RESULTS OF GENETIC RESEARCH IN PATIENTS HAVINGTEMPOROMANDIBULAR JOINT DISEASES

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Annotation. *A hereditary defect of connective tissue formation is one of the factors causing development of the temporomandibular joint pathology. This defect may be identified based on genetic markers. Genetic markers, characterizing a predisposition and a type of course of temporomandibular joint diseases (TMJ) include: changes in collagen gene of type II (COL2A1); a mutation in matrix metalloproteinase gene – 1, mutation in genes coordinating the state of estrogen receptors ER - α and ER - b in osteoblasts and their physiological activity; cytokine expression, first of all of IL-1 and TNF–α, which play a major role in the development of morphological changes; GSTM gene mutation.*

Changes in genes characterizing the development or predisposition to the development of TMJ pathologies were defined based on scrapes of the buccal epithelium of oral mucosa of patients. 10 patients: 5 women and 5 men at the ages from 25 to 50 took part in the research.

The analysis was made in the DNA testing laboratory "Germedtekh", Odessa. The prognosis of the pathologic behavior was made and further treatment measures were taken following the genetic research based on the definite set of genetic markers and their digital values.

Key words: *temporomandibular joint diseases, patients, results of genetic research.*

Diagnosis of joint diseases and articular syndromes is an urgent task for modern therapy. Arthritis, arthrosis, pain dysfunction are the most common diseases of the temporomandibular joint (TMJ). A prerequisite for the occurrence of pathological changes in the joint is a primary focus (foci) of inflammation of an infectious or traumatic nature.

Arthritis is a heterogeneous group of diseases with inflammatory and degenerative changes in the entire tissue complex of the joint: cartilaginous tissue, subchondral bone, synovial membrane, ligaments, capsules, periarticular tendons and muscles, but the most serious changes occur in the cartilaginous tissue. The cartilaginous tissue, which undergoes significant mechanical loads, is constantly forced to self-regeneration, which is provided by the chondrocyte system. Their function is to regenerate the connective tissue matrix, the main components of which are collagen and proteoglycans. In the case of arthritis, the regeneration of chondrocytes is affected and, as a result, the destructive processes in the matrix predominate over regenerative processes.[8,9]

Arthrosis develops in cases where the cartilage and subchondral bone are not able to adequately withstand the mechanical load that is due to the restriction reparative abilities

of these tissues. The hyaline cartilage is the main lodgement of pathological changes development. This is the place where not only the number of chondrocytes is reduced, but the decrease of their metabolic activity occurs. This leads to a decrease in collagen synthesis in the matrix of cartilage and sulphated proteoglycans – chondroitin sulphate, keratan sulphate, proteoglycan-hyaluronic aggregates, as well as hyaluronic acid.

The most important constituent of these changes is a deficit in the synthesis of proteoglycans, which are the main structural component of the cartilage matrix. In the case of arthrosis not only quantitative synthesis of proteoglycans is decreased, but their qualitative composition is also changed, namely production of complete proteoglycans with high molecular weight. [8, 9].

Temporomandibular pain-dysfunction syndrome is a temporomandibular joint pathology, characterized by the disorder of neuromuscular mechanism that regulates all articular movements. Occlusal (joining of teeth) disharmonies, which may occur due to lack of teeth, abnormal bite, improper denture treatment and other factors. In the case of occlusiven pathologies, changes in the nature chewing are observed. And this leads to a constant overloading of chewing muscles on the one side and their unsynchronous activity. All this causes imbalance in the joint. Psychogenic factors (stresses), as a result of which a state with a strong compression of teeth and, accordingly, muscle spasm develops. Scientists have proved that more than a half of patients suffering from the temporomandibular pain-dysfunction syndrome have a psychogenic or neurogenic disorders. Grinding of teeth (bruxism), as a result of which an increased activity of chewing muscles is observed and clenching of teeth occurs is a common cause of this disease development.

A panoramic X-ray of the TMJ is to be made for every patient complaining about the pain in the joint. This pathology is not characterized by radiological changes in the bone tissue of articular heads of the lower jaw, however their asymmetric position and different width of the joint space to the left and right is often found [8, 9].

Rheumatoid arthritis is an autoimmune disease of unknown etiology. It is characterized by a symmetrical cartilage and bone tissue damage. This systemic disease of connective tissue with a predominant damage of small joints in terms of erosive and destructive polyarthritis of unknown etiology with a complex autoimmune pathogenesis. The disease is frequently accompanied by the development of a wide range of systemic symptoms. In most cases the rheumatoid arthritis has chronic course and in case of the lack of timely treatment it leads to the deformation and malfunctioning of joints, and deterioration of the quality of life. The disease may occur in any age and it is mostly spread among women. More often the disease affects hands, fingers, knees, feet, and elbows.

The exact cause of the disease is unknown. It is known that in the case of autoimmune diseases, to which the rheumatoid arthritis is also referred, the immune system perceives healthy tissues as alien agents and combats its own body.

In addition, the role of a wide range of infectious and non-communicable factors that may indirectly participate in the development of the rheumatoid arthritis in connection

with the genetic predisposition is being studied. These factors include: Epstein-Barr virus, parvovirus B19, retroviruses, antigens and stress-related bacteria proteins, smoking, coal dust, drugs, some components of mineral oils, and various chemical compounds [9].

A genetic research was conducted to refine the clinical diagnosis. Changes in genes characterizing the development or predisposition to the development of TMJ pathologies were defined based on scrapings of buccal epithelium from the oral mucousa of patients.

The objective of this research is: to confirm the clinical diagnoses of patients based on the results of a genetic study and their numerical expression for the further development of a Preventive and Curative Intervention Pla

Research Materials and Methods. 10 patients: 5 women and 5 men at the ages from 25 to 50 took part in the research. Buccal epithelium of the oral mucosa was scraped in every patient. The epithelium was collected into an Eppendorf tube containing sterile saline solution. All received biomaterials were transported to the laboratory in specialthermocontainers at the temperature of 4 °C.

Isolation and purification of DNA from buccal cells was made according to the method of Dellaporta (Dellaporta S.L., Wood J., Hicks J.B. A Plant DNA Mini Preparation: Version II // Plant Mol. Biol. Rep. 1983. V. 1. P. 19-21). The collectedmaterialwas carefullystirred, 100 µl were put into a sterilemicrotube, 1000µl of Dellaporta lysingsolution were added. Then the material was vortexed (vortexmicrospinFV – 2400) and incubated at 65°C for 40 min. Afterincubation285 µl of 5M potassiumacetate were added and the mixture was vortexed (vortexmicrospin FV – 2400). Incubation for 10 mininice.It was centrifuged for 5 minutes at υ = 13,000 rpm (at centrifuge: eppendorf Centrifuge 5424). Then the whole of supernatant was transferred into a newmicrotube and an equalquantity ofisopropanol was added, the mixture was carefullyvortexed (vortexmicrospin FV – 2400). Than it was incubated for 30 minutes in a deep-freeze chamber (-20°C) for DNA precipitation. It was centrifuged for 15 minutes at υ = 13,000 rpm to precipitate DNA (at centrifuge: eppendorf Centrifuge 5424). The supernatant was removed.500 µl of 70% ethyl alcohol was added to the DNA sediment. It was vortexed (vortexmicrospin FV-2400). It was centrifuged for 5 minutes at υ = 13,000 rpm (at centrifuge: eppendorf Centrifuge 5424). The supernatant was removed. 300 µl of acetone were added. It was vortexed (vortexmicrospin FV-2400). It was centrifuged for 1 minutes at υ = 13,000 rpm (at centrifuge: eppendorf Centrifuge 5424). The acetone was removed, ascompletely as possibleand the tube was left open. The sediment was dried a little in DryBlock for 1-1.5 min at t = 50°C. The DNA sediment was dissolved in 100 µl of deionized H2O. It was vortexed (vortexmicrospin FV-2400). The content of DNA was definedat a spectrophotometer (Nanophotometr, Implen), bytaking5 µlofaliquot directlyfrom the tube with DNA solution.

The allelic variants of genes Col2A16846C>A, MMP1-1607insG, IL1B C3954T rs1143634, TNF G (-308)A Rs1800629 were assessed by the allele method with a specific polymerase chain reaction (PCR). Investigated sections of genes were amplified in parallel in two eppendorfs for the normal and mutant variant of the gene in 20 µl of a buffer solution (Fermentas firm) and 100 nm of each oligonucleotide primer, 100-150 ng of DNA.

The allelic variants of ER-alpha gene rs2234693, rs9340799 revealed polymerase chain reaction-RFLP by processing the amplifications with PvuII, XbaI restriction enzymes.

Table 1

Sequence of primers and conditions for PCR analysis

Name	Gene	Polymorphism	Sequence oligonucleotides	T °C annealing	Fragments (p.o.)
Glutathione-S-transferase	GSTM1	deletion	f-TGCTTCACGTGT TATGGAGGTTC r-GTTGGGCTCAA ATATACGGTGG	60	219, deletion
Collagen of bone tissue	Col2A1	6846C>A	f-GTTGTCTAGGTG CTGGAGGTT r-GGCGAGGGAGGA GAGAAGG Ar-CCCGCCCACATT CCCTGG Cr-CCCGCCCCCATT CCCTGG	63	350-общ., 154 N/M
Matrix metalloproteinase 1	MMP1	-1607insG	Test-system "SNP-express" Liteh		
Estradiol alpha receptor	ER	Pvu II – A/G	F-ATCCAGGGTTATGTGGCAATGAC R-ACCCTGGCGTCGATTATCTGA	60	PP-527,pp-427, 100, Pp 527,427, 100 п.о
Estradiol alpha receptor	ER	XbaI rs9340799	F-ATCCAGGGTTATGTGGCAATGAC R-ACCCTGGCGTCGATTATCTGA	63	XX-527 xx-382,145 Xx-27,382,145
Tumor necrosis factor alpha	TNF	(-308)A Rs1800629	G-ATAGGTTTTGAGG GGCATGG A-AATAGGTTTTGA GGGGCATGA R-TCTCGGTTTCTT CTCCATCG	55	184
Interleukin 1B	IL1B	C3954Trs1143634	Cf-GCT TTT TTG CTG TGA GTC CCG Tf-CTC AGG TGT CCT CGA AGA AAT CAA R-GAATTAGCAAG CTGCCAGGAG	60	C-230 T-240

A polymorphic version of the glutathione-S-transferase M 1 gene (GSTM1 gene) - the availability or lack of a deletion was determined by PCR method with the appropriate

primers.

The PCR was carried out at BIO-RAD amplifier (USA), the required program for changing the temperature and duration of each reaction step to determine the polymorphism of the studied genes were selected experimentally. The initial denaturation was 95 °C for 10 min. The PCR during 40 cycles: denaturation at 95 °C for 30 seconds, annealing at the temperature of 55 to 65 °C, depending on the locus of specific oligonucleotide primers for 30 seconds and elongation at 72 °C for 30 sec, final elongation for 3 min at 72 °C. The amplified products were fractioned in a horizontal 2% agarosegel, prepared on a singletris-boratebuffer (1xTBE), at a voltage of 100 Vduring 45minutes.Molecular weight markeris DNA pUC19: Msp1.

The agarose gel was stainedwith ethydium bromide and visualized in the transmitted ultraviolet light (Table 1).

Results and their discussion. The genetic risk of osteoarthritis and osteoarthrosis development was defined based on the results of the analysis of GSTM1, Col2A1, MMP1, ER, IL1B, TNF genes, involved in the metabolism of osteochondral tissue of the joint. Alleles of the norm were given 1 score, heterozygotes – 2 scores, mutation – 3 scores. The results are shown in Table 2.

Table 2

Assessment of polymorphism in genes involved in the metabolism of osseous tissue of the joint, genes of inflammation and detoxification markers.

Genes	GSTM1	Col2A1	MMP1	ER	ER	IL1B	TNF	Scores
Polymorphism	+ (0)	6846C>A	1607insG	Pvu II -A/G	Xba1	3954 C/T	308G/A	Mutations
Rheumatoid arthritis	3	3	1	3	3	3	1	15
Temporomandibular pain-dysfunction syndrome	3	2	2	2	3	1	1	6
Arthrosis	1	2	3	2	3	3	1	9
Arthritis	1	3	1	2	2	3	2	6

The following combination of genetic markers indicators is characteristic for rheumatoid arthritis:mutation in GSTM gene, which is responsible for the synthesis of epoxy hydrolase, glutathione transferase, glucuronyltransferase, acetyltransferase, etc., which transform toxic metabolic intermediates of phase I into the polar water-soluble non-toxic compounds – the second phase of detoxification; mutation in COL2A1 gene; mutation in genes coordinating estrogen receptors ERα and ERβ; mutation in IL1B gene, which is responsible for the activity of cytokines. [1,3,4,5]

The following is characteristic for the pain-dysfunction syndrome of the joint: mutation in GSTM1 gene, responsible for the synthesis of enzymes transforming toxic metabolic intermediates of phase I into the polar water-soluble non-toxic compounds

– the second phase of detoxification;mutation in ER gene leading to poor condition of estrogen receptors in osteoblasts, which affects their physiological activity and thus affects the osseous tissue metabolism. [2]

Arthrosis is characterized by a combination of the following indicators:mutation of MMP-1 gene, which is responsible for the synthesis and activity of metalloproteinases, primary degradation of collagen molecules occurring when they are accumulated;mutation in ER Xbal gene andmutation in IL1B gene responsible for cytokine activity. [4,6,7]

A combination of the following indicators is characteristic for arthritis: mutation of COL2A1 gene, which is responsible for the qualitative composition of collagen; mutation in IL1B gene, which is responsible for the activity of cytokines.Proinflammatory cytokines that depress the formation of cartilage matrix, stimulate the synthesis of metalloproteinases and reduce products of tissueinhibitors of matrixproteinases. [3]

The analysis of the total set of mutated genes allows us to distribute patients and to identify the reversibility and irreversibility of pathological changes on the part of the pathological process development, which further allows us to create an individual minutes of preventive and curative interventions in patients havingTMJ pathology. The results of gene mutations are shown on the histogram: Figure 1.

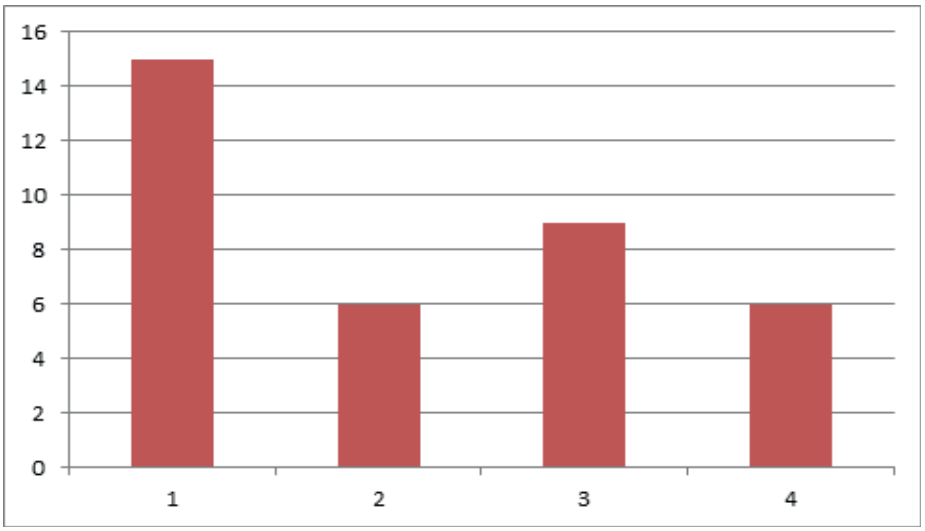

Fig. 1. Mutation of genes related to TMJ diseases

1. Rheumatoid arthritis: 7 genes analyzed, 5 mutations, 15 scores.

2. Temporomandibular pain-dysfunction syndrome: 7 genes analyzed, 2 mutations, 6 scores.

3. Arthrosis: 7 genes analyzed, 3 mutations, 9 scores.

4. Arthritis: 7 genes analyzed, 2 mutations, 6 scores.

According to clinical observations, the availability of mutations scoring from 6 to 9 indicates the reversibility of processes and persistent remission ifpreventive and

curative interventions in the dentoalveolar apparatus are taken. When mutations get min. 9 scores, an advice ofrelated professionals is required to normalize the mineral andmetabolic processes of the body.

The percentage of patients from the examined group having rheumatoid arthritis made 20 %, temporomandibular pain-dysfunction syndrome – 20 %, arthrosis – 30 %, and arthritis – 30 %.

Conclusions. 1. Clinical diagnoses were refined and confirmed based on the genetic research for a specific set of genetic markers and their analysis.

2. A certain set of genetic markers and their numerical value allows us to make a prognosis for the course of the pathological process and take preventive and curative interventions based on them, as well as to develop the individual minutes for treatment of patients having TMJ diseases.

References:

1. Baranov V. S., Baranova E. V., Ivashchenko T. E., Aseev M. V. Genom cheloveka i geny "predraspolozhennosti" (vvedenie v prediktivnuju medicinu). [The human genome and the genes of "predisposition" (an introduction to predictive medicine)]. SPb.: Intermedika; 2000:272.

2. Zhelenina L. A., Ivashchenko T. E., Efimova N. S. Polymorphism of the genes of the family of glutathione-S-transferase (GST) in bronchial asthma in children. Allergology. 2003;2:13-16.

3. Zazerskaja I. E., Aseev M. V., Kuznecova L. V., Moskalenko M. V. Analysis of the association of COL1A1 gene alleles with the development of osteoporosis. Genetics. 2002;12(38):1699-1703.

4. Pantsulaia I., Trofimov S., Kobyliansky E., Livshits G. Pantsulaia I. Genetic and environmental influences on IL-6 and TNF-alpha plasma levels in apparently healthy general population. Cytokine. 2002;l9 (3):138-146.

5. Rovetta G., Grignolo M.C., Buffiini L.[et al. Prevalence of C282Y mutation in patients with rheumatoid arthritis and spondylarthritis. Int. J. Tissue. React. 2002;.24(3):105-109.

6. Alvim-Pereira F., Alvim-Pereira, P. Trevilatto. The Current Knowledge of Genetic Susceptibility Influencing Dental Implant Outcomes. The International journal of oral & maxillofacial implants. 2011:347-367.

7. Grant S. F., Reid D. M., Blake G., [et al.] Reduced bone density and osteoporosis associated with a polymorphic Sp1 binding site in the collagen type I alpha 1 gene. Nat Genet. 1996;14:203–205.

8. Kleinrock M. Functional disorders in the moving parts of the masticatory apparatus. Lviv: “GalDent”; 2015:256.

9. Timofeev A. A. Guidance on maxillofacial surgery and surgical dentistry. Kyiv: LLC “Chervona Ruta-Tours”; 2004:1062.