32.0	0.0001
Females ¤	89 (35)	160 (63)	5 (2.0)	338 (66)	170 (33)	43.6	0.0001
Hypertensives	26 (35)	44 (59)	4 (5.4)	96 (64)	52 (35)	6.8	0.008
Nonhypertensives	165 (36)	11 (2.4)	278 (61)	341 (37)	567 (62)	408.0	0.0001
Diabetics ¤	13 (39)	19 (57)	1 (3.0)	45 (68)	21 (31)	3.5	0.06*
Non-diabetic ¤	177 (36)	301 (61)	14 (2.8)	655 (66)	329 (33)	68.9	0.0001
Asthmatics ¤	21 (37)	33 (58)	2 (3.6)	75 (67)	37 (33)	6.1	0.01
Non-asthmatics ¤	168 (36)	13 (2.8)	285 (61)	349 (37)	583 (62)	412.1	0.0001
SBP<140mmHg ¤	175 (36)	290 (60)	12 (2.5)	640 (67)	314 (32)	67.6	0.0001
SBP>140mmHg ¤	12 (28)	27 (64)	3 (7.1)	51 (60)	33 (39)	5.07	0.02
DBP<90mmHg ¤	167 (36)	283 (61)	12 (2.6)	617 (66)	307 (33)	66.8	0.0001
DBP>90mmHg ¤	20 (35)	33 (59)	3 (5.4)	73 (65)	39 (34)	4.9	0.02

n: observed number, SBP: systolic blood pressure, DBP: diastolic blood pressure; *Falling in concordance with HWE. ¤: Missing values for both genders (1), diabetics and non-diabetics (3), asthmatics and non-asthmatics (6), systolic BP (9) and diastolic BP (10).

Table-3: Logistic regression determinants of main variables with frequencies of allele A and allele G.

Alleles	Comparative frequencies of cases			O R	95% CI	χ^2	p-value
	Hypertensive	**Normotensive**	**Sum**				
Allele- A	96	341	437				
Allele - G	52	567	619				
	148	908	1056	3.07	2.10 – 4.49	39.1	0
	Diabetics	**Non-diabetics**	**Sum**				
Allele- A	45	655	700				
Allele - G	21	329	350				
	66	984	1050	1.08	0.61 – 1.90	0.07	0.787
	Asthmatics	**Non-asthmatics**	**Sum**				
Allele- A	75	349	424				
Allele - G	37	583	620				
	112	932	1044	3.39	2.19 – 5.24	36.12	0
	SBP>140mmHg	**SBP<140mmHg**	**Sum**				
Allele- A	51	640	691				
Allele - G	33	314	347				
	84	954	1038	0.76	0.47 – 1.23	1.41	0.235
	DBP>90mmHg	**DBP<90mmHg**	**Sum**				
Allele- A	73	617	690				
Allele - G	39	307	346				
	112	924	1036	0.93	0.61 – 1.44	0.11	0.735

OR: Odds Ratio, SBP: systolic blood pressure, DBP: diastolic blood pressure.

Table-4: Comparative analysis of effect size of STK39 variant rs35929607 with the effect size of risk factors.

Subject's Phenotype	Model	β-value*	95% Confidence Limit	p-value
Hypertensives BP>140/90mmHg (Both SBP & DBP raised) GG-genotype				
	M1	0.062	-0.097, 0.618	0.153
	M2	0.328	0.352, 0.642	0.000
	M3	0.564	0.583, 10.043	0.000
	M4	0.291	-0.432, -0.208	0.000
	M5	0.747	0.104, 0.216	0.000
Hypertensives BP>140/90mmHg (Both SBP & DBP raised) AG-genotype				
	M1	-0.013	-070, 0.052	0.772
	M2	0.257	0.360, 0.650	0.000
	M3	0.542	0.579, 10.040	0.000
	M4	0.214	-0.427, -0.203	0.000
	M5	0.469	0.102, 0.213	0.000
Normotensives BP<140/90mmHg (Both SBP & DBP normal) GG-genotype				
	M1	-0.062	-0.309, 0.048	0.153
	M2	-0.328	-0.321, -0.176	0.000
	M3	-0.564	-0.521, -0.291	0.000
	M4	-0.292	0.104, 0.216	0.000
	M5	-0.747	-0.108, -0.052	0.000
Normotensives BP<140/90mmHg (Both SBP & DBP normal) AG-genotype				
	M1	0.013	-0.026, 0.035	0.772
	M2	-0.257	-0.325, -0.180	0.000
	M3	-0.498	-0.520, -0.290	0.000
	M4	-0.214	0.102, 0.213	0.000
	M5	-0.662	-0.107, -0.051	0.000
Raised SBP(>140mmHg) GG-genotype				
	M1	0.075	-0.035, 0.526	0.086
	M2	0.385	0.340, 0.565	0.000
	M3	0.469	0.041, 0.411	0.017
	M4	0.390	-0.182, 0.001	0.052
	M5	0.556	0.000, 0.091	0.052
Raised SBP(>140mmHg) AG-genotype				
	M1	0.020	-0.037, 0.059	0.657
	M2	0.333	0.344, 0.569	0.000
	M3	0.419	0.040, 0.410	0.017
	M4	0.340	-0.177, 0.005	0.065
	M5	0.488	-0.003, 0.089	0.065
Raised DBP(>90mmHg) GG-genotype				
	M1	0.051	-0.131, 0.509	0.246
	M2	0.253	0.204, 0.469	0.000
	M3	0.296	-0.087, 0.351	0.238
	M4	0.117	-0.301, -0.082	0.001
	M5	0.421	0.041, 0.150	0.001
Raised DBP(>90mmHg) AG-genotype				
	M1	-0.015	-0.064, 0.046	0.737
	M2	0.191	0.210, 0.475	0.000
	M3	0.234	-0.090, 0.348	0.249
	M4	0.055	-0.298, -0.079	0.001
	M5	0.353	0.039, 0.149	0.001
Normal SBP(<140mmHg) GG-genotype				
	M1	-0.075	-0.263, 0.018	0.086
	M2	0.385	-0.282, -0.170	0.000
	M3	0.469	-0.205, -0.020	0.017
	M4	-0.390	0.000, 0.091	0.052
	M5	0.556	-0.045, 0.000	0.052

Normal SBP(<140mmHg) AG-genotype				
	M1	-0.020	-0.030, 0.019	0.657
	M2	-0.333	-0.285, -0.172	0.000
	M3	0.419	-0.205, -0.020	0.017
	M4	0.340	-0.003, 0.089	0.065
	M5	0.498	-0.044, 0.001	0.065
Normal DBP(<90mmHg) GG-genotype				
	M1	-0.051	-0.255, 0.065	0.246
	M2	-0.253	-0.235, -0.102	0.000
	M3	-0.296	-0.175, 0.044	0.238
	M4	-0.117	0.041, 0.150	0.001
	M5	-0.291	-0.075, -0.020	0.001
Normal DBP(<90mmHg) AG-genotype				
	M1	0.015	-0.023, 0.032	0.737
	M2	-0.191	-0.238, -0.105	0.000
	M3	-0.234	-0.174, 0.045	0.249
	M4	-0.055	0.039, 0.149	0.001
	M5	-0.353	-0.074, -0.020	0.001

M (Model) 1= Effect size due to alleles only (i.e. GG or AG respectively), M2= Alleles GG or AG + Age>50 years M3= Alleles GG or AG + Age>50 years + diabetes, M4= Alleles GG or AG + Age >50 years + diabetes + BMI<23 (Asian Criteria), M5= Alleles GG or AG + Age>50 years +diabetes + BMI>23 (Asian Criteria).
SBP=Systolic blood pressure, DBP=Diastolic blood pressure. *= Cohen's rule (?-values of 0.2-0.3 shows small effect, 0.4-0.5 medium effect and 0.6-0.8 large effect).

one causing most profound rise of blood pressure. It was found that the most significant effect was due to BMI>23 followed by diabetes and advanced age (β= 0.747, 0.564, 0.328 respectively in case of GG homozygotes).

Discussion

Essential hypertension (EHTN) is a known complex multifactorial disorder in which genetic and environmental risk factors play a part in etio-pathogenesis.[20] Prevalence of hypertension varies from region to region throughout the world. It is lowest in the Indo-Pak subcontinent (India, 3.4% in men and 6.8% in women) and the highest in Poland (68.9% in men and 72.5% in women).[21] Coronary artery disease is also a notable cause of morbidity and mortality in the US (specially African-Americans) and South Asians.[22-24] The mortality rates from coronary artery disease in South Asians are reported to be two to three times higher than those in Caucasians irrespective of gender, religion, social class, dietary practices or the country of residence.[24] In Pakistan, prevalence of EHTN is found to be 22.7% (as obtained from the WHO website i.e. Info Base: All data, Pakistan). The presently studied population from Tharparkar, Pakistan, showed prevalence of EHTN to be 14.3%.

In a European study carried out in 2009, the STK39 gene SNP rs35929607 were coincidently associated significantly with systolic and diastolic blood pressure, (p <0.05).[5] In the present study, we found an insignificant association of this polymorphism with EHTN (p= 0.153). We looked at the prevalence of STK39 SNP and observed the prevalence of frequencies of reference and rare alleles and also observed the concordance with HWE.

STK39 locus lies within a genomic region in which multiple blood pressure, obesity, and diabetes-related rodent quantitative trait loci have been mapped. This close proximity of the EHTN, obesity and diabetes-related loci may explain the tendency of hypertensive subjects to develop diabetes.[25] Individuals with an inherent genetic susceptibility to develop EHTN when exposed to the hypertension-inducing risk factors develop the onset and progression of the disease. Hence, minimising these risk factors will tend to prevent such onset and complications of EHTN. Studies have shown that physical activity and exercise decrease blood pressure in about 75% of hypertensive individuals.[24] With exercise, patients also improve other important parametres like lipoproteins and lipid levels as well as insulin sensitivity.[24]

The variant allele A→G which is characteristic of this SNP ideally showed a high prevalence of the genotype AG to be associated with EHTN. The reference allele A showed higher frequency than the rare allele G (p 0.0001). These findings highlight the weaker association of STK39 gene with EHTN in the study population. It is anticipated that in this case, the environmental risk factors might have played a predominant role in increasing the blood pressure.

We confirmed the significance of the effect size of the environmental risk factors after adjusting for raised systolic and diastolic blood pressure. We found that the effect size increased with addition of tested risk factor (i.e. advanced age, diabetes and raised BMI). With the low BMI (<23), the β-value was less (i.e. 0.291), but its value was found to drastically increase with high levels of BMI (>23)

to 0.747 in hypertensive phenotype. In case of the normotensives with GG homozygotes, though the β-value came out to be high, it gave an insignificant p value for all the tested risk factors except age>50 years. This showed that GG homozygote individuals, though they show a normal phenotype, are showing tendency to be hypertensive on addition of risk factors. We also report that the analyses carried out separately for the male and female genders revealed similar pattern of the effect size of genetic and risk factors.

Linear and logistic regression analyses showed that the main blood pressure raising effect was found to be due to BMI which itself is regulated by discrete genes implicated in its control. BMI was also found to follow a rising trend with advancing age, but age itself was an independent factor separate from BMI in this study. In the light of this, age factor may be ruled out, but BMI could be deemed as the most significant factor which in turn is to be impacted by discrete set of its own regulating genes.

An aggressive management of the raised BMI, dyslipidaemia and hypertension is, therefore, warranted to lower the risks of EHTN and cardiovascular disease. Future research should focus on the development of inexpensive and efficient systems to improve blood pressure and lipid profiles.

Conclusions

In this study, essential hypertension risk conferred by STK39 rs35929607 polymorphism (A/G) was different from the previously reported European Population, suggesting that the underlying variant G allele in heterozygotes in the Pakistani population remain unidentified (less associated) in the absence of the risk factors. Interaction of the genetic and environmental risk factors was essential for significant association of GG homozygous alleles in the study population.

References

1. Powles JW, Zatonski W, Vander Hoorn S, Ezzati M. The contribution of leading diseases and risk factors to excess losses of healthy life in Eastern Europe: burden of disease study. BMC Public Health 2005; 5: 116.
2. Kearney PM, Whelton M, Reynolds K. Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. Lancet 2005; 365: 217-23.
3. Anand SS, Yusuf S, Vuksan V, Devanesen S, Teo KK, Montague PA, et al. Differences in risk factors, atherosclerosis, and cardiovascular disease between ethnic groups in Canada: the Study of Health Assessment and Risk in Ethnic groups (SHARE). Lancet 2000; 356: 279-84.
4. Pickering G. Hypertension in general practice. J R Soc Med 1978; 71: 885-9.
5. Wang Y, O'Connell JR, McArdle PF, Wade JB, Dorff SE, Shah SJ, et al, From the Cover: Whole-genome association study identifies STK39 as a hypertension susceptibility gene. Proc Natl Acad Sci USA 2009; 106: 226-31.
6. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005; 21: 263-5.
7. Coleman R A, Liu J, Wade J B. Use of anti-fluorophore antibody to achieve high-sensitivity immunolocalizations of transporters and ion channels. J Histochem Cytochem 2006; 54: 817-27.
8. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, et al. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 2007; 316: 1341-5.
9. Siepel A, Bejerano G, Pedersen JS, Hinrichs AS, Hou M, Rosenbloom K, et al. Evolutionarily conserved elements in vertebrate, insect, worm, and yeast genomes. Genome Res 2005; 15: 1034-50.
10. Aghasadeghi K, Zarei-Nezhad M, Keshavarzi A, Mehrabani D. The prevalence of coronary risk factors in Iranian lor migrating tribe. Arch Iran Med 2008; 11: 322-5.
11. Stergiou GS, Salgami EV; World Health Organization-International Society of Hypertension (WHO-ISH), USA Joint National Committee on Prevention, Detection,Evaluation, and Treatment of High Blood Pressure (JNC-7), European Society of Hypertension-European Society of Cardiology (ESH-ESC). New European, American and International guidelines for hypertension management: agreement and disagreement. Expert Rev Cardiovasc Ther 2004; 2: 359-68.
12. Grundy SM. Obesity, metabolic syndrome, and coronary atherosclerosis. Circulation 2002; 105: 2696-8.
13. Grundy SM, Abate N, Chandalia M. Diet composition and the metabolic syndrome: what is the optimal fat intake? Am J Med 2002; 113 Suppl 9B: 25S-9S.
14. McKeigue PM. Mapping genes underlying ethnic differences in disease risk by linkage disequilibrium in recently admixed populations. Am J Hum Genet 1997; 60: 188-96.
15. Sattar N. The metabolic syndrome: should current criteria influence clinical practice? Curr Opin Lipidol 2006; 17: 404-11.
16. Akbartabartoori M, Lean ME, Hankey CR. The associations between current recommendation for physical activity and cardiovascular risks associated with obesity. Eur J Clin Nutr 2008; 62: 1-9.
17. WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet 2004; 363: 157-63.
18. Sayers EW, Barrett T, Benson DA, Boltan E, Beyant SH, Canese K, et al. Database resources of National Center for Biotechnology Information Nucleic Acid Res 2011, 39: D38-D51.
19. Shu Y, Dhillon S, Ke X, Collins AR, Day INM. An efficient procedure for genotyping single nucleotide polymorphisms. Nucl Acids Res 2001; 29: e88.
20. Gong M, Hubner N. Molecular genetics of human hypertension. Clin Sci (Lond) 2006; 110: 315-26.
21. Kearney PM, Whelton M, Reynolds K, Whelton PK, He J. Worldwide prevalence of hypertension: a systematic review. J Hypertens 2004; 22: 11-9.
22. Uppaluri CR. Heart disease and its related risk factors in Asian Indians. Ethn Dis 2002; 12: 45-53.
23. Enas EA, Yusuf S, Mehta JL. Prevalence of coronary artery disease in Asian Indians. Am J Cardiol 1992; 70: 945-9.
24. Dodani S. Excess coronary artery disease risk in South Asian immigrants: can dysfunctional high-density lipoprotein explain increased risk? Vasc Health Risk Manag 2008; 4: 953-61.
25. Zinner SH, Levy PS, Kass EH. Familial aggregation of blood pressure in childhood. N Engl J Med 1971; 284: 401-4.