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Auto Inflammation Candidate Genes in Juvenile Idiopathic Arthritis

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Abstract- Juvenile idiopathic arthritis (JIA) is a heterogeneous pathology with uncertain causative factors and prognosis, stemming from an immune system dysfunction with the development of autoimmune reactions. The most distinctive and potentially most severe of these is systemic JIA (sJIA), a disease characterized by sharp rises in temperature and rash. A thorough understanding of the complex of immune regulatory mechanisms along with genetic analysis reveals complex relationships between autoimmune reactions and auto inflammation. Sequencing of 15 auto inflammatory genes was performed in 62 patients with JIA: 26 – oligoarthritis, 20 – polyarthritis, 16 – systemic. Studies have shown that 16 (25.8%) patients with the clinical JIA phenotype had changes in nucleotide sequence in the genes encoding auto inflammatory immune response proteins. NOD2 changes were in 12 (19.3%) of them and 1 change in each of the 4 patients NLRP12 (heterozygote, c.1343G> C (p.Gly448Ala)), MEFV (pathogenic heterozygous, c.2082G> A (p.Met694Ile)), ADA2 (heterozygote, c.145C> T). Arg49Trp)), PSTPIP1 (heterozygote, c.806T> A (p.Ile269A)) in the group of studied children with JIA. The study will allow identifying individual genetic loci of JIA risk, expand understanding of the pathogenesis and spectrum of phenotypic manifestations of the disease, improve diagnosis and prediction of its course, as well as reveal new opportunities for monitoring patients with JIA and their personalized therapy.

Keywords: *children, arthritis, genes, autoinflammatory.*

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Autoinflammation Candidate Genes in Juvenile Idiopathic Arthritis

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Abstract- Juvenile idiopathic arthritis (JIA) is a heterogeneous pathology with uncertain causative factors and prognosis, stemming from an immune system dysfunction with the development of autoimmune reactions. The most distinctive and potentially most severe of these is systemic JIA (sJIA), a disease characterized by sharp rises in temperature and rash. A thorough understanding of the complex of immune regulatory mechanisms along with genetic analysis reveals complex relationships between autoimmune reactions and auto inflammation. Sequencing of 15 auto inflammatory genes was performed in 62 patients with JIA: 26 – oligoarthritis, 20 – polyarthritis, 16 – systemic. Studies have shown that 16 (25.8%) patients with the clinical JIA phenotype had changes in nucleotide sequence in the genes encoding auto inflammatory immune response proteins. NOD2 changes were in 12 (19.3%) of them and 1 change in each of the 4 patients NLRP12 (heterozygote, c.1343G> C (p.Gly448Ala)), MEFV (pathogenic heterozygous, c.2082G> A (p.Met694Ile)), ADA2 (heterozygote, c.145C> T). Arg49Trp)), PSTPIP1 (heterozygote, c.806T> A (p.Ile269A)) in the group of studied children with JIA. The study will allow identifying individual genetic loci of JIA risk, expand understanding of the pathogenesis and spectrum of phenotypic manifestations of the disease, improve diagnosis and prediction of its course, as well as reveal new opportunities for monitoring patients with JIA and their personalized therapy.

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I. Introduction

In modern rheumatology, juvenile idiopathic arthritis (JIA) is defined as a heterogeneous pathology of uncertain aetiology, which is based on the immune system dysfunction along with the formation of autoimmune reactions [Ringold S. and Angeles-Han S.T., 2019]. The existing criteria for the classification of JIA are based on the phenotypic, genetic and molecular heterogeneity of the disease, which requires further pathogenesis studying [Martini A. and Ravelli A, 2019]. Rheumatological tests are often negative in clinical symptomatology of JIA, and children without clinical symptomatology of arthritis may have false-positive specific antibodies, which contributes to the difficulty in diagnosis. Modern treatment technologies do not always make it possible to achieve the desired effect since the mechanisms of molecular remission and the ability to restore immunological tolerance remain unclear; sustained remission is achieved infrequently, requiring long-term pharmacological therapy without reliable predictive biomarkers for therapy response [Savic S, Wilson AG, et al, 2017]. Currently, there is increasing evidence that the concept of "juvenile idiopathic arthritis" involves a number of clinical and immunological syndromes with different development mechanisms, prognosis and therapeutic approaches [Savic S , Wilson , et al, 2017].

Recent studies indicate an important role of heredity in initiating autoimmune responses in JIA. The increase in the prevalence of familial risk to 40-50% among seropositive arthritis (especially in first degree relatives) is determined by the presence of a set of allelic genes in the HLA-DRB1 locus; the expression of these genes results in production of proteins on the membrane of immune cells containing "shared epitope" [Frisell T.,2016;Guseva V., 2019]. However, HLA genes determine only 17.0% to 56.0% of heredity, so the search for candidate genes is constantly expanding [Hollenbach J.A, 2010].

A genome-wide association studies in the GWAS study has identified 377 genes in 100 risk loci based on data from 29,880 patients with rheumatoid arthritis (RA); 98 of them were associated with a twofold increase in risk of its development, and 15 were identical to immunodeficiency syndromes, involved in the regulation of inflammatory processes, including caspase-8 and -10, autoimmune regulator (AIRE), IL-2 receptor α (CD25), protein tyrosine phosphatase receptor type C (CD45), protein 1, which activates VDJ recombination (RAG1), RAG2, CD40, serine-protein kinase ATM, non-receptor tyrosine-protein kinase TYK2 (TYK2), IFNγ-2 receptor, interferon regulatory factor 8, NFKB, TLR4 and others. [McAllister and Orozco G., 2011].

Mutations in the *TNFRSF1A, NLRP3*, *MEFV*, *NOD2* genes, etc, which determine the inflammatory processes due to abnormal activation of the innate immune system without the synthesis of highly active auto antibodies and antigen-specific T and B cells (synthesis of signaling proteins, interleukins, cytokines, TNF, NOD-like receptors) associated with arthritis, are conventionally allocated to a separate group of systemic auto inflammatory diseases (SAIDs). However, there is

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increasing evidence that innate immunity plays a significant role in the pathogenesis of autoimmune diseases, including JIA [Angelotti F., 2017]. We have also noticed that some patients with juvenile arthritis have symptoms as patients with systemic auto inflammatory disorders have (fever, severe joint swelling, erythematous rash, increased ESR, C-reactive protein). These patients do not respond or do not respond adequately to protocol therapy, have frequent aggravations and an intermediate prognosis. Identification of such patients from a heterogeneous group of children with arthritis for personalized therapy is an urgent task of modern Rheumatology.

Objective: to determine the features of the clinical progression of JIA on the background of changes in the nucleotide sequence of innate immunity genes.

II. Materials and Methods

Studies were conducted in 62 children diagnosed with JIA from 1 to 17 years (27 boys, 35 girls), who were observed in the Department of Connective Tissue Diseases of the Institute. The average duration of the disease was (4.3±3.3) years. The diagnosis of JIA has been established according to the criteria of the International League of Associations for Rheumatology, ILAR [Ringold S., 2020]. Genetic changes have been verified and decoded in the ExAC genetic database(A MedGen ID is a unique identifier referring to an article in MedGen, NCBI's centralized database of information about genetic disorders and phenotypes. Search by MedGen ID at http://www.ncbi.nlm.nih.gov/medgen. An OMIM number is a unique identifier referring to a comprehensive entry in Online Mendelian Inheritance of Man (OMIM). Search by OMIM number at http://omim.org/)

Parents of patients under 12 years and patients over 12 years have received informed consent for the examination and have provided written consent. The study was performed in compliance with the provisions of the GCP (1996), the Convention on Human Rights and Biomedicine (04.04.1997), the World Medical Association Declaration of Helsinki (1964-2002), the Order of the Ministry of Health of Ukraine № 281 dated 01.11.2001.

Patients were stratified by clinical subtypes of JIA: 26 (41.9 %) patients with oligoarthritis, 20 (32.2 %) with polyarthritis, 16 (25.8 %) with systemic arthritis. 19 (30.6%) children were positive for HLA-B27-antigen (JIA enthesitis-related, HLA-B27-positive – JIA-B27), and 4 (6.4%) children were with anterior uveitis (JIA-uveitis). At the onset of the disease, 35 (56.4 %) children had increased acute phase indicators (stage II activity). All children were negative for rheumatoid factor (RF) and did not have antibodies to citrullinated vimentin (A-CCP), 35 (56.4%) children were positive for antinuclear factor (ANF+) (table 1). 4 (6.4%) patients were diagnosed with selective IgA immunodeficiency.

Table 1: General characteristics of patients with JIA

Indicator	Indicator values
Number of patients, abs.number	62
Age, years Me	9 (1-17)
Duration of illness, years	(4,3±3,3)
Gender (boys/girls), abs.number	27/35
JIA subtype, abs.number (%): Oligoarthritis JIA Polyarthritis JIA Systemic JIA	 26 (41,9) 20 (32,2) 16 (25,8)
ANA (+), abs. n. (%)	35 (56,4)
ANA (-), abs. n. (%)	27 (43,5)
HLA-B27 (+), abs.n. (%)	19 (30,6)
HLA-B27 (-), abs. n. (%)	43 (69,3)
ACCP (-),abs. n (%)	62 (100)
RF (-),abs. n (%)	62(100)
Selective IgA deficiency, abs. n (%)	4 (6,4)

ANA-antinuclear antibodies, IgA-immunoglobulin A; HLA-B27-Human leukocyte antigen B27; ACCP- anti-citrullinated protein antibody; RF - rheumatoid factor

For the genetic sequencing, a sample of the condensed epithelium of the oral cavity was taken into a Saliva Collection Kit Oragene TM test tube (DNA Genotek Inc.3000-500 Palladium Drive Ottawa, ON, Canada K2V 1C2). A high-performance panel exomic new generation sequencing (NGS), based on the decoding of fragments of the DNA molecule, has been performed on the Illumina's HiSeq device (manufactured in the USA) in the Invitae laboratory (USA), each change in the nucleotide sequence is sequenced by Sanger. The target enrichment was applied to coding gene sequences of auto inflammatory syndromes: NOD2, NLRC4, NLRP12, NLRP3, PLCG2, MEFV, ADA2, ELANE, LPIN2, MVK, NLRC4, PSMB8, PSTPIP1, TNFRSF1A, TRNT1.

III. Results

The results have shown that 16 (25.8 %) patients with the clinical JIA phenotype had changes in the nucleotide sequence in the genes encoding auto inflammatory immune response proteins, of which 12 (19.3%) children had changes in the NOD2 [c.2104C>T (p.Arg702Trp)] This sequence change replaces arginine with tryptophan at codon 702 of the NOD2 protein (p.Arg702Trp). The arginine residue is moderately conserved and there is a moderate physicochemical difference between arginine and tryptophan. This variant is present in population databases (rs2066844, ExAC 3%), including multiple homozygous individuals. Numerous population-based case-control studies have shown that this variant confers an elevated risk of Crohn's disease [Peter I, Mitchell AA,2011; Hradsky O, 2008; Naderi N., 2011; Lakatos PL., 2005; Tukel T.,

2004] In a large meta-analysis involving 75 case-control studies with 18,727 cases and 17,102 controls [Yazdanyar S., 2009], individuals carrying this variant had an increased overall risk of Crohn's disease (OR = 2.2, 95% CI 2.0-2.5). (Table 2).

Table 2: Auto inflammation candidate genes in juvenile idiopathic arthritis

Gene	Variant	Zygosity	Variantclassification
NOD2	c.2104C>T (p.Arg702Trp)	heterozygous	IncreasedRiskAllele
ADA2	c.145C>T (p.Arg49Trp)	heterozygous	UncertainSignificance
NLRP12	c.1343G>C (p.Gly448Ala)	heterozygous	UncertainSignificance
PSTPIP1	c.806T>A (p.Ile269Asn)	heterozygous	UncertainSignificance
MEFV	c.1341G>C (p.Lys447Asn)	heterozygous	UncertainSignificance

When all three NOD2 genotypes were combined (p.Arg702Trp, p.Gly908Arg, and p.Leu1007Profs*2), the odds ratios for Crohn's disease were 2.4 (95% CI, 2.0-2.8) for simple heterozygotes, 9.0 (95% CI 6.0-13.5) for compound heterozygotes, and 6.7 (95% CI 4.1-10.9) for homozygotes, compared with noncarriers. This variant is also referred to as R675W and SNP8 in the literature. ClinVar contains an entry for this variant (Variation ID: 4693). Experimental studies have shown that this missense change results in decreased NFkB activity and decreased response to lipopolysaccharide, muramyl dipeptide, and peptidoglycan compared to wildtype protein [Bonen DK., 2003; Li J, Moran T., 2004; Stevens C, 2013]. In summary, this is a frequently observed variant that is associated with approximately a 2.2-fold increased risk of Crohn's disease in population studies. Therefore, it has been classified as an Increased Risk Allele. and one mutation in 4 patients – in genes NLRP12 [c.1343G>C (p.Gly448Ala)]. This sequence change replaces glycine with alanine at cod on 448 of the NLRP12 protein (p.Gly448Ala). The glycine residue is highly conserved and there is a small physicochemical difference between glycine and alanine. This variant is present in population databases (rs104895566, ExAC 0.01%). This variant has been reported in an individual affected with chronic NLRP12-autoinflammatory disorder [Vitale A., 2013]. ClinVar contains an entry for this variant (Variation ID: 97886). Algorithms developed to predict the effect of missense changes on protein structure and function do not agree on the potential impact of this missense change (SIFT: "Tolerated"; PolyPhen-2: "Possibly Damaging"; AlignGVGD: "Class C0"). In summary, the available evidence is currently insufficient to determine the role of this variant in disease. Therefore, it has been classified as a Variant of Uncertain Significance, MEFV [c.1341G>C (p.Lys447Asn)]. This sequence change replaces lysine with asparagine at codon 447 of the MEFV protein (p.Lys447Asn). The lysine residue is highly conserved and there is a moderate physicochemical difference between lysine and asparagine. This variant is present in population databases (rs756322372, ExAC 0.003%). This variant has not been reported in the literature in individuals with MEFV-related conditions. ClinVar contains an entry for this variant (Variation ID: 279849). Algorithms developed to predict the effect of missense changes on protein structure and function output the following: SIFT: "Deleterious"; PolyPhen-2: "Benign"; Align-GVGD: "Class C0". The asparagine amino acid residue is found in multiple mammalian species, suggesting that this missense change does not adversely affect protein function. These predictions have not been confirmed by published functional studies and their clinical significance is uncertain. In summary, the available evidence is currently insufficient to determine the role of this variant in disease. Therefore, it has been classified as a Variant of Uncertain Significance, ADA2 [c.145C>T (p.Arg49Trp)] This sequence change replaces arginine with tryptophan at codon 49 of the ADA2 protein (p.Arg49Trp). The arginine residue is weakly conserved and there is a moderate physicochemical difference between arginine and tryptophan. The frequency data for this variant in the population databases is considered unreliable, as metrics indicate poor data quality at this position in the ExAC database. This variant has been observed in an individual affected with Behçet's disease [Burillo-Sanz S., 2017]. ClinVar contains an entry for this variant (Variation ID: 375246). Algorithms developed to predict the effect of missense changes on protein structure and function do not agree on the potential impact of this missense change (SIFT: "Deleterious"; PolyPhen-2: "Benign"; Align-GVGD: "Class C0"). In summary, the available evidence is currently insufficient to determine the role of this variant in disease. Therefore, it has been classified as a Variant of Uncertain Significance. and PSTPIP1 [c.806T>A (p.Ile269Asn)] This sequence change replaces isoleucine with asparagine at codon 269 of the PSTPIP1 protein (p.Ile269Asn). The isoleucine residue is highly conserved and there is a large physicochemical difference between isoleucine and asparagine. The frequency data for this variant in the population databases is considered unreliable, as metrics indicate poor data quality at this position in the ExAC database. This variant has not been reported in the literature in individuals with PSTPIP1-related conditions. Algorithms developed to predict the effect of missense changes on protein structure and function are either unavailable or do not agree on the potential impact of this missense change (SIFT: "Deleterious";

PolyPhen-2: "Probably Damaging"; Align-GVGD: "Class C0"). In summary, the available evidence is currently insufficient to determine the role of this variant in disease. Therefore, it has been classified as a Variant of Uncertain Significance. (*Table 2*)

All examined patients had a burdened family history (missed miscarriages, autoimmune diseases in first degree relatives), frequent bacterial (*Campylobacter, Citrobacter, Escherichia, Helicobacter, Pseudomonas, Staphylococcus, Yersinia*) and/or viral infections during their lifetime. 80.0% of children had an early onset of joint syndrome (in the first 3-4 years of life) with varying degrees of activity and/or frequent relapses. Tothisday, JIA isconsidered a polygenicautoimmune disease with a heterogeneous clinical pattern, without defined clear bio markers of the disease and in sufficient response to existing therapies (nonsteroidalanti-inflammatory drugs, gluco corticosteroids, biologictreatment), associated within ability to obtains table clinical laboratory remission. Searching for new genetic changes not only ingenes associated with adaptive immunity, but also in innateimmunegenes, which are characterized by changes in the functions of the corresponding protein sencoded by the corresponding auto inflammatory genes (NOD2, NLRP12, MEFV, ADA2 and PSTPIP1) is of great interest. This paper presents studied mutations in 32 genes of auto inflammation in children with different phenotypes of JIA (n = 62), which were obtained from 62 children under thestudy.

Mutations in auto-inflammatory genes have been detected in 9 (56.2 %) children who were negative for ANF (-) and in 7 (43.7%) ANF (+) positive children. There were more HLA-B27(-) negative patients in this group than positive ones: 10 (62.5 %) and 6 (37.5%), respectively.

Non-steroidal anti-inflammatory drugs (NSAIDs) therapy was ineffective for this children and positive results were obtained when the anti-inflammatory effect was increased due to gluco corticoids (GCs). All children received basic disease-modifying therapy, which did not allow them to get a stable clinical and laboratory remission, which was manifested in a slow progression of the pathological process.

4 patients (25,0%) with systemic JIA, 3 (18,75%) patients with polyarthritis and 9 (56,2%) patients with oligoarthritis had changes in the nucleotide sequence in auto inflammatory genes. The data obtained indicate that in oligoarthritis changes in the innate Immunity functioning are observed more often compared to the systemic form and polyarticular form of JIA. Mutations in auto inflammatory genes were detected in 6 (37.5 %) children negative for ANA and HLA-B27 and in 4 (25.0 %) children positive for ANA and HLA-B27 [OR=0,55;CI (0,12-2,53)].

Four children with systemic JIA and eight with oligoarthritis had heterozygous changes in NOD2: c.2104C> T (p.Arg702Trp). It is known that NOD2 gene mutations are classically relatable to an increased risk of Crohn's disease (PMID: 19713276), autosomal dominant Blau syndrome (MedGen UID: 348835). The most common NOD2 variants are: c.2104C> T (p.Arg702 Trp), c.2722G> C (p.Gly908Arg) and c.3019dupC (p.Leu1007Profs*2). But none of our patients at the time of the examination had clinical and laboratory data that indicated the presence of inflammatory bowel disease.

NOD2 gene polymorphism exists in 1-3% of the population, which increases the risk of clinical manifestation 2-4 times in heterozygous and 7-9 times in homozygous conditions. 19.3% of patients with JIA have changes in the nucleotide sequence in the NOD2 gene, which determines a statistically significant difference with the population frequency [OR=11,76, CI (2,53-54,59), p<0,001]. This sequence change replaces arginine with tryptophan at cod on 702 of the NOD2 protein (p.Arg702Trp). The arginine residue is moderately conserved and there is a moderate physicochemical difference between arginine and tryptophan. This variant is present in population databases (rs2066844, ExAC 3%), including multiple homozygous individuals.

Numerous population-based case-control studies have shown that this variant confers an elevated risk of Crohn's disease [Peter I, Mitchell AA,2011; Hradsky O, 2008; Naderi N., 2011; Lakatos PL., 2005; Tukel T., 2004]. In a large meta-an alysis involving 75 case-control studies with 18,727 cases and 17,102 controls [Yazdanyar S., 2009], individuals carrying this variant had an increased overall risk of Crohn's disease (OR = 2.2, 95% CI 2.0-2.5). When all three NOD2 genotypes were combined (p.Arg702Trp, p.Gly908Arg, and p.Leu1007Profs*2), the odds ratios for Crohn's disease were 2.4 (95% CI, 2.0-2.8) for simple heterozygotes, 9.0 (95% CI 6.0-13.5) for compound heterozygotes, and 6.7 (95% CI 4.1-10.9) for homozygotes, compared with noncarriers. This variant is also referred to as R675W and SNP8 in the literature. ClinVar contains an entry for this variant (Variation ID: 4693). Experimental studies have shown that this missense change results in decreased NFkB activity and decreased response to lipopolysaccharide, muramyl dipeptide, and peptidoglycan compared to wild type protein [Bonen DK., 2003; Li J, Moran T., 2004; Stevens C, 2013] . In summary, this is a frequently observed variant that is associated with approximately a 2.2-fold increased risk of Crohn's disease in population studies. Therefore, it has been classified as an Increased Risk Allele.

A 2-year-old girl of Arab origin is being observed in the children`s connective tissue disorder clinic. She was born in 5th pregnancy, complicated by toxicosis and the threatened miscarriage; the previous 2 pregnancies ended in miscarriages, 2 children were born, one of them died at the age of 1 month because

of congenital heart defect. The girl has been ill since the age of 10 months, when a long-term fever, anemia (Hb 70-100 g/l), increased ESR (25-40 mm/h) and CRP (24-31 mg/l) occurred HLA-B27 (+) , АНФ - 1:1000. She received a course of antibacterial therapy, NSAIDs, which was ineffective. The character of disease was progressive, often recurrent with bouts of fever, arthralgias and edema of the knee, ankle, and wrist joints. Ultrasonography has revealed exudative and proliferative changes in the joints. The girl was diagnosed with juvenile idiopathic polyarthritis. Against the background of GCs therapy and methotrexate, a positive dynamics was obtained, but when the dose of GCs was reduced, the inflammatory process activated, which required the intensification of therapeutic measures.

Genetic sequencing revealed a heterozygous change in the ADA2 gene c.145C>T (p.Arg49Trp), which encodes the adenosine deaminase 2 enzyme. This sequence change replaces arginine with tryptophan at the codon unit 49 of the ADA2 protein (p.Arg49Trp).

The onset of systemic JIA in a girl was at the age of 8 years and was characterized by fever, skin rash, arthritis of the knee and hip joints, and myositis. Laboratory tests showed an increase in CRP (24-20 mg/l), ESR(68-20 mm/h), CPK (7086-145 U/l), LDH (707-645 U/l), ANA 1:1000, negative RF, HLA-B27 (+). In the treatment with systemic GCs, hydroxychlorhynine and methotrexate, positive dynamics were obtained, but with a decrease in GCs therapy, there is a constant disease recurrence, which required changes in the baseline therapy and initiation of biological immunotherapy.

Genetic sequencing revealed a pathogenic mutation in the NLRP12 gene. Mutations in the NLRP12 gene determine the activation of NF-kB and caspase signaling pathways and are associated with autosomal dominant Familial cold autoinflammatory syndrome (FCAS) (MedGen UID 435869). The clinical significance of this variant remains uncertain. The sequence change replaces glycine with alanine at the 448 NLRP12 protein codon (p.Gly448Ala). This variant has been reported in a person suffering from chronic NLRP12 inflammation [Naderi N., 2011]. ClinVar contains an entry for this variant (variant ID: 97886). The available evidence is still insufficient to determine the role of this variant in the disease. Just as in case with FCAS, patients with NLRP12 experience periodic episodes of high temperature under the influence of cold, which last for 2-10 days every 3-4 weeks. Fever is usually followed by arthralgia, myalgia, abdominal pain, headache, lymphadenopathy, aphthae on the oral mucosa and skin rash.

In a 3-year-old boy, the disease debuted at the age of 1.5 years, when a spotty rash appeared at the height of the temperature, arthralgia, arthritis of the right knee joint. Systemic JIA was established and GCs therapy, methotrexate were prescribed; it gave a short-term positive effect, but after the third administration there was an allergic reaction, so that the therapy was replaced with the administration of tocilizumab, which allowed to get a positive dynamics and cancel the GCs administration. After a year rash had gradually re-appeared in child; it spread all over the body; the GCs were prescribed again with a positive result.

Genetic testing revealed a change in the nucleotide sequence in the PSTPIP1 gene (MedGen UID: 346801), which encodes a cytoskeletal protein. This protein is active in interaction with various inflammatory proteins; it binds to the cytoplasmic tail of CD2, the t-cell activation and adhesion effector, binds PEST type protein tyrosine phosphatases and directs them to c-Abl kinase to mediate c-Abl-dephosphorylation, thereby regulating the activity of c-Abl. It also interacts with pyrin, which is found in association with the cytoskeleton in myeloid/monocyte cells and modulates immunoregulatory functions. Mutations in this gene are associated with PAPA syndrome (Pyogenic Arthritis, Pyoderma gangrenosum and Acne) and disrupt the physiological signaling necessary for maintaining proper inflammatory response. The genetic defect leads to increased binding of pyrin to NLRP, which causes autoinflammatory reactions [Gupta V., 2018]. The clinical significance of the identified variant is uncertain. This sequence change replaces isoleucine with asparagine in the codon 269 of PSTPIP1 protein (p.Ile269Asn). The isoleucine residue is highly conserved and there is a large physical and chemical difference between isoleucine and asparagine.

IV. Discussion

During genetic testing, 25.8 % of patients with JIA (with ILAR/ACR diagnostic criteria) were found to have mutations in the NOD2, NLRP3, ADA2, MEFV, and PSTPIP1 genes, which increases the risk of initiating auto inflammatory syndromes characterized by arthritis, recurrent episodes of inflammation, fever, etc. [Shnappauf O and Aksentijevich, 2019].

This paper did not aim to determine the distribution of all eles in genes at the population level, but only in a limited cohort of children with different JIA phenotypes. Therefore, follow-up studies in this regard are appropriate.

It is difficult to correct the individual clinical phenotypes of JIA that we have presented with protocol treatment, which indicates the possibility of the existence of juvenile arthritis with genetic variants of auto inflammatory syndromes and disorders in certain functions of innate immunity: a burdened family anamnesis of autoimmune diseases in first degree relatives, acute onset of arthritis after bacterial or viral infections, prolonged fever, rash. It should be noted that these children did not have RF and ACCP, and most of them were HLA-B27 negative.

In 19.3 % of children, there were variation changes in the nucleotide sequence in the NOD2 gene, which mutations are classically associated with Crohn's disease, Blau syndrome signs of which were not detected in our patients. A significant difference in the frequency of NOD2 mutations in our patients with JIA compared to the population indicates the significance of this genetic variation in onset initiation of juvenile arthritis, the mechanisms of which have not yet been fully determined [ExAC Database; Negroni A., 2018]. The NOD2 gene encodes a protein that plays an important role in the activities of cells of both the innate and adaptive immune system (including monocytes, macrophages, and dendritic cells). It happens through the regulation of cytokines, chemokines, and antimicrobial peptides that take part in the antibacterial and antiviral response. NOD2 protein is also active in certain types of epithelial cells, including Paneth cells, in the intestinal mucosa; it is also involved in the recognition of bacteria and modulates the protective function of the immune system of the mucous membranes. NOD2 protein takes part in such processes as autophagy, apoptosis and proteolysis, determines innate inflammatory responses to bacteria and viruses through the activation of NF-KB and caspase-1 pathways, which leads to increased expression of pro inflammatory factors, including IL-1β, TNF-α, IL-6, IL-12p40, IL-8, chemokine ligands, antimicrobial factors.

In addition to its main role in the innate immunity, NOD2 is able to activate the adaptive immune system. It is a key regulator of T-helper 2 cells, which leads to the expression of IL-4 and IL-5. Several studies have shown that joint stimulation by NOD2 agonists and TLR receptors (TLR) causes synergistic production of Th1-associated cytokines in various cell types, although the mechanisms of such reactions are unknown. NOD2 activation, in addition, contributes to the formation of Th17 cells and the production of IL-17A, IL-17F, IL-21 and IL-22 [Brembilla NC, 2018].

The involvement of NOD2 in the pathogenesis of various genetic diseases indicates that this protein is a key regulator of immune and inflammatory responses and plays a crucial role in maintaining the balance between bacteria, epithelium, and the innate immune response of the organism. This protective function is absent in case of NOD2 mutation, which leads to an exacerbation of inflammation and the clinical manifestation of various diseases. Many questions remain unanswered, including the relationship between NOD2 mutations and the microbiota as well as understanding the processes through which mutations in NOD2 can be associated with susceptibility to inflammation and the development of diseases.

Recent studies indicate that the NOD2 gene can be activated in idiopathic arthritis with increased production of TNFa, IL-8, and IL-1β by peripheral blood mononuclear cells, while decreased NOD2 regulation reduced the level of proinflammatory cytokines, NF-kB, TRAF6, and IKK [Franca RFO, 2015]. NOD2 protein is expressed in fibroblasts and synovial fluid of patients with RA [Gupta V, 2018]. It is supposed that the NOD2 regulatory pathway is functional, since stimulation of peripheral blood mononuclear cells with muramyl dipeptide (MDP) has induced the production of larger amount of tumor necrosis factor (TNF-α), interleukins (IL-8 and IL-1β) compared to osteoarthritis. Synovial fluid obtained from patients with RA is able to activate the NF-kB signaling pathway [Kim HW, 2017].

Therefore, activation of NOD2-associated auto inflammatory mechanisms can lead to modification and transformation of autoimmunity, which should be taken into account for treatment of juvenile arthritis. Elucidating the mechanisms of regulation and function of NOD2 in juvenile arthritis may lead to the development of an effective therapeutic strategy for inflammation.

The change in the nucleotide sequence in the ADA2 gene in a child with idiopathic arthritis calls attention to itself too. To date, more than 60 mutations of this gene have been identified; they cause changes in the adenosine deaminase 2 enzyme, the deficiency or activity loss of which is characterized by abnormal inflammation of organs and tissues with various clinical phenotypes, including vasculopathy, stroke, hematological and multisystemic disorders that can occur along with arthritis. An enzyme deficiency is assumed to be able to disrupt the balance between proinflammatory and anti-inflammatory macrophages in tissues and lead to the accumulation of proinflammatory macrophages resulting in abnormal inflammation [Lee PY, 2020]. ADA is considered a probable candidate gene for susceptibility to common immune-mediated diseases. ADA polymorphism in the clinical modification of RA and response to methotrexate treatment has been less studied [Lee PY, 2020].

The mutation found in the child in our study does not correspond to an autoinflammatory disease, which requires further research for the possibility of new prospects for the treatment of this category of patients. Frequency data for this variant in population databases is considered unreliable, and the ExAC database indicates poor data quality in this position. The variant was registered in a person with Behcet's disease [Burillo-Sanz S., 2017]. ClinVar contains an entry for this variant (option ID: 375246).

The protein, which is encoded by NLRP12, inhibits the activation of nuclear transcription factor NF-kB, which regulates the expression of proinflammatory cytokine genes, activates caspase 1. Inadequate functional activity of NLRP12 can lead to the development of cryopyrin-associated periodic syndrome (CAPS) [Gupta V., 2018]. In our patient, who has changes in the nucleotide sequence in this gene, the clinical picture does not correspond to CASP, but

requires differential diagnosis between systemic JIA and systemic connective tissue diseases.

Our data correspond to the results obtained during the study, involved a large group of patients from the UK, which has also found associations between psoriatic JIA and mutations in NLRP3, NOD2, MEFV and PSTPIP1 [Brembilla NC, 2018]. These data confirm the significance of extrapolation from monogenic syndromes to identify candidate genes for susceptibility to such a complex disease as JIA.

Thus, mutations of four genes that are presented in our work encode proteins that are the main protective components of the innate immune system against intracellular pathogens, and increase the secretion of IL-1β by activating caspase 1. Perhaps, polymorphisms in the studied genes may have an effect on the expression of IL-1β, and subsequently affect the onset of JIA. In case if our findings are confirmed in other studies, we will be able to provide evidence for the advisability of treatment of specific variants of JIA with IL-1β antagonists. However, the sample size in this cohort was too small for adequate results verification, and further studies with a larger cohort of JIA patients are required to confirm them.

V. Conclusion

Nucleotide sequence changes in NOD2, NLRP3, MEFV and PSTPIP1 genes were detected in 25.8% of patients with JIA, whose clinical phenotypes are characterized by arthritis with recurrent episodes of inflammation, fever, rash, lackof RF, ACCPand who are more often HLA-B27 negative, are poorly treated by protocol therapy, which determines the possibility of the existence of juvenile arthritis with variation sininnate immunity genes.

In 19.3% of children, variable changes in the nucleotide sequence in the NOD2 gene were detected. A significant difference in the frequency of NOD2 mutations in patients with JIA compared with the population indicates the importance of this genetic variation for the initiation of juvenile arthritis.

Therefore, insomecases, juvenil earthritis may have a mixed clinical phenotype of auto immune-auto inflammatory overlap, which should be considered when choosing a personalized therapeutictactic.

JIA is a relatively rare pediatric disease, in which few full-genome studies have been conducted, especially at the population level. Therefore, follow-up research in this regard is appropriate.

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