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Investigation of the Frequency of *IL27* Gene -964 A>G Polymorphism (rs153109) in Infants with a History of Heart Wall Defects in Yazd

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ABSTRACT

Background: Congenital Heart Disease (CHD) is one of the leading causes of infant mortality with some problems in the heart's structure at birth. One of the most common congenital heart diseases is the septal defect, in which there is a hole in the wall (septum). Although the etiology of CHD is mainly unknown, numerous studies have suggested both genetic and environmental factors contribute to the development of this disease. This study aims to investigate the frequency of -964 A>G polymorphism (rs153109) in the *IL27* gene in infants with CHD in Yazd province, Iran.

Methods: The study included 30 infants with CHD. We genotyped the *IL27* polymorphism by using the PCR- Sequencing technique.

Results: Data revealed that the frequencies of AA, AG, and GG among the population of Yazd province were 40%, 40%, and 20%, respectively. The frequency of A and G alleles were 60% and 40%, respectively.

Conclusion: The higher frequency of the A allele in patients with CHD compared to the G allele suggests that the A allele may increase atrial septal defect and ventricular septal defect susceptibility in Yazd province. It is recommended that the presence of the A allele and AG genotype be used as a predictor for the development of septal defects.

Introduction

Congenital heart diseases (CHDs) are referred to abnormal structure and function of the cardiovascular system at birth. Data has reported that cardiovascular diseases have the highest incidence of birth defects and have increased annually since 2005.[1] This malformation accounts for

approximately one-third of all congenital disorders. Every year, nearly 1% of births (40,000 infants) are diagnosed with CHD in the United States, and around 100-200 death reports are caused by unrecognized heart disease in infants (https://www.cdc.gov). Asia has the highest incidence of CHD with 9.3 per thousand live births.[2]

One of the most common congenital heart diseases is a defect in the interventricular and atrial wall or a hole in the heart. Depending on the hole location in the heart, CHDs can be divided into ventricular septal defect (VSD), atrial septal defect (ASD), and atrioventricular Septal Defect (AVSD).[3] Currently, the cause of CHD is not fully recognized. Numerous studies have demonstrated that CHDs are affected by genetic and environmental factors.[4] It is well known that environmental factors during fetal development increase the risk of CHD. These factors can include viral infections such as rubella, exposure to environmental teratogens, and congenital diseases such as diabetes and hypertension.[5,6] In addition, genetic factors such as chromosome structure disorders, gene mutations, polymorphisms, RNA disorders, and epigenetic factors are effective in causing CHD.[7] It is estimated that around 400 genes such as *NKX2.5, NKX2.6, GATA6, IRX4, TBX20,* and *ZIC3* are related to the pathogenesis of CHDs. Mutations in genes encoding transcription factors, cell signaling effectors, and chromatin modifiers are critical in characteristics, differentiation, and patterns of cardiac development, structure, and function. Nevertheless, due to genetic heterogeneity, the cause of almost 60% of cases with CHD is unknown, which leads to variable phenotype and penetrance. Therefore, CHD is best described as having complex and, in some cases, mendelian patterns. CHD mutations are inherited as autosomal dominant, autosomal recessive, and X-linked phenotypes with high penetrance.[1]

Previous research suggests that cytokines may contribute to the development of human birth defects during embryogenesis.[8] IL27, as a new member of the IL12 family, is a heterodimer cytokine that is composed of two subunits, p28 and Epstein-Barr virus-induced gene 3 (EBI3), which are encoded by *IL27* and *EBI3* genes, respectively. It is produced by antigen-presenting cells (APCs) such as dendritic cells and macrophages, and acts on other immune cells to modulate the immune responses. It promotes differentiation of native T cells to T helper 1, as well as IFN-γ production by T cells and natural killer cells in contribution to IL12. IL27 implicates its biological effects through a receptor complex consisting of IL27Rα (also known as WSX-1 or TCCR) and glycoprotein 130 (gp130).[9,10] Binding IL27 to its receptor activates the JAK/STAT signaling pathway, which eventually regulates the expression of genes involved in immune cell proliferation, differentiation, and function.[11] Previous reports have shown that IL27 plays a critical role in mother-fetus tolerance and successful pregnancy. The *IL27* gene is located on chromosome 16p11.[9] Previous reports have shown the association of *IL27* genetic variations with different diseases such as chronic obstructive pulmonary disease[12-15] and allergic rhinitis.[16-19] One of the most common polymorphisms of the *IL27* gene is the A-964G polymorphism (rs153109), which is located in the promoter region of the gene. The population frequency of A-964G homozygosity is more variable among Europeans. The study conducted by Danyan Zhang analyzed the relationship between IL27 gene polymorphism and the risk of CHD. The association between *IL27* gene polymorphism and the risk of CHD was first analyzed by Danyan Zhang et al.[9] In recent years, several articles have reported the association between the polymorphism (A-964G) and the risk of CHD.[9,20,21] However, the results and conclusions are still contradictory. The aim of this study is the detection of rs153109 polymorphism and its frequency in patients with CHD in Yazd province, Iran. Also, this research evaluates the association of rs153109

polymorphism with the risk of CHD development in this population.

Materials and Methods

Subjects: In the present study, 30 blood samples of Patients suffering from CHD with the type of heart cavity (ASD, VSD, AVSD) were collected after clinical evaluation. The samples were recruited from the heart center of Afshar Hospital, Yazd, Iran, in 2023. Demographic characteristics such as gender, type of CHD (ASD, VSD, AVSD), gestational age of the mother (range = 13-43), and mother's underlying disease such as diabetes, hypertension, anemia, infection, hypothyroid and miscarriage history, and others were collected by reviewing patients' medical records (Table 1).

DNA extraction and genotyping analysis: Genomic DNA was extracted from whole blood for polymerase chain reaction (PCR) using the BehPrep G-Plus DNA Extraction Kit (Behgene Co, Iran).

Genotyping of *IL27* -964 A/G: *IL27* genotyping was performed using the polymerase chain reaction (PCR) method followed by sequencing. The A>G conversion in the promoter region in -964 was amplified to form a fragment of 450 bp by using designed primers 5´CGCCTGGTTTCTATCTCACAC3´ and 5´GTGTTTAGGGTCAGAGCTATCAG3´ as forward and reverse primers, respectively.

Table 1. Demographic Characteristics of Patients with CHD

Type of CHD	Gender	Gestational age of the mother	Mother's underlying disease
VSD, PDA	Male	16	-
VSD	Male	35	Urolithiasis
VSD	Male	24	Hypothyroid
ASD	Female	14	Miscarriage history
ASD	Male	38	-
VSD	Female	25	-
ASD	Male	17	-
ASD	Female	30	-
VSD	Male	41	Miscarriage history and Hypertension
VSD	Female	43	Hypothyroid and Diabetes
ASD	Female	36	Hypothyroid, Diabetes, HLP
ASD	Male	18	Anemia
VSD	Male	23	Addiction
VSD	Male	17	-
VSD, PDA, PH	Female	35	Miscarriage history
ASD	Female	37	History of death after the birth
ASD, VSD, PAPVC	Male	27	Hypothyroid
VSD	Female	19	-
ASD	Male	30	-
ASD	Female	25	Miscarriage history
ASD	Female	32	-
VSD	Female	33	Miscarriage history and Urinary tract infection
VSD, PDA	Male	37	Diabetes and anemia
VSD	Female	38	Miscarriage history
ASD	Female	13	-
ASD	Male	22	Miscarriage history
VSD, PDA, PH	Female	32	-
ASD	Male	17	-
VSD, PDA	Female	19	Blood infection
VSD	Male	22	-

The PCR condition was; 94°C for 5 min, followed by 35 cycles of 94°C for 45s, 61°C for 45s, 72°C for 45s, and a final extension step at 72°C for 5 min. The 450 bp fragment as a PCR product was detected on a 1.2% agarose gel.

Sequencing of the amplified fragment: Sequencing is a method used to determine the sequence of nucleotides of a desired piece of DNA. The sequence of nucleotides A, G, C, and T in DNA can be determined through various methods and technologies. Sequencing of the target fragments was done using the Sanger sequencing method in the ABI 3730 XL capillary sequencer system (Research Center of Genetics, Meybod, Iran). The obtained results were analyzed by the Finch TV program (1.4 version) and the Align Nucleotide Sequences BLAST site (http://blast.ncbi.nlm.nih.gov).

Detection of genotypic and allelic frequency of IL27 -964 A/G polymorphism in the studied population: Genotypic and allelic frequencies were calculated using the following formulas, and the results were calculated separately (Tables 2 and 3).

Table 2. The Frequency of Genotypic Distribution for IL27 -964 A/G Polymorphism in the Studied Population

Genotype	Number	Frequency (%)
A/A	12	40
G/G	6	20
A/G	12	40

Genotypic frequency

$$f(AA) = \frac{n(AA)}{N}$$

$$f(AG) = \frac{n(AG)}{N}$$

$$f(GG) = \frac{n(GG)}{N}$$

Allelic frequency

$$f(A) = \frac{2n(AA)+n(AG)}{2N}$$

$$f(G) = \frac{2n(GG)+n(AG)}{2N}$$

Statistical analysis: Statistical analysis was performed by SPSS for Windows (version 26, IBM Corporation, Armonk, NY). Deviation from Hardy-Weinberg Equilibrium (HWE) for *IL-27* SNP was calculated using the chi-squared test (https://gene-calc.pl/hardy-weinberg-page). The data were presented in the form of allelic and genotypic frequencies in the studied population. Statistical tests of the χ^2-test were used to evaluate the genotypic and allelic frequency of rs153109 in this study group.

Table 3. The Frequency of Allelic Distribution for IL27 -964 A/G Polymorphism in the Studied Population

Allele	Number	Frequency (%)
A	36	60
G	24	40

Results

***The results of PCR on electrophoresis*:** The desired fragment of the *IL27* gene was amplified by PCR. The results showed a fragment with a length of 450 bp for all 30 samples (Figure 1).

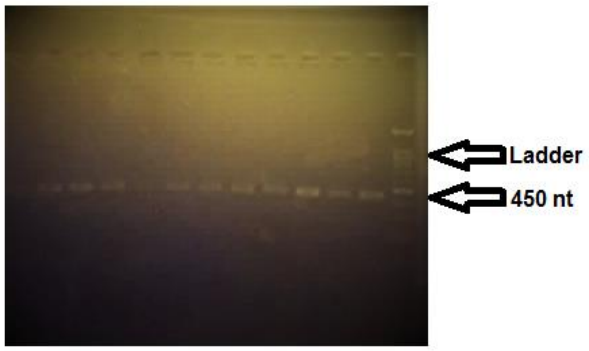

Figure 1. The amplified fragments of the IL27 gene with the size of 450 bp for a number of samples

The results of sequencing in the study group: The PCR product was sequenced by Sanger sequencing and different genotypes of *IL27* -964 A/G (AA, AG, and GG), AA and GG as homozygote, and AG as heterozygote were detected. An allele is a wild-type allele in rs153109. Figures 2A, B, and C present different genotypes of *IL27*-964 A/G with Finch TV software. Table 4 presents the results of sequencing for each patient.

Detection of genotypic and allelic frequency of IL27 -964 A/G polymorphism in the studied population: Genotypic and allelic frequencies of *IL27 -964 A/G polymorphism* were calculated in the studied population.

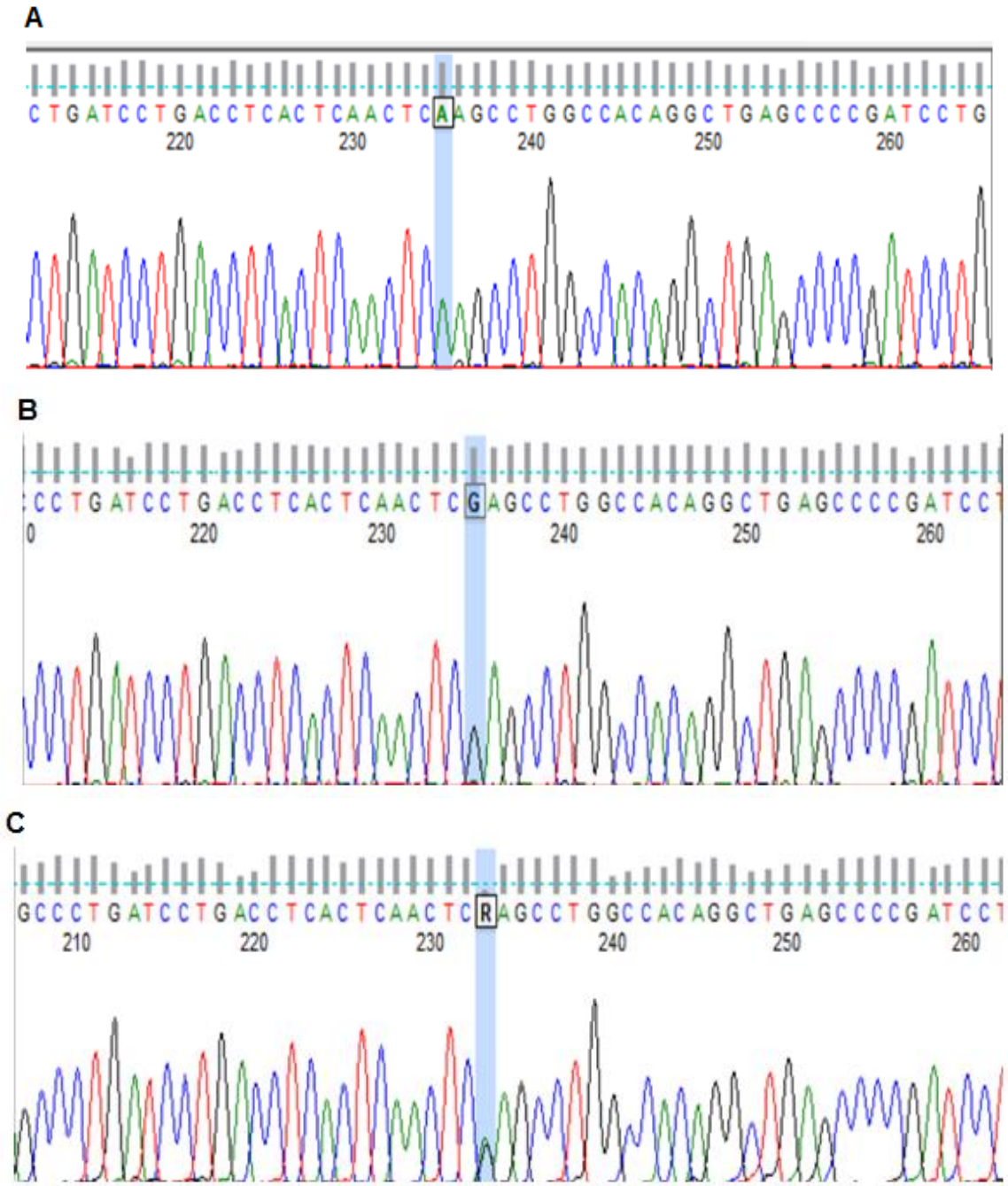

Figure 2. A) The result of the sequencing of the studied sample without IL27 -964 A/G polymorphism and in wild type homozygote (A/A), B) The result of the sequencing of the studied sample with IL27 -964 A/G polymorphism in a GG homozygote status. C) The result of the sequencing of the studied sample with IL27 -964 A/G polymorphism in an AG heterozygote status

The results revealed that among the patients, 12 cases had AA and 12 cases had AG genotypes, while the number of patients with GG genotypes was 6. Also, the frequency of the A allele (60%) was more than the G allele (40%) (Tables 2 and 3).

Discussion

CHD is the most common cause of infant mortality in the world and is the first cause of death among congenital defects. It affects the structure and function of the heart.

Table 4. The Results of Sequencing for Each Patient

Patient	Genotype rs153109 (-964 A>G)	Patient	Genotype rs153109 (-964 A>G)
P1	AG	P16	AG
P2	AG	P17	AG
P3	AA	P18	AA
P4	AG	P19	AA
P5	GG	P20	AA
P6	AA	P21	GG
P7	AA	P22	AA
P8	AG	P23	GG
P9	AA	P24	AG
P10	AG	P25	GG
P11	AA	P26	AA
P12	GG	P27	AG
P13	GG	P28	AA
P14	AA	P29	AG
P15	AG	P30	AG

Two types of CHD with high prevalence are VSD and ASD. The incidence rate of CHDs varies in different populations. While some cases of CHD are due to genetic or environmental factors, the exact cause of many cases is unknown.[22] Syndromic and non-syndromic gene mutations are among the genetic factors of CHD. Genetic variations can affect the mRNA expression level and its stability, as well as the activity and stability of the encoded protein.[23] Advances in genomic technologies have revealed the genetic causes of CHD. Recent studies have shown that genes involved in developmental pathways and organogenesis, as well as transcription factors are widely contributed to CHD.[24] In addition, it has been reported that immune cytokines can play an influential role in a successful pregnancy.[8] The *IL27* gene, encoding a heterodimeric and pro-inflammatory cytokine, is one of the cytokines involved in immune responses and increases the sensitivity during embryogenesis. It has been reported that IL27 acts directly on endothelial cells and inhibits angiogenesis by inducing anti-angiogenic chemokines, interferon-inducible protein 10 (IP-10), and monokine induced by interferon-gamma (MIG).[25] Furthermore, IL27 produced by extravillous trophoblasts may prevent excessive angiogenic activity through a negative feedback mechanism. Therefore, it is assumed that there is a potential link between IL27 and angiogenesis during embryogenesis, and it may be involved in the pathogenesis of CHD.[25,26] Previous reports have shown the association of *IL27* genetic polymorphisms with the risk of CHD.[9,20,21] The genetic polymorphisms may affect the expression level and stability of mRNA, as well as the stability and activity of the encoded protein.[27,28] A polymorphism (rs153109) has been recognized in the promoter region of IL27 (*IL27* -964 A/G) that may affect the mRNA expression level of *IL27*. Previous studies related to CHD have focused more on Chinese and European populations. The *IL27* gene in patients with CHD has not been evaluated specifically in the Iranian population. Considering the different genetic backgrounds and different epidemic trends, it is important to know the occurrence and predict the risk of CHD among the Iranian population and Yazd province. The main aim of this study is to detect the frequency of *IL27* -964 A>G (rs153109) polymorphism in newborns with CHD in Yazd province, Iran. Results of this study revealed that the frequency of the A allele was higher in patients with CHD (60%), suggesting the A allele may increase atrial septal defect and ventricular septal defect susceptibility. However, it has been reported that there is a significant association between the G allele of rs153109 polymorphism with an increased risk of atrial septal defect and ventricular septal defect in the Chinese population.[9,20] In addition, it has been shown that the other polymorphisms of the *IL27* gene have a significant association with CHD. T allele of rs26528 and A allele of rs40837 polymorphisms in the *IL27* gene demonstrate significant differences in patients with CHD in comparison with the healthy group in the Mexican population.[21] On the other hand, previous reports have shown the association of *IL27* genetic polymorphisms with different diseases, such as chronic obstructive pulmonary disease[12-15] and allergic rhinitis.[16-19] There is a significant association

between the AG genotype and the A allele of rs153109 polymorphism with the risk of chronic obstructive pulmonary disease and allergic rhinitis, respectively.[13,17]

This study had limitations due to the small population studied. G and GG allele genotype of rs153109 polymorphism in the *IL27* gene increases the susceptibility to CHD, including atrial septal defect and ventricular septal defect, in the Iranian population. The presence of this polymorphism in the promoter region of the *IL27* gene can make changes in mRNA transcription and mRNA expression levels.

Conclusion

The current study showed that the A allele and AA/AG increase the susceptibility to CHD, including atrial septal defect and ventricular septal defect, in the Yazd population which can be referred to the Iranian population. Therefore, this polymorphism may be one of the potential factors of CHD, and it can be useful as a prognostic biomarker to predict CHD occurrence.

Conflict of Interest

The authors declare no conflicts of interest.

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Ethical Considerations

The present study was approved by Mashhad Academic Center for Education, Culture and Research Ethics Committee (IR.ACECR.JDM.REC.1402.026).

Author's Contribution

Conceptualization, M.M.; methodology, M.M., M.Y; formal analysis, M.Z. and A.J.; investigation, M.Z., A.J.; resources, M.Z.; writing-original draft preparation, M.Z.; writing-review and editing, M.Y.; supervision, M.M. All authors have read and agreed to the published version of the manuscript.

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References

1. Zaidi S, Brueckner M. Genetics and genomics of congenital heart disease. Circ Res 2017; 120(6): 923-40.
2. Zhao L, Chen L, Yang T, Wang T, Zhang S, Chen L, et al. Birth prevalence of congenital heart disease in China, 1980–2019: a systematic review and meta-analysis of 617 studies. Eur J Epidemiol 2020; 35(7): 631-42.
3. Blue GM, Kirk EP, Sholler GF, Harvey RP, Winlaw DS. Congenital heart disease: current knowledge about causes and inheritance. Med J Aust 2012; 197(3): 155-9.
4. Fahed AC, Gelb BD, Seidman J, Seidman CE. Genetics of congenital heart disease: the glass half empty. Circ Res 2013; 112(4): 707-20.
5. Boyd R, McMullen H, Beqaj H, Kalfa D. Environmental exposures and congenital heart disease. Pediatrics 2022; 149(1): e2021052151.
6. Kalisch-Smith JI, Ved N, Sparrow DB. Environmental risk factors for congenital heart disease. Cold Spring Harb Perspect Biol 2020; 12(3): a037234.
7. Shabana NA, Shahid SU, Irfan U. Genetic contribution to congenital heart disease (CHD). Pediatr Cardiol 2020; 41(1): 12-23.
8. Adamczak R, Ukleja-Sokołowska N, Lis K, Dubiel M. Function of follicular cytokines: roles played during maturation, development and implantation of embryo. Medicina (Kaunas) 2021; 57(11): 1251.
9. Zhang D, Ma M, Yang Y, Wan L, Yang Z, Lv J, et al. Association between polymorphisms in IL27 and risk for CHD in a Chinese population. Cardiol Young 2016; 26(2): 237-43.
10. Wang Q, Liu J. Regulation and immune function of IL-27. Regulation and Immune Function of IL-27. Adv Exp Med Biol 2016: 191-211.
11. Jafarizade M, Kahe F, Sharfaei S, Momenzadeh K, Pitliya A, Zahedi Tajrishi F, et al. The role of interleukin-27 in atherosclerosis: A

contemporary review. Cardiology 2021; 146(4): 517-30.
12. Hobbs BD, Parker MM, Chen H, Lao T, Hardin M, Qiao D, et al. Exome array analysis identifies a common variant in IL27 associated with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2016; 194(1): 48-57.
13. Huang N, Liu L, Wang X-Z, Liu D, Yin S-Y, Yang X-D. Association of interleukin (IL)-12 and IL-27 gene polymorphisms with chronic obstructive pulmonary disease in a Chinese population. DNA Cell Biol 2008; 27(9): 527-31.
14. Masjedy A, Salesi M, Ahmadi A, Salimian J, Jamalkandi SA. Association between single-nucleotide polymorphism of cytokines genes and chronic obstructive pulmonary disease: A systematic review and meta-analysis. Cytokine 2023; 171: 156352.
15. Chen Y, Zhang R, Zeng L, Wei H, Chen Y, Zeng J. IL-27 genetic variation and susceptibility of dilated cardiomyopathy in Chinese Han population. Per Med 2017; 14(5): 401-8.
16. Koohyanizadeh F, Falahi S, Mortazavi SHR, Salari F, Rezaiemanesh A, Karaji AG. Correlation between IL-27p28 Genetic Polymorphisms and Risk of Allergic Rhinitis. Gene Expression 2023; 22(4): 281-7.
17. Shen Y, Yuan X-D, Hu D, Ke X, Wang X-Q, Hu G-H, et al. Association between interleukin-27 gene polymorphisms and susceptibility to allergic rhinitis. Hum Immunol 2014; 75(9): 991-5.
18. Wei P, Kou W, Sun R, Hu G-h, Hu D, Feng J, et al. Association study between interleukin-12 receptor β1/β2 genes and allergic rhinitis in the Chinese Han population. Eur Arch Otorhinolaryngol 2015; 272(4): 889-93.
19. Yu JI, Han WC, Lee JH, Kim HS, Yun KJ, Lee JH, et al. Human IL-27p28 gene polymorphisms are associated with the serum total IgE levels of allergic rhinitis patients. Journal of Life Science 2009; 19(3): 299-304.
20. Fan Q, Nie S, Li S, Liao Y, Zhang H, Zha L, et al. Analysis of the genetic association between IL27 variants and coronary artery disease in a Chinese Han population. S Sci Rep 2016; 6(1): 25782.
21. Posadas-Sánchez R, Pérez-Hernández N, Rodríguez-Pérez J, Ángeles-Martínez J, Posadas-Romero C, Cardoso-Saldaña G, et al. PS230 Interleukin-27 Polymorphisms are Associated With Premature Coronary Artery Disease and Insulin Resistance. The Genetics of Atherosclerotic Disease (GEA) Mexican Study. Global Heart 2016; 11(2): e51.
22. Zimmerman MS, Smith AGC, Sable CA, Echko MM, Wilner LB, Olsen HE, et al. Global, regional, and national burden of congenital heart disease, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Child Adolesc Health 2020; 4(3): 185-200.
23. Damayantie V, Rahayuningsih SE, Afriandi I. Congenital heart disease characteristics in low birth weight infants at Dr. Hasan Sadikin General Hospital in 2010-2014. Althea Medical Journal 2019; 6(3): 115-22.
24. Suluba E, Shuwei L, Xia Q, Mwanga A. Congenital heart diseases: genetics, non-inherited risk factors, and signaling pathways. Egypt J Med Hum Genet 2020; 21(1): 1-12.
25. Shimizu M, Shimamura M, Owaki T, Asakawa M, Fujita K, Kudo M, et al. Antiangiogenic and antitumor activities of IL-27. J Immunol 2006; 176(12): 7317-24.
26. Vignali DAA, Kuchroo VK. IL-12 family cytokines: immunological playmakers. Nat Immunol 2012; 13(8):722-8.
27. Asadi S, Abkar M, Zamanzadeh Z, Taghipour Kamalabad S, Sedghi M, Yousefnia S. Association of SOD2 rs2758339, rs5746136 and rs2842980 polymorphisms with increased risk of breast cancer: a haplotype-based case-control study. Genes Genomics 2023: 45(9): 1165-78.
28. Yousefnia S. Association between genetic polymorphism of catalase (CAT) C-262T, Cu/Zn superoxide dismutase (SOD1) A251G and risk of age-related macular degeneration. Molecular and Biochemical Diagnosis Journal 2014; 1(2): 77-88.