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GENETIC POLYMORPHISMS AS A RISK FACTOR FOR TYPE 1 DIABETES MELLITUS AND ORAL DISEASES

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The study analyzed the association between toll-like receptor-2 (TLR2 rs5743708 Arg753Gln), interleukin-10 (IL-10 rs3024491 G-1082A), vitamin D receptor (VDR rs1544410 283A/G(BsmI), osteopontin (OPN rs1126616 C6982T) of single nucleotide polymorphisms and the type 1 diabetes, oral diseases susceptibility in the Ukrainian children. The study included 27 children divided into two groups (17 children with type 1 diabetes and 10 children without concomitant diseases). The state of oral health was assessed according to generally accepted methods. As a result of the studies, it was found that IL-10 gene polymorphism was associated with the risk of the type 1 diabetes in the heterozygous and mutant alleles. In the dominant GG allele, IL-10 significantly reduces the risk of the the type 1 diabetes (OR=5.05, 95% CI, 1.79–221.62, P=0.004). No significant differences were found in the frequency of genotypes of the genes for the toll-like receptor-2 (TLR2 rs5743708 Arg753Gln), vitamin D receptor (VDR rs1544410 283A/G (BsmI), osteopontin (OPN rs1126616 C6982T) and variations in their alleles in groups. The study of the association between gene polymorphism and the main indicators of oral health in children did not demonstrate any statistically significant indices.

Key words: oral health, caries, periodontitis, type 1 diabetes mellitus, children, genetic polymorphism.

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ПОЛІМОРФІЗМ ГЕНІВ ЯК ЧИННИК РИЗИКУ РОЗВИТКУ ЦУКРОВОГО ДІАБЕТУ 1 ТИПУ ТА СТОМАТОЛОГІЧНИХ ХВОРОБ

У дослідженні вивчався асоціативний зв'язок однонуклеотидних поліморфізмів тол-подібного рецептору-2 (TLR2 rs5743708 Arg753Gln), інтерлейкіну-10 (IL-10 rs3024491 G-1082A), рецептору вітаміну D (VDR rs1544410 283A/G(BsmI), остеопонтину (OPN rs1126616 C6982T) з розвитком цукрового діабету 1 типу та стоматологічних хвороб в українській популяції дітей. До дослідження залучено 27 дітей, що були розподілені на дві групи (17 дітей з цукровим діабетом 1 типу та 10 – без супутньої патології). Стан здоров'я ротової порожнини оцінювали згідно загальноприйнятих методик. В результаті проведених досліджень було встановлено, що поліморфізм гену ІЛ-10 асоціювався з ризиком цукрового діабету 1 типу в гетерозигодній та мутантній алелі. В домінантній алелі GG ІЛ-10 вірогідно зменшує ризик захворювання (OR=15.05, 95% ДІ, 1.79–221.62, P=0.004). Достовірних відмінностей частоти генотипів генів тол-подібного рецептору-2 (TLR2 rs5743708 Arg753Gln), рецептору вітаміну D (VDR rs1544410 283A/G(BsmI), остеопонтину (OPN rs1126616 C6982T) та варіацій їх алелей не виявлено в групах дослідження. Вивчення асоціативного зв'язку поліморфізму генів та основних показників стоматологічного здоров'я у дітей не продемонстрував вірогідно значущих показників.

Ключові слова: здоров'я ротової порожнини, карієс, пародонтит, цукровий діабет 1 типу, діти, генетичний поліморфізм.

The study is a fragment of the research project "Stratification of risk factors and pathogenetic aspects of periodontal diseases and their prevention and treatment in adolescents and young adults with type 1 diabetes", state registration No. 0122U201639.

Type 1 diabetes mellitus (T1DM) is a common endocrinological disease caused by autoimmune destruction of pancreatic beta cells and insufficient insulin production. The disease is most common among Europeans and has a tendency to increase in annual incidence. Genetic predisposition plays a crucial role in the development of T1DM, so a significant number of researchers are studying gene polymorphisms and epigenetic mechanisms of changes in the expression of genes that control key metabolic pathways in the human body [6].

Genetic polymorphism is quite common (the threshold frequency is usually 1 % in the population). A single nucleotide polymorphism (SNP) is associated with the replacement of a genetic component – a nucleotide, which is very common in the genome, from one nucleotide base to another [11].

The new genetic techniques aim to identify associations of genotypes with phenotypes by studying the human genome. Based on the detection of DNA sequence changes, the so-called single-nucleotide polymorphism (SNPs), genome-wide association studies (GWAS) involve the study of associations between genomic variants and human phenotype and diseases. GWAS test hundreds of thousands of genetic variants in many genomes to find those that are statistically associated with a particular trait or disease. Based on the identification of genetic risk factors, it is possible to make a reasonable prediction of disease susceptibility (heritability assessment), predict clinical risks, determine biological susceptibility to a particular disease or to drugs, and develop new directions for prevention and treatment strategies [11].

Two main hypotheses have been put forward regarding the genetic basis of complex diseases. The first hypothesis, the "common disease-common variant" hypothesis, suggests that the greatest impact on genetic susceptibility to complex disorders comes from genetic variants that are common in the general population but have a weak effect individually. The second hypothesis is the "common disease-rare variant" hypothesis, according to which the main factor of susceptibility to complex diseases is rare variants (minor allele frequency < 1 %) present in the genome [2].

The results of GWAS studies and whole-genome linkage analysis have made a significant contribution to current knowledge about the influence of genetics on the key pathways in the pathogenesis of T1DM and oral diseases [6].

At the same time, the results of other studies indicate the relationship of gene polymorphisms in the development of vascular, neurological and other complications of diabetes. The vast majority of patients with diabetes have a complicated course of oral diseases, which causes premature tooth loss [1, 2].

The pathogenesis of oral diseases is complex and includes genetic, environmental and autoimmune factors. Numerous studies have shown that genetic factors play a significant role in the progression of periodontal disease [2, 3].

Therefore, the study of the relationship between the polymorphism of certain genes and dental diseases and T1DM is important for determining the key pathways in the pathogenesis and complicated course of the disease, as well as for pre-diagnosis.

The purpose of the study was to analyze the association between toll-like receptor-2 (TLR2 rs5743708 Arg753Gln), interleukin-10 (IL-10 rs3024491 G-1082A), vitamin D receptor (VDR rs1544410 283A/G(BsmI), osteopontin (OPN rs1126616 C6982T) polymorphisms and type 1 diabetes mellitus, oral diseases susceptibility in children.

Materials and methods. The clinical trial involved 27 children aged 9–17 years. Approval for the study was obtained based on the decision of the Ethics Committee Shupyk National Healthcare University of Ukraine (protocol N8, 07.11.2022). In this framework 17 children with T1DM were included in the study group, while 10 children were included in the control group. Children were examined and diagnosed with T1DM at the Komisarenko V.P. Institute of Endocrinology and Metabolism of the National Academy of Medical Sciences of Ukraine in the 2023 year.

The dental examination were calculated as caries index DMF in the permanent dentition and DMF mixed dentition. Oral hygiene was assessed using the oral hygiene index-simplified (OHI-S). The periodontal conditions were assessed according to the Basic Periodontal Examination (BPE) and Simplified Basic Periodontal Examination (sBPE) index as a screening tool for periodontal disease in children with recommendations for treatment and management [12].

The molecular genetic study was conducted at the SE "The Institute of stomatology and maxilla-facial surgery National academy of medical sciences of Ukraine". The material for genotyping was DNA isolated from buccal epithelial cells of the oral cavity. The scraping of epithelial tissues together with the applicator was placed in an Eppendorf with 200 µl of a 5 % solution of Chelex 100 in sterile distilled water (Chelex in sodium form, 100–200 mesh, Bio-Rad). DNA was extracted using a modified method with Chelex [13].

The concentration and purity of the DNA preparation were determined using a spectrophotometer (NanoGenius), for which up to 5 µL of liquid was taken.

Genotyping for the toll-like receptor-2 (TLR2 rs5743708 Arg753Gln) and interleukin-10 (IL 10 rs3024491 G-1082A) genes was performed by allele-specific polymerase chain reaction (PCR).

Metabion primers (Germany) were used for amplification of the studied allelic gene variants. The osteopontin gene polymorphism (OPN rs1126616 C6982T) was detected by PCR-PCR with appropriate primers (Metabion, Germany). The vitamin D receptor gene polymorphism (VDR rs1544410 283A/G(BsmI)) was determined by real-time PCR using a DTlite amplifier (DNA Technology LLC).

The software environment for statistical computing and graphics R-3.6.3 for Windows (GNU General Public Licence) was used for the statistical evaluation of the research data.

Results of the study and their discussion. A comparative analysis of glycated haemoglobin level and the main dental parameters in the study groups was carried out. Predictably, significant differences in glycated haemoglobin were recorded in the study groups ($p<0.001$). In the study group, the median DMF index was almost twice as high as in the group of healthy children, however, there was no significant difference ($p=0.781$) (Table 1).

This may be due to a small sample and a significant variation in the results in the study group. Inflammatory processes in periodontal tissues and significantly higher rates of BPE and sBPE ($p=0.015$) were recorded in the study group. The results obtained indicate the need for regular professional hygiene and periodontal treatment in children with T1DM.

Table 1

Comparative analysis of glycated haemoglobin level and caries intensity in the study groups

	Study group Me (IQR)	Control group Me (IQR)	P-value
HbA1c	9.20 (7.70–1.00)	3.65 (3.52–4.10)	<0.001
DMF index	8.00 (1.00–11.00)	4.50 (4.00–5.00)	0.781

A comparative analysis of gene polymorphism in the study group of children with type 1 diabetes and the control group was performed. The results of the Fisher's exact test and the estimation of the odds of developing the disease are presented in Table 2.

Table 2

Comparative analysis of genetic polymorphism in patients with T1DM and healthy controls and odds ratio for each allele

	Genotype	Study group N (%)	Control group N (%)	P-value	Estimating the odds for each pair of alleles
VDR rs1544410 283A/G (BsmI)	GG	4(23.5)	4(40)	p-value =1	OR=2.1, 95 % CI, 0.29–6.02, P=0.415
	GA	11(64.7)	6(60)		OR=0.82, 95 % CI, 0.13–5.67, P=1)
	AA	2(11.8)	0(0)		OR=0.95 % CI, 0.00–9.12, P=0.516
IL10 Rs 3024491 G-1082A	GG	2(11.8)	7(70)	p-value =0.008	OR=15.05, 95 % CI, 1.79–221.62, P=0.004
	GA	10(58.8)	3(30)		OR=0.31, 95 % CI, 0.04–2.01, P=0.237
	AA	5(29.4)	0(0)		OR=0.95 % CI, 0.00–1.70, P=0.124
OPN Rs 1126616 C6982T	SS	13(76.5)	9(90)	p-value =1	OR=2.68, 95 % CI, 0.21–151.15, P=0.621
	CT	3(17.6)	1(10)		OR=0.53, 95 % CI, 0.01–7.87, P=1
	TT	1(5.9)	0		OR=0,95 % CI, 0.00–66.23, P=1
TLR2 Rs 5743708 G>A Arg753Gln	GG	15(88.2)	9(90)	p-value =0.578	OR=1.19, 95 % CI, 0.06–78.52, P=1
	GA	2(11.8)	1(10)		

A high level of mutation of the vitamin D receptor gene was detected, however, there was no significant difference between the two study groups. Therefore, the VDR rs1544410 283A/G (BsmI) polymorphism has no effect on the risk of developing and progressing T1DM.

The association of gene polymorphisms with oral diseases was determined. The Mann-Whitney U-test was used to assess differences between independent samples. No statistically significant associations of gene polymorphism with caries intensity were found, which may be due to the high prevalence of caries in the Ukrainian population and the small sample size of the study groups.

Qualitative indicators of the intensity of inflammatory processes and basic periodontal examination were evaluated using Fisher's exact test for two independent groups. According to the results of statistical analysis, no significant associations of gene polymorphism with inflammatory processes in periodontal tissues were found. The results demonstrated the absence of associations between gene polymorphism and oral health by caries intensity and BPE (sBPE).

Cytokine gene polymorphism often affects the cytokine expression profile and may partially regulate susceptibility to infection by affecting the colonization of pathogenic bacteria or the activity of the immune-inflammatory response to microbial invasion [8].

Over the past two decades, the influence of VDR gene polymorphism on the development of various autoimmune disorders has been studied [7].

According to the results of numerous clinical trials, systematic reviews and meta-analyses, there are conflicting data on the association between VDR gene SNPs and T1DM, which may be due to small sample sizes, clinical heterogeneity and low statistical power [14].

The results are consistent with a systematic review and meta-analysis that included 1116 studies and analyzed VDR gene polymorphisms including FokI (rs2228570), TaqI (rs731236), BsmI (rs1544410) and ApaI (rs7975232) and their associations with T1DM susceptibility. This meta-analysis suggested a significant association between VDR gene polymorphism and T1DM susceptibility in ethnic-specific analysis. To examine the association between BsmI polymorphism and T1DM risk, 34 case-control studies

with 4826 cases and 7159 control subjects were included. According to meta-analysis BsmI SNP was not the risk factor for T1D susceptibility in the general population [14].

Osteopontin (OPN) is a pleiotropic bone protein synthesized by bone cells and plays an important role in skeletal bone remodeling. OPN is found in immune cells (B- and T-lymphocytes, natural killer cells, natural killer cells, macrophages, neutrophils, and dendritic cells). Many researchers have linked OPN gene expression to the pathogenesis of many autoimmune diseases, including T1DM. Some studies indicate that OPN promotes the destruction of pancreatic β-cells, induces inflammation of adipose tissue, upregulates pro-inflammatory cytokines and stimulates B-lymphocytes to produce antibodies [4].

Clinical studies have shown increased levels of OPN in children with T1DM compared to the control group (p=0.023), but no significant association between the OPN rs1126772 polymorphism (p=0.79) and T1DM was found [4].

The results of this study revealed no significant difference in the polymorphism of the genes encoding osteopontin.

Innate immunity is the first defense system against invading pathogens through a complex system of toll-like receptors (TLRs). Microbial structures (bacterial lipopolysaccharides, peptidoglycans, lipoproteins, bacterial DNA, etc.) interact with TLRs, which activate innate immune cells (neutrophils, macrophages/monocytes) through intracellular signaling pathways [8].

Data on the association between TLR2 polymorphism and T1DM do not conclusively confirm a correlation due to sample sizes, the interaction of other genes, or specific environmental factors [10, 15].

The study did not demonstrate a significant association (p=0.578) between the TLR2 polymorphism and T1DM.

IL-10 is an anti-inflammatory cytokine with broad biological effects. The cytokine is secreted by CD4+ cells (Th1, Th2), macrophages, thymocytes, B-lymphocytes, mast cells, and keratinocytes [9].

The results of studies of potential relationships of IL-10 gene expression revealed a significant negative correlation with age, duration of T1DM, and diagnosis. IL-10 gene expression was significantly increased in patients with ketoacidosis, and a positive correlation was found between IL-10 levels and glycated haemoglobin (HbA1c). Increased expression of IL-10 in T1DM may be due to metabolic stress as a result of ketoacidosis or hyperglycaemia [9].

In the study group of patients with T1DM, the vast majority of children (58.8 %) were registered with the heterozygous type of the gene encoding interleukin 10 and 29.4 % of children with the mutant IL-10 AA gene. A significant difference (p=0.008) between the study groups was recorded. An analysis of the odds ratio of developing diabetes mellitus for each pair of alleles was performed. The GG allele has a protective effect against T1DM and significantly reduces the risk of developing the disease (OR=15.05, 95 % CI, 1.79–221.62, P=0.004).

At the same time, the IL-10 AA gene mutation may have a negative risk for diabetes mellitus, but due to the small sample size of this study, no reliable results were obtained. The frequency of the IL-10 gene allele A in the study group was found in 88.23 % of patients with T1DM, while in the control group it was 30 % of cases ($p<0.05$). The results obtained indicate that the IL-10 allele A contributes to the high risk of T1DM and may be a T1DM's genetic predisposition in children.

Genetic factors can influence both the etiological factors of oral diseases, such as the colonization of microflora, and various links in the pathogenesis [5].

Further study of the relationship and influence of molecular genetic markers of genes on the pathogenesis of oral diseases is an important area of research. The search for reliable genetic markers for the development and progression of caries and periodontal diseases will contribute to the understanding of the pathogenetic mechanisms of oral pathology, preclinical diagnosis, and will also allow predicting, monitoring and modifying the severity of oral disease in children with diabetes.

Conclusions

1. The genetic polymorphisms could possibly contribute to the T1DM's genetic predisposition in children: IL-10 gene polymorphism, heterozygous GA and AA mutant alleles modulate the risk of the disease. The GG allele of IL-10 has a protective effect against the development of T1DM and significantly reduces the risk of the disease OR=15.05, 95 % CI, 1.79–221.62, P=0.004.

2. There were no significant differences in the frequency of genotypes of the toll-like receptor-2 (TLR2 rs5743708 Arg753Gln), vitamin D receptor (VDR rs1544410 283A/G(BsmI), osteopontin (OPN rs1126616 C6982T) genes or variations in their alleles in the study groups.

3. The TLR2 rs5743708, IL-10 rs3024491, VDR rs1544410 283A/G(BsmI), OPN rs1126616 gene polymorphism were not significantly associated with dental caries and periodontal diseases susceptibility in children.

4. The high prevalence of caries and periodontal diseases in the Ukrainian population complicates the analysis of the association between gene polymorphism and oral morbidity and requires a significant increase in the number of subjects in the samples and a more careful selection of inclusion/exclusion criteria for the formation of study groups.

References

1. Ahmad R, Haque M. Oral Health Messengers: Diabetes Mellitus Relevance. Diabetes Metab Syndr Obes. 2021; 1:14:3001–3015. doi: 10.2147/DMSO.S318972.
2. de Coo A, Quintela I, Blanco J, Diz P, Carracedo Á. Assessment of genotyping tools applied in genetic susceptibility studies of periodontal disease: A systematic review. Arch Oral Biol. 2018; 92:38–50. doi: 10.1016/j.archoralbio.2018.04.012.
3. Dienha OV, Pyndus VB, Litovkin KV, Shnaider SA, Džupa P, Pochtar VM, et al. DEFB1, MMP9 AND COX2 gene polymorphisms and periodontitis. World of medicine and biology. 2023; 1(83). doi: 10.26724/2079-8334-2023-1-83-53-56.
4. Karamizadeh Z, Kamali Sarvestani E, Saki F, Karamifar H, Amirhakimi GH, Namavar Shooshtarian MH, et al. Investigation of osteopontin levels and genomic variation of osteopontin and its receptors in Type 1 diabetes mellitus. J Endocrinol Invest. 2013; 36(11):1090–3. doi: 10.3275/9098.
5. Kuz IO, Sheshukova OV, Tkachenko IM, Kazakova KS, Bauman SS, Trufanova VP, et al. Periodontal indices in children with insulin-dependent diabetes mellitus during the treatment of gingivitis. World of medicine and biology. 2021 :4 (78): 80–84. doi:10.26724/2079-8334-2021-4-78-80-84.
6. Lee HS, Hwang JS. Genetic aspects of type 1 diabetes. Ann Pediatr Endocrinol Metab. 2019; 24(3):143–148. doi: 10.6065/apem.2019.24.3.143.
7. Makoui MH, Imani D, Motallebnezhad M, Azimi M, Razi B. Vitamin D receptor gene polymorphism and susceptibility to asthma: A meta-analysis based on 17 case-control studies. Ann Allergy Asthma Immunol. 2020; 124(1):57–69. doi: 10.1016/j.anai.2019.10.014.
8. Novoshytskyy V, Mazur I. Vitamin D in periodontology. Oral and General Health. 2021; 2(3): 13–16. https://doi.org/10.22141/ogh.2.3.2021.240723.
9. Santos D, Haber JF, Barbalho SM, Sgarbi JA, de Argollo Haber RS, de Labio RW, et al. The Relationship between Type 1 Diabetes Mellitus, TNF-α, and IL-10 Gene Expression. Biomedicines. 2023; 7:11(4):1120. doi: 10.3390/biomedicines11041120.
10. Sun C, Zhi D, Shen S, Luo F, Sanjeevi CB. SNPs in the exons of Toll-like receptors are associated with susceptibility to type 1 diabetes in Chinese population. Hum Immunol. 2014; 75(11):1084–8. doi: 10.1016/j.humimm.2014.09.008.
11. Uffelmann E, Huang QQ, Munung NS, de Vries J, Okada Y, Martin A. et al. Genome-wide association studies. Nat Rev Methods Primers. 2021; 1:56–59. https://doi.org/10.1038/s43586-021-00056-9.
12. Updated periodontal guidelines for under-18s. Br Dent J. 2021; 231(8):434. doi: 10.1038/s41415-021-3588-5. PMID: 34686801.
13. Walsh PS, Metzger DA, Higuchi R. Chelex 100 as a Medium for Simple Extraction of DNA for PCR-Based Typing from Forensic Material. BioTechniques. 2013; 54(3):134–139. https://doi.org/10.2144/000114018.
14. Zhai N, Bidares R, Makoui MH, Aslani S, Mohammadi P, Razi B, et al. Vitamin D receptor gene polymorphisms and the risk of type 1 diabetes: a meta-regression and updated meta-analysis. BMC Endocr Disord. 2020; 8;20(1):121. doi: 10.1186/s12902-020-00575-8.
15. Zhang Y, Liu J, Wang C, Liu J, Lu W. Toll-Like Receptors Gene Polymorphisms in Autoimmune Disease. Front Immunol. 2021; 26;12:672346. doi: 10.3389/fimmu.2021.672346.

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