REVIEW

# Associations between interleukin-10 polymorphisms and susceptibility to juvenile idiopathic arthritis: a systematic review and meta-analysis

Sara Harsini[1,2], Amene Saghazadeh[1,2], Saharnaz Nedjat[3], Nima Rezaei[1,2,4,5]

[1] Research Center for Immunodeficiencies, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran
[2] Network of Immunity in Infection, Malignancy and Autoimmunity (NIIMA), Universal Scientific Education and Research Network (USERN), Tehran, Iran
[3] Epidemology and Biostatistics Deparment, School of Public Health, Knowledge Utilization Research Center, Tehran University of medical Sciences, Tehran, Iran
[4] Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[5] Systematic Review and Meta-analysis Expert Group (SRMEG), Universal Scientific Education and Research Network (USERN), Boston, MA, USA

Correspondence
<rezaei_nima@tums.ac.ir>

Accepted for publication February 16, 2018

To cite this article: Harsini S, Saghazadeh A, Nedjat S, Rezaei N. Associations between interleukin-10 polymorphisms and susceptibility to juvenile idiopathic arthritis: a systematic review and meta-analysis. *Eur. Cytokine Netw.* 2018; 29(1): 16-26 doi:10.1684/ecn.2018.0404

**ABSTRACT.** ***Background:*** **Cytokine genes, including interleukin-10 (*IL-10*), are known to play important roles in the pathogenesis of juvenile idiopathic arthritis (JIA). This study aims to determine whether the *IL-10* polymorphisms confer susceptibility to JIA.** ***Methods:*** **A meta-analysis was performed on the associations between the *IL-10 -1082 G/A*, *-592 C/A,* and *-819 C/T* polymorphisms and JIA. A total number of 7 studies involving 1,785 patients and 6,142 controls were considered in the meta-analysis.** ***Results:*** **Meta-analysis of the *IL-10 -592* C/A and *-819* C/T polymorphisms showed no association with JIA in the study participants, or in Caucasian or Middle Eastern participants. Meta-analysis of the *IL-10 -1082* A allele in all study participants, Caucasian and Middle Eastern, showed significant associations with RA (overall ORs were 1.17, 1.15, and 1.41, respectively). Meta-analysis of the AA versus GG genotype of the *IL-10 -1082* G/A polymorphism revealed significant associations with JIA (OR = 3.66, 95% CI = 1.44-9.29, *P* = 0.006) in participants from Middle Eastern countries. Additionally, meta-analysis of the GG versus AA+GA genotypes of the *IL-10 -1082* G/A polymorphism revealed the GG genotype as the protective factor against JIA in the Middle Eastern subgroup (OR = 0.44, 95% CI = 0.20-0.94, *P* = 0,04). Moreover, meta-analysis of the *IL-10 -1082 A* allele in 4 studies on Hardy-Weinberg equilibrium showed a significant association with JIA (OR = 1.17, 95% CI = 1.07-1.28, *P* = 0.0009). No association was found between the *IL-10 (-1082, -819, -592)* ACC, ATA, and GCC haplotypes and JIA.** ***Conclusions:*** **These results suggest that the *IL-10 -1082 G/A* polymorphism confers susceptibility to JIA.**

**Key words:** juvenile idiopathic arthritis, interleukin-10, polymorphism, meta-analysis

Juvenile idiopathic arthritis (JIA), characterized by chronic joint inflammation with an onset prior to the age of sixteen years, is a most frequent rheumatic disorder of childhood, with an approximate worldwide prevalence of 1 in every 1,000 children [1]. Although the etiology of RA remains unknown, it is widely accepted that both environmental and genetic components contribute to its initiation and progression [2]. Considering the presence of chronic inflammation within synovial joints as the common feature of all JIA subtypes, it has been previously propounded that certain single nucleotide polymorphisms (SNPs) within the promoter and coding sequences of various cytokines' genes, including interleukin-10 (*IL-10*), could be associated with the susceptibility to JIA [3-9].

IL-10, a major immunoregulatory cytokine, exerts its anti-inflammatory effect by ameliorating proinflammatory cytokine synthesis [10], diminishing inflammation in the collagen-induced arthritis animal model [11], reversing cartilage degradation by mononuclear cells from RA patients [12], hindering mononuclear cell traffic into synovial tissue by downregulating intercellular adhesion molecule 1 expression by synovial cells [13, 14], and impeding the production of proinflammatory cytokines by mononuclear cells from synovial cells, synovial fluid, and peripheral blood of patients with rheumatoid arthritis (RA) [12]. *IL-10* gene, assumed as an attractive candidate gene according to its chromosomal location and functional relevance, maps to chromosome 1q32 and exhibits polymorphisms in its promoter region, which results in variations in transcription [15]. Three functional *IL-10* SNPs, namely, at -1082A/G (rs1800896), -819C/T (rs3021097), and -592A/C (rs1800872), which are located at putative regulatory regions in *IL-10* promoter, have been a focus of intensive research recently [16]. The

doi: 10.1684/ecn.2018.0404

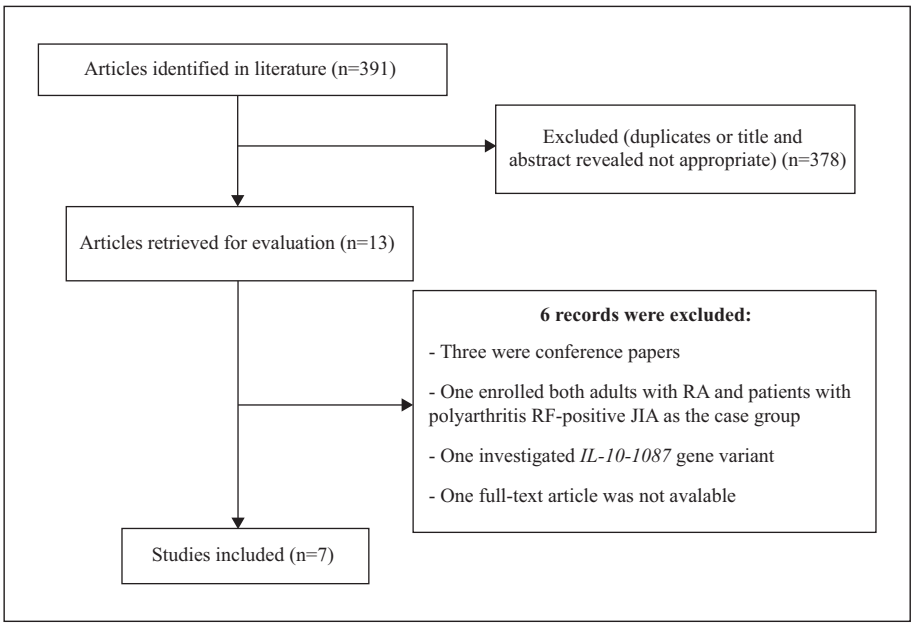


**Figure 1**
Flow diagram of all studies included in this meta-analysis; of 391 studies identified *via* electronic and hand searching, a total of 7 studies were included.

**Table 1**
The HWE test for *IL-10 -1082, -819*, and *-592* genotypes distribution among eligible studies.

| SNP | Study | Control | | | HWE |
|---|---|---|---|---|---|
| | | AA | AB | BB | |
| IL-10 -592 | Crawley E., 1999 | 18 | 92 | 164 | Yes (0.303) |
| IL-10 -592 | Donn R.P., 2001 | 10 | 81 | 148 | Yes (0.795) |
| IL-10 -592 | Fathy M.M., 2017 | 19 | 71 | 10 | No (0.000) |
| IL-10 -592 | Harsini S., 2016 | 12 | 57 | 71 | Yes (0.907) |
| IL-10 -819 | Cinek O., 2003 | 48 | 50 | 5 | Yes (0.075) |
| IL-10 -819 | Crawley E., 1999 | 164 | 92 | 18 | Yes (0.303) |
| IL-10 -819 | Donn R.P., 2001 | 148 | 81 | 10 | Yes (0.795) |
| IL-10 -819 | Fathy M.M., 2017 | 10 | 71 | 19 | No (0.000) |
| IL-10 -819 | Harsini S., 2016 | 71 | 57 | 12 | Yes (0.907) |
| IL-10 -1082 | Cinek O., 2003 | 21 | 71 | 11 | No (0.0001) |
| IL-10 -1082 | Crawley E., 1999 | 80 | 124 | 70 | Yes (0.121) |
| IL-10 -1082 | Donn R.P., 2001 | 50 | 118 | 71 | Yes (0.940) |
| IL-10 -1082 | Fathy M.M., 2017 | 10 | 73 | 17 | No (0.000) |
| IL-10 -1082 | Harsini S., 2016 | 53 | 75 | 12 | No (0.042) |

SNP: Single nucleotide polymorphism; HWE: Hardy-Weinberg equilibrium.

aforementioned polymorphisms are, therefore, known to alter the binding sites of transcription factors that could affect IL-10 synthesis.

A multitude of studies have examined the association between the *IL-10 -1082* G/A, *-819* C/T, and *-592* C/A polymorphisms and the risk of JIA [15, 17-22], but the results were contradictory. This is partly due to the low statistical powers of individual studies. Therefore, we performed a meta-analysis of all eligible studies, so as to derive a better estimation of the association and to resolve inconsistencies. In the current study, we conducted meta-analysis to investigate whether the *IL-10 -1082* G/A, *-819* C/T, and *-592* C/A polymorphisms contribute to JIA vulnerability. To our knowledge, this is the first genetic meta-analysis performed with respect to the association between the aforementioned polymorphisms and the risk of JIA.

## MATERIALS AND METHODS

### *Search strategy and study selection*

To find relevant studies, both electronic searches and manual reference retrievals were performed. Combinations of keywords, such as, “interleukin-10”, “IL-10”, “polymorphism”, “juvenile rheumatoid arthritis”, “juvenile idiopathic arthritis”, “juvenile arthritis”, “JIA”, and

**Table 2**
Characteristics of studies included in meta-analysis.

| First author | Year of publication | Country | Ethnicity | JIA/ Control | Gene variant | Allele frequency | | | Genotype Frequency | | | Haplotype frequency | | | Newcastle-Ottawa Scale |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | JIA | Control | | JIA | Control | | JIA | Control | |
| Crawley E. | 1999 | United Kingdom | Caucasian | 435/274 | -1082 | A | 437 | 274 | AA | 120 | 80 | GCC | 433 | 264 | 5 |
| | | | | | | | | | GA | 197 | 124 | | | | |
| | | | | | | G | 433 | 264 | GG | 118 | 70 | | | | |
| | | | | | -819 | C | 664 | 420 | CC | 254 | 164 | | | | |
| | | | | | | | | | CT | 156 | 92 | ACC | 231 | 156 | |
| | | | | | | T | 206 | 128 | TT | 25 | 18 | | | | |
| | | | | | -592 | A | 206 | 128 | AA | 25 | 18 | | | | |
| | | | | | | | | | CA | 156 | 92 | | | | |
| | | | | | | C | 664 | 420 | CC | 254 | 164 | ATA | 206 | 128 | |
| Donn R.P. | 2001 | United Kingdom | Caucasian | 348/239 | -1082 | A | 349 | 218 | AA | 104 | 50 | GCC | 347 | 260 | 8 |
| | | | | | | | | | GA | 141 | 118 | | | | |
| | | | | | | G | 347 | 260 | GG | 103 | 71 | | | | |
| | | | | | -819 | C | 532 | 377 | CC | 196 | 148 | | | | |
| | | | | | | | | | CT | 141 | 81 | ACC | 185 | 117 | |
| | | | | | | T | 164 | 101 | TT | 12 | 10 | | | | |
| | | | | | -592 | A | 164 | 101 | AA | 12 | 10 | | | | |
| | | | | | | | | | CA | 140 | 81 | | | | |
| | | | | | | C | 532 | 377 | CC | 196 | 148 | ATA | 164 | 101 | |
| Cinek O. | 2003 | Czech Republic | Caucasian | 130/103 | -1082 | A | 133 | 113 | AA | 28 | 21 | | | | 8 |
| | | | | | | | | | GA | 77 | 71 | | | | |
| | | | | | | G | 127 | 93 | GG | 25 | 11 | | | | |
| | | | | | -819 | C | 201 | 146 | CC | 75 | 48 | | | | |
| | | | | | | | | | CT | 51 | 50 | | | | |
| | | | | | | T | 59 | 60 | TT | 4 | 5 | | | | |

**Table 2**
Characteristics of studies included in meta-analysis (*continued*).

| First author | Year of publication | Country | Ethnicity | JIA/ Control | Gene variant | Allele frequency | | | Genotype Frequency | | | Haplotype frequency | | | Newcastle-Ottawa Scale |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | JIA | Control | | JIA | Control | | JIA | Control | |
| Fife M.S. | 2006 | United Kingdom | Caucasian | 172/473 | -1082 | A | 169 | 387 | | | | | | | 7 |
| | | | | | | G | 139 | 425 | | | | | | | |
| | | | | | -592 | A | 92 | 205 | | | | | | | |
| | | | | | | C | 222 | 647 | | | | | | | |
| Omoyinmi E. | 2012 | United Kingdom | Caucasian | 545/4813 | -1082 | A | 572 | 4,620 | | | | | | | 7 |
| | | | | | | G | 518 | 5,006 | | | | | | | |
| Harsini S. | 2016 | Iran | Persian | 55/140 | -1082 | A | 66 | 181 | AA | 20 | 53 | GCC | 30 | 99 | 8 |
| | | | | | | | | | GA | 26 | 75 | | | | |
| | | | | | | G | 30 | 99 | GG | 2 | 12 | | | | |
| | | | | | -819 | C | 62 | 199 | CC | 20 | 71 | | | | |
| | | | | | | | | | CT | 22 | 57 | ACC | 32 | 100 | |
| | | | | | | T | 36 | 81 | TT | 7 | 12 | | | | |
| | | | | | -592 | A | 36 | 81 | AA | 7 | 12 | | | | |
| | | | | | | | | | CA | 22 | 57 | | | | |
| | | | | | | C | 62 | 199 | CC | 20 | 71 | ATA | 34 | 81 | |
| Fathy M.M. | 2017 | Egypt | Egyptian | 100/100 | -1082 | A | 115 | 93 | AA | 23 | 10 | | | | 7 |
| | | | | | | | | | GA | 69 | 73 | | | | |
| | | | | | | G | 85 | 107 | GG | 8 | 17 | | | | |
| | | | | | -819 | C | 89 | 91 | CC | 12 | 10 | | | | |
| | | | | | | | | | CT | 65 | 71 | | | | |
| | | | | | | T | 111 | 109 | TT | 23 | 19 | | | | |
| | | | | | -592 | A | 111 | 109 | AA | 23 | 19 | | | | |
| | | | | | | | | | CA | 65 | 71 | | | | |
| | | | | | | C | 89 | 91 | CC | 12 | 10 | | | | |

**Table 3**
Meta-analysis of associations between the *IL-10 -592* C/A polymorphisms and JIA.

| | JIA | Controls | OR (95% CI) | Overall effect, *P* value | Heterogeneity (d.f.) |
|---|---|---|---|---|---|
| *Overall population* | | | | | |
| A *versus* C | 2,178 | 2,358 | 1.15 [1.00, 1.32] | Z = 1.91, *P* = 0.06 | $I^2$ = 0% (4) |
| AA *versus* CC | 549 | 452 | 1.04 [0.68, 1.59] | Z = 0.18, *P* = 0.86 | $I^2$ = 0% (3) |
| AA *versus* CA+CC | 932 | 753 | 1.06 [0.73, 1.55] | Z = 0.32, *P* = 0.75 | $I^2$ = 0% (3) |
| CC *versus* AA+CA | 932 | 753 | 0.86 [0.70, 1.06] | Z = 1.38, *P* = 0.17 | $I^2$ = 0% (3) |
| *Caucasian ethnicity* | | | | | |
| A *versus* C | 1,880 | 1,878 | 1.14 [0.97, 1.33] | Z = 1.61, *P* = 0.11 | $I^2$ = 0% (2) |
| AA *versus* CC | 487 | 340 | 0.90 [0.54, 1.50] | Z = 0.40, *P* = 0.69 | $I^2$ = 0% (1) |
| AA *versus* CA+CC | 783 | 513 | 0.85 [0.51, 1.41] | Z = 0.63, *P* = 0.53 | $I^2$ = 0% (1) |
| CC *versus* AA+CA | 783 | 513 | 0.87 [0.69, 1.09] | Z = 1.20, *P* = 0.23 | $I^2$ = 0% (1) |
| *Middle East ethnicity* | | | | | |
| A *versus* C | 298 | 480 | 1.18 [0.87, 1.60] | Z = 1.05, *P* = 0.30 | $I^2$ = 0% (1) |
| AA *versus* CC | 62 | 112 | 1.41 [0.67, 2.97] | Z = 0.91, *P* = 0.36 | $I^2$ = 0% (1) |
| AA *versus* CA+CC | 149 | 240 | 1.41 [0.80, 2.48] | Z = 1.19, *P* = 0.24 | $I^2$ = 0% (1) |
| CC *versus* AA+CA | 149 | 240 | 0.83 [0.49, 1.40] | Z = 0.69, *P* = 0.49 | $I^2$ = 13% (1) |

**Table 4**
Meta-analysis of associations between the *IL-10 -819* C/T polymorphisms and JIA.

| | JIA | Controls | OR (95% CI) | Overall effect, *P* value | Heterogeneity (d.f.) |
|---|---|---|---|---|---|
| *Overall population* | | | | | |
| C *versus* T | 2,124 | 1,712 | 0.96 [0.83, 1.11] | Z = 0.55, *P* = 0.58 | $I^2$ = 24% (4) |
| CC *versus* TT | 628 | 505 | 1.02 [0.68, 1.53] | Z = 0.11, *P* = 0.91 | $I^2$ = 0% (4) |
| CC *versus* CT+TT | 1,063 | 856 | 0.94 [0.77, 1.13] | Z = 0.67, *P* = 0.50 | $I^2$ = 34% (4) |
| TT *versus* CC+CT | 1,063 | 856 | 1.02 [0.71, 1.47] | Z = 0.11, *P* = 0.91 | $I^2$ = 0% (4) |
| *Caucasian ethnicity* | | | | | |
| C *versus* T | 1,826 | 1,232 | 1.00 [0.84, 1.18] | Z = 0.05, *P* = 0.96 | $I^2$ = 42% (2) |
| CC *versus* TT | 566 | 393 | 1.19 [0.74, 1.92] | Z = 0.72, *P* = 0.47 | $I^2$ = 0% (2) |
| CC *versus* CT+TT | 914 | 616 | 1.00 [0.71, 1.39]$^R$ | Z = 0.02, *P* = 0.98 | $I^2$ = 58% (2) |
| TT *versus* CC+CT | 914 | 616 | 0.82 [0.51, 1.31] | Z = 0.84, *P* = 0.40 | $I^2$ = 0% (2) |
| *Middle East ethnicity* | | | | | |
| C *versus* T | 298 | 480 | 0.85 [0.63, 1.15] | Z = 1.05, *P* = 0.30 | $I^2$ = 0% (1) |
| CC *versus* TT | 62 | 112 | 0.71 [0.34, 1.49] | Z = 0.91, *P* = 0.36 | $I^2$ = 0% (1) |
| CC *versus* CT+TT | 149 | 240 | 0.83 [0.49, 1.40] | Z = 0.69, *P* = 0.49 | $I^2$ = 13% (1) |
| TT *versus* CC+CT | 149 | 240 | 1.41 [0.80, 2.48] | Z = 1.19, *P* = 0.24 | $I^2$ = 0% (1) |

"JRA", were entered as Medical Subject Heading (MeSH) components and as text words. We searched databases of Medline (Pubmed and Embase), Scopus (ScienceDirect), and Science Citation Index (ISI Web of Knowledge) up to July 2017. Whenever the results of an eligible study were inaccessible, we contacted the first or corresponding authors. The bibliographies of eligible studies were also screened to identify additional relevant publications. Articles in English were just retrieved.

In the present meta-analysis, the original association studies were included if they met all of the following inclusion criteria: (a) it was designed as a case-control association study, (b) it diagnosed patients with juvenile idiopathic arthritis (JIA) without any other rheumatologic complications, (c) it enrolled healthy controls, (d) it assessed the association of -1082 A/G, -892 C/T, and -592 C/A polymorphisms in the *IL-10* gene with susceptibility to JIA, (e) it provided adequate data including genotype/allele frequency in both case and control groups to calculate the pooled odds ratio (OR), and (f) the paper must have been published in peer-reviewed journal as full article. The flow diagram of the study selection is shown in *figure 1*.

### *Data extraction and quality assessment*

Totally, seven studies were selected for this meta-analysis [15, 17-22]. The following data were extracted from each included publication: (a) first author, (b) year of publication, (c) country of origin, (d) ethnicity, (e) investigated *IL-10* gene variant, (f) number of genotyped cases and controls, and (g) allele/genotype/haplotype frequency in cases and controls. Using the Newcastle-Ottawa Quality Assessment Scale for case-control studies, the quality of included investigations was evaluated independently by two authors [23]. This scale comprised two different instruments for evaluating case-control and cohort studies, both of which include measures of quality in the following three different domains: selection, comparability, and exposure. Up to one point can be assigned for each of the four areas measured within the selection domain and for each of three areas

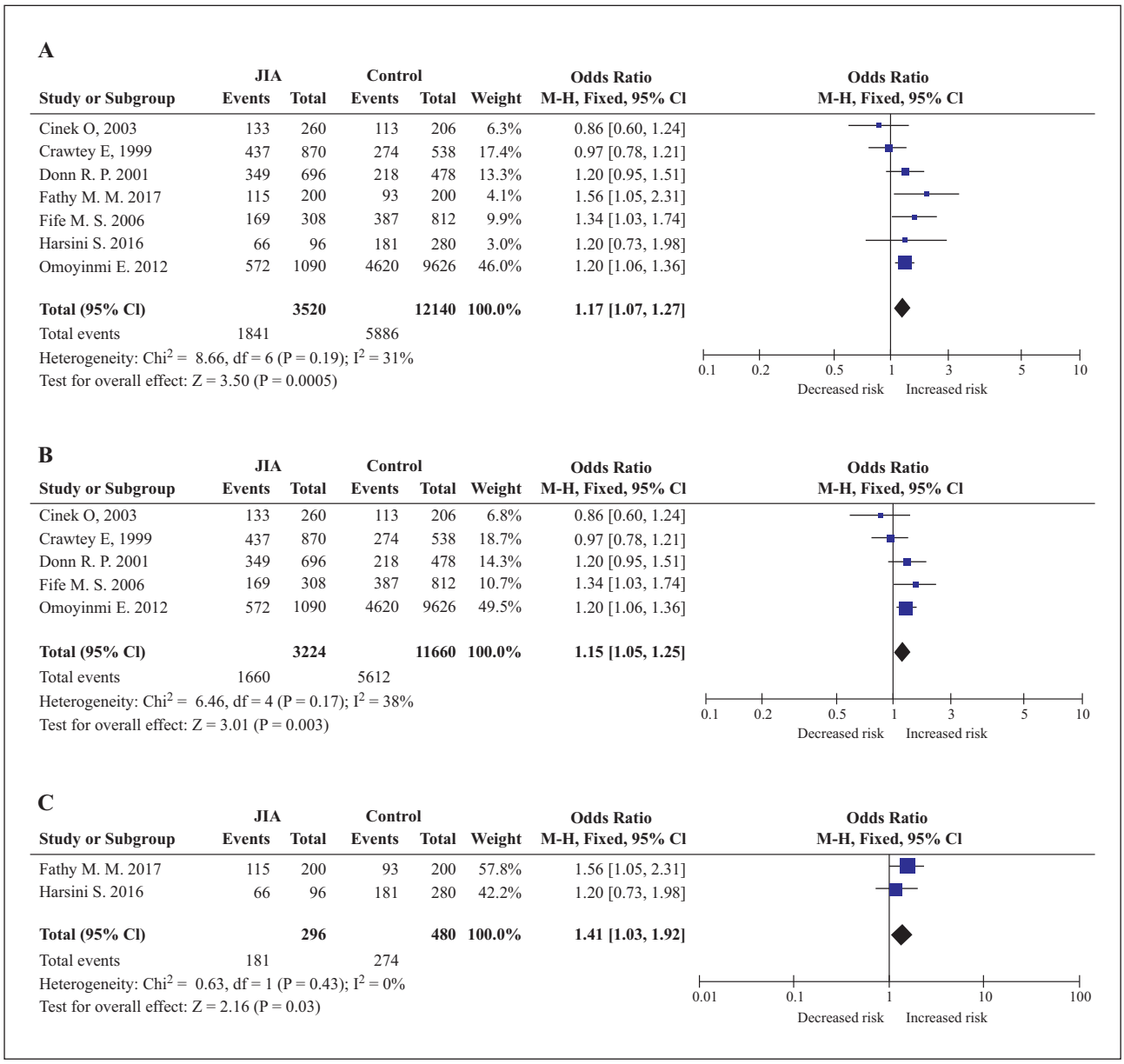


**Figure 2**

ORs and 95% CI of individual studies and pooled data for the association between the A *versus* G alleles of the *IL-10-1082* G/A polymorphism and JIA **(A)** in the overall population, **(B)** in Caucasian ethnicity, and **(C)** in Middle East ethnicity. M-H: Mantel-Haenszel; CI: confidence interval.

measured within the exposure domain. While a study can receive a maximum of two points within the comparability domain, the highest score could be nine. Low-quality studies were considered to have a score of four or less, moderate-quality studies to have a score of four up to six, and high-quality studies to have a score of seven or greater. A joint reevaluation of the original article was the solution in case of any discrepancies between the two authors.

### *Statistical analyses*

The combined OR with its 95% CI was estimated in a fixed effect or random effect model to determine the strength of the associations between *IL-10* gene polymorphisms and susceptibility to JIA. The $I^2$ statistic was applied to assess the heterogeneity among studies, which represented the percentage of total variation contributed by a between-study variation that ranged from 0% to 100% [24]. If there was no significant heterogeneity, a fixed effect model would be applied to pool data. Otherwise, a random effect model was applied. *P* value of less than 0.1 was considered statistically significant for the heterogeneity test. The publication bias was evaluated using funnel plots, Egger's test, and Begg's test. The Hardy-Weinberg equilibrium (HWE) has been also assessed, using the Stata version 12 software (Stata Statistical Software: Release 12, College Station, TX: StataCorp: LP), so as to evaluate the genotype frequencies in the included studies. As the deviation from HWE in controls has been associated with problems in the population stratification, genotyping error, or selection bias [25, 26], the magnitude of deviations from HWE and its statistical significance are demonstrated. HWE analysis was performed among the healthy controls using the $\chi^2$, as the $\chi^2 < 3.84$ indicated the allele frequency to be in HWE. In case of having the studies with a significant deviation of HWE, sensitivity analyses have been conducted, excluding the studies that deviated from HWE as recommended [27]. All analyses were performed, using Review Manager,

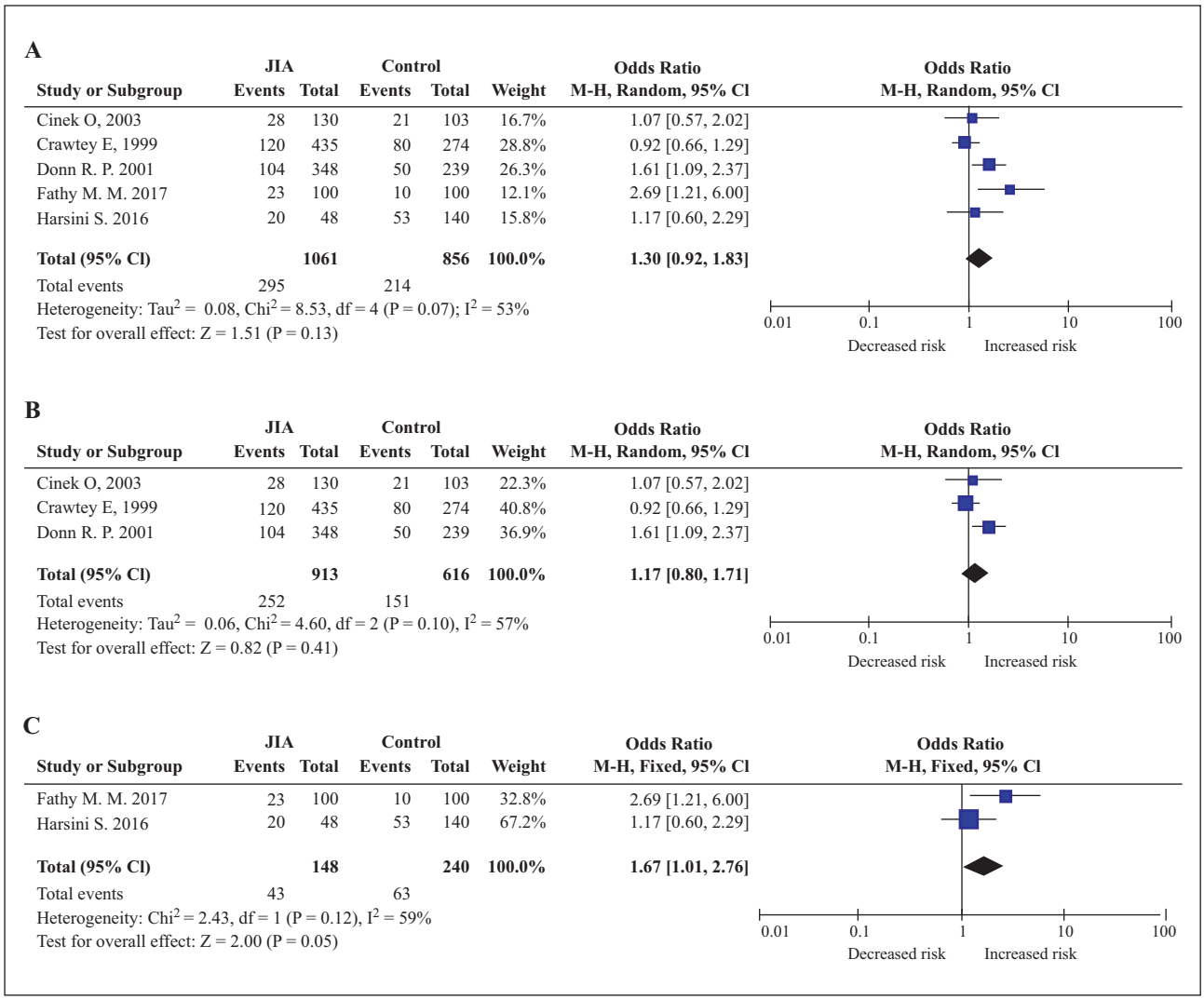


**Figure 3**

ORs and 95% CI of individual studies and pooled data for the association between the AA *versus* GA+GG genotypes of the *IL-10-1082* G/A polymorphism and JIA **(A)** in the overall population, **(B)** in Caucasian ethnicity, and **(C)** in Middle East ethnicity. M-H: Mantel-Haenszel; CI: confidence interval.

version 5.3 (The Nordiac Cochrane Center, Copenhagen, Denmark) software, and the final results were presented as forest plots. *P* value of less than 0.05 was considered statistically significant.

## RESULTS

### *Study characteristics*

A total of 391 records were initially identified from the selected databases. A total of 378 duplicates, as well as irrelevant articles, were excluded after titles and abstracts were screened. The full texts of the remaining 13 articles were carefully evaluated. Eventually, 7 articles were included in the current meta-analysis [15, 17-22]. All of the 7 articles were published in English. Of the 7 eligible studies, 5 were carried out among Caucasian populations, and 2 were among Middle Eastern populations. Except for three studies [17, 20, 22], the genotype distributions in the controls for all studies were consistent with the Hardy-Weinberg equilibrium (*table 1*). According to the Newcastle-Ottawa Quality Assessment Scale, the results of quality assessment indicated that 3 studies scored 8 stars, 3 studies scored 7 stars, and 1 study scored 5 stars. The characteristics of all eligible studies are depicted in *table 2*.

### *Meta-analysis of the association between the IL-10 -592 C/A polymorphism and JIA*

The meta-analysis did not show any association of the *IL-10 -592* C/A polymorphism with JIA. Stratification by ethnicity did not indicate any association of the aforementioned SNP with JIA, neither in Caucasians nor in Middle Eastern participants (*table 3*).

### *Meta-analysis of the association between the IL-10 -819 C/T polymorphism and JIA*

A summary of meta-analyses results concerning associations between *IL-10 -819* C/T polymorphisms and JIA is demonstrated in *table 4*. The meta-analysis did not reveal any association of the *IL-10 -819* C/T polymorphism with JIA in any of the overall, Caucasian or Middle Eastern, populations.

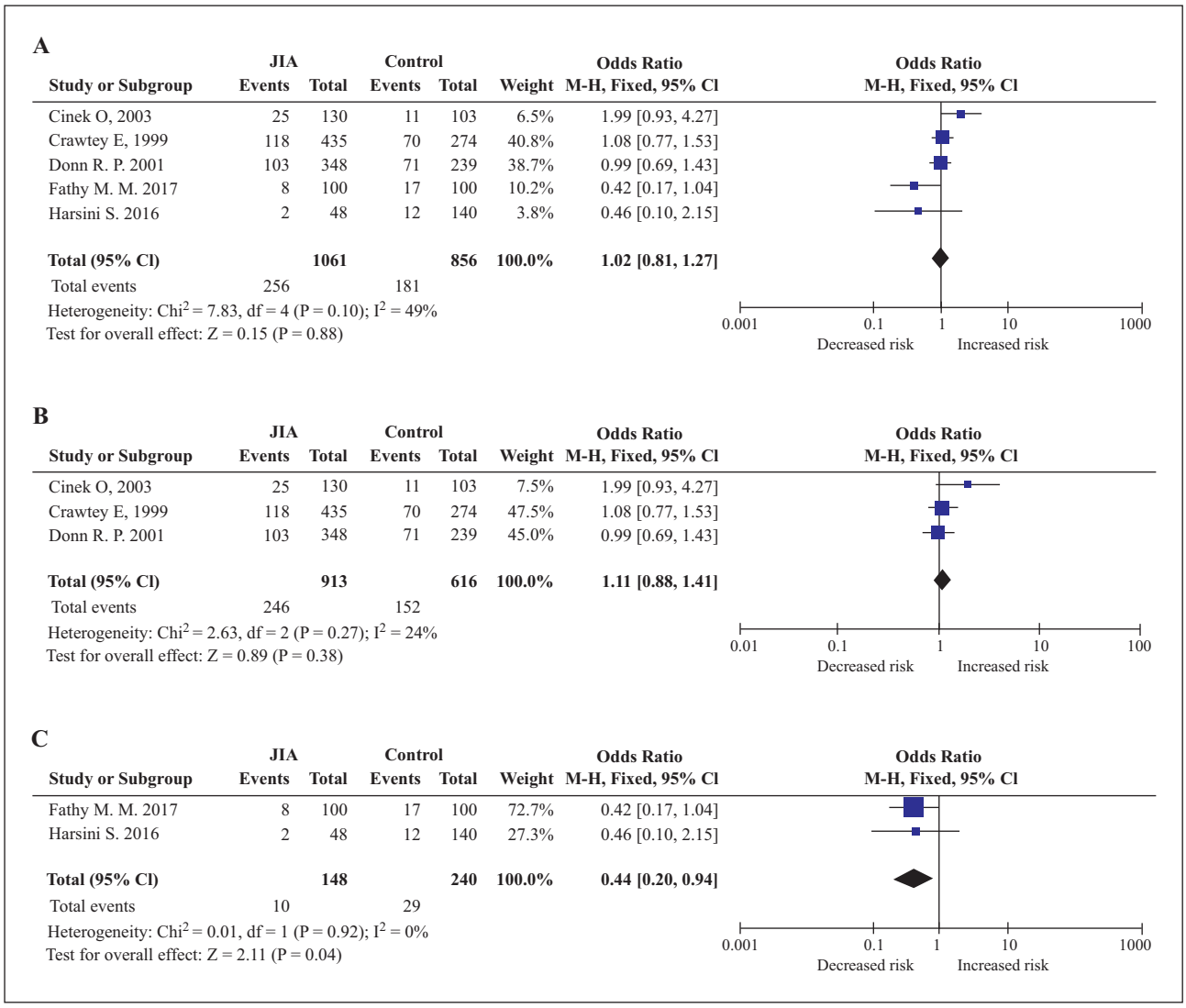


**Figure 4**

ORs and 95% CI of individual studies and pooled data for the association between the GG *versus* GA+AA genotypes of the *IL-10-1082* G/A polymorphism and JIA **(A)** in the overall population, **(B)** in Caucasian ethnicity, and **(C)** in Middle East ethnicity. M-H: Mantel-Haenszel; CI: confidence interval.

**Table 5**

Meta-analysis of associations between the *IL-10 -1082* G/A polymorphisms and JIA.

| | JIA | Controls | OR (95% CI) | Overall effect, *P* value | Heterogeneity (d.f.) |
|---|---|---|---|---|---|
| *Overall population* | | | | | |
| A *versus* G | 3,520 | 12,140 | 1.17 [1.07, 1.27] | Z = 3.50, *P* = 0.0005 | $I^2$ = 31% (6) |
| AA *versus* GG | 551 | 395 | 1.32 [0.75, 2.30]$^R$ | Z = 0.97, *P* = 0.33 | $I^2$ = 66% (4) |
| AA *versus* GA+GG | 1,061 | 856 | 1.30 [0.92, 1.83]$^R$ | Z = 1.51, *P* = 0.13 | $I^2$ = 53% (4) |
| GG *versus* AA+GA | 1,061 | 856 | 1.02 [0.81, 1.27] | Z = 0.15, *P* = 0.88 | $I^2$ = 49% (4) |
| *Caucasian ethnicity* | | | | | |
| A *versus* G | 3,224 | 11,660 | 1.15 [1.05, 1.25] | Z = 3.01, *P* = 0.003 | $I^2$ = 38% (4) |
| AA *versus* GG | 498 | 303 | 1.03 [0.78, 1.38] | Z = 0.23, *P* = 0.82 | $I^2$ = 50% (2) |
| AA *versus* GA+GG | 913 | 616 | 1.17 [0.80, 1.71]$^R$ | Z = 0.82, *P* = 0.41 | $I^2$ = 57% (2) |
| GG *versus* AA+GA | 913 | 616 | 1.11 [0.88, 1.41] | Z = 0.89, *P* = 0.38 | $I^2$ = 24% (2) |
| *Middle East ethnicity* | | | | | |
| A *versus* G | 296 | 480 | 1.41 [1.03, 1.92] | Z = 2.16, *P* = 0.03 | $I^2$ = 0% (1) |
| AA *versus* GG | 53 | 92 | 3.66 [1.44, 9.29] | Z = 2.73, *P* = 0.006 | $I^2$ = 0% (1) |
| AA *versus* GA+GG | 148 | 240 | 1.67 [1.01, 2.76] | Z = 2.00, *P* = 0.05 | $I^2$ = 59% (1) |
| GG *versus* AA+GA | 148 | 240 | 0.44 [0.20, 0.94] | Z = 2.11, *P* = 0.04 | $I^2$ = 0% (1) |

**Table 6**
Meta-analysis of associations between the *IL-10 (-1082, -819, and -592)* haplotypes and JIA.

| | JIA | Controls | OR (95% CI) | Overall effect, *P* value | Heterogeneity (d.f.) |
|---|---|---|---|---|---|
| *Haplotype* | | | | | |
| GCC | 1,676 | 1,306 | 0.92 [0.79, 1.07] | Z = 1.06, *P* = 0.29 | $I^2$ = 49% (2) |
| ACC | 1,676 | 1,306 | 0.96 [0.81, 1.13] | Z = 0.49, *P* = 0.63 | $I^2$ = 23% (2) |
| ATA | 1,676 | 1,306 | 1.08 [0.91, 1.28] | Z = 0.85, *P* = 0.40 | $I^2$ = 0% (2) |

**Table 7**
Meta-analysis of associations between the *IL-10 -1082* G/A, *-819* C/T, and *-592* C/A polymorphisms and JIA in the overall population in HWE.

| | JIA | Controls | OR (95% CI) | Overall effect, *P* value | Heterogeneity (d.f.) |
|---|---|---|---|---|---|
| *IL-10 -592 C/A* | | | | | |
| A *versus* C | 1,978 | 2,158 | 1.16 [1.00, 1.35] | Z = 1.95, *P* = 0.05 | = 0 % (3) |
| AA *versus* CC | 514 | 423 | 1.05 [0.66, 1.66] | Z = 0.19, *P* = 0.85 | = 0 % (2) |
| AA *versus* CA+CC | 832 | 653 | 0.98 [0.62, 1.55] | Z = 0.08, *P* = 0.94 | = 0 % (2) |
| CC *versus* AA+CA | 832 | 653 | 0.85 [0.68, 1.05] | Z = 1.52, *P* = 0.13 | = 0 % (2) |
| *IL-10 -819 C/T* | | | | | |
| C *versus* T | 1,924 | 1,512 | 0.96 [0.82, 1.13] | Z = 0.51, *P* = 0.61 | = 43 % (3) |
| CC *versus* TT | 593 | 476 | 1.03 [0.66, 1.59] | Z = 0.13, *P* = 0.90 | = 0 % (3) |
| CC *versus* CT+TT | 963 | 756 | 0.92 [0.76, 1.13] | Z = 0.79, *P* = 0.43 | = 47 % (3) |
| TT *versus* CC+CT | 963 | 756 | 0.94 [0.61, 1.44] | Z = 0.30, *P* = 0.76 | = 0 % (3) |
| *IL-10 -1082 G/A* | | | | | |
| A *versus* G | 2,964 | 11,454 | 1.17 [1.07, 1.28] | Z = 3.31, *P* = 0.0009 | = 25 % (3) |
| AA *versus* GG | 445 | 271 | 1.10 [0.82, 1.49] | Z = 0.64, *P* = 0.52 | = 57 % (1) |
| AA *versus* GA+GG | 783 | 513 | 1.21 [0.70, 2.09]$^R$ | Z = 0.68, *P* = 0.49 | $I^2$ = 78% (1) |
| GG *versus* AA+GA | 783 | 513 | 1.04 [0.81, 1.34] | Z = 0.32, *P* = 0.75 | = 0% (1) |

$^R$ indicates random-effects analysis.

### *Meta-analysis of the association between the IL-10 -1082 G/A polymorphism and JIA*

Meta-analyses findings concerning associations between *IL-10 -1082* G/A polymorphisms and JIA are shown in *table 5* and *figures 2-4*.

Meta-analysis of the *IL-10 -1082* A allele revealed significant associations with JIA in all study participants (OR = 1.17, 95% CI = 1.07-1.27, *P* = 0.0005). Such positive association between *IL-10 -1082* A allele with JIA was also observed in the Caucasian ethnicity (OR = 1.15, 95% CI = 1.05-1.25, *P* = 0.003). Two studies were included for the subgroup analysis of the Middle East ethnicity [20, 22]. The *IL-10 -1082* A allele was associated with JIA in the Middle Eastern participants (OR = 1.41, 95% CI = 1.03-1.92, *P* = 0.03). Meta-analysis of the AA versus GG genotypes of the *IL-10 -1082* G/A polymorphism revealed significant associations with JIA (OR = 3.66, 95% CI = 1.44-9.29, *P* = 0.006) in Middle Eastern participants. Additionally, meta-analysis of the GG versus AA+GA genotypes of the *IL-10 -1082* G/A polymorphism revealed the GG genotype as the protective factor against JIA in the same subgroup (OR = 0.44, 95% CI = 0.20-0.94, *P* = 0.04). In addition, meta-analysis of the AA versus GG+GA genotypes of the *IL-10 -1082* G/A polymorphism divulged a marginal significance, as the AA genotype could be associated with JIA (OR = 1.67, 95% CI = 1.01-2.76, *P* = 0.05).

### *Meta-analysis of the association between the IL-10 (-1082, -819, -592) haplotypes and JIA*

As depicted in *table 6*, no association of the *IL-10 (-1082, -819, -592)* ACC, ATA, and GCC haplotypes with JIA was found through the meta-analysis.

### *Heterogeneity, sensitivity analysis, and publication bias*

Moderate heterogeneity was found in a few meta-analyses of *IL-10 -819* C/T and *-1082* G/A polymorphisms. Publication bias was examined by Begg's funnel plot and Egger's test. The shape of the generated funnel plots seemed symmetrical, signifying the absence of publication bias. Then, the Egger's test was conducted to provide statistical evidence of funnel plot asymmetry. The results revealed no significant evidence of publication bias of the present meta-analysis (Egger's test *P*-values >0.1).
Sensitivity analysis, which has been performed to assess the stability of the results of the meta-analysis by excluding studies inconsistent with HWE, did not affect our results, suggesting the stability of this meta-analysis (*table 7*).

## DISCUSSION

In view of the multifactorial nature of JIA, genetic factors, regarded as the strong determinants of this disease, have been a focus of research in recent years. Several genes

involved in the pathogenesis of JIA were considered as probable JIA risk factors, among which, the *IL-10* gene is one of the most extensively studied. The production of IL-10, which is known to be a potent anti-inflammatory cytokine that acts through the inhibition of the proinflammatory cytokines' synthesis, upregulation of B-cell production and differentiation [28], suppression of the joint swelling and deformation, as well as the cartilage necrosis, is genetically regulated and is controlled at the transcription level by means of certain regulatory regions in its promoter [29]. An increasing number of studies have recommended that the *IL-10* -1082 G/A, -819 C/T, and -592 C/A polymorphisms are associated with individuals' susceptibility to JIA. Nonetheless, the results are unconvincing. Due to the insufficient statistical power of individual studies with a small sample size to successfully establish the reliable association, there is a need for meta-analysis to detect the relationship of these polymorphisms with JIA proneness.

In this meta-analysis, including 1,785 JIA patients and 6,142 controls from 7 case-control studies, we addressed the association between IL-10 polymorphisms and JIA proneness. Data from published studies were pooled to examine genetic associations between the most frequently studied polymorphisms of the *IL-10* gene and JIA. Meta-analysis of the *IL-10* -592 C/A and -819 C/T polymorphisms displayed no association with JIA in all study participants, Caucasian or Middle Eastern. Conversely, meta-analysis of the *IL-10* -1082 A allele revealed a significant association with JIA in all study participants, as well as in both the Caucasian and Middle Eastern subgroups. In this meta-analysis, we also assessed the association between this polymorphism by using the additive, recessive, and dominant genetic models. In all of these three models, we observed significant associations while analyzing the Middle East population. According to these results, we could conclude that -1082 A allele carriers might be more susceptible to JIA in the Middle East ethnic group.

The *IL-10* -1082 G/A polymorphism is positioned within a putative Ets transcription factor-binding site, modifying the level of IL-10 protein production [30]. It has been previously indicated that the -1082 A allele could be associated with lower IL-10 synthesis, while the -1082 G allele might be associated with elevated *in vitro* IL-10 production [31]. Recent investigations on patients with JIA have shown the reduced production of IL-10 from whole blood culture [32], which may recommend a correlation between the -1082 A allele and individuals' vulnerability to JIA. The result of the present meta-analysis, indicating the -1082 A allele as a probable risk factor for JIA, was in line with the data provided earlier. However, bearing in mind the complex etiology of JIA, which involves both the genetic and environmental factors, further studies are required to be performed to assess the exact mechanisms.

Our results should be interpreted with caution due to the restricted number of studies included, which also constrained further subgroup analyses. In addition, the distributions of the -592 C/A as well as the -819 C/T genotypes in the normal control groups did not meet the requirement for HWE in one study. Moreover, the distributions of the -1082 G/A genotypes in the normal control groups were found to be inconsistent with the HWE in three investigations. Subgroup analysis for studies in HWE has been reperformed, as a deviation from HWE among controls indicates genotyping errors or potential bias during control selection. For the remaining studies in HWE, meta-analysis depicted significant associations between the -1082 A allele and JIA in the overall population. However, it should be noted that this association was based on the results of a few studies only.

Previous studies have indicated linkage disequilibrium for the -1082 G/A, -819 C/T, and -592 C/A polymorphisms. The common GCC haplotype was found to be associated with an elevated level of Il-10 secretion, while the least common ATA haplotype has been correlated with reduced IL-10 production [28]. Thus, the meta-analysis of haplotypes was performed in this study and culminated in no significant association between the well-known GCC, ACC, and ATA haplotypes and JIA. However, this result should be taken into account with caution due to the inadequacy of haplotype data.

The current study has some limitations that should be acknowledged when explaining the results. First, as the studies producing negative results may have been missed or may not have been published, publication bias may have influenced the analysis, though we conducted Begg's and Egger's tests. Hence, we could not disregard the possibility of bias. Additionally, confounding factors and heterogeneity might place a bias on our findings. Second, only publications in English were included in this meta-analysis. Therefore, it is possible that some relevant studies or publications in other languages were unexploited, which may have misled the meta-analysis. Third, all studies except two were performed in Caucasian ethnicity, so our results are not applicable to other ethnicities. Fourth, as the insufficient data available did not allow us to perform further meta-analysis, data were not stratified by other factors such as JIA severity.

## CONCLUSION

In conclusion, this study, to the best of our knowledge, is the first meta-analysis to consider the association between the -1082 G/A, -819 C/T, and -592 C/A polymorphisms in the *IL-10* gene and the susceptibility to JIA. This study advocates that the *IL-10* -1082 G/A polymorphism confers susceptibility to JIA, supporting the hypothesis that the *IL-10* -1082 A allele may act as a risk factor for JIA. Nevertheless, our findings should be interpreted with caution owing to a small number of investigations included. Additional larger-scale studies in populations with different ethnicities are warranted to validate our results.

***Disclosure.*** *Financial support: none. Conflict of interest: none.*

## REFERENCES

1. Symmons D, Jones M, Osborne J, Sills J, Southwood T, Woo P. Pediatric rheumatology in the United Kingdom: data from the British Pediatric Rheumatology Group National Diagnostic Register. *J Rheumatol* 1996; 23: 1975.

2. Petty RE, Southwood TR, Baum J, *et al*. Revision of the proposed classification criteria for juvenile idiopathic arthritis: Durban, 1997. *J Rheumatol* 1998; 25: 1991.

3. Donn R, Barrett J, Farhan A, *et al*. Cytokine gene polymorphisms and susceptibility to juvenile idiopathic arthritis. *Arthritis Rheumatol* 2001; 44: 802.

4. Harsini S, Ziaee V, Maddah M, *et al*. Interleukin 10 and transforming growth factor beta 1 gene polymorphisms in juvenile idiopathic arthritis. *Bratisl Lek Listy* 2016; 117: 258.

5. Maddah M, Harsini S, Rezaei A, *et al*. Association of interleukin-2, but not interferon-gamma, single nucleotide polymorphisms with juvenile idiopathic arthritis. *Allergol Immunopathol (Madr)* 2016; 44: 303.

6. Maddah M, Harsini S, Ziaee V, *et al*. Association of tumour necrosis factor-alpha G/A -238 and G/A -308 single nucleotide polymorphisms with juvenile idiopathic arthritis. *Int J Immunogenet* 2016; 43: 391.

7. Ziaee V, Maddah M, Harsini S, *et al*. Association of interleukin-1 family gene polymorphisms with juvenile idiopathic arthritis in Iranian population. *Allergol Immunopathol (Madr)* 2016; 44: 542.

8. Ziaee V, Maddah M, Moradinejad MH, *et al*. Association of interleukin-6 single nucleotide polymorphisms with juvenile idiopathic arthritis. *Clin Rheumatol* 2017; 36: 77.

9. Ziaee V, Rezaei A, Harsini S, *et al*. Polymorphisms of genes encoding interleukin-4 and its receptor in Iranian patients with juvenile idiopathic arthritis. *Clin Rheumatol* 2016; 35: 1943.

10. Cassatella MA, Meda L, Bonora S, Ceska M, Constantin G. Interleukin 10 (IL-10) inhibits the release of proinflammatory cytokines from human polymorphonuclear leukocytes. Evidence for an autocrine role of tumor necrosis factor and IL-1 beta in mediating the production of IL-8 triggered by lipopolysaccharide. *J Exp Med* 1993; 178: 2207.

11. Joosten LA, Lubberts E, Durez P, *et al*. Role of interleukin-4 and interleukin-10 in murine collagen-induced arthritis. Protective effect of interleukin-4 and interleukin-10 treatment on cartilage destruction. *Arthritis Rheum* 1997; 40: 249.

12. van Roon JA, van Roy JL, Gmelig-Meyling FH, Lafeber FP, Bijlsma JW. Prevention and reversal of cartilage degradation in rheumatoid arthritis by interleukin-10 and interleukin-4. *Arthritis Rheum* 1996; 39: 829.

13. Jorgensen C, Apparailly F, Couret I, Canovas F, Jacquet C, Sany J. Interleukin-4 and interleukin-10 are chondroprotective and decrease mononuclear cell recruitment in human rheumatoid synovium *in vivo*. *Immunology* 1998; 93: 518.

14. Kawakami A, Eguchi K, Matsuoka N, *et al*. Inhibitory effects of interleukin-10 on synovial cells of rheumatoid arthritis. *Immunology* 1997; 91: 252.

15. Omoyinmi E, Forabosco P, Hamaoui R, *et al*. Association of the IL-10 gene family locus on chromosome 1 with juvenile idiopathic arthritis (JIA). *PLoS One* 2012; 7: e47673.

16. Eskdale J, Wordsworth P, Bowman S, Field M, Gallagher G. Association between polymorphisms at the human IL-10 locus and systemic lupus erythematosus. *Tissue Antigens* 1997; 49: 635.

17. Cinek O, Vavřincová P, Striz I, *et al*. Association of single nucleotide polymorphisms within cytokine genes with juvenile idiopathic arthritis in the Czech population. *J Rheumatol* 2004; 31: 1206.

18. Crawley E, Kay R, Sillibourne J, Patel P, Hutchinson I, Woo P. Polymorphic haplotypes of the interleukin-10 5' flanking region determine variable interleukin-10 transcription and are associated with particular phenotypes of juvenile rheumatoid arthritis. *Arthritis Rheum* 1999; 42: 1101.

19. Donn RP, Barrett JH, Farhan A, *et al*. Cytokine gene polymorphisms and susceptibility to juvenile idiopathic arthritis. *Arthritis Rheum* 2001; 44: 802.

20. Fathy MM, Elsaadany HF, Ali YF, *et al*. Association of IL-10 gene polymorphisms and susceptibility to juvenile idiopathic arthritis in Egyptian children and adolescents: a case-control study. *Ital J Pediatr* 2017; 43: 9.

21. Fife MS, Gutierrez A, Ogilvie EM, *et al*. Novel IL10 gene family associations with systemic juvenile idiopathic arthritis. *Arthritis Res Ther* 2006; 8: R148.

22. Harsini S, Ziaee V, Maddah M, *et al*. Interleukin 10 and transforming growth factor beta 1 gene polymorphisms in juvenile idiopathic arthritis. *Bratisl Lek Listy* 2016; 117: 258.

23. Wells G, Shea B, O'connell D, *et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses*. Oxford: Ottawa Hospital Research Institute, 2014, 2015.

24. Higgins J, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002; 21: 1539.

25. Minelli C, Thompson JR, Abrams KR, Thakkinstian A, Attia J. How should we use information about HWE in the meta-analyses of genetic association studies? *Int J Epidemiol* 2007; 37: 136.

26. Rodriguez S, Gaunt TR, Day IN. Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. *Am J Epidemiol* 2009; 169: 505.

27. Thakkinstian A, McElduff P, D'Este C, Duffy D, Attia J. A method for meta-analysis of molecular association studies. *Stat Med* 2005; 24: 1291.

28. Taga K, Tosato G. IL-10 inhibits human T cell proliferation and IL-2 production. *J Immunol* 1992; 148: 1143.

29. Tarzi M, Klunker S, Texier C, *et al*. Induction of interleukin-10 and suppressor of cytokine signalling-3 gene expression following peptide immunotherapy. *Clin Exp Allergy* 2006; 36: 465.

30. Hee CS, Gun SC, Naidu R, Gupta E, Somnath SD, Radhakrishnan AK. Comparison of single nucleotide polymorphisms in the human interleukin-10 gene promoter between rheumatoid arthritis patients and normal subjects in Malaysia. *Mod Rheumatol* 2007; 17: 429.

31. Martinez A, Pascual M, Pascual-Salcedo D, Balsa A, Martin J, De la Concha E. Genetic polymorphisms in Spanish rheumatoid arthritis patients: an association and linkage study. *Genes Immun* 2003; 4: 117.

32. Müller K, Herner E, Stagg A, Bendtzen K, Woo P. Inflammatory cytokines and cytokine antagonists in whole blood cultures of patients with systemic juvenile chronic arthritis. *Br J Rheumatol* 1998; 37: 562.