The role of the immune response mediator genes polymorphism in the predisposition to juvenile idiopathic arthritis

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Abstract: Objective — The aim of the work was to study the contribution of the immune response mediator genes polymorphism (TNFA rs1800629, LTA rs909253, IL1B rs16944, IL2-IL21 rs6822844, IL2RA rs2104286, IL6 rs1800795, IL10 rs1800872, MIF rs755622, CTLA4 rs3087243, NFKB1 rs28362491, PTPN22 rs2476601, PADI4 rs2240336) to the formation of the predisposition to juvenile idiopathic arthritis (JIA) and its clinical variants.

Material and Methods — The JIA group included 330 patients and the control group – 342 volunteers without autoimmune diseases from the Republic of Bashkortostan, Russia. Genotyping was conducted by the real-time polymerase chain reaction.

Results — Taking into account the differences by sex, it was established, that the alleles/genotypes of the TNFA rs1800629, LTA rs909253, IL2-IL21 rs6822844, PTPN22 rs2476601 polymorphic loci and the TNFA rs1800629*G – LTA rs909253*G haplotype are associated with the development of JIA as a whole (p<0.05); alleles/genotypes of the LTA rs909253, IL1B rs16944, IL2-IL21 rs6822844, IL2RA rs2104286, IL6 rs1800795, IL10 rs1800872, MIF rs755622, CTLA4 rs3087243, NFKB1 rs28362491, PTPN22 rs2476601 polymorphic loci and the TNFA rs1800629*G – LTA rs909253*G haplotype — with some of JIA clinical variants (p<0.05).

Conclusion — In this work, the relationship of the alleles, genotypes and haplotypes of a number of the immune response mediator genes polymorphic loci with the risk of the development of JIA and its clinical variants was established. Specific associations were observed for girls and boys, which indicates the existence of sexual dimorphism in the JIA pathogenesis.

Keywords: juvenile idiopathic arthritis, predisposition, polymorphic loci, association, sexual dimorphism.

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Introduction

Juvenile idiopathic arthritis (JIA) is one of the most common chronic rheumatic diseases in children [1]. An important role in the JIA development is given to the immune response disorders, arising in genetically predisposed individuals [2-4].

Among the key mediators of the immune response, the cell surface molecules (including proteins of the major histocompatibility complex), pro- and anti-inflammatory cytokines, transcription factors, enzymes and other regulatory molecules can be particularly highlighted. Polymorphism, which is characteristic for many of the corresponding genes, causes pronounced interindividual variability, including the variability in the predisposition to JIA [2-4].

In recent years, a relatively large number of studies, including genome-wide association studies (GWAS), have been performed to detect the specific JIA risk markers. Nevertheless, the question is still open [3, 4]. Only for a small number of candidate genes polymorphic variants the association was confirmed in independent studies, and their total contribution to the explanation of the hereditary predisposition to JIA is rather small [3, 4]. In addition, the results of replicative studies are often contradictory, which may be due to a variety of factors, such as the use of different approaches for describing JIA phenotypes and for patients grouping, incorrect selection criteria and insufficient sample size, genotyping errors, and true population differences [4].

The aim of the work was to study the contribution of the immune response mediator genes polymorphism (TNFA rs1800629, LTA rs909253, IL1B rs16944, IL2-IL21 rs6822844, IL2RA rs2104286, IL6 rs1800795, IL10 rs1800872, MIF rs755622, CTLA4 rs3087243, NFKB1 rs28362491, PTPN22 rs2476601, PADI4 rs2240336) to the formation of the predisposition to JIA and its clinical variants.

Material and Methods

Study design and subjects

A case-control study was conducted. The study was approved by the expert council on biomedical ethics of Bashkire State Medical University (Ufa, Russia). The JIA group included 330 patients who...
underwent examination and treatment in the cardio-
rheumatological department of the Republican Children's Clinical
Hospital in 2011-2017. The JIA diagnosis was established according
to the International League of Associations for Rheumatology
(ILAR) criteria [5]. The presented JIA clinical variants and their ratio
in our sample are shown in Table 1. As a control group, 342
volunteers without autoimmune diseases were selected. All
participants of the study (for the JIA group – parents of all
patients) signed the voluntary informed consent. The age of the
examined patients was 9.05 (4.99, 13.30) years, and of the
controls – 18.00 (18.00, 19.00) years (data presented as median
with low and upper quartiles). The ratio of males and females in
the JIA and control groups was 34.24%/65.76% and
30.99%/69.01%, respectively. All the individuals included in the
study were residents of the Republic of Bashkortostan (Russia) and
belonged to the following ethnic groups: Tatars (25.54%), Russians
(21.72%), Bashkirs (13.13%), mixed and others (39.62%).

Genotyping

DNA isolation from the lymphocytes of the whole blood
samples was performed using a standard phenol-chloroform
method [6]. Genotyping of all the individuals for the 12
polymorphic loci (TNFA rs1800629, LTA rs909253, IL1B rs16944,
IL2-IL21 rs6822844, IL2RA rs2104286, IL6 rs1800795, IL10
rs1800872, MIF rs755622, CTLA4 rs3087243, NFκB1 rs28362491,
PTPN22 rs2476601, PADI4 rs2240336) was conducted by the real-
time polymerase chain reaction (StepOnePlus™ Real-Time PCR
System, Applied Biosystems, USA). Sequence-specific primers and
allele-specific probes were designed and synthesized by the “DNK
syntez” company (Russia). The distribution of the polymorphic loci
variants in patients with JIA and in the control group is shown in the
Supplementary Tables 1-5 (Appendix 1).

Statistical analysis

Statistical processing of the results was carried out using
Microsoft Excel, SNPsStats, R v.3.4.2, PowerMarker v.3.25,
STATISTICA v.10 (StatSoft, Inc.) [7-9].

To compare the genotype and allele frequency distribution in the
JIA patients group and in the control group the two-tailed
Fisher's Exact test was used. The differences were considered
statistically significant at p<0.05. A similar analysis was also
performed separately for boys and girls and for specific clinical
variants of the disease. The multiple testing correction of the p-
values was carried out by applying a permutation test with a 10000
permutations (p<0.05). Therefore, the haplotypes of these loci have
also been studied as the potential risk markers for the
development of JIA and its clinical variants.

Testing for the deviations from the Hardy-Weinberg
equilibrium was carried out in the SNPsStats package. There were
no significant deviations from the Hardy-Weinberg equilibrium for
the TNFA rs1800629, LTA rs909253, IL1B rs16944, IL2RA
rs2104286, IL6 rs1800795, IL10 rs1800872, MIF rs755622, CTLA4
rs3087243, NFκB1 rs28362491, PTPN22 rs2476601, PADI4
rs2240336 polymorphic loci in both groups (JIA and control)
(p>0.05). A slight deviation from the Hardy-Weinberg equilibrium
was established for the IL2-IL21 rs6822844 polymorphic locus in the
control group (p=0.019), but considering that the controls
were selected according to the specified criteria (age, sex, the
absence of autoimmune diseases), this locus was kept for the
subsequent analysis.

Results

The established relationship of the alleles, genotypes and
haplotypes of a number of the immune response mediator genes
polymorphic loci with the risk of the development of JIA and its
clinical variants is shown in the Table 2.

Taking into account the differences by sex, the risk predictors
of the development of JIA as a whole were identified among the
alleles/genotypes of the loci TNFA rs1800629 (for girls), LTA
rs909253 (for boys), IL2-IL21 rs6822844 (for girls), PTPN22
rs2476601 (for girls), as well as among the haplotypes of the
TNFA rs1800629 – LTA rs909253 loci (for boys). In addition, the
predictors of the formation of some JIA clinical variants were established:

- Rheumatoid factor positive polyarthritis (alleles/genotypes
  of the locus IL6 rs1800795 (for both the general group of boys and girls),
  MIF rs755622 (only for the general group of boys and girls));
- Rheumatoid factor negative polyarthritis (alleles/genotypes
  of the loci LTA rs909253 (for boys), IL2RA rs2104286 (for boys),
  IL10 rs1800872 (for boys) and the haplotype TNFA rs1800629*G –
  LTA rs909253*G (for boys));
- Persistent oligoarthritis (alleles/genotypes of the loci LTA
  rs909253 (only for the general group of boys and girls),
  IL1B rs16944 (for boys), IL2-IL21 rs6822844 (for boys), IL6 rs1800795
  (both for girls and for boys), IL10 rs1800872 (only for the general group
  of boys and girls), NFκB1 rs28362491 (only for the general group
  of boys and girls), PTPN22 rs2476601 (for girls) and the haplotype
  TNFA rs1800629*G – LTA rs909253*G (for boys));
- Extended oligoarthritis (alleles/genotypes of the loci IL2-IL21
  rs6822844 (for girls), CTLA4 rs3087243 (for girls), PTPN22
  rs2476601 (for girls));
- Enthesitis related arthritis (alleles/genotypes of the loci LTA
  rs909253 (for boys), IL6 rs1800795 (only for the general group
  of boys and girls), NFκB1 rs28362491 (for boys), PTPN22 rs2476601
  (for boys) and the haplotype TNFA rs1800629*G – LTA rs909253*G
  (for boys));

Table 1. Clinical characteristics of the JIA group

| JIA clinical variants                | Total (%) | Boys / Girls |
|--------------------------------------|-----------|--------------|
|                                     | n (p<0.05) | % (p<0.05)   |
| Systemic arthritis                  | 29 (8.79)  | 14/15 (48.28/51.72) |
| Rheumatoid factor positive          | 6 (1.82)   | 1/5 (16.67/83.33)  |
| polyarthritis                       |           |              |
| Rheumatoid factor negative          | 86 (26.06) | 17/69 (19.77/80.23) |
| polyarthritis                       |           |              |
| Persistent oligoarthritis           | 98 (29.70) | 33/65 (33.67/66.33) |
| Extended oligoarthritis             | 46 (13.94) | 5/41 (12.74/87.26)  |
| Enthesitis related arthritis        | 35 (10.61) | 29/6 (82.67/17.34)  |
| Psoriatic arthritis                 | 8 (2.42)   | 3/5 (60/40)       |
| Undifferentiated arthritis          | 22 (6.77)  | 11/11 (50.00/50.00) |
| The whole group                     | 330 (100)  | 113/217 (34.24/65.76) |

Hereinafter: n, number of patients in the groups; p<0.05, frequency in the corresponding JIA clinical variant group.

Table 2. Haplotype frequencies of immune response mediator genes

| Loci                  | Boys / Girls | OR (95% CI) |
|-----------------------|--------------|-------------|
| IL1B                  | IL2-IL21     | IL6         | IL10       | TNFA       | LTA         |
| rs909253              | rs1800629    | rs1800795   | rs1800872  | rs755622   | rs909253    |
| 0.027 (0.100 - 0.590) | 0.234 (0.078 - 0.703) | 0.156 (0.053 - 0.490) | 0.147 (0.049 - 0.481) | 0.202 (0.066 - 0.618) |

In addition, the odds ratio (OR) with the Baptista-Pike exact
conditional 95% confidence interval (95% CI) were calculated [11].

Given that the TNFA and LTA genes are located in the same
cluster on chromosome 6, the linkage disequilibrium test for the
TNFA rs1800629 and LTA rs909253 polymorphic loci was
performed in the SNPsStats package, which showed almost
complete linkage disequilibrium at 99.94% (D=0.0807, D'=0.9994, r=0.5438, p=0.000). Therefore, the haplotypes of these loci have
also been studied as the potential risk markers for the
development of JIA and its clinical variants.

The whole group

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### Table 2. The relationship between the immune response mediator genes polymorphic loci variants and the risk of development of JIA and its clinical variants

| JIA and its clinical variants | The sex | The risk predictors |
|------------------------------|---------|---------------------|
| **JIA as a whole**           |         |                     |
| the general group of boys and girls | TNFA rs1800629*AA (p=0.021, OR=0.10, 95% CI 0.00-0.581), PTPN22 rs2476601*GA (p=0.029, OR=0.48, 95% CI 0.21-1.04), IL6 rs1800829*AA (p=0.016, OR=1.41, 95% CI 1.07-1.85) ||
| the general group of girls    | haplotype TNFA rs1800629*G - LTA rs909253*G (p=0.016, OR=1.41, 95% CI 1.07-1.85) ||
| boys                         | TNFA rs1800629*AA (p=0.031, OR=0.032, 95% CI 0.00-0.075), IL2-RA rs6828444*TT (p=0.039, OR=0.037, 95% CI 0.012-0.861) ||
| girls                        | PTPN22 rs2476601*GG (p=0.039, OR=0.041, 95% CI 0.001-0.952), PTPN22 rs2476601*AA (p=0.029, OR=0.031, 95% CI 1.048-2.392) ||
|                | haplotype TNFA rs1800629*G - LTA rs909253*G (p=0.018, OR=1.79, 95% CI 1.11-2.89) ||
| **Rheumatoid factor positive polyarthritis** |         |                     |
| the general group of boys and girls | LTA rs909253*AG (p=0.007, OR=0.007, 95% CI 0.001-0.194), IL6 rs1800795*CC (p=0.002, OR=0.003, 95% CI 1.00-1.01) ||
| boys                         | MIF rs756522*CC (p=0.031, OR=0.028, 95% CI 0.001-0.075), IL2-RA rs24104286*GA (p=0.039, OR=0.037, 95% CI 0.012-0.87) ||
| girls                        | IL10 rs1800872*CC (p=0.040, OR=0.037, 95% CI 0.001-0.194), IL10 rs1800872*TA (p=0.047, OR=0.048, 95% CI 1.205-3.475) ||
|               | haplotype TNFA rs1800629*G - LTA rs909253*G (p=0.041, OR=0.001, 95% CI 0.000-0.075) ||
| **Rheumatoid factor negative polyarthritis** |         |                     |
| the general group of boys and girls | LTA rs909253*GA (p=0.030, OR=0.031, 95% CI 0.000-0.745), MIF rs756522*CC (p=0.031, OR=0.028, 95% CI 0.001-0.075) ||
| boys                         | LTA rs909253*AA (p=0.034, OR=0.035, 95% CI 0.090-0.887), IL2-RA rs24104286*GA (p=0.039, OR=0.037, 95% CI 0.012-0.87) ||
| girls                        | IL10 rs1800872*CC (p=0.040, OR=0.037, 95% CI 0.001-0.194), IL10 rs1800872*TA (p=0.047, OR=0.048, 95% CI 1.205-3.475) ||
|               | haplotype TNFA rs1800629*G - LTA rs909253*G (p=0.041, OR=0.001, 95% CI 0.000-0.075) ||
| **Persistent oligoarthritis** |         |                     |
| the general group of boys and girls | IL6 rs1800795*GC (p=0.046, OR=0.048, 95% CI 0.295-0.988), PTPN22 rs2476601*GA (p=0.012, OR=0.012, 95% CI 1.250-4.204) ||
| boys                         | IL6 rs1800795*GC (p=0.010, OR=0.008, 95% CI 1.250-4.204), IL6 rs1800795*GC (p=0.019, OR=0.019, 95% CI 0.000-0.745) ||
| girls                        | IL6 rs1800795*GC (p=0.019, OR=0.019, 95% CI 0.000-0.745), IL6 rs1800795*GC (p=0.019, OR=0.019, 95% CI 0.000-0.745) ||
|               | haplotype TNFA rs1800629*G - LTA rs909253*G (p=0.002, OR=0.003, 95% CI 1.00-1.01) ||
| **Extended oligoarthritis**  |         |                     |
| the general group of boys and girls | IL6 rs1800795*GC (p=0.002, OR=0.003, 95% CI 1.00-1.01), IL6 rs1800795*GC (p=0.019, OR=0.019, 95% CI 0.000-0.745) ||
| boys                         | IL6 rs1800795*GC (p=0.002, OR=0.003, 95% CI 1.00-1.01), IL6 rs1800795*GC (p=0.019, OR=0.019, 95% CI 0.000-0.745) ||
| girls                        | IL6 rs1800795*GC (p=0.019, OR=0.019, 95% CI 0.000-0.745), IL6 rs1800795*GC (p=0.019, OR=0.019, 95% CI 0.000-0.745) ||
|               | haplotype TNFA rs1800629*G - LTA rs909253*G (p=0.002, OR=0.003, 95% CI 1.00-1.01) ||
| **Enthesitis related arthritis** |         |                     |
| the general group of boys and girls | LTA rs909253*AG (p=0.002, OR=0.003, 95% CI 1.00-1.01), LTA rs909253*AG (p=0.002, OR=0.003, 95% CI 1.00-1.01) ||
| boys                         | LTA rs909253*AG (p=0.002, OR=0.003, 95% CI 1.00-1.01), LTA rs909253*AG (p=0.002, OR=0.003, 95% CI 1.00-1.01) ||
| girls                        | LTA rs909253*AG (p=0.002, OR=0.003, 95% CI 1.00-1.01), LTA rs909253*AG (p=0.002, OR=0.003, 95% CI 1.00-1.01) ||
|               | haplotype TNFA rs1800629*G - LTA rs909253*G (p=0.004, OR=0.007, 95% CI 1.44-4.56) ||
| **Psoriatic arthritis**      |         |                     |
| the general group of boys and girls | LTA rs909253*AG (p=0.001, OR=0.001, 95% CI 1.00-3.87) ||
| boys                         | LTA rs909253*AG (p=0.001, OR=0.001, 95% CI 1.00-3.87), LTA rs909253*AG (p=0.002, OR=0.026, 95% CI 0.001-0.194) ||
| girls                        | LTA rs909253*AG (p=0.002, OR=0.026, 95% CI 0.001-0.194), LTA rs909253*AG (p=0.002, OR=0.026, 95% CI 0.001-0.194) ||
|               | haplotype TNFA rs1800629*G - LTA rs909253*G (p=0.008, OR=0.087, 95% CI 1.05-4.54) ||
Psoriatic arthritis (the haplotype TNFA rs1800629*G - LTA rs909253*G only for the general group of boys and girls).

It should be noted that the Rheumatoid factor positive polyarthritis and Psoriatic arthritis patients samples were small, which is why the sex stratification was not carried out. Associations with the development of the Systemic arthritis for the studied polymorphic variants of the immune response mediator genes were not detected, including in the sex-stratified analysis (p>0.05).

**Discussion**

As a result of this work, the relationship of the alleles, genotypes and haplotypes of a number of the immune response mediator genes polymorphic loci with the risk of the development of JIA and its clinical variants – Rheumatoid factor positive polyarthritis, Rheumatoid factor negative polyarthritis (only in boys), Persistent oligoarthritis, Extended oligoarthritis, Psoriatic arthritis – was established. Specific associations were observed for girls and boys, which indicates the existence of sexual dimorphism in the JIA pathogenesis. For girls, the risk markers of JIA in general, as well as of Persistent oligoarthritis and Extended oligoarthritis were established, and for boys – of JIA in general and of Rheumatoid factor negative polyarthritis, Persistent oligoarthritis, Psoriatic arthritis (Table 3).

Some of the examined polymorphic variants of the immune response mediator genes have previously been studied for a relationship with the JIA development in separate ethnic groups, but the results are contradictory. Nevertheless, the data of a number of papers are generally consistent with the results of the present study. The protective effect on the development of JIA and/or its clinical variants was shown for the TNFA rs1800629*A allele in the works of Schmeling H. et al. (2006), Kaalla M.J. et al. (2013), Reinards T.H. et al. (2015); for the IL2-IL21 rs6822844*T allele – in the works of Albers H.M. et al. (2009), Hinks A. et al. (2010); for the IL2RA rs2104286*G allele – in the works of Hinks A. et al. (2009), Thompson S.D. et al. (2010) [12-18]. According to Crawley E. et al. (1999), the presence of ATA-containing genotypes of the IL10 gene rs1800896, rs1800871 and rs1800872 polymorphic loci haplotypes was significantly more characteristic for patients with Extended oligoarthritis, than for those with Persistent oligoarthritis [19]. A number of authors have shown that the PTPN22 rs2476601*A allele marks an increased risk of the development of JIA in general and of some of its variants [2, 13, 18, 20-22]. According to the latest data, the association of the PTPN22 rs2476601*A allele with the JIA development is characteristic only for girls [23].

At the same time, according to a number of studies, the TNFA rs1800629*A allele marks an increased risk of the development of JIA in general (in the Mexican population) or its polyarticular (in the Serbian population) and oligoarticular (in the British population) variants [24-26]. Several studies have reported the absence of a relationship between the TNFA rs1800629 polymorphic locus variants and the risk of the JIA development in the Portuguese, Spanish, Turkish, Czech, German, French and Italian populations [27-32]. A replicative study of Ellis et al. (2013) did not reveal the relationship of the IL2-IL21 rs6822844 polymorphic locus alleles with the JIA development in the Australian population [33]. Prahalad et al. (2009) and Reinards et al. (2015) reported the absence of a relationship of the IL2RA rs2104286 polymorphic locus alleles with the development of JIA or its variants in children of European descent, and Ellis et al. (2013) – with the development of JIA in the Australian population [14, 33, 34]. Oen et al. (2005) also reported the absence of association of the IL10 rs1800896, rs1800871 and rs1800872 polymorphic locus genotypes and the genotypes of their haplotypes with the development of JIA and its variants in children of European descent [35]. In the Chinese and Hungarian populations, no relationship was found between the PTPN22 rs2476601 polymorphic locus variants and the development of JIA, however, the sample size in these studies was relatively small [36, 37].
When analyzing the MIF rs755622 polymorphic locus, Donn et al. (2002) found that the MIF rs755622*C allele marks an increased risk of the JIA development in children from the UK [38]. Several studies (on samples of European origin [from the USA and Germany], as well as in the Turkish population) have reported on the absence of a relationship of the MIF rs755622 polymorphic locus alleles and genotypes with the development of JIA and / or its variants [13, 27, 39]. At the same time, Reinders et al. (2015) found that the MIF rs755622*C allele marks a protective effect on the JIA development in children of European descent [14].

The CTLA4 rs3087243 polymorphic locus has been studied in JIA by several authors groups. Suppiah et al. (2006), Prahalad et al. (2008) and Ellis et al. (2013) did not reveal any independent associations of the CTLA4 rs3087243 polymorphic locus variants (in isolated analysis, excluding haplotypes) with the JIA development in individuals from Northern Ireland, the USA (predominantly of Northern European ancestry) and Australia, respectively [33, 40, 41]. However, Hinks et al. (2010) on a sample of European origin from the UK, as well as in a meta-analysis with the inclusion of the Prahalad et al. (2008) data showed a borderline significance level (p=0.05) for the rarer occurrence of the CTLA4 rs3087243*A allele in JIA patients than in controls [16].

The observed inconsistency of the results is probably related to the samples characteristics (including sample size, ethnic factors), the pronounced clinical heterogeneity of JIA and the presence of sexual dimorphism in the disease pathogenesis, which indicates the need to consider these aspects when studying the molecular genetic basis of JIA.

**Conclusion**

In this work, the relationship of the alleles, genotypes and haplotypes of a number of the immune response mediator genes polymorphic loci with the risk of the development of JIA and its clinical variants was established. Specific associations were observed for girls and boys, which indicates the existence of sexual dimorphism in the JIA pathogenesis.

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**Conflict of interest**

The authors declare that they have no conflict of interest.

**Ethical approval**

All procedures performed in studies involving human participants were in accordance with the standards of the Local ethical committee of Bashkir State Medical University (Ufa, Russia) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Supplementary Table 1. Analysis of the distribution of the polymorphic loci alleles and genotypes in patients with JIA and in the control group

| Gene      | Polymorphic locus | Alleles | Subjects | The whole group (f+m) | Female (f) | Male (m) | n (f+m) | f+m : f : m |
|-----------|-------------------|---------|----------|-----------------------|------------|----------|---------|-------------|
| TNFA      |                   | G/A     | patients | Alleles and frequencies, % | Alleles and frequencies, % | Alleles and frequencies, % | n (f+m) |
|           |                   |         | controls | (1/2/2) | (1/2/2) | (1/2/2) |          |
| LTA       |                   | A/G     | patients | 10.2 | 80.1/19.0/7.0 | 10.4 | 79.3/20.0/7.0 | 11.8 | 76.5/23.5/0.0 | 86.9/17.2/0.9 | 342 : 236 : 106 | 342 : 236 : 106 |
| IL18      |                   | C/T     | patients | 32.4 | 45.3/54.7/0.0 | 30.4 | 48.4/51.6/0.0 | 32.8 | 49.0/51.0/0.0 | 86.9/17.2/0.9 | 342 : 236 : 106 | 342 : 236 : 106 |
| IL2-1     |                   | G/T     | patients | 31.8 | 47.6/52.4/0.0 | 31.8 | 46.2/53.8/0.0 | 32.8 | 49.0/51.0/0.0 | 86.9/17.2/0.9 | 342 : 236 : 106 | 342 : 236 : 106 |
| IL2RA     |                   | A/G     | patients | 17.9 | 65.3/30.9/3.0 | 16.7 | 65.3/30.9/3.0 | 19.0 | 65.3/30.9/3.0 | 86.9/17.2/0.9 | 342 : 236 : 106 | 342 : 236 : 106 |
| IL6       |                   | G/C     | patients | 35.4 | 37.6/47.9/14.3 | 36.0 | 35.0/53.9/11.1 | 36.8 | 42.3/57.7/9.9 | 86.9/17.2/0.9 | 342 : 236 : 106 | 342 : 236 : 106 |
| IL10      |                   | C/A     | patients | 37.1 | 47.4/51.5/0.0 | 36.0 | 39.4/60.6/1.0 | 39.4 | 49.9/50.1/0.5 | 86.9/17.2/0.9 | 342 : 236 : 106 | 342 : 236 : 106 |
| CTLA4     |                   | G/A     | patients | 25.0 | 49.9/49.1/0.0 | 25.5 | 49.9/49.1/0.0 | 26.5 | 49.9/49.1/0.0 | 86.9/17.2/0.9 | 342 : 236 : 106 | 342 : 236 : 106 |
| NFKB1     |                   | I/D     | patients | 17.8 | 35.6/47.5/6.5 | 17.8 | 35.6/47.5/6.5 | 17.8 | 35.6/47.5/6.5 | 86.9/17.2/0.9 | 342 : 236 : 106 | 342 : 236 : 106 |
| PTPN22    |                   | G/T     | patients | 21.9 | 46.8/53.2/0.0 | 21.9 | 47.1/52.9/0.0 | 21.9 | 47.1/52.9/0.0 | 86.9/17.2/0.9 | 342 : 236 : 106 | 342 : 236 : 106 |
| PADI4     |                   | A/G     | patients | 13.6 | 63.0/37.0/0.0 | 13.6 | 63.0/37.0/0.0 | 13.6 | 63.0/37.0/0.0 | 86.9/17.2/0.9 | 342 : 236 : 106 | 342 : 236 : 106 |

Hereinafter: (1), the major allele; (2), the minor allele; (11), (12), (22), genotypes homozygous for the major and minor alleles, respectively; (12), heterozygous genotype; f+m, female and male; f, female; m, male.

Supplementary Table 2. Analysis of the distribution of the polymorphic loci alleles and genotypes in patients with Rheumatoid factor negative polyarthritis and in the control group

| Gene      | Polymorphic locus | Alleles | Subjects | The whole group (f+m) | Female (f) | Male (m) | n (f+m) | f+m : f : m |
|-----------|-------------------|---------|----------|-----------------------|------------|----------|---------|-------------|
| TNFA      |                   | G/A     | patients | Alleles and frequencies, % | Alleles and frequencies, % | Alleles and frequencies, % | n (f+m) |
|           |                   |         | controls | (1/2/2) | (1/2/2) | (1/2/2) |          |
| LTA       |                   | A/G     | patients | 17.4 | 68.4/28.8/3.0 | 16.7 | 69.9/28.6/3.0 | 18.9 | 65.3/31.2/3.0 | 86.9/17.2/0.9 | 342 : 236 : 106 | 342 : 236 : 106 |
| IL18      |                   | C/T     | patients | 35.4 | 37.6/47.9/14.3 | 35.4 | 37.6/47.9/14.3 | 35.4 | 37.6/47.9/14.3 | 86.9/17.2/0.9 | 342 : 236 : 106 | 342 : 236 : 106 |
| IL2-1     |                   | G/T     | patients | 41.0 | 47.6/52.4/0.0 | 37.3 | 47.8/52.2/0.0 | 39.4 | 47.8/52.2/0.0 | 86.9/17.2/0.9 | 342 : 236 : 106 | 342 : 236 : 106 |
| IL2RA     |                   | A/G     | patients | 18.4 | 65.3/30.9/3.0 | 17.8 | 65.3/30.9/3.0 | 17.8 | 65.3/30.9/3.0 | 86.9/17.2/0.9 | 342 : 236 : 106 | 342 : 236 : 106 |
| IL6       |                   | G/C     | patients | 32.8 | 42.3/57.7/9.9 | 31.9 | 40.9/59.1/9.3 | 34.0 | 42.3/57.7/9.9 | 86.9/17.2/0.9 | 342 : 236 : 106 | 342 : 236 : 106 |
| IL10      |                   | C/A     | patients | 29.4 | 49.9/49.1/0.0 | 29.4 | 49.9/49.1/0.0 | 29.4 | 49.9/49.1/0.0 | 86.9/17.2/0.9 | 342 : 236 : 106 | 342 : 236 : 106 |
| CTLA4     |                   | G/A     | patients | 33.3 | 47.4/51.5/0.0 | 33.3 | 47.4/51.5/0.0 | 33.3 | 47.4/51.5/0.0 | 86.9/17.2/0.9 | 342 : 236 : 106 | 342 : 236 : 106 |
| NFKB1     |                   | I/D     | patients | 13.6 | 63.0/37.0/0.0 | 13.6 | 63.0/37.0/0.0 | 13.6 | 63.0/37.0/0.0 | 86.9/17.2/0.9 | 342 : 236 : 106 | 342 : 236 : 106 |
| PTPN22    |                   | G/T     | patients | 19.9 | 68.4/28.8/3.0 | 19.9 | 68.4/28.8/3.0 | 19.9 | 68.4/28.8/3.0 | 86.9/17.2/0.9 | 342 : 236 : 106 | 342 : 236 : 106 |
| PADI4     |                   | A/G     | patients | 22.6 | 47.4/52.6/0.0 | 22.6 | 47.4/52.6/0.0 | 22.6 | 47.4/52.6/0.0 | 86.9/17.2/0.9 | 342 : 236 : 106 | 342 : 236 : 106 |

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### Supplementary Table 3. Analysis of the distribution of the polymorphic loci alleles and genotypes in patients with Persistent oligoarthritis and in the control group

| Polymorphic locus | Alleles | Subjects | The whole group (f+m) | Female (f) | Male (m) | n1 | n0 | f+m : f : m |
|-------------------|---------|----------|-----------------------|------------|----------|-----|-----|-------------|
|                   |         |          | Alleles and genotypes, % | Alleles and genotypes, % | Alleles and genotypes, % |     |     |             |
|                   |         |          | (2) | (11)/(12)/(22) | (2) | (11)/(12)/(22) | (2) | (11)/(12)/(22) | (2) | (11)/(12)/(22) | (f+m : f : m) |
| TNFA        | 1800629 | G/A      | 11.2 | 78.2/20.4/1.0  | 10.0 | 80.0/20.0/0.0  | 13.6 | 75.8/12.3/1.0  | 98 | 65/33 |             |
| LTA         | 909253  | A/G      | 13.2 | 76.3/21.1/2.6  | 14.4 | 73.7/23.7/2.5  | 10.4 | 82.1/15.1/2.8  | 134 | 236/106 |             |
| IL1B        | 16944   | C/T      | 35.7 | 43.9/40.8/15.3 | 36.2 | 40.0/47.7/12.3 | 34.8 | 51.5/7.7/3.2   | 98 | 65/33 |             |
| IL2-21      | 6826844 | G/T      | 29.4 | 49.1/43.0/7.9  | 30.3 | 47.0/45.7/3.6  | 27.4 | 53.8/37.8/2.8  | 134 | 236/106 |             |
| IL2RA       | 2104286 | G/C      | 32.7 | 43.9/46.9/9.2  | 37.7 | 35.4/54.8/10.8 | 22.7 | 60.6/33.7/1.6  | 98 | 65/33 |             |
| IL6         | 1800795 | G/C      | 37.9 | 38.9/46.5/14.6 | 37.1 | 39.0/47.9/13.1 | 39.6 | 38.7/34.1/17.9 | 134 | 236/106 |             |
| IL10        | 1800872 | C/A      | 37.2 | 83.7/16.3/0.0  | 10.8 | 78.5/21.5/0.0  | 2.0  | 99.6/0.0/0.0   | 98 | 65/33 |             |
| MIF          | 755622  | G/C      | 12.1 | 78.7/18.4/2.9  | 12.3 | 78.8/17.3/1.8  | 11.8 | 79.0/18.1/1.9  | 236 | 236/106 |             |
| CTLA4       | 3087243 | G/A      | 24.0 | 56.3/28.5/12.8 | 23.8 | 56.9/27.5/12.1 | 24.2 | 60.9/30.5/12.1 | 98 | 65/33 |             |
| NFKB1       | 23862491| I/D      | 24.0 | 56.3/28.5/12.8 | 23.8 | 56.9/27.5/12.1 | 24.2 | 60.9/30.5/12.1 | 98 | 65/33 |             |
| PTPN22      | 2476601 | G/A      | 26.1 | 77.4/22.6/0.0  | 27.6 | 75.7/24.3/0.0  | 28.1 | 74.2/23.8/0.0  | 98 | 65/33 |             |
| PADI4       | 2240336 | G/A      | 26.1 | 77.4/22.6/0.0  | 27.6 | 75.7/24.3/0.0  | 28.1 | 74.2/23.8/0.0  | 98 | 65/33 |             |

### Supplementary Table 4. Analysis of the distribution of the polymorphic loci alleles and genotypes in patients with Extended oligoarthritis and in the control group

| Polymorphic locus | Alleles | Subjects | The whole group (f+m) | Female (f) | Male (m) | n1 | n0 | f+m : f : m |
|-------------------|---------|----------|-----------------------|------------|----------|-----|-----|-------------|
|                   |         |          | Alleles and genotypes, % | Alleles and genotypes, % | Alleles and genotypes, % |     |     |             |
|                   |         |          | (2) | (11)/(12)/(22) | (2) | (11)/(12)/(22) | (2) | (11)/(12)/(22) | (f+m : f : m) |
| TNFA        | 1800629 | G/A      | 7.6  | 84.8/15.2/0.0  | 8.5  | 82.9/17.1/0.0  | 0.0  | 100.0/0.0/0.0  | 46  | 41/5       |
| LTA         | 909253  | A/G      | 32.5 | 76.3/21.1/2.5  | 32.8 | 73.7/23.7/2.5  | 37.8 | 66.6/15.4/12.5 | 134 | 236/106 |             |
| IL1B        | 16944   | C/T      | 29.4 | 49.1/43.0/7.9  | 30.3 | 47.0/45.7/3.6  | 27.4 | 53.8/37.8/2.8  | 98 | 65/33 |             |
| IL2-21      | 6826844 | G/T      | 39.1 | 37.0/47.8/15.2 | 35.4 | 41.5/46.3/12.2 | 7.0  | 0.0/60.0/40.0  | 46  | 41/5       |
| IL2RA       | 2104286 | G/C      | 39.1 | 37.0/47.8/15.2 | 35.4 | 41.5/46.3/12.2 | 7.0  | 0.0/60.0/40.0  | 46  | 41/5       |
| IL6         | 1800795 | G/C      | 36.7 | 69.6/20.3/9.8  | 37.7 | 69.0/21.9/9.2  | 37.3 | 39.6/62.4/12.2 | 98 | 65/33 |             |
| IL10        | 1800872 | C/A      | 37.2 | 68.4/28.4/3.2  | 32.6 | 66.6/24.4/11.1 | 23.4 | 38.8/43.1/13.8 | 34  | 30/5/15.0  |
| MIF          | 755622  | G/C      | 38.0 | 34.8/53.4/10.9 | 40.2 | 31.8/67.7/1.1  | 18.9 | 69.0/21.9/1.1  | 98 | 65/33 |             |
| CTLA4       | 3087243 | G/A      | 36.4 | 42.7/45.3/12.0 | 36.4 | 39.4/48.3/12.3 | 30.7 | 50.3/38.7/11.6 | 134 | 236/106 |             |
| NFKB1       | 23862491| I/D      | 36.4 | 42.7/45.3/12.0 | 36.4 | 39.4/48.3/12.3 | 30.7 | 50.3/38.7/11.6 | 134 | 236/106 |             |
| PTPN22      | 2476601 | G/A      | 35.4 | 42.7/45.3/12.0 | 36.4 | 39.4/48.3/12.3 | 30.7 | 50.3/38.7/11.6 | 134 | 236/106 |             |
| PADI4       | 2240336 | G/A      | 39.1 | 71.2/27.3/2.0  | 19.9 | 68.3/17.8/1.3  | 0.0  | 100.0/0.0/0.0  | 98 | 65/33 |             |
Supplementary Table 5. Analysis of the distribution of the polymorphic loci alleles and genotypes in patients with Enthesitis related arthritis and in the control group

| Gene | rs  | Alleles | Subjects | The whole group (f+m) | Female (f) | Male (m) | n<sub>f+m</sub> |
|------|-----|---------|----------|----------------------|------------|----------|---------------|
|      |     |         | Alleles and genotypes frequencies, % | Alleles and genotypes frequencies, % | Alleles and genotypes frequencies, % |          |               |
|     |     |         | (1)/(2)/(3) | (1)/(2)/(3) | (1)/(2)/(3) |          |               |
| TNFA | 1800629 | G/A | patients | 71.7 | 85.7/14.3/0.0 | 16.7 | 66.7/33.3/0.0 | 5.2 | 89.7/10.3/0.0 | 35.6 : 29 |
|      |     |       | controls | 41.4 | 22.9/71.4/5.7 | 33.3 | 33.3/66.7/0.0 | 43.1 | 20.7/77.2/6.9 | 35.6 : 29 |
| IL1B | 16944 | C/T | patients | 40.0 | 42.9/34.3/22.9 | 33.3 | 50.0/33.3/16.7 | 41.4 | 41.4/34.5/24.1 | 35.6 : 29 |
|      |     |       | controls | 39.9 | 38.9/48.5/14.6 | 37.1 | 39.0/47.9/13.1 | 39.6 | 38.7/43.4/17.9 | 35.6 : 29 |
| IL2-21 | 6822844 | G/T | patients | 7.7  | 88.6/11.4/0.0 | 8.3  | 83.3/16.7/0.0 | 5.2  | 89.7/10.3/0.0 | 35.6 : 29 |
|      |     |       | controls | 7.1  | 78.7/18.4/2.9  | 12.3 | 78.8/17.8/3.4 | 11.8 | 78.3/19.8/1.9 | 35.6 : 29 |
| IL2RA | 2104286 | A/G | patients | 17.1 | 68.6/28.6/2.9 | 25.0 | 50.0/50.0/0.0 | 15.5 | 72.4/24.1/3.4 | 35.6 : 29 |
|      |     |       | controls | 17.4 | 68.4/28.4/3.2 | 16.7 | 69.9/26.7/3.4 | 18.9 | 65.1/32.1/2.8 | 35.6 : 29 |
| IL6  | 1800795 | G/C | patients | 38.6 | 45.7/31.4/22.9 | 33.3 | 50.0/33.3/16.7 | 39.7 | 44.8/31.0/24.1 | 35.6 : 29 |
|      |     |       | controls | 34.2 | 42.1/47.4/10.5 | 32.8 | 43.2/47.9/8.9 | 37.3 | 39.6/46.3/14.2 | 35.6 : 29 |
| IL10 | 1800872 | C/A | patients | 37.1 | 42.9/40.0/17.1 | 33.3 | 50.0/33.3/16.7 | 37.9 | 41.4/41.7/17.2 | 35.6 : 29 |
|      |     |       | controls | 31.6 | 47.7/41.5/10.8 | 32.6 | 46.2/42.4/11.4 | 29.2 | 50.9/39.6/9.4 | 35.6 : 29 |
| MIF  | 755622 | G/C | patients | 14.3 | 74.3/22.9/2.9 | 8.3  | 83.3/16.7/0.0 | 15.5 | 72.4/24.1/3.4 | 35.6 : 29 |
|      |     |       | controls | 21.8 | 60.2/36.0/3.8 | 22.2 | 59.7/36.0/4.2 | 20.8 | 61.3/35.8/2.8 | 35.6 : 29 |
| CTLA4 | 3087243 | G/A | patients | 32.9 | 45.7/42.9/11.4 | 16.7 | 66.7/33.3/0.0 | 36.2 | 41.4/44.8/13.8 | 35.6 : 29 |
|      |     |       | controls | 34.6 | 42.7/45.3/12.0 | 36.4 | 39.4/48.3/12.3 | 30.7 | 50.0/38.7/11.3 | 35.6 : 29 |
| NFKB1 | 28362491 | I/D | patients | 52.9 | 28.6/37.1/34.3 | 50.0 | 33.3/33.3/33.3 | 53.4 | 27.6/37.9/34.5 | 35.6 : 29 |
|      |     |       | controls | 44.9 | 31.0/48.2/20.8 | 47.2 | 28.4/48.7/22.9 | 39.6 | 36.8/47.2/16.0 | 35.6 : 29 |
| PTPN22 | 2476601 | G/A | patients | 18.6 | 65.7/34.3/0.0 | 25.0 | 66.7/16.7/16.7 | 17.2 | 65.5/34.5/0.0 | 35.6 : 29 |
|      |     |       | controls | 9.4  | 83.0/15.2/1.8 | 9.4  | 82.6/16.2/1.3 | 9.4  | 84.0/13.2/2.8 | 341 : 235 : 106 |
| PADI4 | 2240336 | G/A | patients | 51.4 | 22.9/51.4/25.7 | 38.3 | 16.7/50.0/33.3 | 50.0 | 24.1/51.7/24.1 | 35.6 : 29 |
|      |     |       | controls | 43.1 | 32.3/49.3/18.5 | 43.2 | 32.6/48.3/19.1 | 42.9 | 31.4/51.7/17.1 | 341 : 235 : 106 |