Association of MTHFR C677T and A1298C gene polymorphisms with hypertension

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Abstract:

Objectives. To check for the association of genetic polymorphisms related to the methylenetetrahydrofolate reductase (MTHFR) gene namely C677T and A1298C with hypertension in Saudi affected subjects from Qassim region.

Subjects and methods: Participants included 123 Saudi hypertensive cases (83 males and 40 females) in addition to 250 (142 males and 108 females) unrelated healthy controls from the same locality. Their age mean ±SD was 50.93 ± 15.43 years. For all subjects, DNA was extracted followed by real-time PCR amplifications for characterization of genotypes and alleles related to MTHFR C677T and A1298C gene polymorphisms

Results: Total cases showed significantly higher carriage rate for the mutant allele 677T compared to controls (40.7% vs. 26%, OR=1.9, 95% CI= 1.2-3.1) with a lower frequency of the wild type 677CC genotype (59.3% vs. 74%, p=0.004). The same was observed among cases-subgroups of hypertension associated with obesity with a notably higher odds ratio (OR=2.6, 95% CI=1.3-5.01, p=0.004). Total cases showed also significantly higher frequency of mutant 1298 C allele carriage rate compared to controls (59.3% vs. 42.4%, OR=1.98, 95% CI= 1.3-3.1) with a lower frequency of the normal AA genotype (40.7% vs. 57.6%, p=0.003). The same was observed among cases-subgroups of hypertension associated with both diabetes and obesity and among cases of hypertension with obesity, also with higher odds ratio (OR=2.6 and 2.2 respectively).

Conclusion. This work showed that genetic polymorphisms related to the MTHFR gene are associated with the risk of hypertension particularly when accompanied with obesity and diabetes among Saudi subjects.

Keywords: Methylenetetrahydrofolate reductase, hypertension, Saudi, Genetic polymorphisms

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Introduction

The development of hypertension is believed to originate from an interaction of lifestyle exposures, such as high dietary sodium, excess weight, and alcohol consumption, with multiple genetic factors. [1, 2] In addition, in many hypertensive patients, there is strong inherited evidence derived from twin, adoption and family studies indicating that about one third to one-half of the inter-individual variation of blood pressure levels is heritable. [1, 3]

Despite considerable efforts to study the molecular genetics of hypertension, the inherently complex nature is somewhat an obstacle in elucidating the involved genes. [4] The case-control association study of candidate genes remain an efficient approach for detecting the "modest-effect" genetic variants underlying disease susceptibility provided the study is adequately powered. [5] Many case-control studies have addressed in particular the putative role of C to T transition at nucleotide 677 (C677T) and A to C transition at nucleotide 1298 (A1298C) in the methylenetetrahydrofolate reductase (MTHFR) gene in the development of hypertension. MTHFR is partly controlling the folate metabolism through catalyzing the reduction of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate; [6] a co substrate for the conversion of homocysteine to methionine; [5] thus resulting into a state of hyperhomocysteinemia; [3, 7] particularly in subjects with low plasma folate levels. [8]

Both homozygosity for MTHFR C677T (TT) and A1298C (CC), and compound heterozygosity for C677T and A1298C (677CT/1298AC) genotypes were found to be associated with a reduced MTHFR enzyme activity. [3,7,9] Homozygous MTHFR TT genotype has also been found to be associated with an increased risk of hypertension, [8] which may due to in increased homocysteine levels that were also found in patients with complicated type I and II diabetes mellitus. [10, 11]

Hypertension is a well known prevalent disorder among Saudi population especially in Qassim Region which is a tribal region characteristically having high frequency of consanguinity plus a high aggregation rate of familial diseases as hypertension, obesity, and diabetes. [12-14] In spite of this fact, little data were -so far- published concerning the genetic background of Saudi subjects in terms of their susceptibility to hypertension.

The objective of this study was to test for the association of the MTHFR (C667T and A1298C) gene polymorphisms with hypertension, obesity and diabetes among Saudi subjects from Qassim region.

Subjects and Methods

Participants included 123 cases (83 males and 40 females) with an age mean ±SD of 50.93 ± 15.43 years. They were known to have longstanding essential hypertension i.e. with systolic blood pressure (SBP) between 140 and 200 mmHg, or diastolic blood pressure (DBP) between 90 and 120 mmHg. Their minimum duration of hypertension is one year that was confirmed during at least two visits at the outpatient clinic of Buraidah Central Hospital, Qassim region, Saudi Arabia. Of these cases, 24.1% had a positive family history of hypertension, 32.4% had positive parental consanguinity, 12.8 % were smokers, 84.6% were obese and 46.2% had diabetes. For comparison, 250 normal healthy unrelated subjects (142 males and 108 females) with an age mean and SD of 47.65 ± 11.15 were taken from the same locality as controls.

Obesity was diagnosed on the basis of the most commonly used definitions, established by the World Health Organization (WHO) in 1997 and published in 2000, that defines the body mass index (BMI) of obesity as being 30 or greater. [15] On the other hand, diabetes - type 2 was diagnosed on the basis of blood sugar during fasting time (12-14hr), being higher than 126 mg/dL at least for two times; or with symptoms of hyperglycemia and a casual (random) plasma glucose is higher than 200 mg/dl (11.1 mmol/l). [16] An informed consent was obtained from all participants as well as an ethical approval from the local Ethics and Scientific Committees.

DNA extraction and amplification

The isolation of genomic DNA was done on a MagNA Pure LC instrument (Roche Molecular Biochemicals, Mannheim, Germany), using the manufacturer's standard protocol. Oligonucleotide primers and fluorescence-labeled hybridization probes (Table 1.) were designed for amplification and sequence-specific detection of corresponding polymorphism (TIB MolBiol; Berlin, Germany) as described before. [17, 18]

Table 1. Oligonucleotide primers and fluorescence-labeled hybridization probes used for characterization of polymorphic variants of MTHFR gene.

	MTHFR C677T [A223V]		**AF105980**	**Tm**
MTHFR se	AggCCAgCCTCTCCTgACTg	S	514-533	61,1°C
MTHFR R	AggACggTgCggTgAgAgTg	A	850-831	63,0°C
MTHFR Sensor	CgggAg**C**CgATTTCATCA—FL	S	671-688	57,1°C
MTHFR_640	640-CgCAgCTTTTCTTTgAggCTgACA—PH	S	692-715	64,9°C
	MTHFR A1298C [E430A]		**AL953897**	**Tm**
1298 ex8 F	CTTTggggAgCTgAAggACTACTAC	A	80541-517	59,8°C
1298 ex8 R	CACTTTgTgACCATTCCggTTTg	S	80379-408	61,9°C
Sensor [A]	CTTCAAAgACACTT**T**CTTCACTggTC—FL	S	80429-454	58,3°C
Anchor	640-CTCCTCCCCCCACATCTTCAgCAg—PH	S	80457-480	67,4°C

The master mixture contained 2 µl of a 10 X mixture of Light Cycler Fast-Start DNA master hybridization probes (**Roche Diagnostics),** 5 mM MgCl2 , a 1 µM MTHFR primers including 0.075 µM of specific primers, and a 0.2 µM of hybridization probes. Fluorescence curves were analyzed with the LightCycler software (version 3.5.3). Automated calculation of crossing points was done by the second-derivative maximum method. The fluorescence of each capillary was measured at wavelengths of 640 and 705 nm (dual-color option). Each run contained positive control, wild type (WT), mutant type (MT), heterozygous type (HT) and one negative control (blank reagent and water). Each result was confirmed by the specific peak in the corresponding melting curve.

Statistical analysis

Allelic and genotypic frequencies were compared and statistically analyzed using the Fisher's exact test and Odds ratio (OR) with 95% confidence intervals (95% CI). Conformity of genotype distributions with Hardy-Weinberg (HW) equilibrium was evaluated by chi-square analysis. For all tests, a p-value < 0.05 was considered to be statistically significant.

Results

Compared to controls, total hypertensive cases showed significantly higher frequency of mutant allele 677T carriage (TT + CT) (40.7% vs. 26%, OR=1.9, 95% CI= 1.2-3.1) with a lower frequency of the normal 677CC genotype (59.3% vs. 74%, p=0.004). The same was observed among cases-subgroup of hypertension associated with obesity notably with higher odds ratio (OR=2.6, 95% CI=1.3-5.01, p=0.004). Although cases-subgroup of hypertension with obesity and diabetes and

cases-subgroup with hypertension alone showed the same phenomena of higher frequency of mutant T allele carriage and lower normal CC genotype than controls, yet it did not reach a statistical significance (p>0.05) (Table 2).

Compared to controls, total hypertensive cases showed significantly higher frequency of mutant 1298 C allele carriage (CC + CA) (59.3% vs. 42.4%, OR=1.98, 95% CI= 1.3-3.1) with a lower frequency of the normal AA genotype (40.7% vs. 57.6%, p=0.003). The same was observed among cases-subgroup of hypertension associated with diabetes and obesity and cases-subgroup of hypertension and obesity, with higher odds ratio (OR=2.6 and 2.2 respectively). Although cases of hypertension alone showed the same phenomena of higher frequency of mutant C allele carriage and lower normal AA genotype than controls, yet it did not reach a statistical significance (p=1) (Table 3).

Table 2. Genotyping and allele frequency of MTHFR C677T gene polymorphism among hypertensive Saudi cases compared to controls

MTHFR C677T Genotypes	Controls	Total Cases	Hypertension with obesity and diabetes	Hypertension with Obesity	Hypertension Only
	n (%)	n (%)	n (%)	n (%)	n (%)
Total	250 (100)	123 (100)	53 (100)	44 (100)	26 (100)
Mutant (TT + CT)	65 (26)	50 (40.7)	21 (39.6)	21 (47.7)	8 (30.8)
Normal (CC)	185 (74)	73 (59.3)	32 (60.4)	23 (52.3)	18 (69.2)
p	-	0.004*	0.06	0.004*	0.6
OR (95% CI)	-	1.9 (1.2-3.1)	1.9 (1.01-3.5)	2.6 (1.3-5.01)	1.3 (0.51-3.05)

p : cases vs. controls * : p significant <0.05

Table 3. Genotyping and allele frequency of MTHFR A1298C gene polymorphism among hypertensive Saudi cases compared to controls

MTHFR A1298C Genotypes	Controls	Total Cases	Hypertension with obesity and diabetes	Hypertension with Obesity	Hypertension Only
	n (%)	n (%)	n (%)	n (%)	n (%)
Total	250 (100)	123 (100)	53 (100)	44 (100)	26 (100)
Mutant (CC + AC)	106 (42.4)	73 (59.3)	35 (66.0)	27 (61.4)	11 (42.3)
Normal (AA)	144 (57.6)	50 (40.7)	18 (34.0)	17 (38.6)	15 (57.7)
p	-	0.003*	0.002*	0.02*	1
OR (95% CI)		1.98 (1.3-3.1)	2.6 (1.4-4.9)	2.2 (1.1-4.2)	1 (0.44-2.3)

P: cases vs. controls *: p significant <0.05

Comparing case subgroups in terms of gender, age, smoking, consanguinity and family history showed non-significant difference related to all studied genotypes and alleles (Table 3).

Table 3. Genotypes of MTHFR 677 and 1298 polymorphisms among Saudi hypertensive cases related to their potential risk factors

	677 CT				1298AC			
	TT %	CT %	CC %	*p*	AA %	AC %	CC %	*P*
Gender								
Male	.0	40.7	59.3	>0.05	39.0	51.2	9.8	>0.05
Female	5.0	35.0	60.0		42.5	52.5	5.0	
Age group								
-50 years	.0	35.6	64.4	>0.05	39.1	50.0	10.9	>0.05
>50 years	2.7	41.1	56.2		41.1	52.1	6.8	
Smoking								
Positive	0.0	46.2	53.8	>0.05	15.4	76.9	7.7	>0.05
Negative	2.1	42.6	55.3		43.2	48.4	8.4	
Family history								
Positive	.0	35.7	64.3	>0.05	42.9	46.4	10.7	>0.05
Negative	2.3	42.5	55.2		38.6	53.4	8.0	
Consanguinity								
Positive	2.9	45.7	51.4	>0.05	42.9	42.9	14.3	>0.05
Negative	1.3	37.7	61.0		38.5	56.4	5.1	

Discussion

The current results showed that Saudi hypertensive cases had significantly higher frequency of mutant alleles; MTHFR 677T particularly in obese cases; and MTHFR 1298C particularly among obese and diabetic cases. An interesting previous finding was that reported by Settin et al. showing no statistical significance of the association of MTHFR alleles with obesity among healthy Saudi controls. [19] This might give a more confirmation to our finding here of an association of these alleles with hypertension particularly if accompanied by obesity and diabetes among affected Saudi subjects compared to healthy controls.

These results are consistent with those of Markan et al., who stated that MTHFR 677T and 1298C alleles and the co-occurrence of MTHFR 677 CT/MTHFR 1298 CC genotypes were associated with increased risk of hypertension among Indian patients. [20] In another study, Koupepidou et al., have proposed that MTHFR 677TT and 677CT/1298AC genotypes may be predisposing the hypertensive patients to hypertensive nephrosclerosis and chronic renal failure. [21] It was also reported that MTHFR 677C/T polymorphism might be significant risk factors for hypertension in Taiwanese population. [22] A recent large scale study conducted by Inamoto et al. reported that the TT genotype of the MTHFR gene was associated with an increased risk of hypertension among Japanese subjects conferring a small, but significant risk in Caucasians. [23] However, a modest significant association was reported between MTHFR C677T mutation and essential hypertension. [8] Regarding association with diabetes, a

previous study conducted on Czech subjects concluded that the C allele of the 677C/T MTHFR polymorphism is associated with type II diabetes mellitus in women. [24] Previous researches could also elicit positive association of obesity and premature coronary artery disease in hypertensive adolescents with MTHFR C677T mutations. [25, 26]

Conversely, no significant association was reported between C677T variant of MTHFR polymorphism and metabolic syndrome, hypertension, and diabetes in a study among Iranians. [27] Similarly Russo et al. found no association between MTHFR C677T polymorphism and metabolic syndrome in a group of type II diabetic subjects with mild hyperhomocysteinemia. [28] Also, Yamada et al., observed no association between MTHFR C677T polymorphism and metabolic syndrome. [29] This discrepancy may be postulated to be due to the effect of epigenetic mechanisms controlling gene expression influenced by environmental conditions as life style and diet. In this respect, it is hypothesized that folic acid fortification could overcome metabolic block caused by thermolabile MTHFR mutation, and consequently affects DNA methylation and gene expression. [27, 30] Interestingly, comparing case subgroups in terms of gender, age, smoking, consanguinity, and positive family history of hypertension; showed non-significant difference related to the frequency of all studied genotypes and alleles. In fact consanguinity is known to increases the incidence of autosomal and multifactorial disorders. On our series of cases, mutant 677 TT and 1298 CC genotypes were present in only 2.9% and 14.3% of positive consanguineous cases respectively while it was still present in 1.3% and 5.1% of negative consanguineous ones with a final statistically nonsignificant association with the susceptibility for hypertension ($p>0.05$). This implies that these polymorphisms predispose to hypertension regardless of the effect of age, gender or family status. These results were also reported previously by other researchers. [31- 34] However, we recommend another wide scale study including the detailed data of the amount of exposure to smoking or other pollutants and also, the detailed data of diet and exercise to get a big view about the gene environmental interactive factors into the causation of hypertension, diabetes and obesity. In this respect, researchers recommended programs of screening for serum homocystein; serum and RBC folate, vitamin B6, B12 and folate supplementations to subjects with risk genotypes and/or other environmental risk factors. [35-37]

In conclusion; this study demonstrated the association of MTHFR gene polymorphisms with hypertension, obesity and diabetes in Saudi subjects from Qassim Region. These polymorphisms can be used as markers for the susceptibility to hypertension among affected families who can be counseled for potential prevention and management.

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Conflict of Interest

Authors report this work with a complete freedom from any sort of conflict of interest

References

1. Snieder H., Harshfield G.A., Treiber F.A.; Heritability of blood pressure and hemodynamics in African- and European-American youth. Hypertension. 2003; 41: 1196-201.
2. Mein C.A., Caulfield M.J., Dobson R.J., Munroe P.B.; Genetics of essential hypertension. Hum Mol Genet. 2004; 13(Spec No 1):R169–R175.
3. Daniels P.R., Kardia S.L., Hanis C.L., Brown C.A., Hutchinson R., Boerwinkle E., Turner S.T.; Genetic Epidemiology Network of Arteriopathy study. Familial aggregation of hypertension treatment and control in the Genetic Epidemiology Network of Arteriopathy (GENOA) study. Am J Med. 2004; 116: 676-81.
4. Agarwal A., Williams G.H., Fisher N.D.; Genetics of human hypertension. Trends Endocrinol Metab 2005; 16: 127– 133.
5. Slager SL, Schaid DJ. Evaluation of candidate genes in case-control studies: a statistical method to account for related

subjects. Am J Hum Genet 2001; 68: 1457–1462.
6. Frosst P., Blom H.J., Milos R., Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP.; A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995; 10: 111–113.
7. Weisberg I, Tran P, Christensen B, Sibani S, Rozen R.; A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. Mol Genet Metab. 1998; 64: 169-72.
8. Heux S, Morin F, Lea RA, Ovcaric M, Tajouri L, Griffiths LR.; The methylentetrahydrofolate reductase gene variant (C677T) as a risk factor for essential hypertension in Caucasian. Hypotens Res 2004; 27(9): 663-7.
9. Weisberg IS, Jacques PF, Selhub J, Bostom AG, Chen Z, Curtis Ellison R, Eckfeldt JH, Rozen R; The 1298A-->C polymorphism in methylenetetrahydrofolate reductase (MTHFR): in vitro expression and association with homocysteine. Atherosclerosis. 2001; 156: 409-15.
10. Neugebauer S, Tarnow L, Stehouwer C, Teerlink T, Baba T, Watanabe T, Parving HH; Total plasma homocysteine is associated with hypertension in Type I diabetic patients. Diabetologi. 2002 Sep; 45(9):1315-24.
11. Stabler SP, Estacio R, Jeffers BW, Cohen JA, Allen RH, Schrier RW; Total homocysteine is associated with nephropathy in non-insulin-dependent diabetes mellitus. Metabolism 1999; 48:1096–1101.
12. Soyannwo MA, Gadallah M, Kurashi NY, Hams J, El-Essawi O, Khan NA, Singh RG, Alamri A, Beyari TH; Studies on preventive nephrology: Systemic hypertension in the pediatric and adolescent population of Gassim, Saudi Arabia. Ann Saudi Med. 1997; 17: 47-52.
13. Soyannwo MA, Kurashi NY, Gadallah M, Hams J, el-Essawi O, Khan NA, Singh RG, Alamri A, Beyari TH.; Blood pressure pattern in Saudi population of Gassim.Afr J Med Med Sci. 1998; 27: 107-16.
14. Al-Sheikh SO, Aljabri BA, Al-Ansary LA, Al-Khayal LA, Al-Salman MM, Al-Omran MA; Prevalence of and risk factors for peripheral arterial disease in Saudi Arabia. A pilot cross-sectional study. Saudi Med J. 2007; 28: 412-4.
15. WHO http://www.who.int/mediacentre/factsheets/fs311/en/index.html (2000, accessed September, 2011)
16. American Diabetes Association. Standards of medical care in diabetes--2009. Diabetes Care. 2009; 32 Suppl 1:S13-61.
17. Ahsen, N, Oellerisch, M, and Schütz, E. A Method for Homogeneous Color-Compensated Genotyping of Factor V (G1691A) and Methylenetetrahydrofolate Reductase (C677T) Mutations Using Real-Time Multiplex Fluorescence PCR, Clin Biochemistry 33 (2000) 535-539.
18. Etienne MC, Ilc K, Formento JL, Laurent-Puig P, Formento P, Cheradame S, Fischel JL, Milano G. Thymidylate synthase and methylenetetrahydrofolate reductase gene polymorphisms: relationships with 5-fluorouracil sensitivity. Br J Cancer. 2004 Jan 26; 90(2):526-34.
19. Settin AA, Algasham A, Dowaidar M, Ismail H. Methylene tetrahydrofolate reductase and angiotensin converting enzyme gene polymorphisms related to overweight/obesity among Saudi subjects from Qassim Region. Dis Markers. 2009; 27(2):97-102.
20. Markan S, Sachdeva M, Sehrawat BS, Kumari S, Jain S, Khullar M.; MTHFR 677 CT/MTHFR 1298 CC genotypes are associated with increased risk of hypertension in Indians.Mol Cell Biochem. 2007; 302: 125-31.
21. Koupepidou P, Deltas C, Christofides TC, Athanasiou Y, Zouvani I, Pierides A.; The MTHFR 677TT and 677CT/1298AC genotypes in Cypriot patients may be predisposing to hypertensive nephrosclerosis and chronic renal failure Int Angiol. 2005; 24: 287-94.

22. Lin PT, Cheng CH, Wei JC, Huang YC.; Low plasma pyridoxal 5'-phosphate concentration and MTHFR 677C-->T genotypes are associated with increased risk of hypertension. Int J Vitam Nutr Res. 2008 Jan; 78(1):33-40.
23. Inamoto N, Katsuya T, Kokubo Y, Mannami T, Asai T, Baba S, Ogata J, Tomoike H.; Association of methylenetetrahydrofolate reductase gene polymorphism with carotid atherosclerosis depending on smoking status in a Japanese general population. Ogihara T. Stroke. 2003; 34: 1628-33.
24. Benes P, Kanková K, Muzík J, Groch L, Benedík J, Elbl L, Izakovicová-Hollá L, Vasků A, Znojil V, Vácha J. ; Methylenetetrahydrofolate reductase polymorphism, type II diabetes mellitus, coronary artery disease, and essential hypertension in the Czech population. Mol Genet Metab. 2001; 73: 188-95.
25. Fridman O, Porcile R, Vanasco V, Junco MN, Gariglio L, Potenzoni MA, Bañes I, Morales A.; Study on homocysteine levels and methylenetetrahydrofolate reductase gene variant (C677T) in a population of Buenos Aires City. Clin Exp Hypertens. 2008; 30: 574-84.
26. Koo HS, Lee HS, Hong YM.; Methylenetetrahydrofolate reductase TT genotype as a predictor of cardiovascular risk in hypertensive adolescents. Pediatr Cardiol. 2008; 29: 136-41.
27. Fakhrzadeh H, Mirarefin M, Sharifi F, Ghotbi S, Rezaei Hemami M, Mohammad Amoli M, Pourebrabrahim R, Nouri M, Tavakkoly Bazzaz J, shafaee A, Larijani B.; Association of Methylenetetrahydrofolate Reductase gene Polymorphism (C677T) with Metabolic Syndrome in an Iranian Population: Tehran Homocysteine SurveyIranian Journal of Diabetes and Lipid Disorders; 2009 38 (3): 37-46.
28. Russo GT, Di Benedetto A, Alessi E, Ientile R, Antico A, Nicocia G, La Scala R, Di Cesare E, Raimondo G, Cucinotta D.; Mild hyperhomocysteinemia and the common C677T polymorphism of methylenetetrahydrofolate reductase gene are not associated with the metabolic syndrome in Type 2 diabetes. J Endocrinol Invest 2006; 29: 201-7.
29. Yamada Y, Kato K, Hibino T, Yokoi K, Matsuo H, Segawa T, Watanabe S, Ichihara S, Yoshida H, Satoh K, Nozawa Y.; Prediction of genetic risk for metabolic syndrome. Atheroscler 2007; 191(2): 298-304.
30. Jablonka E, and Lamb MJ.; The changing concept of epigenetics. Ann NY Acad Sci 2002; 981:82.
31. Qian X, Lu Z, Tan M, Liu H, Lu D.; A meta-analysis of association between C677T polymorphism in the methylenetetrahydrofolate reductase gene and hypertension. Eur J Hum Genet. 2007 Dec; 15(12):1239-45.
32. Takeuchi F, Isono M, Katsuya T, Yamamoto K, Yokota M, Sugiyama T, Nabika T, Fujioka A, Ohnaka K, Asano H, Yamori Y, Yamaguchi S, Kobayashi S, Takayanagi R, Ogihara T, Kato N.; Blood pressure and hypertension are associated with 7 loci in the Japanese population. Circulation. 2010 Jun 1; 121(21):2302-9.
33. Maitland-van der Zee AH, Lynch A, Boerwinkle E, Arnett DK, Davis BR, Leiendecker-Foster C, Ford CE, Eckfeldt JH.; Interactions between the single nucleotide polymorphisms in the homocysteine pathway (MTHFR 677C>T, MTHFR 1298 A>C, and CBSins) and the efficacy of HMG-CoA reductase inhibitors in preventing cardiovascular disease in high-risk patients of hypertension: the GenHAT study. Pharmacogenet Genomics. 2008 Aug; 18(8):651-6.
34. Zhang YL, Zhou SX, Lei J, Zhang JM.; Association of angiotensin I-converting enzyme gene polymorphism with ACE and PAI-1 levels in Guangdong Chinese Han patients with essential hypertension] Nan Fang Yi Ke Da Xue Xue Bao. 2007 Nov; 27(11):1681-4. Chinese
35. Ilhan N, Kucuksu M, Kaman D, Ilhan N, Ozbay Y.; The 677 C/T MTHFR polymorphism is associated with essential hypertension, coronary artery disease, and higher homocysteine levels. Arch Med Res. 2008 Jan; 39(1):125-30.

36. Biswas A, Ranjan R, Meena A, Akhter MS, Yadav BK, Munisamy M, Subbiah V, Behari M, Saxena R.; Homocystine levels, polymorphisms and the risk of ischemic stroke in young Asian Indians. J Stroke Cerebrovasc Dis. 2009 Mar-Apr; 18(2):103-10.
37. Ozbek Z, Kucukali CI, Ozkok E, Orhan N, Aydin M, Kilic G, Sazci A, Kara I.; Effect of the methylenetetrahydrofolate reductase gene polymorphisms on homocysteine, folate and vitamin B12 in patients with bipolar disorder and relatives. Prog Neuropsychopharmacol Biol Psychiatry. 2008 Jul 1; 32(5):1331-7.