# Relationship between serine/threonine kinase 39 gene polymorphisms with some cardiac biomarkers in hypertensive patients

Fadhil Jawad Al-Tu'ma,[a] Zena Abdul-Ameer Mahdi[a] & Hassan Mahmood Abo Almaali[b]

[a]Deparment of Biochemistry, College of Medicine, University of Karbala, Holy Karbala city, Iraq.
[b]Branch of Clinical Laboratory Science, College of Pharmacy, University of Karbala, Holy Kerbala city, Iraq.
Correspondence to Zena Abdul-Ameer Mahdi (email: z.al-hadedy@outlook.com).
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**Aim** This study aimed to evaluate the association between STK39 SNP rs35929607 and some cardiovascular risk factors in hypertension patients in holy Kerbala city, Iraq.
**Materials and Methods** We included 74 hypertensive patients with no signs and symptoms of renal impairment and another 30 control subjects. The links between genotype and hypertension were examined. Then, the SNP related variances in the blood pressure and remaining cardiovascular risk factors were studied.
**Results** There is no significant association between STK39 rs35929607 and hypertension in current study. However Allele A showed a significant association in hypertensive patient compared with control group particularly in female gender. The hypertensive patients showed a significant higher result in age, BMI, FBS, total cholesterol, and STG, LDL-C and lower level in HDL-C.
**Conclusion** The association between the SNP rs35929607ofSTK3 and hypertension was not significant in current study in Kerbala population of Iraq. Furthermore, only Allele A showed a significant association with hypertension in females group. Further studies needed to clarify the effect of other STK39 variants on these cardiovascular risk factors.
**Keywords** hypertension, BMI, STK39 , LDL-C, polymorphism

## Introduction

The pathogenesis of essential hypertension is believed to be multifactorial and under the influence of multiple genetic and environmental factors.[1] Genes associated with blood pressure (BP) are not sufficiently known as yet.[2] A recent genome wide association study examined single nucleotide polymorphisms (SNPs) in American old order Amish and identified SNPs in the serine/threonine kinase 39 (STK39) that were associated with hypertension.[3] The STK39 encodes a serine/threonine kinase known as a STE20-related proline rich kinase (SPAK), one of STE20 family. These kinases were shown to interact with a new discovered serine/threonine kinases (WNK kinases) and cation-chloride co-transporters, and it was suggested that their functional change may cause BP dysregulation.[4] Furthermore the SPAK is also related to cytoskeletal rearrangement, mitogen activated protein (MAP) kinases, and inflammation. The associations between the above-mentioned SNPs and different STK39 SNPs and BP were confirmed in other Caucasian and Chinese cohorts.[5,6] However, the influence of STK39 polymorphism on BP has been inconsistent, and the effects of the variants have not been found in other large studies.[7,8] Although a few studies have been conducted to replicate the results in the Asian population, the association between STK39 variants and hypertension is still unclear among the Asians.

Here we aimed to examine the effect of rs35929607 of STK39 on hypertension in a population of Karbala province, Iraq, using 74 hypertensive and 30 control subjects. The primary purpose was to evaluate the association between the SNPs and the risk of hypertension in females and males. In addition, we investigated the effects of the SNPs on other cardiovascular risk factors.

## Materials and Methods

### *Subjects*

In current study, 74 hypertensive patients were studied who were all diagnosed previously and some were taking treatment along with the 30 healthy controls. And the control subjects had to have systolic BP <135 mmHg and diastolic BP <85 mmHg, without using any blood pressure lowering agents. Patients were selected from the out-patient department of Al-Hussein Teaching Hospital, Al-Hussein Medical City, Holy Karbala, Iraq. The history, blood pressure, cardiac biomarkers and body mass index of all the subjects were investigated and measured.

On the day of enrolment, clinical data including demographic variables, medical history, and antihypertensive medications were recorded for all study samples. BP was measured by trained nurses at the right brachial artery using a mercury sphygmomanometer. Subjects had 10 minutes of rest in the supine position before the measurements, and the average of at least three measurements were used in the study. Venous blood samples were collected after an overnight fast, and samples were analyzed within 4 hours of collection. All analyses were conducted by the hospital laboratory.

### *Genotyping*

Genomic deoxyribonucleic acid (DNA) was extracted from 2 mL of peripheral venous blood by Genomic DNA Extraction Kit, from BIONEER, South Korea. In order to detect allele A, tetra primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) has been used. The detection of the allele G was carried out by using ARMS PCR reaction for STK39 rs35929607 (Figs. 1, 2). The PCR was carried out using an eppendorf gradient

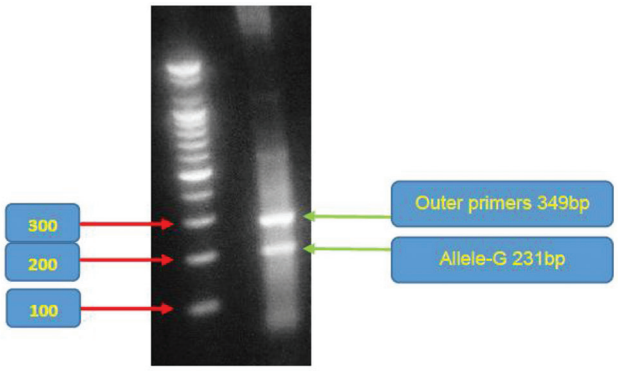


Fig. 1 **Electrophoresis band for allele G of STK39 gene.**

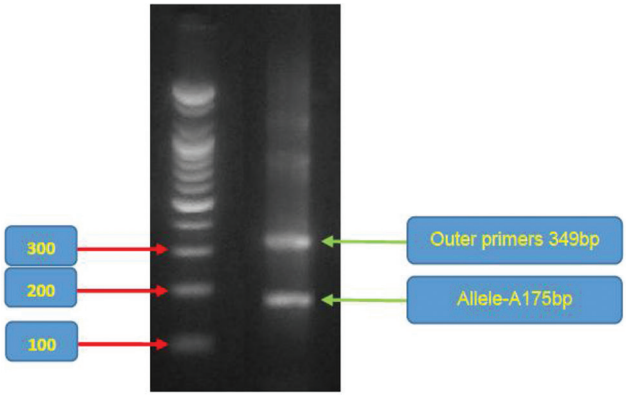


Fig. 2 **Electrophoresis band for allele A of STK39 gene.**

Table 1. **Four primers for STK39rs 35929607**[9]

| | | |
|---|---|---|
| STK-1 | CTCATGGAATTA AAGGATTATTAGGATAACG | Inner forward |
| STK-2 | AACACTCTCACAAGAAGAGATCCCAGTG | Outer forward |
| STK-3 | CACATTTTGGCAGTGTTTGGACAGCT | Inner reverse |
| STK-4 | CTCCCAGGTCGTTTTCAAACAAAAATAA | Outer reverse |

thermocycler. The reaction was carried out according to the following program:

- 95°C for 7 minutes (initial denaturation), 40 cycles of the following:
- 95°C for 45 seconds (denaturation)
- 64°C for 45 seconds (annealing)
- 72°C for 45 second (extension)
- 72°C for 7 minutes (final extension) and 4°C (hold phase)

The PCR products were separated by 1.5% agarose gel electrophoresis and the run at 120 V for 30–40 minutes and the gel was transferred to UV trans-illuminator for visualization under ultraviolet light. Bands for the required product sizes were obtained. Bands for the required product sizes of STK39 gene were obtained as outer primers 349 bp, allele-A 175 bp and allele-G 231 bp (Table 1).

### *Biochemical Parameters*

Fasting blood sample of (3 ml) collected by venipuncture placed into plain tubes and allowed to clot for 10–15 minutes,

Table 2. **Characteristics of the study subjects**

| Parameters | Hypertension Mean ± SD | Control Mean ± SD | p value |
|---|---|---|---|
| Age (years) | 51.07 ± 10.60 | 46.67 ± 12.62 | 0.07 |
| BMI (kg/m$^2$) | 29.62 ± 5.42 | 24.06 ± 1.63 | 0.00 |
| SBP (mmHg) | 144.69 ± 21.46 | 117.50 ± 13.57 | 0.00 |
| DBP (mmHg) | 88.51 ± 10.97 | 71.83 ± 9.42 | 0.00 |
| FBS (mg/dl) | 131.07 ± 63.25 | 97.27 ± 9.73 | 0.00 |
| Serum urea (mg/dl) | 29.00 ± 8.32 | 27.00 ± 6.32 | 0.23 |
| S. creatinine (mg/dl) | 0.71 ± 0.25 | 0.68 ± 0.24 | 0.49 |
| S. albumin (g/L) | 4.37 ± 0.24 | 4.33 ± 0.23 | 0.43 |
| S. cholesterol (mg/dl) | 182.88 ± 38.71 | 178.90 ± 35.85 | 0.62 |
| S. TG (mg/dl) | 181.93 ± 108.46 | 147.50 ± 74.87 | 0.11 |
| S. HDL-C (mg/dl) | 39.65 ± 23.03 | 41.07 ± 15.48 | 0.75 |
| S. LDL-C (mg/dl) | 108.79 ± 40.51 | 108.73 ± 36.06 | 0.99 |
| S. VLDL-C (mg/dl) | 42.75 ± 60.47 | 32.33 ± 20.44 | 0.36 |

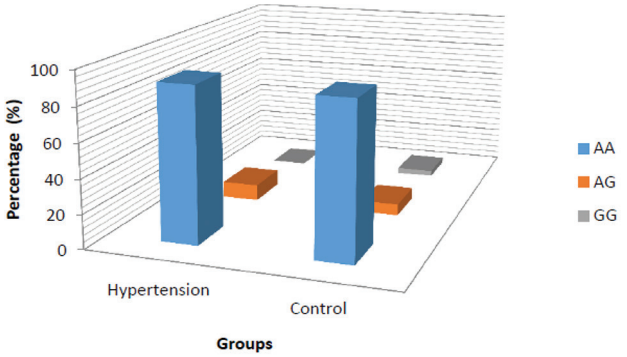


Fig. 3 **The STK39 rs35929607 genotype in patients and control groups.**

centrifuged, and the separated serum was used for further measurement of fasting blood sugar, urea, creatinine, albumin, and lipid profile.

### *Statistical Analysis*

Group differences in the categorical variables, genotypes, were assessed by chi-square test, and continuous variables were examined by Student's t-test. Results were expressed as mean ± SD and 2-tailed value of $p < 0.05$ was considered statistically significant. All data were analyzed using Statistical Package for the Social Sciences (SPSS) 17.0 version.

## Results

The characteristics of population of this study are presented in Table 2. The average age was 51.07 ± 10.60 years for the patients and 46.67 ± 12.62 years for the controls. The hypertensive patients have higher BMI, FBS, with significant P = 0.00 and high total cholesterol, triglyceride, LDL-C, and low high density lipoprotein-cholesterol (HDL-C) levels.

### *Relationship between Genotype of STK39 and Hypertension (Fig. 3)*

Genotype distributions of STK39 polymorphisms were almost the same between normotensive controls and hypertensive patients (Table 3).

Table 3. **The STK39 rs35929607 genotypes distribution in hypertension**

| | | AA N (%) | AG N (%) | GG N (%) | p value |
|---|---|---|---|---|---|
| Sex | Female | 60 (92.3) | 5 (7.7) | 0 (0.0) | 0.38 |
| | Male | 34 (87.2) | 4 (10.3) | 1 (2.6) | |
| Chronic Diseases | Hypertension | 67 (90.5) | 7 (9.5) | 0 (0.0) | 0.27 |
| | Control | 27 (90.0) | 2 (6.7) | 1 (3.3) | |
| Systolic | SBP > 140 mmHg | 43 (84.3) | 7 (13.7) | 1 (2.0) | 0.11 |
| | SBP < 140 mmHg | 51 (96.2) | 2 (3.8) | 0 (0.0) | |
| Diastolic | DBP > 90 mmHg | 44 (84.6) | 7 (13.5) | 1 (1.9) | 0.13 |
| | DBP < 90 mmHg | 50 (96.2) | 2 (3.8) | 0 (0.0) | |

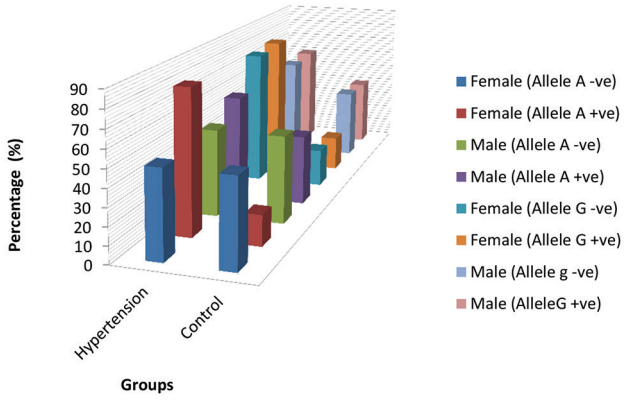


Fig. 4 **The STK39 alleles in hypertension and control groups.**

Table 4. **The STK39 alleles in hypertension and control groups**

| Sex | Alleles | Result | Hypertension N (%) | Control N (%) | p value |
|---|---|---|---|---|---|
| Female | Allele A | negative | 4 (50.0) | 4 (50.0) | 0.05 |
| | | positive | 47 (82.5) | 10 (17.5) | |
| Male | Allele A | negative | 2 (50.0) | 2 (50.0) | 0.54 |
| | | positive | 21 (60.0) | 14 (40.0) | |
| Female | Allele G | negative | 47 (78.3) | 13 (21.7) | 0.70 |
| | | positive | 4 (80.0) | 1 (20.0) | |
| Male | Allele G | negative | 20 (58.8) | 14 (41.2) | 0.67 |
| | | positive | 3 (60.0) | 2 (40.0) | |

### *Relationship between Studied Alleles and Hypertension (Fig. 4)*

The relationship between hypertension risk and alleles of STK39 in males is presented in Table 4. Indicated no association between genotypes and hypertension in any group which reached statistical significance. The only significant association was in females with allele A.

### *Relation among Studied Variants, Blood Pressure, and Cardiovascular Risk Factors*

To start with, we have to clarify that variants GG has been detected in one sample hence all this variants statistics depended on one sample which would have been much better having more than one case for accurate outcome.

Although it has not been statistically proven, all AG variants in total sample and hypertensive patients have higher systolic BP than other variants.

Also, an obvious relationship has been clarified for the AG variants with elevated S. urea, S. creatinine, S. total cholesterol, S. TG and S. LDL-C compared to other variants in total, hypertensive, and control groups with significant P value in the control group of serum urea.

Moreover, AG variant patients have lower S. HDL-C in comparison to the other variants in all groups (Table 5).

## Discussion

In the current study, we found no significant association between SNP STK39 rs35929607and hypertension in the case control study of 104 Iraqi populations. Although there was no association of this SNP on adjusted BPs, there was a significant association between allele A of STK39 rs35929607 and hypertension group. Also, there was a significant association between STK39 genotype and age, systolic BP and albumin this relationship was more obvious in females.

On the other hand, the polymorphisms of STK39 were reported to be associated with BP first in Caucasian samples.[3] In a meta-analysis combining four studies, Wang et al.,[3,4] showed the effect of SNPs of rs6749447 and rs3754777 on BP. Another SNP of STK39, rs35929607, was also related to the hypertension prevalence in women of two cohorts in Swedes.[5] In the Chinese population, two SNPs of STK39 (rs6433027 and 3754777) were found to be associated with hypertension.[6]

In the results of current study, the associations between STK39 SNPs and hypertension or BP were not significant. Although the reason for the difference is not clear, several possibilities may be suggested.

First, all the samples were of Iraqis resident in Karbala holy city, and the STK39-BP relationship may be weak in this population. The influence of STK39 polymorphism on BP has not been consistently confirmed. In two large GWAS, using more than 60,000 people, the effects of STK39 SNPs on BP were not found.[7,8]

In addition, in one British[10] and one Chinese[11] study, variants of STK39 were not associated with BP. Recently, Rhee et al.[12] reported four previously reported SNPs associated with salt-sensitivity in 101 Koreans. However, in that study, no polymorphism of STK39 showed significant effects after adjusting of confounding factors.

Second, although STK39 gene encodes for a protein that plays a role in BP regulation, its final effects may be too small to be detected consistently in every cohort study. Then, as the population size of this study is not big enough, it cannot be completely ruled out that some variants with impacts may not have obtained statistical significance.

In this study, the influence of STK39 variants on the risk factors was clearer in women. This finding is in accordance with prior studies that have shown gender dependency of STK39 effect. In a study by Fava et al.[5] the association of STK39 rs35929607 variant and hypertension was evident only in women. Sex differences in hypertension may be attributed to multiple factors such as lifestyle, diet, and genetic polymorphism.[13] Interestingly, it has been documented that SPAK expression is affected by both androgen and oestrogens in

Table 5. **Genotype of the studied variants and cardiovascular risk factors**

| Risk factors | Alleles | Total | Hypertension | Control |
|---|---|---|---|---|
| | | Mean ± S.D. | Mean ± S.D. | Mean ± S.D. |
| Age (years) | | 0.35 | 0.11 | 0.91 |
| | AA | 49.28 ± 11.28 | 50.43 ± 10.69 | 46.41 ± 12.36 |
| | AG | 55.00 ± 11.93 | 57.14 ± 7.97 | 47.50 ± 24.75 |
| | GG | 52.00 ± 0.00 | 0.00 | 52.00 ± |
| BMI | | 0.87 | 0.93 | 0.1 |
| | AA | 27.95 ± 5.44 | 29.60 ± 5.57 | 23.86 ± 1.55 |
| | AG | 28.83 ± 4.00 | 29.79 ± 4.02 | 25.49 ± 1.53 |
| | GG | 26.70 ± 0.00 | 0.00 | 26.70 ± |
| Systolic BP | | 0.11 | 0.07 | 0.08 |
| | AA | 135.34 ± 23.06 | 143.24 ± 21.48 | 115.74 ± 13.06 |
| | AG | 152.22 ± 19.22 | 158.57 ± 16.76 | 130.00 ± .00 |
| | GG | 140.00 ± 0.00 | 0.00 | 140.00 ± |
| Diastolic BP | | 0.54 | 0.71 | 0.05 |
| | AA | 83.24 ± 13.33 | 88.36 ± 11.39 | 70.56 ± 8.47 |
| | AG | 87.78 ± 8.33 | 90.00 ± 5.77 | 80.00 ± 14.14 |
| | GG | 90.00 ± 0.00 | 0.00 | 90.00 ± |
| FBS | | 0.84 | 0.8 | 0.96 |
| | AA | 120.98 ± 57.64 | 130.46 ± 65.75 | 97.44 ± 10.07 |
| | AG | 127.78 ± 33.87 | 136.86 ± 32.87 | 96.00 ± 9.90 |
| | GG | 95.00 ± 0.00 | 0.00 | 95.00 ± |
| S. Urea | | 0.19 | 0.37 | 0.04 |
| | AA | 27.99 ± 7.63 | 28.72 ± 8.14 | 26.19 ± 5.95 |
| | AG | 33.00 ± 9.15 | 31.71 ± 10.14 | 37.50 ±.71 |
| | GG | 28.00 ± 0.00 | 0.00 | 28.00 ± |
| S. Creatinine | | 0.03 | 0.03 | 0.38 |
| | AA | .67 ± .23 | .66 ± .23 | .69 ± .24 |
| | AG | .89 ± .31 | .87 ± .35 | .94 ± .19 |
| | GG | .80 ± 0.00 | 0.00 | .80 ± |
| S. Albumin | | 0.08 | 0.24 | 0.03 |
| | AA | 4.37 ± .23 | 4.38 ± .24 | 4.35 ± .21 |
| | AG | 4.20 ± .23 | 4.27 ± .21 | 3.95 ± .07 |
| | GG | 4.50 ± 0.00 | 0.00 | 4.50 ± |
| S. Cholesterol | | 0.31 | 0.18 | 0.8 |
| | AA | 179.97 ± 36.88 | 180.91 ± 37.04 | 177.63 ± 37.08 |
| | AG | 200.33 ± 46.20 | 201.71 ± 51.79 | 195.50 ± 30.41 |
| | GG | 180.00 ± 0.00 | 0.00 | 180.00 ± |
| S. TG | | 0.11 | 0.02 | 0.7 |
| | AA | 166.67 ± 98.00 | 172.83 ± 104.92 | 151.37 ± 77.88 |
| | AG | 235.67 ± 116.79 | 269.00 ± 110.71 | 119.00 ± 24.04 |
| | GG | 100.00 ± 0.00 | 0.00 | 100.00 ± |
| S. HDL-C | | 0.54 | 0.4 | 0.59 |
| | AA | 40.75 ± 21.77 | 40.38 ± 23.83 | 41.67 ± 15.89 |
| | AG | 34.52 ± 11.72 | 32.64 ± 12.01 | 41.10 ±11.03 |
| | GG | 25.00 ± 0.00 | 0.00 | 25.00 ± |

*Continued*

Table 5. **Continued**

| Risk factors | Alleles | Total | Hypertension | Control |
|---|---|---|---|---|
| | | Mean ± S.D. | Mean ± S.D. | Mean ± S.D. |
| | | 0.59 | 0.68 | 0.51 |
| S. LDL-C | AA | 107.58 ± 39.67 | 108.16 ± 41.14 | 106.14 ± 36.45 |
| | AG | 118.34 ± 34.49 | 114.84 ± 36.03 | 130.60 ± 36.63 |
| | GG | 135.00 ±0.00 | 0.00 | 135.00 ± |
| | | 0.85 | 0.61 | 0.69 |
| S. VLDL-C | AA | 39.24 ± 54.49 | 41.59 ± 63.13 | 33.42 ± 21.27 |
| | AG | 47.13 ± 23.36 | 53.80 ± 22.14 | 23.80 ± 4.81 |
| | GG | 20.00 ± 0.00 | 0.00 | 20.00 ± |

human prostate cancer cells.[14] Nevertheless, further studies are needed to clarify the underlying mechanism of gender dependency in STK39 effect on cardiovascular risk factors.

The population studied in the initial GWAS from Wang et al.[3] is American Old Order Amish, which is a closed founder population emigrated from Switzerland in the early 1700s. The study populations in articles of Fava et al.[5] and Donner et al. are from Sweden and Finland. Those populations are all Caucasians and close in geographical location. When we checked the details of the positive SNPs (rs6749447, rs3754777, and rs4977950) in Wang's GWAS, we found that the minor allele frequency differs greatly between Europeans and Asians. To determine whether STK39 is a common candidate gene for hypertension, we set to find out whether it is associated with hypertension in Iraqi population or not.

There were studies for two SNPs (rs35929607 and rs12692877) within STK39 in Chinese population, which were assumed functional SNPs based on a multispecies sequence arrangement.[3] However, neither SNP was significantly associated with hypertension in the Chinese population. As the result was quite different from Fava's replication study[5] and these result confirmed our result. Five genome-wide significant SNPs genotyped in this study did not show a positive association with hypertension in the Chinese population. Similarly, Cunnington et al.[10] also did not find any association between the three SNPs (rs6749447, rs3754777, and rs35929607) within STK39 and systolic BP or diastolic BP from a cohort of 1372 British Caucasians.

Furthermore, in Fava's study, and after exclusion of 2398 individuals from Malmo preventive project cohort, who also participated in the Malmo diet and cancer study, G allele of rs35929607 was no longer associated with hypertension.[5] Thus, it is still questionable whether STK39 can be accepted as a common susceptibility gene for hypertension. In a European study carried out in 2009, the STK39 gene SNP rs35929607 were coincidently associated significantly with systolic and diastolic blood pressure, ($p < 0.05$).[3] In another study in Tharparkar, Pakistan population, they found an insignificant association of this polymorphism with EHTN ($p = 0.153$). They 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.[15]

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.[15]

## Conclusion

It was found that a presence of significant association between the studied SNP and hypertension, particularly in females, through allele A. However, the SNP showed genotype-related differences in blood urea, creatinine, albumin and lipid profile levels. The current study is the first to systemically analyse the association between STK39 variants and multiple cardiovascular risk factors. ■

## References

1. J. Kuneš, J. Zicha. The interaction of genetic and environmental factors in the etiology of hypertension. Center for cardiovascular research and institute of physiology, academy of sciences of the Czech republic, Prague, Czech republic. Physiol Res. 2009;58 (Suppl. 2):S33–S41.
2. Huan T, Esko T, Peters MJ, Pilling LC, Schramm K. Schurmann C, et al. A meta-analysis of gene expression signatures of blood pressure and hypertension. PLoS Genet. 2015 Mar;11(3):e1005035. doi: 10.1371/journal.pgen.1005035. PMID: 25785607
3. 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 U S A. 2009 Jan;106(1):226–231. doi: 10.1073/pnas.0808358106 PMID: 19114657
4. Delpire E, Gagnon KB. SPAK and OSR1: STE20 kinases involved in the regulation of ion homoeostasis and volume control in mammalian cells. Biochem J. 2008 Jan;409(2):321–331. doi: http://dx.doi.org/10.1042/bj20071324 PMID: 18092945
5. Fava C, Danese E, Montagnana M, Sjögren M, Almgren P, Engström G, et al. Serine/threonine kinase 39 is a candidate gene for primary hypertension especially in women: results from two cohort studies in Swedes. J Hypertens. 2011 Mar;29(3):484–491. doi: 10.1097/HJH.0b013e3283-42b2c1 PMID: 21178783
6. Chen LY, Zhao WH, Tian W, Guo J, Jiang F, Jin LJ, et al. STK39 is an independent risk factor for male hypertension in Han Chinese. Int J Cardiol. 2012 Jan; 154(2):122–127. doi: 10.1016/j.ijcard.2010.09.007 PMID: 20889219
7. Newton-Cheh C, Johnson T, Gateva V, Tobin MD, Bochud M, Coin L, et al. Genome-wide association study identifies eight loci associated with blood pressure. Nat Genet. 2009 Jun;41(6):666–676. doi: 10.1038/ng.361 PMID: 19430483
8. Levy D, Ehret GB, Rice K, Verwoert GC, Launer LJ, Dehghan A, et al. Genome-wide association study of blood pressure and hypertension. Nat Genet. 2009 Jun;41(6):677–687. doi: 10.1038/ng.384 PMID: 19430479
9. Sayers EW, Barrett T, Benson DA, Boltan E, Bryant SH, Canese K, et al. Database resources of national center for biotechnology information. Nucleic Acids Res. 2011 Jan;39:D38–D51. doi: 10.1093/nar/gkq1172 PMID: 21097890
10. Cunnington MS, Kay C, Avery PJ, Mayosi BM, Koref MS, Keavney B. STK39 polymorphisms and blood pressure: an association study in British Caucasians and assessment of cis-acting influences on gene expression. BMC Med Genet. 2009 Dec;10:135. doi: 10.1186/1471-2350-10-135 PMID: 20003416

11. Niu WQ, Zhang Y, Ji KD, Gao PJ, Zhu DL. Contribution of five top whole-genome association signals to hypertension in Han Chinese. J Hum Hypertens. 2011 Apr;25(4):278–280. doi: 10.1038/jhh.2010.114 PMID: 21150932
12. Rhee MY, Yang SJ, Oh SW, Park Y, Kim CI, Park HK, et al. Novel genetic variations associated with salt sensitivity in the Korean population. Hypertens Res. 2011 May;34(5):606–611. doi: 10.1038/hr.2010.278 PMID: 21228780
13. Ruixing Y, Jinzhen W, Shangling P, Weixiong L, Dezhai Y, Yuming C. Sex differences in environmental and genetic factors for hypertension. Am J Med. 2008 Sep;121(9):811–819. doi: 10.1016/j.amjmed.2008.04.026 PMID: 18724972
14. Qi H, Labrie Y, Grenier J, Fournier A, Fillion C, Labrie C. Androgens induce expression of SPAK, a STE20/SPS1-related kinase, in LNCaP human prostate cancer cells. Mol Cell Endocrinol. 2001 Sep;182(2):181–192. doi: http://dx.doi.org/10.1016/S0303-7207(01)00560-3 PMID: 11514053
15. Loung Vasandas Umedani, Bushra Chaudhry (Department of Biological and Biomedical Sciences, Aga Khan University, Karachi, Pakistan),Vikram Mehraj (Unite Mixte de Recherche 6236, Centre National de laRechercheScientifique, France), Muhammad Ishaq (Department of Biochemistry, United Medical and Dental College, Ibrahim Hydri, Korangi Creek, Karachi, Pakistan) 2010.