JKMU

Increased Risk of Hypertension in Low aged-carriers with rs2596542 Risk-allele

Mohammad Amin Monsef, M.Sc. [1], Seyed-Morteza Javadirad, Ph.D. [2], Sorour Eslami, M.Sc. [1]

1. Department of Cell and Molecular Biology and Microbiology, Faculty of Biological Science and Technology, University of Isfahan, Isfahan, Iran

2. Assistant Professor, Department of Cell and Molecular Biology and Microbiology, Faculty of Biological Science and Technology, University of Isfahan, Isfahan, Iran (Corresponding author: Email: javadirad@yahoo.com)

ARTICLE INFO

Abstract

Background: Hypertension with its related disorders is one of the most common health problems among the Iranian population. Hypertension can be developed by chronic stress and a positive association between stress and rs2596542 has been confirmed.

Methods: A Total number of 112 hypertensive patients and 97 healthy individuals were involved in the study. Total blood genomic-DNA was extracted and PASA (PCR amplification of specific alleles) method was used to amplify MICA-rs2596542 polymorphic site. Different genotypes were visualized. The normality of the data was assessed and the binary logistic regression was used for OR and 95%CI calculations.

Results: A-risk allele of rs2596542 increased the risk of hypertension development significantly (OR=1.734, p=0.006). Females were significantly more potent to hypertension development than males (OR=2.015, p=0.013). Risk-allele homozygotes (AA) showed a higher risk of hypertension development than GG (OR=2.132, p=0.020) and AG individuals (OR=3.206, p=0.006). Age adjustments at 70 years old, further increased the risk of hypertension development in GG (OR=3.772, p=0.011) and AG (OR=6.531, p=0.009) individuals.

Conclusion: A-risk allele of rs2596542 could increase the risk of hypertension up to 3.2 folds and this risk could be upraised after sex and age adjustments.

Introduction

Hypertension (HT) is a condition that can strain the heart because of the excessive force of blood flow throughout the blood vessels. According to the 2017 report of the global burden of hypertension, the number of individuals with HT and its associated deaths is on the rise (1). A systematic review from 1980-2012 was declared HT as one of the most common health problems among the Iranian population (2). It has been clarified that ethnicity could be a factor with high impacts on the occurrence of HT and its related disorders such as heart attack, stroke, kidney problems, and finally death (3, 4). Despite the numerous loci that have been identified and the linkage analysis that has been performed, there is a lot of uncertainty about the genetics of HT (5-9). Nevertheless, genetic polymorphic sites are one of the

significances that could be differentially associated with HT.

MHC class I polypeptide-related sequence A (*MICA*, 6p21.33) gene is transcribed especially in the epitheliums of the blood vessels (10, 11). As a self-antigen and a responder to stress, *MICA* expression was also reported in the epithelium of intestinal gamma-delta T cells and in tumor cells (12, 13). Our previous study also showed that MICA polymorphisms could increase the risk of breast cancer development in the Iranian population (14).

It has been revealed that chronic psychological stress can increase blood pressure (12, 15). Genome-wide association studies have showed a strong linkage of rs2596542 polymorphic site (in the 5′ flanking region of *MICA* gene, MAF: 0.42) and chronic psychological stresses such as hepatocellular carcinoma (16-19). Therefore, in the present study, we tried to explore the possible role of MICA-rs2596542 in the Iranian population tackling HT.

Materials and Methods
Subjects

A case-control observational study was performed in the Isfahan province of Iran. A Total number of 112 HT patients and 97 healthy individuals were involved in the study. HT was confirmed at least after three times reading of blood pressure by the same expert physician. Written informed consent forms were obtained before total blood collection.

MICA genotyping

Genomic DNA extraction was performed using the miller salting-out method (20). Polymerase chain reaction (PCR) amplification of specific alleles (PASA) was used for MICA rs2596542 polymorphic site amplification. PASA primers were as follows: GGTTATCTGCCTGCCATAG as the common reverse primer, TCCCAAAGAACAGCTACAAG for detecting ancestral-G allele and TCCCAAAGAACAGCTACAAA for amplifying mutated-A allele. PCR amplification condition was as follow: 1) one step of initial denaturation at 95°C for 5 min, 2) 35 cycles of denaturation at 95°C for 1 min, annealing at 60°C for 1 min and extension at 72°C for 1 min, 3) final extension at 72°C for 10 min. Different genotypes were visualized on 1% agarose gel electrophoresis.

Statistical analysis

Genotype distributions and frequencies were calculated using IBM SPSS statistics v21.0. The normality of the data was assessed using the Kolmogorov-Smirnov test. Binary logistic regression was used for odd ratio (OR) and 95% confidence interval (95%CI) calculation. p-value less than 0.05 was considered as significant.

Results
Population information

The total population consisted of 112 (53.6%) patients and 97 (46.4%) control individuals. The mean age of the patients was 74.38±1.17 years, and the mean age of our control individuals was 71.68±1.40 years.

Among 209 individuals included in this study, 97 (46.4%) were males and 112 (53.6%) were females with the mean age of 71.46±1.37 and 74.57±1.18 respectively (Table 1).

Table 1. Population information of the studied individuals

Gender	Frequency No (%)	Mean age ± SEM
Male	97 (46.4%)	71.46±1.37
Female	112 (53.6%)	74.57±1.18
Total	209 (100%)	73.13±0.90
Patients	112 (53.6%)	74.38±1.17
Control individuals	97 (46.4%)	71.68±1.40

SEM: standard error of mean

MICA rs2596542 allele amplification and genotyping

Two amplification reactions were performed separately in two tubes and genotypes were visualized (Figure 1). Samples 1 and 4 were heterozygote as both G allele and A allele were amplified simultaneously. Sample 2 was GG homozygous and sample 3 was AA homozygous as the former was shown amplification for only G allele and the later for A allele only.
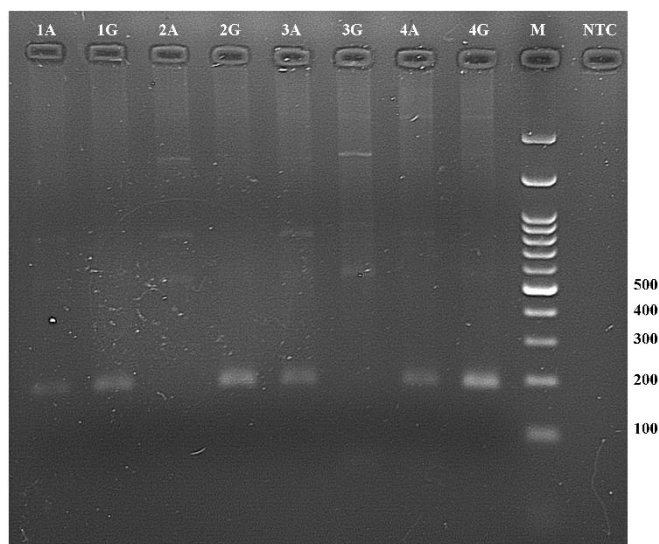

Figure 1. PASA driven genotypes

Two separate amplifications were done in discrete tubes for each sample. Homozygotes (sample 2 and 3) showed one band and heterozygotes (samples 1 and 4) were represented by two bands. A: mutated-A allele, G: ancestral G-allele, M: 100bp DNA ladder, NTC: non-template control.

Genotype distribution of HT patients and control individuals

Three genotypes were observed in our total population designated as GG, AA, and AG (Table 2). The frequencies of the genotypes were 49.3% for GG, 32.5% for AA and 18.2% for AG. Homozygous AA genotype was the predominant genotype in patients with 67.6% frequency. In control individuals, AG genotype with 60.5% frequency was the most frequent genotype followed by GG genotype with 50.5% frequency. The lowest frequent genotype in control individuals was AA genotype with 32.4% frequency. GG genotype was selected as the reference genotype because it showed a similar frequency between HT patients and control individuals.

Table 2. Genotype distribution of the studied individuals

	Genotypes	Frequency No (%)
Total population	AA	68 (32.5)
	AG	38 (18.2)
	GG	103 (49.3)
	total	209 (100)
Patients	AA	46 (67.6)
	AG	15 (39.5)
	GG	51 (49.5)
	total	112 (100)
Control individuals	AA	22 (32.4)
	AG	23 (60.5)
	GG	52 (50.5)
	total	97 (100)

HT development, alleles, genotypes, sex and age adjustments

The association of HT development with alleles, genotypes, age and sex of the individuals were presented in Table 3. A-allele of MICA-rs2596542 increased the risk of HT development 1.7 times more than G-allele and it was significant (OR=1.7, p=0.006). The risk of HT development was 2.132 times higher (p=0.020) when AA genotype was compared with the GG reference genotype. Homozygous AA genotype also increased the risk of HT more than 3 times (OR =3.206, p=0.006), when it was compared to AG heterozygotes.

Table 3. The association of HT risk with sex and genotypes

	Unadjusted Odd ratio (OR)		Adjusted OR	
	OR (p-value)	95% CI	OR (p-value)	95% CI
Female \ male	2.015 (0.013)	1.152-3.502		
A \ G	1.734 (0.006)	1.167 - 2.574		
AA \ GG	2.132 (0.020)	1.126-4.036	3.772 (0.011)[*]	1.353- 10.513
AA \ GG			2.783 (0.034)[†]	1.079-7.176
AA \ AG	3.206 (0.006)	1.404-7.319	6.531 (0.009)[*]	1.592-26.788
AA \ AG			4.030 (0.024)[†]	1.201-13.526

*Age cut-off of 70 was used for HT risk assessment

†Female were compared with men for HT risk assessment

The risk of HT development in females was 2 folds greater than that in males and the difference was significant (OR=2.015, p=0.013). Comparison of AA patients respectively with GG and AG ones, showed OR=3.772 (p=0.011) and OR=6.531 (p=0.009) after age adjustment. Sex adjusted-ORs for AA patients were 2.783 (p=0.034) and 4.030 (p=0.024) when they were compared with GG and AG ones respectively.

Discussion

Hypertension as a traditional cause of cardiovascular diseases has been refocused because of its new embedded roles in dementia, physical disability, and falls/fractures (21-23). Common biological aging as the foremost cause of HT could induce the condition by several mechanisms such as inflammation, oxidative stress induction, and endothelial dysfunction (24). However the influence of genetics on this classical complex trait is just explored via genome-wide association studies, but a lot of uncertainties remain to be clarified (25).

Sixteen genetic loci on chromosomes 1, 2, 3, 4, 5, 7, 12, 15, 17 and 20, have impacts on the development of HT. Striatin, adducin-1, beta 2 adrenergic receptor, cytochrome P450 family 11 subfamily B member 2 (*CYP11B2*), *CYP17A1*, Endothelin-1, Estrogen receptor beta, Lysine-specific demethylase 1, Serum- and glucocorticoid-inducible kinase 1, Bradykinin receptor B2, Electrogenic sodium bicarbonate cotransporter 4, Plasma membrane calcium-transporting ATPase 1, Serine/threonine 39, WNK lysine-deficient protein kinase 1, Angiotensin receptor type I, Fibroblast growth factor 5, G protein beta 3 subunit, genes polymorphisms and also another 41 genes with insufficient evidences were also reported to be associated with HT (26). The most effective genes with strong correlation and with supportive cohort evidences are caveolin-1 (*CAV1*), angiotensinogen (*AGT*), renin enzyme (*REN* gene), angiotensin I (*ANG I*) and angiotensin II (*ANG II*). These genes are the members of renin-angiotensin system that directly control the vascular resistance and regulate blood pressure (27). *CAV1* gene variants (rs926198 and rs3807989) were in linkage disequilibrium with HT and the prevalence of the disease in Japanese men was associated with *CAV1*-rs2255991 and rs1799998 (28, 29). Null mice experiments candidated *CAV1* gene as the regulators of eNOS activity, the key developer of HT (30, 31). *AGT* gene variants were also in positive association with HT and an Iranian population study presented a

relationship between an AGT-TT genotype and hypertension (32). *AGT* gene variant (rs699) was associated with HT development in Caucasian-Brazilians (33). Different *AGT* gene haplotypes and polymorphisms were shown to be associated with HT in Indians, but not with Hellenic population (34, 35). Finally, AGT-rs5051 was reported to act synergistically with β1-adrenergic receptor blocker and angiotensin-converting enzyme inhibitors, in order to fight HT (36, 37). Renin enzyme converts AGT to ANG I and ANG II and all these three related genes were considered with the risk of HT in women (38, 39). REN-rs5707 and two haplotypes were correlated with HT in women aged from 40-70 years old (38). Therefore it seems that gene polymorphisms behave in a sex dependent manner to influence blood pressure.

New genes with impacts on the development of HT could be beneficial to uncover the molecular dark side of blood pressure. *MICA* gene polymorphisms that have been extensively studied and mutated "rs2596542-A" allele, was introduced as a risk factor of hepatocellular carcinoma (16, 40, 41). Interestingly in the present study, we also showed MICA-A-allele as the risk allele of HT development. Accordingly, patients with AA genotypes showed an elevated risk of HT development. In accordance to the previous studies, we showed that being female could also increase the risk of HT development. According to our knowledge, it is the first time that females' carriers of two "A" risk alleles of the *MICA* gene were shown to be at higher risk of HT development. Heterozygote advantage also observed in our studied population because AG heterozygotes showed higher OR than AA ones, but it is not clear why over-dominance is

seen and why being AG heterozygote, could even increase the risk of HT. The highest risk of HT development achieved when age adjustment was considered; as being younger than 70 years old could dramatically upraise the risk of HT development up to 6.5 folds significantly.

MICA-A-allele is a membrane-bounded anti-tumor ligand for natural killer cell receptors (NKG2D), while rs2596542-G allele produces a soluble form of MICA (42, 43). Owing to the vicinity of MICA locus to HLA-B gene, and because the HLA-B gene is linked with numerous autoimmune diseases, a lot of efforts have been done to discover the role of MICA in the development of autoimmune diseases (44, 45). It is postulated that *MICA* gene purposed its effects via T cells interaction with NKG2D (44). Moreover, a comprehensive study of patients carrying *MICA*-A-allele opened the eyes to the role of TGF-β in liver fibrosis (46). Consequently it seems that the *MICA*-A-allele is not only able to lower the level of its soluble form in serum, but also is able to reduce the mRNA of its membranous bound isoform. Therefore *MICA*-A-allele is a negative regulator of itself, meanwhile it could elevate TGF-β levels in cells and TGF-β instead, could decrease the expression of *MICA*-mRNA (47, 48). We proposed here that A-risk allele might elevate the cell stress because of i) the elevation of TGF-β mRNA that in turn diminishes cell death and ii) the loss of its protein content and therefore the loss of an stress response product in cells (41, 49). Elevation of cell stress alongside immune suppression, because of the lack of MICA\NKG2D interaction, could lead to the development of HT (50).

In conclusion, MICA-rs2596542 is composed of the ancestral "G" allele and the mutated "A" allele with an influence on HT development. HT patients were compared with control individuals and it was cleared that AA genotype significantly could increase the risk of HT more than 4 times in women, and even more elevated risk of HT could be achieved when age cut-of 70 was considered.

Competing Interests

The authors declared no competing interests.

Author statement

Each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in Journal of Kerman University of Medical Sciences.

References

1. Forouzanfar MH, Liu P, Roth GA, Ng M, Biryukov S, Marczak L, et al. Global burden of hypertension and systolic blood pressure of at least 110 to 115 mm hg, 1990-2015. JAMA 2017; 317(2):165-82.

2. Mirzaei M, Moayedallaie S, Jabbari L, Mohammadi M. Prevalence of hypertension in Iran 1980–2012: a systematic review. J Tehran Heart Cent 2016; 11(4):159-67.

3. Carson AP, Howard G, Burke GL, Shea S, Levitan EB, Muntner P. Ethnic differences in hypertension incidence among middle-aged and older U. S. adults: the multi-ethnic study of atherosclerosis. Hypertension 2011; 57(6):1101-7.

4. Lackland DT. Racial differences in hypertension: implications for high blood pressure management. Am J Med Sci 2014; 348(2):135-8.

5. Beevers G, Lip GY, O'Brien E. ABC of hypertension: The pathophysiology of hypertension. BMJ 2001; 322(7291):912-6.

6. Justice AE, Howard AG, Chittoor G, Fernandez-Rhodes L, Graff M, Voruganti VS, et al. Genome-wide association of trajectories of systolic blood pressure change. BMC Proc 2016; 10(Suppl 7):321-7.

7. 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; 41(6):677-87.

8. Wain LV, Vaez A, Jansen R, Joehanes R, van der Most PJ, Erzurumluoglu AM, et al. Novel blood pressure locus and gene discovery using genome-wide association study and expression data sets from blood and the kidney. Hypertension 2017.

9. Warren HR, Evangelou E, Cabrera CP, Gao H, Ren M, Mifsud B, et al. Genome-wide association analysis identifies novel blood pressure loci and offers biological insights into cardiovascular risk. Nat Genet 2017; 49(3):403-15.

10. Ghadially H, Brown L, Lloyd C, Lewis L, Lewis A, Dillon J, et al. MHC class I chain-related protein A and B (MICA and MICB) are predominantly expressed intracellularly in tumour and normal tissue. Br J Cancer 2017; 116(9):1208-17.

11. Leelayuwat C, Townend DC, Degli-Esposti MA, Abraham LJ, Dawkins RL. A new polymorphic and multicopy MHC gene family related to nonmammalian class I. Immunogenetics 1994; 40(5):339-51.

12. Groh V, Steinle A, Bauer S, Spies T. Recognition of stress-induced MHC molecules by intestinal epithelial gammadelta T cells. Science 1998; 279(5357):1737-40.

13. Schrambach S, Ardizzone M, Leymarie V, Sibilia J, Bahram S. In vivo expression pattern of MICA and MICB and Its relevance to auto-immunity and cancer. PLoS One 2007; 2(6):e518.

14. Vallian S, Rad MJ, Tavallaei M, Tavassoli M. Correlation of major histocompatibility complex class I related A (MICA) polymorphism with the risk of developing breast cancer. Med Oncol 2012; 29(1):5-9.

15. Spruill TM. Chronic psychosocial stress and hypertension. Curr Hypertens Rep 2010; 12(1):10-6.

16. Augello G, Balasus D, Fusilli C, Mazza T, Emma MR, Giannitrapani L, et al. Association between MICA gene variants and the risk of hepatitis c virus-induced hepatocellular cancer in a sicilian population sample. OMICS 2018; 22(4):274-82.

17. Lo PH, Urabe Y, Kumar V, Tanikawa C, Koike K, Kato N, et al. Identification of a functional variant in the MICA promoter which regulates MICA expression and increases HCV-related hepatocellular carcinoma risk. PLoS One 2013; 8(4):e61279.

18. Mohamed AA, Elsaid OM, Amer EA, Elosaily HH, Sleem MI, Gerges SS, et al. Clinical significance of SNP (rs2596542) in histocompatibility complex class I-related gene A promoter region among hepatitis C virus related hepatocellular carcinoma cases. J Adv Res 2017; 8(4):343-9.

19. Marangon CG, de Bitencorte JT, Michita RT, Lunge VR, Dos Santos DC, Álvares-da-Silva MR, et al. Association between MICA rs2596542 Polymorphism with the risk of hepatocellular carcinoma in chronic hepatitis C patients. Pathol Oncol Res 2020; 26(3):1519-25.

20. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16(3):1215.

21. Erickson SR, Kornexl K. Blood pressure screening, control, and treatment for patients with developmental disabilities in general medicine practices. J Pharm Technol 2016; 32(6):234-9.

22. Margolis KL, Palermo L, Vittinghoff E, Evans GW, Atkinson HH, Hamilton BP, et al. Intensive blood pressure control, falls, and fractures in patients with type 2 diabetes: the ACCORD trial. J Gen Intern Med 2014; 29(12):1599-606.

23. Zhou D, Xi B, Zhao M, Wang L, Veeranki SP. Uncontrolled hypertension increases risk of all-cause and cardiovascular disease mortality in US adults: the NHANES III Linked Mortality Study. Scientific Reports 2018; 8(1):9418.

24. Buford TW. Hypertension and aging. Ageing Res Rev 2016; 26:96-111.

25. Ehret GB, Caulfield MJ. Genes for blood pressure: an opportunity to understand hypertension. Eur Heart J 2013; 34(13):951-61.

26. Manosroi W, Williams G. Genetics of Human Primary Hypertension: Focus on Hormonal Mechanisms. Endocrine Reviews 2018; 40(3):825-56.

27. Sparks MA, Crowley SD, Gurley SB, Mirotsou M, Coffman TM. Classical renin-angiotensin system in kidney physiology. Compr Physiol 2014; 4(3):1201-28.

28. Pojoga LH, Underwood PC, Goodarzi MO, Williams JS, Adler GK, Jeunemaitre X, et al. Variants of the Caveolin-1 Gene: A Translational Investigation Linking Insulin Resistance and Hypertension. J Clin Endocrinol Metab 2011; 96(8):E1288-92.

29. Yamada Y, Ando F, Shimokata H. Association of gene polymorphisms with blood pressure and the prevalence of hypertension in community-dwelling Japanese individuals. Int J Mol Med 2007; 19(4):675-83.

30. Grilo A, Fernandez ML, Beltrán M, Ramirez-Lorca R, González MA, Royo JL, et al. Genetic analysis of CAV1 gene in hypertension and metabolic syndrome. Thromb Haemost 2006; 95(4):696-701.

31. Duplain H, Burcelin R, Sartori C, Cook S, Egli M, Lepori M, et al. Insulin resistance, hyperlipidemia, and hypertension in mice lacking endothelial nitric oxide synthase. Circulation 2001; 104(3):342-5.

32. Tabei SM, Nariman A, Daliri K, Roozbeh J, Khezri A, Goodarzi HR, et al. Simple renal cysts and hypertension are associated with angiotensinogen (AGT) gene variant in Shiraz population (Iran). J Renin Angiotensin Aldosterone Syst 2013; 16(2):409-14.

33. Bonfim-Silva R, Guimarães LO, Santos JS, Pereira JF, Barbosa AA, Rios DL. Case-control association study of polymorphisms in the angiotensinogen and angiotensin-converting enzyme genes and coronary artery disease and systemic artery hypertension in African- and Caucasian-Brazilians. Journal of Genetics 2016; 95(1):63-9.

34. Tsezou A, Karayannis G, Giannatou E, Papanikolaou V, Triposkiadis F. Association of renin-angiotensin system and natriuretic peptide receptor A gene polymorphisms with hypertension in a Hellenic population. J Renin Angiotensin Aldosterone Syst 2008; 9(4):202-7.

35. Purkait P, Halder K, Thakur S, Ghosh Roy A, Raychaudhuri P, Bhattacharya S, et al. Association of angiotensinogen gene SNPs and haplotypes with risk of hypertension in eastern Indian population. Clin Hypertens 2017; 23:12.

36. Kurland L, Liljedahl U, Karlsson J, Kahan T, Malmqvist K, Melhus H, et al. Angiotensinogen gene polymorphisms: relationship to blood pressure response to antihypertensive treatment. Results from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation vs Atenolol (SILVHIA) trial. Am J Hypertens 2004; 17(1):8-13.

37. Yu H, Lin S, Zhong J, He M, Jin L, Zhang Y, et al. A core promoter variant of angiotensinogen gene and interindividual variation in response to angiotensin-converting enzyme inhibitors. J Renin Angiotensin Aldosterone Syst 2014; 15(4):540-6.

38. Mansego ML, Redon J, Marín R, González-Albert V, Martin-Escudero JC, Fabia MJ, et al. Renin polymorphisms and haplotypes are associated with blood pressure levels and hypertension risk in postmenopausal women. J Hypertens 2008; 26(2):230-7.

39. Park J, Song K, Jang Y, Yoon SK. A polymorphism of the renin gene rs6682082 is associated with essential hypertension risk and blood pressure levels in Korean women. Yonsei Med J 2015; 56(1):227-34.

40. Goto K, Kato N. MICA SNPs and the NKG2D system in virus-induced HCC. J Gastroenterol 2015; 50(3):261-72.

41. Sharkawy RE, Bayoumi A, Metwally M, Mangia A, Berg T, Romero-Gomez M, et al. A variant in the MICA gene is associated with liver fibrosis progression in chronic hepatitis C through TGF-β1

dependent mechanisms. Scientific Reports 2019; 9(1):1439.

42. Kumar V, Yi Lo PH, Sawai H, Kato N, Takahashi A, Deng Z, et al. Soluble MICA and a MICA variation as possible prognostic biomarkers for HBV-induced hepatocellular carcinoma. PLoS One 2012; 7(9):e44743.

43. Shi J, Szmania S, Zhan F, Shaughnessy J, Barlogie B, Tricot G, et al. Soluble and membrane bound MICA in myeloma do not adversely effect regulation of natural killer cell function via NKG2D. Blood 2004; 104(11):2462.

44. Caillat-Zucman S. How NKG2D ligands trigger autoimmunity? Hum Immunol 2006; 67(3):204-7.

45. Choy MK, Phipps ME. MICA polymorphism: biology and importance in immunity and disease. Trends Mol Med 2010; 16(3):97-106.

46. Sharkawy RE, Bayoumi A, Metwally M, Mangia A, Berg T, Romero-Gomez M, et al. A variant in the MICA gene is associated with liver fibrosis progression in chronic hepatitis C through TGF-beta1 dependent mechanisms. Sci Rep 2019; 9(1):1439.

47. Friese MA, Wischhusen J, Wick W, Weiler M, Eisele G, Steinle A, et al. RNA interference targeting transforming growth factor-beta enhances NKG2D-mediated antiglioma immune response, inhibits glioma cell migration and invasiveness, and abrogates tumorigenicity in vivo. Cancer Res 2004; 64(20):7596-603.

48. Siegel PM, Massagué J. Cytostatic and apoptotic actions of TGF-β in homeostasis and cancer. Nat Rev Cancer 2003; 3(11):807-21.

49. Walshe TE, Saint-Geniez M, Maharaj AS, Sekiyama E, Maldonado AE, D'Amore PA. TGF-beta is required for vascular barrier function, endothelial survival and homeostasis of the adult microvasculature. PLoS One 2009; 4(4):e5149.

50. Xia M, Guerra N, Sukhova GK, Yang K, Miller CK, Shi GP, et al. Immune activation resulting from NKG2D/ligand interaction promotes atherosclerosis. Circulation 2011; 124(25):2933-43.