



Significant ACE Gene Haplotype Stipulates Development of Type 2 Diabetes in Women with Gestational Diabetes Mellitus

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Authors' contributions

This work was carried out in collaboration between all authors. Author PA managed the literature searches, managed and performed all the experimental process. Authors NA and KD provided samples. Author MMJ designed the study, analyzed the results and drafted manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Angiotensin-converting enzyme (ACE) plays a key role in glucose and insulin regulation, and in the onset of diabetes. ACE gene polymorphisms A240T, C1237T, G2350A and I/D located in the promoter, coding and non-coding regions have been studied in both type-2 diabetes and gestational diabetes mellitus (GDM). However, their impact on the development of type-2 diabetes post GDM remains unknown, especially in under-represented population.

Aim: We examined possible associations and networking between ACE gene polymorphism susceptibility / protection towards/against progression of type-2 diabetes post GDM in North Indian women.

Methods: Two hundred and twenty four women ($n = 224$) were recruited in this study and genotyped for four ACE gene polymorphisms using polymerase chain reaction (PCR), followed by

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digestion through restriction endonuclease enzymes.

Results: Study results suggest a significant association of *ACE* genes SNPs *A240T*, *C1237T*, *G2350A* and *I/D* haplotype with GDM cases progressing to type-2 diabetes later in life ($P = .02$). Individuals possessing haplotype “*CAA*” derived from these SNPs had a 3.65 fold increased risk of type-2 diabetes development in GDM cases later in life relative to other haplotypes.

Conclusion: Due to its pivotal role in the pathogenesis of both diseases, the current finding might be of future therapeutic value. Larger-scale studies are required to confirm this novel finding in multi-ethnic populations.

Keywords: Haplotype; GDM; type-2 diabetes; Single Nucleotide Polymorphism (SNP); ACE.

ABBREVIATIONS

CDKAL1-cyclin-dependent kinase 5 regulatory subunit-associated protein 1-like 1; **CDKN2A**-cyclin-dependent kinase inhibitor 2A; **HHEX**-hematopoietically expressed homeobox, **IGF2BP2**-insulin-like growth factor 2 mRNA-binding protein 2; **SLC30A8a** zinc transporter and member of solute carrier family 30; **TCF7L2**- transcription factor-7-like 2; **GCK** -glucokinase; **KCNJ11**-potassium inwardly rectifying channel subfamily J member 11; **MTNR1B**- melatonin receptor 1B; **IRS1**-insulin receptor substrate 1.

1. INTRODUCTION

Angiotensin-converting enzyme (ACE) is a dipeptidyl carboxypeptidase enzyme encoded by the *ACE* gene. This gene is located on chromosome 17q23 and contains 26 exons and 25 introns [1,2]. ACE plays a key role in circulating the renin-angiotensin system (RAS) pathway that regulates blood pressure and electrolyte balance [2]. ACE converts angiotensin-1 (ANG I), an inactive 10 amino-acid peptide to the biologically active angiotensin-II (8 amino acids) [2]. Several studies show that the *Alu Insertion /Deletion (ACE I/D)* polymorphism present on intron 16 of the *ACE* gene is associated with high concentrations of ACE levels in plasma [2], as well as with gestational diabetes mellitus (GDM), type-2 diabetes, and diabetes-related complications [3-7]. Previous studies involving *ACE* polymorphisms have provided contradictory results when assessing the effect on GDM [3,4], type-2 diabetes, and type-2 diabetes complications in different populations [5-7]. ACE plays an important role in developing diabetic complications in type-2 diabetes cases [6,7], however, study involving the role of *ACE* polymorphisms with progression to type-2 diabetes post GDM has not yet been reported to the best of our knowledge.

GDM is defined as any degree of glucose intolerance with onset or first recognition during pregnancy [8]. Women diagnosed with GDM are at an increased risk of developing type-2 diabetes in later life (usually within 10–15 years), with incidence increasing markedly in the first 5

years and plateauing after 10 years [8]. The rates of progression of type-2 diabetes post GDM ranges from 2.6–70% over follow-up periods of 6 weeks to 28 years [8]. A revision by *Bellamy et al.* assessed 20 cohort studies and over 600,000 women diagnosed with type-2 diabetes; of these, 30,000 were diagnosed with GDM [9]. This finding suggests that, women with a GDM diagnosis had at least a sevenfold increase in the risk of type-2 diabetes incidence in later life when compared to those with normo-glycemic pregnancies [9]. This association, together with ethnicity and a family history of type-2 diabetes ratify similar risks in developing both GDM and type-2 diabetes. Accumulative studies also indicate that a similar pathogenesis and genetic variants associations exist among GDM and type-2 diabetes patients [10-13]. Many studies showed that SNPs present on [§]*CDKAL1*, [§]*CDKN2A*, [§]*HHEX*, [§]*IGF2BP2*, [§]*SLC30A8*, [§]*TCF7L2*, [§]*GCK*, [§]*KCNJ11*, [§]*MTNR1B*, [§]*IRS1* genes which are strongly associated with type 2 diabetes are significantly associated with a higher risk of GDM too [10-13].

Patho-physiology of GDM is similar to type 2 diabetes pathophysiology [14]. Women undergo major physiological changes during pregnancy that allow the fetus to survive and thrive in the intrauterine environment. One important physiological change that occurs in normal pregnancies is an increase in insulin resistance throughout pregnancy [14]. By the second trimester of pregnancy, women's insulin sensitivity declines to one third that of their non-pregnant state. This increased insulin resistance

facilitates continuous glucose transfer to the fetus, and when insulin resistance is accompanied by pancreatic β -cell insufficiency, GDM may develop [15]. Peripheral insulin resistances followed by an insulin-secretory defect are some of the pathophysiological changes of GDM similar to those observed in type 2 diabetes [13].

Studies have shown that genetic networking affects disease susceptibility [16-19]. There are reports suggesting that due to interactions between several loci, an increase in significant genetic susceptibility between ACE predispositions facilitates the onset of hypertension and coronary artery disease [20-22]. For example, a haplotype construction among ACE gene polymorphisms has shown synergistic effects on hypertension and coronary artery disease; however, no such association was observed in some ACE gene polymorphisms individually with hypertension [20,22]. On the other hand, detecting gene-to-gene interactions remains a challenge due to a large number of possible SNP haplotype configurations [16-22].

A linkage disequilibrium (LD) block difference of haplotypes varies with populations. For example, in Africans haplotypes have been shorter and more diverse, while Europeans reported longer and fewer [18-22]. This suggests that different LD of the SNPs located in the ACE gene may vary with geographically distributed populations [20-22]. Consequently, this article evaluated ACE polymorphism networking with type-2 diabetes progression in GDM cases from North India.

2. MATERIALS AND METHODS

Out of 10,000 subjects who attended obstetrics, gynecology and endocrinology wards or outpatient departments of All India Institute of Medical Sciences (AIIMS), New Delhi, India, during the study period (May 2009 – January 2014), only 300 women met the selection criteria (Tables 1 and 2) of which only 224 gave blood sample and written consent. The institute ethics committee of AIIMS approved the study protocol (Ref. No.: IESC/T-130/2010). Written consent was obtained from all cases and controls enrolled in this study. A qualified physician, according to American Diabetes Association (ADA) guidelines [23], completed screening and management of diabetes during pregnancy. Ethylene Diamine Tetra Acetic acid (EDTA) tubes were used to collect 2-ml of blood samples from participating women.

2.1 Determination of ACE Polymorphisms

Analysis of ACE gene polymorphisms A-240-T (ACE 4), C-1237-T (ACE 6), A-2350-G (ACE 8), and I/D, were achieved as per the methodology specified by Zhu et al. [22]. ACE SNPs listed in Table 2 were amplified using PCR, followed by digestion of PCR products following manufacturer protocol (see Table 2). The digested products were viewed under UV visualization after running them on the agarose gel stained with ethidium bromide. SNPs ACE 6 and ACE 8 were amplified in a 15 μ l reaction containing 25 ng of genomic DNA, 0.3 mM of each primer, 7.5 μ l of 2X PCR master-mix (Fermentas, USA), with MgCl₂ concentrations (Table 3). Cycling conditions were 95°C for 15 min; followed by 35 cycles of 94°C for 30s, annealing at the appropriate temperature (TA) (see Table 3) for 30s, and 72°C for 30s. Finally, a 10-minute extension at 72°C.

For ACE 4, ACE 5 amplification was conducted, followed by nested PCR of ACE 4 taking ACE 5 PCR product as a template. The following procedure was used to amplify ACE 4: ACE 5 amplified by its primers (Table 3); in a 50 μ l -ml reaction containing 100 ng of genomic DNA, 0.3mM of each primer, 25 μ l of 2 X PCR master mix (Fermentas, USA), 2.5 mM MgCl₂ and 10% glycerol. Cycling conditions for touchdown PCR for ACE 5 were 95°C for 1 min 30s; then 10 cycles of 95°C for 1 min, 65°C (0.5°C/ cycle) for 1 min, and 72°C for 3 min; and then 30 cycles of 95°C for 1 min, 60°C for 1 min, and 72°C for 3 mins. A 1:25 dilution of the resulting products was then used as a template to amplify the region containing ACE 4 in a 25 μ l reaction containing 0.3 mM of each primer, 1 mM MgCl₂, and 12.5 μ l of 2X PCR master-mix (Fermentas, USA). Cycling conditions were 95°C for 1 min 30s; then 35 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 3 mins; this was followed by 72°C for 10 mins (Table 3). For I/D polymorphism, the primers listed in Table 3 were used to amplify the region of Alu insertion or deletion. PCR amplification was performed in a 15 μ l reaction tube containing 25 ng of DNA, 0.45 mM of each primer, 7.5 μ l of 2 X PCR master-mix (Fermentas, USA), and 3 mM MgCl₂. The cycling conditions were 95°C for 15 mins; then 35 cycles of 94°C for 1 min, 62°C for 45s, and 72°C for 1 min, respectively. A final run was performed at 72°C for 10 mins. All samples sequence analysis was done using ABI3730XI (Applied Biosystem, Texas, USA), which confirmed our genotyping results.

Table 1. Inclusion and exclusion criteria for GDM and type 2 diabetes cases

Characteristics	GDM (n=105)		Type 2 diabetes (n=119)	
	Included	Excluded	Included	Excluded
Type	Pregnant women who develop Diabetes \geq 20 weeks of pregnancy [8]	Women with any other type of diabetes or complications	Women with type 2 diabetes	Women with any other type of diabetes or diabetes complications
Age	18-44 yrs	<18 & >44 yrs	>44 yrs*	\leq 40 yrs
Glucose level	Two or more values \geq threshold values mentioned in Table 1	Only 1 value \geq threshold values mentioned in Table 1	Fasting \geq 126 mg /dl or PP^ after 2 hrs \geq 200 mg/dl ; Two or more values \geq threshold values mentioned in Table 1	Fasting \geq 126 mg /dl or PP^ after 2 hrs \geq 200 mg/dl ; Only 1 value \geq threshold values mentioned in Table 1
Other	---	Any kind of <ul style="list-style-type: none"> • Essential / chronic hypertension • Cardiac Diseases • Endocrinological /Neurological disorders, • Infectious or noninfectious diseases 	---	1.Pregnant women 2.Any kind of <ul style="list-style-type: none"> • Essential / chronic hypertension, • Cardiac Diseases • Endocrinological /Neurological disorders • Infectious or noninfectious diseases

GDM- Gestational Diabetes Mellitus; * Average age of menopausal Indian women [24]

Table 2. Demographic and clinical parameters

	GDM	Type 2 Diabetes	p-values* #
Age (Mean\pmSD)	28.9 \pm 4.2 (21-40)	52.84 \pm 8.9 (41-75)	<0.001
GDM onset [weeks]			
Mean \pm SD (95% of CI)	21.0 \pm 7.94 (19.43-22.51)		
No. of Children before [median(Range)]	0(0-2)	3(0-8)	
No. of Caesareans [n (%)]	14(13.33%)	5(4.2%)	
GDM in previous Pregnancies [n (%)]	13(12.3%)	1(0.84%)	
No. of Foetal Deaths/pt[median(Range)]	1(0-8)	1(0-4)	
Glucose levels (Mean \pmSD) [in mg/dl]			
Fasting	120.13 \pm 48.54	150.42 \pm 53.57	<0.001
PP	185.83 \pm 51.01	215.03 \pm 78.31	<0.001

Key: Pt, patient * p-value of GDM Vs type 2 diabetes; # p-values< 0.05 were considered significant

2.2 Statistical Analysis

Demographics and clinical variables between active groups and controls were analyzed with Stata 15.0 (College Station, Texas, USA) for windows. Numerical variables not normally distributed were presented as mean \pm standard deviation and median (range) and were compared using ANOVA/ Krushkal–Wallis test,

followed by Bonferroni correction. Logistic regression analyses were used to test for the association of polymorphisms or haplotypes with GDM and Type 2 diabetes mellitus. Statistical significance was set at p<0.05. Two/three and four loci haplotypes were constructed as per Shi and He, 2005 [25]. All data analyses, research design and manuscript writing was performed at Dasman Diabetes Institute, Kuwait.

Table 3. Polymorphisms positions and PCR-RFLP conditions for detection of 4 SNPs in the ACE Gene [&]

Polymorphisms (location)	Position	Primers sequences (5' → 3')	T _A (°C)	MgCl ₂ (mM)	RFLP Enzyme	T (°C)
ACE4 (5UTR)	A240T	ACE4F: TGTCACTCCGGAGGCGGGAGGCT GAGAAAGGGCCTCCTCTCTCT 55 ACE4R: b	57	1	<i>XbaI</i>	37
ACE5 (5UTR)	C93T	ACE5F: ACCATGGCCTGGTGAAGAAGC ACE5R: CGGCTCTGCCCTTCTCCTGCGC	TD [*]	2.5	ACE 5 was amplified for nested PCR of ACE 4	
ACE6 (exon 8)	C1237T	ACE6F: AGTGCACACGGGTCACGATG ACE6R: CACCAAGTAGCCAAAGGGCAG	65	1.5	<i>BsmBI</i>	60
ACE I/D (intron 16)		ACEIDF: CTGGAGACCACTCCCATCCTTTCT ACEIDR: GATGTGGCCATCACATTCGTCAGAT	60	3		
ACE8 (exon 17)	A2350G	ACE8F: CTGACGAATGTGATGGCCGC ACE8R: TTGATGAGTTCCACGTATTTTCG	60	2	<i>BstUI</i>	60

[&] Described by Zhu et al. (2001) [21]; ^{*} Touchdown PCR

3. RESULTS

Detailed analysis of genotype and allele frequencies of each *ACE gene polymorphisms* A-240-T (*ACE 4*), C-1237-T (*ACE 6*), A-2350-G (*ACE 8*), and *ACE I/D*, with GDM and type 2 diabetes has been in our previously published paper [26].

The linkage disequilibrium mapping of four *ACE* polymorphisms is shown in Fig. 1. Haplotype analyses of *ACE* gene polymorphisms were carried out by combining them in two, three and four loci respectively. Of 64 haplotypes combinations observed, only 13 haplotypes were significant. Only polymorphisms with high linkage disequilibrium (LD' values = 0.9 and r^2 values = 0.8) were considered.

Table 4 illustrates two, three and four loci haplotype combinations of four *ACE gene* polymorphisms. Haplotype combinations, *D-1*, *D-2*, *D-4*, *DD-2*, *DD-3*, *DD-5*, *DD-6*, and *DDI-2* prevented progression to type 2 diabetes post GDM cases. On the contrary, haplotype combinations *D-3*, *DD-1*, *DD-4* and *DDI-1* were shown to increase susceptibility of type-2 diabetes onset post GDM in women from the studied sample.

4. DISCUSSION AND CONCLUSION

It is widely accepted that GDM is one of the strongest predictors for the long-term development of type-2 diabetes in at-risk women [8-13]. In order to prevent progress of GDM to chronic diabetic state later in life it is necessary to screen women with GDM for those high-risk genetic factors. This allows the timely introduction of measures to delay or prevent

diabetes onset. ACE is a zinc metalloprotease that is widely distributed on the surface of epithelial and endothelial cells [22]. The *ACE gene* is one of the most intensely studied genes because of the key role it plays in the RAS. This study therefore calculated the haplotype configurations of four polymorphisms in the coding and non-coding region of the *ACE gene* in order to assess the future progression of GDM to type 2 diabetes in 224 North-Indian women.

Our results indicate that haplotypes *D-1 D-2*, *D-4* in two loci, *DD-2*, *DD-3*, *DD-5*, *DD-6* in three loci, and *DDI-2* in four loci were protective haplotypes, i.e. GDM cases comprising these haplotypes are not susceptible to future onset of type 2 diabetes. GDM cases having haplotype combinations *D-3*, *DD-1*, *DD-4* and *DDI-1* were susceptible to type-2 diabetes post GDM. Of these, haplotype *DDI-1* in GDM cases posed the highest risk for type 2 diabetes development than other haplotypes (Table 4).

This is the first study to demonstrate the role of haplotype configurations of *ACE gene* polymorphisms with regard to development of type-2 diabetes post GDM. Considering the critical role of *ACE gene* interactions during GDM and the risk this poses for type-2 diabetes onset in life, these findings could therefore be of potential therapeutic value in the future. A confirmatory larger scale population with different ethnicities studies is recommended to validate the results of the present study. Although specific predictive factors for the development of type-2 diabetes post-GDM can be identified in the index pregnancy, women with a history of GDM and *ACE DDI-1* haplotype, as a group, are worthy of long-term follow-up to ameliorate type 2 diabetes risk.

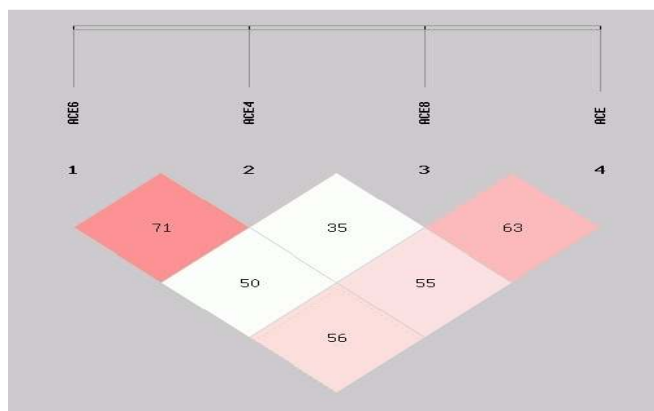


Fig. 1. Graphical overview of pair-wise linkage disequilibrium analysis of the investigated polymorphisms GDM and type 2 diabetes combined

Table 4. Two, three and four loci haplotype analysis of 4 SNPS of ACE gene

Polymorphisms	Hapcode	Loci	Type 2 diabetes	GDM	p value*	O.R. (95% of CI)*
Two Loci						
ACE 4 & 8	D-1	TA	18(7.6%)	35(16.4%)	0.004	0.42(0.23-0.77)
ACE 6& 8	D-2	TA	22(9.1%)	40(19.1%)	0.002	0.42(0.24-0.74)
ACE 6& I/D	D-3	CI	121(50.9%)	85(40.8%)	0.031	1.50(1.04-2.19)
ACE 8 & I/D	D-4	AD	27(11.6%)	43(20.4%)	0.009	0.51(0.30-0.85)
Three Loci						
ACE 6, 4 & 8	DD-1	CAA	18(7.7%)	7(3.3%)	0.044	2.43(1.03-5.93)
	DD-2	TTA	15(6.6%)	30 (14.4%)	0.006	0.42(0.22-0.79)
ACE 4.8 & ID	DD-3	TAD	17(7.1%)	30 (14.4%)	0.012	0.45(0.24-0.85)
ACE 6, 8 & ID	DD-4	CAI	15(6.4%)	3(1.5%)	0.01	4.28(1.26-14.50)
	DD-5	TAD	22 (9.2%)	34(16.4%)	0.01	0.50(0.28-0.88)
	DD-6	TAI	0	6(2.6%)	0.001	0.01 (0.001-0.035)
Four Loci						
	DDI-1	CAAI	14(5.8%)	3(1.6%)	0.023	3.65(1.11-12.05)
	DDI-2	TTAI	0	6(2.5%)	0.024	0.002(<0.001-0.027)

* GDM versus Type 2 Diabetes; + p-value <0.05 were considered significant

Key: DDI – Dasman Diabetes Institute - as manuscript was prepared under their guidance

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sayed-Tabatabaei FA, Oostra BA, Isaacs A, van Duijn CM, Witteman JCM. ACE polymorphism. *Circulation Research*. 2006;98:1123-1133.
2. Salimi S, Mokhtari M, Yaghmaei M, Jamshidi M, Naghavi A. Association of angiotensin-converting enzyme intron 16 insertion/deletion and angiotensin II type 1 receptor A1166C gene polymorphisms with preeclampsia in South East of Iran. *Journal of Biomedicine and Biotechnology*; 2011. Article ID 941515.
3. Khan IA, Jahan P, Hasan Q, Rao P. Angiotensin-converting enzyme gene insertion/deletion polymorphism studies in Asian Indian pregnant women biochemically identifies gestational diabetes mellitus. *J Renin Angiotensin Aldosterone Syst*. 2013;1-6.
4. Dostálová Z, Bienertová-Vasku AJ, Vasku A, Gerychová R, Unzeitig V. Insertion-deletion polymorphism in the gene for

5. angiotensin- converting enzyme (I/D ACE) in pregnant women with gestational diabetes. *Ceska Gynekol*. 2006;71(5):369-73.
5. Zhou JB, Yang JK, Lu JK, An YH. Angiotensin-converting enzyme gene polymorphism is associated with type 2 diabetes: A meta-analysis. *Molecular Biology Reports*. 2010;37(1):67-73.
6. Movva S, Alluri RV, Komandur S, Vattam K, Eppa K, et al. Relationship of angiotensin-converting enzyme gene polymorphism with nephropathy associated with type 2 diabetes mellitus in Asian Indians. *Journal of Diabetes and Its Complications* 2007;21(4):237-241.
7. Zhou JB, Yang JK. Angiotensin-converting enzyme gene polymorphism is associated with proliferative diabetic retinopathy: A meta-analysis. *Acta diabetologica*. 2010; 47(Suppl 1):187-193.
8. Kim, Newton, Knopp. Gestational diabetes and the incidence of type 2 diabetes. *Diabetes Care*. 2002;25(10):1862-1866.
9. Bellamy L, Casas JP, Hingorani AD, Williams D, et al. Type 2 diabetes mellitus after gestational diabetes: A systematic review and meta-analysis. *Lancet*. 2009; 373(9677):1773–1779.
10. Cho YM, Kim TH, Lim S, et al. Type 2 diabetes-associated genetic variants discovered in the recent genome-wide association studies are related to gestational diabetes mellitus in the Korean population. *Diabetologia*. 2009;52(2):253–261.

11. Zhang C, Bao W, Rong Y, et al. Genetic variants and the risk of gestational diabetes mellitus: A systematic review. *Human Reproduction Update*. 2013;19(4): 376-390.
12. Robitaille J, Grant AM. The genetics of gestational diabetes mellitus: Evidence for relationship with type 2 diabetes mellitus. *Genetics in Medicine*. 2008;10:240–250.
13. Lauenborg J, Grarup N, Damm P, et al. Common type 2 diabetes risk gene variants associate with gestational diabetes. *J. Clin. Endocrinol. Metab*. 2009;94(1):145–150.
14. Buchanan TA, Xiang AH. Gestational diabetes mellitus. *J Clin Invest*. 2005;115: 485–491.
15. Buchanan TA. Pancreatic B-cell defects in gestational diabetes: Implications for the pathogenesis and prevention of type 2 diabetes. *J Clin Endocrinol Metab*. 2001;86:989–993.
16. Aly TA, A Ide A, Jahromi MM, Barker JM, Fernando MS, Babu SR et al. Extreme genetic risk for type 1A diabetes. *PNAS*. 2006;103(38):14074–14079.
17. Howson JM, Cooper JD, Smyth DJ, Walker NM, Stevens H, et al. Evidence of gene-gene interaction and age-at-diagnosis effects in type 1 diabetes. *Diabetes*. 2012;61(11):3012-3017.
18. Jahromi M, Ahmed A, Behbehani K. Networking between cytokine gene polymorphisms profile and HLA in the pathogenesis of type 1 diabetes (A Pilot Study). *MOJ Immunol*. 2014;1(4):00023.
19. Neuman RJ, Wasson J, Atzmon G, Wainstein J, Yerushalmi Y, et al. Gene-gene interactions lead to higher risk for development of type 2 diabetes in an Ashkenazi Jewish Population. *PLoS One*. 2010;5(3):e9903.
20. Martínez-Rodríguez N, Posadas-Romero C, Villarreal-Molina T, Vallejo M, Del-Valle-Mondragon L, et al. Single nucleotide polymorphisms of the angiotensin-converting enzyme (ACE) gene are associated with essential hypertension and increased ACE enzyme levels in Mexican individuals. *PLoS One*. 2013;8(5):e65700. DOI: 10.1371/journal.pone.0065700
21. Martínez-Ríos MA, Alvarez-León E, Totomoch A, Angeles J, Peña-Duque MA, Delgadillo-Rodríguez H, Martínez-Rodríguez N, et al. Haplotypes of the angiotensin-converting enzyme (ACE) gene are associated with coronary artery disease but not with restenosis after coronary stenting. *Experimental and Molecular Pathology*. 2014;97(1):166-170.
22. Zhu X, Bouzekri N, Southam L, Cooper RS, Adeyemo A, et al. Linkage and association analysis of angiotensin I-converting enzyme (ACE)-gene polymorphisms with ACE concentration and blood pressure. *Am J Hum Genet*. 2001;68: 1139–1148.
23. American Diabetes Association. Classification and diagnosis of diabetes. Sec. 2. In *Standards of Medical Care in Diabetes*. *Diabetes Care*. 2015; 38(Suppl. 1):S8–S16.
24. Mahajan N, Aggarwal M, Bagga, A. Health issues of menopausal women in North India. *Journal of Mid-Life Health*. 2012;3: 84-87.
25. Shi YY, He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res*. 2005;15:97–98.
26. Aggarwal P, Agarwal N, Das N, Dalal K. Association of polymorphisms in angiotensin-converting enzyme gene with gestational diabetes mellitus in Indian women. *International Journal of Applied and Basic Medical Research*. 2016;6(1): 31-37. DOI: 10.4103/2229-516X.174006

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