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INVESTIGATION OF THE POLYMORPHISM PREVALENCE RS1800925, RS20541 GENE IL13 IN BRONCHIAL ASTHMA IN UZBEKISTAN

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XULOSA

Allergik kasalliklarning rivojlanishi ko'plab omillarga asoslanadi. Ko'pgina tadqiqotlardan ma'lumki, atopiya poligen, multifaktorial hisoblanadi va ko'plab genlarning atrof-muhit bilan o'zaro ta'sirida namoyon bo'ladi. Ushbu maqolada atopik kasalliklarda IL13 genining rs1800925, rs20541 polimorfizmlari muhokama qilinadi. IL-13 asosiy sitokin bo'lib, yallig'lanish, teri to'siqni disfunktsiyasi va allergik kasalliklarga xos bo'lgan boshqa alomatlarda muhim rol o'ynaydi. Tadqiqot maqsadi viloyatimizda allergik kasalliklarga chalingan bemorlarda IL-13 genining rs20541 va rs1800925 polimorfizmlarini va ularning ushbu kasalliklarning rivojlanishiga qo'shgan hissasini tekshirishdan iborat edi. Tadqiqot uchun material sifatida allergiya tarixi bo'lmagan 495 bemor va 120 ko'ngillining tomirlaridan periferik qon namunalari olindi. Buyurtma qilingan primerlar Sanger-sequins usuli bilan tekshirildi. Primer tuzilishiga qarab ikki bosqichli kuchaytirish amalga oshirildi. PCR usulidan so'ng natijalarni statistik qayta ishlash amalga oshirildi. Kichik guruhlar tahlili asosida bronxial astma bilan og'rigan bemorlarda IL-13 genining rs1800925 polimorfizmi va C/C genotipi tashuvchilarning eng katta ulushi o'rtasida muhim bog'liqlik aniqlandi. Va atopik dermatit tashhisi qo'yilgan bemorlarda IL-13 genining 1112C/T rs1800925 va R130Q rs20541 polimorfizmlarining C/C va G/G genotiplari mos ravishda nazorat guruhiga qaraganda yuqori.

Kalit so'zlar: *allergik kasalliklar, bronxial astma, IL13 geni, rs1800925, rs20541, multifaktorial.*

РЕЗЮМЕ

Развитие аллергических заболеваний основано на многих факторах. Из многочисленных исследований известно, что атопия считается полигенной, многофакторной и проявляется взаимодействием многих генов с окружающей средой. В этой статье рассматриваются полиморфизмы rs1800925, rs20541 гена IL13 при атопических заболеваниях. IL-13 является основным цитокином, который играет важную роль в воспалении, дисфункции кожного барьера и других симптомах, характерных для аллергических заболеваний. Целью исследования было проверить полиморфизмы rs20541 и rs1800925 гена IL13 у пациентов с аллергическими заболеваниями в нашем регионе и их вклад в развитие этих заболеваний. В качестве материала для исследования были взяты образцы периферической крови из вены 495 пациентов и 120 добровольцев, не имевших в анамнезе аллергии. Упорядоченные праймеры были проверены методом Сэнгера-секвинса. Двухступенчатая амплификация проводилась в зависимости от структуры праймера. После метода ПЦР была проведена статистическая обработка результатов. На основании подгруппового анализа выявлена значимая ассоциация между полиморфизмом rs1800925 гена IL-13 среди пациентов с бронхиальной астмой и наибольшей долей носителей генотипа C/C. А у пациентов с диагнозом атопический дерматит генотипы C/C и G/G полиморфизмов 1112C/T rs1800925 и R130Q rs20541 гена IL-13 были выше, чем в контрольной группе, соответственно.

Ключевые слова: *аллергические заболевания, бронхиальная астма, ген IL13, rs1800925, rs20541, многофакторный.*

The global prevalence of allergic diseases (ADs) has increased significantly in recent years [3,6,19]. According to numerous studies, the atopic march usually begins in children with the development of atopic dermatitis (AD), then atopic asthma (AA), and finally progresses to atopic rhinitis (AR). However, their age distribution varies [3,6,7,10,11,19]. But this is not a definitive theory; however, the atopic march model is currently still under debate [3,6]. The mechanism for the development of atopic march lies primarily in genetic and environmental factors, which leads to type 2 immune reactions, and sometimes to an increase in IgE levels [2,3,6,10,11]. The disease often begins in childhood, but can also occur later in life [3,6]. A characteristic feature of the immunopathogenesis of ADs is dysregulation of type 2 T helper cells (Th2) and type 2 innate lymphoid cells. This leads to a significant increase in type 2 immune cytokines. Cytokines play a crucial role in the regulation of IgE syn-

thesis, the level of which is usually highly elevated in most patients [2,4,6,9,17,18].

Research has shown that interleukin-13 (IL-13) is a major cytokine that plays an important role in the inflammation, skin barrier dysfunction, skin thickening, infections, itching and allergic reactions that characterize atopic dermatitis [4,5,9]. IL-13 messenger RNA (mRNA) expression is increased in both lesional and unlesioned skin compared with healthy control tissues, and IL-13 mRNA levels correlate with disease severity. Circulating IL-13-producing T cells increase disease intensity in AD patients and correlate with disease activity [4,5,9,16,18]. The gene encoding human IL-13 is located on chromosome 5q31-33 in the cluster of genes encoding IL-4, IL-3, IL-5, IL-9 and granulocyte-macrophage colony-stimulating factor (GM-CSF) and the gene encoding IL -13, consists of 4 exons and 3 introns. The genes encoding IL-4 and IL-13 are located very close. IL-13 has 25% homology with IL-4, so it shares some functional properties. [1,5,13,14,15,16,17]. IL-13 is expressed by activated Th2 cells, mast cells, basophils, eosinophils and NK cells and the receptors are IL-13Ra1 and IL-13Ra2. Signal transduction occurs through the IL-4R type II complex, which consists of IL-4Ra and IL-13Ra1 [1,5,18]. Both interleukins of the tail subunits of intracellular receptors interact with tyrosine kinases of the Janus family (JAK 1-3, TYK2). IL-4R binds to JAK1, whereas IL-13R1 interacts with either JAK2 or TYK2. Following activation of JAK1, tyrosine residues of IL-4R in the cytoplasmic domain are phosphorylated, allowing binding to the transcription factor STAT6. Once phosphorylated, the two STAT6 molecules dimerize and translocate to the nucleus, where the complex influences the transcription of many IL-13 dependent genes [7,13,17]. The concentration of IL-13 in peripheral blood increases in bronchial asthma both in a stable state and during exacerbations. Increased IL-13 expression is taken into account when comparing asthma and non-asthmatic eosinophilic bronchitis [1,5,13,16].

THE AIM OF OUR WORK was to determine the occurrence of two polymorphisms rs1800925, rs20541 of the IL13 gene and their significance in ADs in our republic.

MATERIALS AND METHODS

The object of our research was the venous blood of 495 patients diagnosed with ADs aged from 2 to 72 years (average age 30 years), sent to the allergy center of the Republic of Uzbekistan, the diagnosis was established by an allergist during examination and as a control 120 conditionally healthy people (no manifestations of allergic diseases at the time of examination and in the anamnesis). The general sample of patients was divided into three subgroups: 1. diagnosed with allergic rhinitis (AR) (239); 2. Allergic asthma (AA) (148); 3. Atopic dermatitis (AD) (108).

Whole blood DNA extraction was performed using QIAamp DNA Blood Kits 250 (QIAGEN Inc., Valencia, CA, USA).

Total reaction volume 16 µl: primer mixture 1 µl (Primer design (rs1800925: f-c ttctggaggacttctaggaaaac, f-t ttctggaggacttctaggaaaat, f-com ggagatggggtctcactatg; rs20541: f-g actttttcgcgagggacg, f-a actttttcgcga gggaca, r-com tgaggtcggctaggctga) and their verification using Primer-BLAST programs (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) and Oligo Analyzer 3.1 (http://eu.idtdna.com/calc/analyzer). The melting temperature was determined in silico using the interactive program "Calculate DNA oligo melting temperature" (https://zendto.bioneer.co.kr/e-calculateTm.html). The primer was ordered from Integrated DNA Technologies, primer concentration 2.0 OE/ml), buffer 10 µl (QuantiNova™ SYBR Green PCR Kit, Qiagen, Venlo, Netherlands), and test DNA 5 µl (1-5ng per µl).

Allele-specific polymerase chain reaction (AS-PCR) was performed on a Bio-Rad CFX96 real-time PCR system (Bio Rad Laboratories, Hercules, California, USA). Amplification was carried out according to the structure of the primer according to the following protocol: initial denaturation (2 min at 95°C) and two-stage amplification: 1st stage: denaturation (15 seconds at 95°C), annealing (from 40 seconds to 1 minute at 63 to 66°C), elongation (40 seconds at 72°C) from 7 to 10 cycles and 2nd stage: denaturation (20 seconds at 95°C), annealing (40 seconds to 1 minute at 59 to 60°C), elongation (40 s at 72°C) 40-43 cycles. Analysis of AS-PCR results was carried out using the SYBR detector program. The results obtained were documented in the form of growth curves of the SYBR detectors in graphical mode using the appropriate program.

Sequencing of the PCR fragment was carried out with the following consequence: amplicons were purified using Exo SAP (GMLAG, Switzerland). Sequencing reactions were performed using Big Dye Terminators 3.1 (TFS, USA). Sequencing reaction products were purified using Big Dye Xterminator (TFS, USA). Sequencing reaction products were separated on a 3500 Genetic Analyzer (TFS).

Data analysis was carried out using standard statistical methods. Statistical processing of the results was carried out using MS Office Excel 2016 (Microsoft). The correspondence of the distribution of observed frequencies of genotypes of the studied genes to the theoretically expected Hardy-Weinberg equilibrium was checked using the χ^2 criterion (Rebrova O.Yu., 2002). The calculation was carried out using a calculator for calculating statistics in case-control studies on the Gene Expert website (Russia, https://calc.pcr24.ru/index.php). Analysis of interactions between polymorphisms was carried out using MDR (Multifactor Dimensionality Reduction (mdr-2.0_beta_8.3; http://www.multifactor Dimensionalityreduction.org/)) software. The nucleotide sequences of the analyzed regions of the IL13 gene were deposited in GenBank (NCBI) under accession numbers: AF377331.2, U31120.1, U10307.1, L13029.1, X69079.1, L06801.1, NM_002188.3, NG_012090. 1, AC004039.1, L42079.1.

RESULTS of an analysis of the frequency distribution of allele-genotype variants (rs1800925) of the IL-13 gene polymorphism among patients with ADs, carriers of the C/C genotype amounted to 17.0%, and heterozygous genotype C/T - 67%, at the same time carriers of the T/T IL-13 gene genotype was 16%. Consequently, the results of the study on the R130Q (rs20541) polymorphism of the IL-13 gene in patients were distributed as follows: the A/A allele - 13% and the heterozygous A/G genotype - 41%, and the homozygous G/G genotype occurred in 46%.

When analyzing the genotypic distribution in disease subgroups 1112C/T (rs1800925) of the IL-13 gene in patients with AR, the C/C allele was 13%, the heterozygous C/T genotype was 74%, and the homozygous T/T genotype was 13%. In patients with AA, the C/C allele was 26% and the heterozygous C/T genotype was 60%, and the homozygous T/T genotype was found in 14%. And in AD, the C/C allele was 12% and the heterozygous genotype C/T was 64%, and the homozygous T/T genotype was found in 24%.

According to the R130Q rs20541 polymorphism in patients with AR, the A/A genotype is 11%, A/G heterozygous genotype 41%, G/G homozygous genotype 48%. In patients with astma, the A/A genotype is 14%, A/G heterozygous genotype 41%, G/G homozygous genotype 45%. In patients diagnosed with AD, the A/A genotype is 17%, A/G heterozygous genotype 38%, G/G homozygous genotype 45%.

In the control group, analysis of the frequency distribution of genotypes CC, CT and TT of the 1112C/T polymorphism (rs1800925) of the IL-13 gene was 16%, 78% and 6%, respectively. And for polymorphism R130Q rs20541 A/A genotype 6%, A/G heterozygous genotype 42%, G/G homozygous genotype 52%.

When discussing, the results of studies conducted on two polymorphisms of the IL 13 gene indicate that between the main and control groups there was no significant difference between the main and control groups of rs1800925 (with dominant (OR = 1.08, 95% CI: 0.63 – 1.85, P = 0.79), recessive (OR = 0.36) = 2.75, 95% CI: 1.28 – 5.90, P = 0.07), and additive (OR = 1.08, 0.59, 2.75, 95% CI: 0.63 –5.90, P = 0.14)) and rs20541 (with dominant (OR = 0.81, 1.23, 95% CI: 0.55 – 1.82, P = 0.3), recessive (OR = 0.46, = 2.16, 95% CI: 0.22 – 4.64, P = 0.04), and additive (OR = 0.81, 0.95, 2.16, 95% CI: 0.55 –4.64, P = 0.09)).

When comparing the results of genotypic distribution in disease subgroups (AR, AA, AD) and the control group, the following values were revealed. When comparing AR with control groups for the studied polymorphisms of the IL-13 gene and AA for the R130Q rs20541 polymorphism of the IL-13 gene with control groups, no significant was found. At the same time, significant was found in the rs1800925 polymorphism of the IL-13 gene between patients with AA and the control group (dominant (OR = 0.34, 2.97, 95% CI: 0.20 – 5.06, P = 0.0005), recessive (OR = 0.44 = 2.28, 95% CI: 0.18 – 5.50, P = 0.06), and additive (OR = 0.34, 2.08, 2.28, 95% CI: 0.20 –5.50, P = 0.0006)) as well as those studied for both polymorphisms in patients with AD and the control group, rs1800925 (dominant (OR = 0.72, 1.39, 95% CI: 0.33 – 3.06, P = 0.0041), recessive (OR = 0.22 = 4.64, 95% CI: 0.22 – 4.64, P = 0.0002), and additive (OR = 0.72, 0.51, 4.64, 95% CI: 0.33 –8.97, P = 0.003)), rs20541 (dominant (OR = 0.76, 1.31, 95% CI: 0.45–2.20, P = 0.31), recessive (OR = 0.34, = 2.93, 95% CI: 0.14 – 7.05, P = 0.01), and additive (OR = 0.76, 0.86, 2.93, 95% CI: 0.45 –7.05, P = 0.06)).

Numerous case-control studies have examined the association between IL13 gene polymorphisms and the risk of developing ADs. For example, Gaceja K.V. et al. [8] conducted a meta-analysis and included 11 publications in Asian populations on the 1111C/T polymorphism of the IL-13 gene rs1800925 in patients with AA. According to their findings, the study found a correlation between this polymorphism and AA. However, Qurashi T.A. et al. [15] conducted a study and concluded that there is no direct correlation between the polymorphism of the IL13 gene and the polymorphism of other genes with atopy.

Despite the numerous studies conducted, insufficient statistical power, this may be due to limited sample sizes, and the most important reason is that atopic disease is considered multifactorial and therefore requires continued research on a larger scale, that is, taking into account age, place of residence, collecting genealogical history, pathogenic factors and the interaction of genes of these pathogenic factors.

CONCLUSIONS

Thus, based on our data, we can conclude that among AA, the highest proportion of carriers of the C/C genotype is the rs1800925 polymorphism of the IL-13 gene. In patients diagnosed with AD, genotypes C/C and G/G of polymorphisms 1112C/T rs1800925 and R130Q rs20541 of the IL-13 gene are higher than in the control group, respectively.

REFERENCES

1. Akdis M. Aab, A., Altunbulakli, C. et al. Interleukins (from IL-1 to IL-38), interferons, transforming growth factor β, and TNF-α: Receptors, functions, and roles in diseases //Journal of Allergy and Clinical Immunology. – 2016. – T. 138. – №. 4. – C. 984-1010. https://doi.org/10.1016/j.jaci.2016.06.033.
2. Al-Shobaili H. A. Ahmed, A. A., Alnomair, N., Alobead, Z. A., & Rasheed, Z. Molecular genetic of atopic dermatitis: an update //International journal of health sciences. – 2016. – T. 10. – №. 1. – C. 96. PMID: 27004062 PMCID: PMC4791162
3. Aw M. Penn, J., Gauvreau, G. M., Lima, H., & Sehmi, R. Atopic march: collegium internationale allergologicum update 2020 //International Archives of Allergy and Immunology. – 2020. – T. 181. – №. 1. – C. 1-10. https://doi.org/10.1159/000502958
4. Bieber T. Interleukin-13: targeting an underestimat-

ed cytokine in atopic dermatitis //Allergy. – 2020. – T. 75. – №. 1. – C. 54-62. DOI: 10.1111/all.13954
5. Brightling C. E., Saha S., Hollins F. Interleukin-13: prospects for new treatments //Clinical & Experimental Allergy. – 2010. – T. 40. – №. 1. – C. 42-49. doi: 10.1111/j.1365-2222.2009.03383.x
6. Davidson W. F. Leung, D. Y., Beck, L. A. et al. Report from the National Institute of Allergy and Infectious Diseases workshop on "Atopic dermatitis and the atopic march: Mechanisms and interventions" //Journal of Allergy and Clinical Immunology. – 2019. – T. 143. – №. 3. – C. 894-913. https://doi.org/10.1016/j.jaci.2019.01.003
7. Fujii M. Current understanding of pathophysiological mechanisms of atopic dermatitis: Interactions among skin barrier dysfunction, immune abnormalities and pruritus //Biological and Pharmaceutical Bulletin. – 2020. – T. 43. – №. 1. – C. 12-19. https://doi.org/10.1248/bpb.b19-00088
8. Gaceja K. V. Ancheta, Z. F. R., Buna, A. C. A., et al. Association of interleukin-13 gene single nucleotide polymorphism rs1800925 with allergic asthma in Asian population: A meta-analysis //Asia Pacific Allergy. – 2023. – T. 13. – №. 4. – C. 148-157. DOI: 10.5415/apallergy.0000000000000119
9. Guttman-Yassky E. Blauvelt, A., Eichenfield, L. F. et al. Efficacy and safety of lebrikizumab, a high-affinity interleukin 13 inhibitor, in adults with moderate to severe atopic dermatitis: a phase 2b randomized clinical trial //JAMA dermatology. – 2020. – T. 156. – №. 4. – C. 411-420. doi:10.1001/jamadermatol.2020.0079.
10. Han H., Roan F., Ziegler S. F. The atopic march: current insights into skin barrier dysfunction and epithelial cell-derived cytokines //Immunological reviews. – 2017. – T. 278. – №. 1. – C. 116-130. https://doi.org/10.1111/imr.12546
11. Klonowska J. Gleń, J., Nowicki, R. J., & Trzeciak, M. New cytokines in the pathogenesis of atopic dermatitis—new therapeutic targets //International journal of molecular sciences. – 2018. – T. 19. – №. 10. – C. 3086. https://doi.org/10.3390/ijms19103086
12. Kocaaga A., Kocaaga M. Psoriasis: An Immunogenetic Perspective //Global Medical Genetics. – 2022. – T. 9. – №. 02. – C. 082-089. DOI: 10.1055/s-0042-1743259
13. Marone G. Granata, F., Pucino, V. et al. The intriguing role of interleukin 13 in the pathophysiology of asthma //Frontiers in pharmacology. – 2019. – T. 10. – C. 1387. https://doi.org/10.3389/fphar.2019.01387
14. Omraninava M. Eslami, M. M., Aslani, S., Razi, B.,et al. Interleukin 13 gene polymorphism and susceptibility to asthma: a meta-regression and meta-analysis //Eur Ann Allergy Clin Immunol. – 2020. Doi 10.23822/EurAnnACI.1764-1489.180
15. Qurashi T. A. Shah, A., Bhat, G. A., Khan, M. S. et al. Atopy in Kashmir-validation from a case control study with respect to IgE and Interleukin genes // Allergy, Asthma & Clinical Immunology. – 2021. – T. 17. – C. 1-14. https://doi.org/10.1186/s13223-021-00623-5
16. Rael E. L., Lockey R. F. Interleukin-13 signaling and its role in asthma //World Allergy Organization Journal. – 2011. – T. 4. – №. 3. – C. 54-64. https://doi.org/10.1097/WOX.0b013e31821188e0
17. Seyfizadeh N. Seyfizadeh, N., Gharibi, T., & Babaloo, Z. Interleukin-13 as an important cytokine: A review on its roles in some human diseases //Acta microbiologica et immunologica Hungarica. – 2015. – T. 62. – №. 4. – C. 341-378. https://doi.org/10.1556/030.62.2015.4.2
18. Silverberg J. I., Kantor R. The role of interleukins 4 and/or 13 in the pathophysiology and treatment of atopic dermatitis //Dermatologic clinics. – 2017. – T. 35. – №. 3. – C. 327-334. https://doi.org/10.1016/j.det.2017.02.005
19. Vaseghi-Shanjani M. Snow, A. L., Margolis, D. J. et al. Atopy as immune dysregulation: offender genes and targets //The Journal of Allergy and Clinical Immunology: In Practice. – 2022. – T. 10. – №. 7. – C. 1737-1756. https://doi.org/10.1016/j.jaip.2022.04.001
