Association between STAT4 polymorphisms and the risk of juvenile idiopathic arthritis in Han Chinese populations

X. Huang[1], Z. Wang[2], N. Jia[1], S. Shangguan[2], J. Lai[3], X. Cui[1], F. Wu[3], L. Wang[2]

[1]Laboratory of Institute, [2]Molecular Immunity Department, [3]Department of Rheumatism and Immunity, Capital Institute of Paediatrics, Beijing, China.

Abstract

Objective

Previous studies have demonstrated a potential role of STAT4 polymorphisms in increased juvenile idiopathic arthritis (JIA) risk in Caucasian populations; however, their role remains unclear in Han Chinese populations. We aimed to investigate single nucleotide polymorphisms (SNPs) of STAT4 and their role in JIA in Han Chinese populations.

Methods

This study included 205 JIA cases and 267 healthy controls. MassArray high-throughput DNA analyser and mass spectrometry were used to analyse 16 STAT4 SNP sites. The relationship between these SNPs and JIA risk was calculated using multiple logistic regressions.

Results

The G allele of rs11893432 was associated with an increased risk of JIA (odds ratio [OR]: 1.73; 95% confidence interval [CI]: 1.03–2.88; p=0.037). This relationship was observed in oligoarticular JIA (OR: 2.75; 95% CI: 1.29–5.83; p=0.026), and not in polyarticular JIA or systemic JIA. The GG motif was significantly correlated with oligoarticular JIA risk, compared to the CC+CG motif (OR: 1.88; 95% CI: 1.06–3.32; p=0.034). The C allele of rs1018981 and the A allele of rs10931481 were associated with a greater risk of polyarticular JIA (C allele: [OR: 7.82; 95% CI: 1.06–57.74; p=0.044]; A allele: [OR: 2.86; 95% CI: 1.23, 6.65; p=0.039).

Conclusion

The G allele of rs11893432 was significantly associated with JIA risk, particularly oligoarticular JIA, in Han Chinese populations. SNPs at rs1018981 and rs10931481 were correlated with higher risk of polyarticular JIA.

Key words

STAT4 polymorphisms, juvenile idiopathic arthritis, Han Chinese populations

*Xiaolan Huang, MS**
*Zhen Wang, MS**
Nan Jia, BS
Shaofang Shangguan, MS
Jianming Lai, MS
Xiaodai Cui, MS
*Fengqi Wu, MS***
*Li Wang, MD***

**Co-first authors and contributed equally to this work.*

***Co-corresponding authors and contributed equally to this work.*

Please address correspondence to:
Dr Fengqi Wu,
Department of Rheumatism and Immunity,
Capital Institute of Paediatrics,
2 Yabao Road, Chaoyang District,
Beijing 100020, China.
E-mail: fengqiwu112@2008.sina.com

Dr Li Wang,
Molecular Immunity Department (address as above)
E-mail: liaosy1980@163.com

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Introduction

Juvenile idiopathic arthritis (JIA) is a common autoimmune disease, characterised by heterogeneous chronic arthritis in children aged ≤16 years (1). The prevalence of JIA is approximately 10 per 100,000 children among Caucasian populations (2). The major subtypes of JIA are oligoarticular JIA, polyarticular JIA, systemic JIA, enthesitis-related arthritis, psoriatic JIA, and other JIA with no single subtype criteria met. Oligoarticular JIA is most common in Europe, while polyarticular JIA is more common in India and New Zealand (3). The aetiology of JIA remains unknown, and both genetic and environmental factors are correlated with the progression of JIA (4).

A linkage peak was observed for rheumatoid arthritis (RA) in chromosome 2q in families of European ancestry. Several polymorphisms in the third intron of the signal transducer and activator of transcription 4 (*STAT4*) gene are correlated with RA. Four SNPs (rs11889341, rs7574865, rs8179673, and rs10181656) are strongly correlated with RA risk in Caucasians (5). Confirmed RA susceptibility loci are excellent JIA candidate susceptibility loci, because RA shares clinical and pathological features with JIA. *STAT4* is expressed in activated peripheral blood monocytes, dendritic cells, and macrophages at the sites of inflammation in humans. *STAT4* is activated by IL-12, leading to Th type 1 and Th type 17 differentiation, monocyte activation, and IFN-ɣ production (6, 7). *STAT4*-deficient mice showed less disease severity and decreased inflammation as compared to wild-type mice in a proteoglycan-induced model of arthritis (8). *STAT4* may play a key regulatory role in the pathogenesis and manifestation of RA.

Numerous studies have illustrated race-related differences in the relationship between non-HLA susceptibility and RA. The PTPN22 risk allele (R620W, rs2476601) is associated with RA in Caucasian RA cohorts, but not in non-white populations (9-11). To evaluate the relationship between *STAT4* SNPs and JIA, we studied 16 SNPs among 205 JIA cases and 267 controls in Han Chinese subjects. Stratified analysis was conducted based on JIA subtypes due to the heterogeneous clinical phenotype of JIA.

Materials and methods

Study design and study subjects

A case-control study was designed to identify possible associations between *STAT4* SNPs and JIA susceptibility. JIA and control samples were obtained from Beijing, China, and all participants were of Han Chinese decent. JIA cases were obtained from Capital Institute of Paediatrics affiliated hospitals in the same area from 2010 to 2014. Diagnosis of JIA was performed by experienced paediatric rheumatologists according to the International Classification of Disease guidelines (1). The subtypes of JIA (n=205) included 39 polyarticular JIA, 76 oligoarticular JIA, 52 systemic JIA, and 38 other subtype. Individuals in the control group (n=267) were selected from children matched by region, gender, and age. Children with a family history of autoimmune and inflammatory diseases were excluded from the control group. This study was reviewed and approved by the Research Ethics Committee of the Capital Institute of Paediatrics (IRB00008963), and written informed consent was obtained from all participants or their parents. The objectives and procedures of this study were explained to all participants and their parents. All potential participants who declined to participate or did not participate were eligible for treatment and were not disadvantaged in any way by not participating in the study.

DNA extraction

All blood samples were stored at -20°C before being shipped on ice to the laboratory. Genomic DNA was extracted from frozen samples using a Maxwell 16 system (Promega, Madison, WI, USA), according to the manufacturer's instructions. The concentration and purity of DNA samples was determined by measuring their absorbance at 260 and 280 nm.

Genotyping

SNPs (n=16), including seven tag SNPs, spanning the *STAT4* gene were

Table I. Baseline characteristics of juvenile idiopathic arthritis (JIA) patients according to subtypes.

Characteristic	Polyarticular JIA	Oligoarticular JIA	Systemic JIA	Other subtype
n	39	76	52	38
Age (year)	8.18±2.50	6.97±3.99	8.71±3.78	8.43±4.15
Gender				
Male	24 (61.54)	41 (53.95)	15 (28.85)	16 (42.11)
Female	15 (38.46)	35 (40.05)	37 (71.15)	22 (57.89)

selected for evaluation using Haploview software (v. 3.32; http://www.broad.mit.edu/mpg/haploview/). Additional informative SNPs (n=9) were selected from the National Center for Biotechnology Information (NCBI) SNP database (www.ncbi.nlm.nih.gov/SNP). The SNPs rs925847, rs3024894, rs6252770, rs7572482, rs10174238, rs10189819, rs10931481, rs11685878, and rs16833215 were selected for analysis. Genotyping was conducted with a MassArray high-throughput DNA analyser and matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry (Sequenom, San Diego, CA, USA). Genotyping was repeated and 10% of samples were sequenced to validate the genotyping consistency. Amplifications were conducted according to the manufacturer's protocol.

Prediction of binding factor

The Transcription Element Search System (TESS, http://www.cbil.upenn.edu/cgi -bin/tess/tess) was employed to predict the binding factor of DNA sequences. This system can identify binding sites using site or consensus strings and positional weight matrices from the TRANSFAC, JASPAR, IMD, and CBIL-GibbsMat databases.

Statistical analysis

The population Hardy-Weinberg equilibrium was assessed using Chi-square tests. Clinical continuous variables are expressed as mean ± SD, and differences between groups were assessed using a Mann-Whitney U-test or Wilcoxon signed-rank test. Patients in each case group were divided into four major types: polyarticular JIA, oligoarticular JIA, systemic JIA, and other subtypes. Genotype and allele frequencies for each SNP were compared between cases and controls using a Chi-squared test. Multiple logistic regression analysis, based on age and gender, was calculated to estimate adjusted odds ratios (ORs) and 5% confidence intervals (CIs). All reported *p*-values are 2-sided, and *p*-values <0.05 were considered statistically significant. Data were analysed with SPSS 16.0 (McGraw-Hill Inc., New York, NY, USA).

Quality control

Negative controls and 60% duplicate samples were included to check the genotyping quality. Analysis was only performed when the genotyping accuracy was >90%.

Results

Subjects

The average age was 7.28 and 8.04 in the case and control groups, respectively. The study cohort contained 108 males and 101 females in the case group, and 142 males and 125 females in the control group. There were no significant differences in age between the two study groups, but the gender distribution was statistically significant (p=0.037). The clinical characteristics of each subtype of JIA are presented in Table I.

Genotype distribution of rs11893432 in STAT4 polymorphisms

The associations between the rs11893432 genotype in *STAT4* and the risk of JIA are listed in Table II. First, we noted that GG was associated with an increased risk of JIA compared with that of CC (OR: 1.73; 95% CI: 1.03–2.88), while no other significant difference of genotype or allele were found between the case and control group. Second, there was no significant association between rs11893432 and the risk of polyarticular JIA. Third, GG was associated with greater risk of oligoarticular JIA compared with that of CC (OR: 2.75; 95% CI: 1.29–5.83) or GC+CC (OR: 1.88; 95% CI: 1.06–3.32). The G allele of rs11893432 was associated with an increased risk of oligoarticular JIA compared with that of the C allele of rs11893432 (OR: 2.13; 95% CI: 1.10–4.11). Finally, rs11893432 had no significant impact on the risk of systemic JIA and other JIA.

Gender stratified analysis was also performed, the results of which are shown in Supplemental material (Table S1). We noted that GG was associated with an increased risk of JIA compared with CC in female subjects (OR: 2.18; 95%

Table II. Genotype and allele counts for rs11893432 variants in the control and juvenile idiopathic arthritis (JIA) participants.

Control	JIA			Polyarticular JIA			Oligoarticular JIA			Systemic JIA			Other subtype		
n	n	*p*-value	OR (95%CI)	n	*p*-value	OR (95%CI)	n	*p*-value	OR (95%CI)	n	*p*-value	OR (95%CI)	n	*p*-value	OR (95%CI)
CC 80	48	0.037	1	11	0.890	1	13	0.026	1	16	0.980	1	8	0.280	1
CG 109	89		1.36 (0.86-2.14)	18		1.20 (0.54-2.68)	32		1.81 (0.89-3.66)	22		1.01 (0.50-2.04)	17		1.56 (0.64-3.79)
GG 56	58		1.73 (1.03-2.88)	8		1.04 (0.39-2.75)	25		2.75 (1.29-5.83)	12		1.07 (0.47-2.44)	12		2.14 (0.82-5.58)
C80	48	0.064	1	11	0.720	1	13	0.018	1	16	0.930	1	8	0.160	1
G	165	147	1.48 (0.97-2.26)	26		1.15 (0.54-2.44)	57		2.13 (1.10-4.11)	34		1.03 (0.54-1.98)	29		1.76 (0.77-4.02)
CC+CG 189	37	0.100	1	29	0.870	1	45	0.034	1	38	0.860	1	25	0.220	1
GG	56	58	1.43 (0.93-2.19)	8		0.93 (0.40-2.15)	25		1.88 (1.06-3.32)	12		1.07 (0.52-2.18)	12		1.62 (0.77-3.43)

Table III. Genotype and allele counts for rs10189819 and rs10931481 variants in control and juvenile idiopathic arthritis (JIA) subtypes.

Control			Polyarticular JIA			Oligoarticular JIA			Systemic JIA			Other subtype		
		n	n	*p*-value	OR (95%CI)	n	*p*-value	OR (95%CI)	n	*p*-value	OR (95%CI)	n	*p*-value	OR (95%CI)
	TT	219	28	0.13	1	62	0.58	1	41	0.55	1	28	0.78	1
rs10189819	CT	25	2		0.63 (0.14-2.78)	6		0.85 (0.33-2.16)	3		0.64 (0.18-2.22)	3		0.94 (0.27-3.31)
	CC	2	2		7.82 (1.06-57.74)	0		0.00 (0.00-NA)	0		0.00 (0.00-NA)	0		0.00 (0.00-NA)
	TT	219	28	0.8	1	62	0.6	1	41	0.38	1	28	0.82	1
	CT-CC	27	4		1.16 (0.38-3.56)	6		0.78 (0.31-1.99)	3		0.59 (0.17-2.05)	3		0.87 (0.25-3.05)
	TT-CT	244	30	0.055	1	68	0.32	1	44	0.42	1	31	0.49	1
	CC	2	2		8.13 (1.10-59.88)	0		0.00 (0.00-NA)	0		0.00 (0.00-NA)	0		0.00 (0.00-NA)
rs10931481	GA	107	9	0.039	1	31	0.96	1	27	0.22	1	20	0.23	1
	AA	79	19		2.86 (1.23-6.65)	21		0.92 (0.49-1.72)	13		0.65 (0.32-1.34)	11		0.74 (0.34-1.64)
	GG	62	8		1.53 (0.56-4.18)	18		1.00 (0.52-1.94)	8		0.51 (0.22-1.19)	5		0.43 (0.15-1.21)
	GA	107	9	0.034	1	31	0.87	1	27	0.096	1	20	0.16	1
	AA-GG	141	27		2.28 (1.03-5.04)	39		0.95 (0.56-1.63)	21		0.59 (0.32-1.10)	16		0.61 (0.30-1.23)
	GA-AA	186	28	0.72	1	52	0.9	1	40	0.2	1	31	0.12	1
	GG	62	2		0.86 (0.37-1.98)	18		1.04 (0.57-1.91)	8		0.60 (0.27-1.35)	5		0.48 (0.18-1.30)

CI: 1.04–4.59), while this significant association was not observed in males. The GC genotype was correlated with greater risk of oligoarticular JIA in females (OR: 2.89; 95% CI: 1.07–7.81), while there was no significant correlation between the GC genotype and the risk of oligoarticular JIA in males.

Genotype distribution of rs10189819 and rs10931481 in STAT4 polymorphisms

The genotype distribution of rs10189819 and rs10931481 in *STAT4* polymorphisms in the case and control group is presented in Table III. Overall, we noted that the CC allele of rs10189819 was related to a greater risk of polyarticular JIA compared with that of TT (OR: 7.82; 95% CI: 1.06–57.74) or TT+CT (OR: 8.13; 95% CI: 1.10–59.88). The AA (OR: 2.86; 95% CI: 1.23–6.65) or AA+GG (OR: 2.28; 95% CI: 1.03–5.04) alleles of rs10931481 were associated with an increased risk of polyarticular JIA as compared with that of GA. No other significant relationships were observed between *STAT4* SNPs and increased risk of a specific JIA subtype. Stratified analysis of polyarticular JIA indicated that the AA allele of rs10931481 was correlated with greater risk of polyarticular JIA compared with that of the GA allele in males, while this significant relationship was not observed in females. There were no significant relationship between rs10189819 and the risk of polyarticular JIA in males and females (Supplementary material [Table S2]).

Table IV. Associations between haplotypes and risk of juvenile idiopathic arthritis (JIA).

Haplotype	Case fre	Control fre	OR(95%CI)	*p*-value
ACT	0.404	0.4017	1	
GGT	0.3794	0.3214	1.19(0.89-1.60)	0.24
AGT	0.1312	0.1457	1.18(0.75-1.85)	0.47
GCT	0.1008	0.0558	0.42(0.23-0.74)	0.0033
ACC	0.0143	0.0151	1.08(0.32-3.64)	0.9

Distribution of STAT4 haplotype frequencies

Each SNP site involved with JIA risk was investigated for linkage disequilibrium. Evidence for linkage disequilibrium was observed for rs10931481 C>T, rs11893432 A>T and rs12618242 G>A. The three SNPs generated five common haplotypes, and the global haplotype distribution was significantly different between the cases and controls. The GCT haplotype was associated with a reduced risk of JIA compared with that of ACT (OR: 0.42; 95% CI: 0.23–0.74; p=0.0033; Table IV).

Discussion

In the present case control study, we recruited a cohort of 205 JIA cases and 267 healthy controls to explore possible correlations between *STAT4* SNPs and the risk of JIA in Han Chinese populations. The primary novel finding of the present study was that the GG allele of rs11893432 was correlated with higher JIA risk compared with that of the CC allele, especially for oligoarticular JIA. The G allele of rs11893432 was correlated with greater risk of oligoarticular JIA as compared with that of the C allele. The most significant correlation between the GG or GC alleles of rs11893432 on JIA or oligoarticular JIA risk was observed in females, while this relationship was not detected in males. Furthermore, the CC allele of rs10189819 was associated with greater risk of polyarticular JIA compared with that of the TT or TT+CT alleles. Similarly, the AA or AA+GG alleles of rs10931481 were related to higher risk of polyarticular JIA compared with that of the GA allele. The AA allele of rs10931481 correlated with a greater polyarticular JIA risk than that of the GA allele, primarily in males. Finally, we noted that the GCT haplotype plays an important protective role on the risk of JIA as compared with that of the ACT haplotype.

There has been abundant research on the impact of *STAT4* on the incidence of JIA. Alberdi-Saugstrup *et al*. found that the TT allele of rs7574865 of *STAT4* was correlated with greater risk of actively inflamed joints and extra-articular damage in Caucasians (12). However, Dimopoulou *et al*. reported no significant association between *STAT4* rs7574865 and JIA risk in a Greek population (13). Prahalad *et al*. found that *STAT4* rs7574865 was correlated with the risk of JIA, RA, and systemic lupus erythematosus in USA (5). Fan *et al*. conducted a case control study including 137 Chinese JIA patients and 150 gender and age frequency-matched healthy volunteers and found that the G/T allele of rs7574865 of *STAT4* was a significant risk factor for enthesitis-related arthritis and hepatomegaly in Han Chinese patients (14). Two meta-analyses have also demonstrated that the T allele of rs7574865 in *STAT4* was associated with autoimmune diseases, including systemic lupus erythematosus, RA, type 1 diabetes, systemic sclerosis, inflammatory bowel diseases, primary Sjögren syndrome, and JIA (15, 16). A possible role of rs7574865 of *STAT4* could be IL-12 signalling, which promotes the differentiation of naive CD4+ T cells into Th 1 cells, which could produce IFN-γ (17). This factor plays an important role in pathogenic T cells during JIA. However, the role of additional *STAT4* SNPs in the progression of JIA remains unknown. We conducted this case control study to evaluate any potential role of *STAT4* SNPs on JIA in Han Chinese populations.

We selected the three SNPs based on an in-depth study of the functions of *STAT4* SNPs and focused on those likely to alter *STAT4* gene transcription or translation. We used AliBaba 2.1 software to predict the putative regulatory elements in the *STAT4* promoter region and found that the 2,000 bp region had several putative transcription factor binding sites. The G allele of rs11893432 was associated with an increased risk of JIA, especially oligoarticular JIA. This relationship was significant in females, with no observed relationship in males. The C allele of rs10189819 was associated with higher polyarticular JIA risk. Finally, the homozygote of rs10931481 was associated with significantly increased polyarticular JIA risk, and this significant association was observed in males only. This may be due to different distributions of cases and controls in each gender group, which were associated with 95% CI. Further, these significantly sex differences between STAT4 polymorphisms and JIA risk might affect by sex hormones CD4 lymphocytes, and Th1 cytokines, which could directly influence immune system (18, 19). This phenomenon has already demonstrated in several other SNPs, which through examining allele expression in peripheral blood mononuclear cells among various populations (20, 21).

In conclusion, the G allele of rs11893432 was associated with greater risk of JIA, especially oligoarticular JIA, in Han Chinese populations. The C allele of rs1018981 and the A allele of rs10931481 were similarly correlated with higher risk of polyarticular JIA. Although the data were reliable and this study had >80% power to replicate previous results, an independent replicate of this study is required in a Han Chinese population to further confirm these findings. Functional studies should also be undertaken to verify the findings of this case control study.

References

1. PETTY RE, SOUTHWOOD TR, MANNERS P *et al*.: International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. *J Rheumatol* 2004; 31: 390.
2. MANNERS PJ, BOWER C: Worldwide prevalence of juvenile arthritis why does it vary so much? *J Rheumatol* 2002; 29: 1520-30.
3. PRAKKEN B, ALBANI S, MARTINI A: Juvenile idiopathic arthritis. *Lancet* 2011; 377: 2138-49.
4. MOROLDO MB, TAGUE BL, SHEAR ES, GLASS DN, GIANNINI EH: Juvenile rheumatoid arthritis in affected sibpairs. *Arthritis Rheum* 1997; 40: 1962-66.
5. PRAHALAD S, HANSEN S, WHITING A *et al*.: Variants in TNFAIP3, STAT4, and C12orf30 loci associated with multiple autoimmune diseases are also associated with juvenile idiopathic arthritis. *Arthritis Rheum* 2009; 60: 2124-30.
6. LEE YH, WOO JH, CHOI SJ, JI JD, SONG GG: Association between the rs7574865 polymorphism of STAT4 and rheumatoid arthritis: a meta-analysis. *Rheumatology Int* 2010; 30: 661-6.
7. WATFORD WT, HISSONG BD, BREAM JY, MUUL L, O'SHEA JJ: Signaling by IL-12 and IL-23 and the immunoregulatory roles of STAT4. *Immunol Rev* 2004; 202: 139.
8. HILDNER KM, SCHIRMACHER P, ATREYA I *et al*.: Targeting of the transcription factor STAT4 by antisense phosphorothioate oligonucleotides suppresses collagen-induced arthritis. *J Immunol* 2007; 178: 3427-36.
9. REMUZGO-MARTINEZ S, GENRE F, CASTANEDA S *et al*.: Protein tyrosine phosphatase non-receptor 22 and C-Src tyrosine kinase genes are down-regulated in patients with rheumatoid arthritis. *Sci Rep* 2017; 7: 10525.
10. GOH LL, YONG MY, SEE WQ *et al*.: NLRP1, PTPN22 and PADI4 gene polymorphisms and rheumatoid arthritis in ACPA-positive Singaporean Chinese. *Rheumatology Int* 2017; 37: 1295-302.
11. LOPEZ-CANO DJ, CADENA-SANDOVAL D, BELTRAN-RAMIREZ O *et al*.: The PTPN22 R263Q polymorphism confers protection against systemic lupus erythematosus and rheumatoid arthritis, while PTPN22 R620W confers susceptibility to Graves' disease in a Mexican population. *Inflamm Res* 2017; 66: 775-81.
12. ALBERDI-SAUGSTRUP M, ENEVOLD C, ZAK M *et al*.: Non-HLA gene polymorphisms in juvenile idiopathic arthritis: associations with disease outcome. *Scand J Rheumatol* 2017; 46: 369-76.
13. DIMOPOULOU DG, ZERVOU MI, TRACHANA M *et al*.: Investigation of juvenile idiopathic arthritis susceptibility loci: results from a Greek population. *Human Immunol* 2013; 74: 1194-8.
14. FAN ZD, WANG FF, HUANG H *et al*.: STAT4 rs7574865 G/T and PTPN22 rs2488457 G/C polymorphisms influence the risk of developing juvenile idiopathic arthritis in Han Chinese patients. *PloS One* 2015; 10: e0117389.
15. LIANG YL, WU H, SHEN X *et al*.: Association of STAT4 rs7574865 polymorphism with autoimmune diseases: a meta-analysis. *Mol Biol Rep* 2012; 39: 8873-82.
16. ZHENG J, YIN J, HUANG R, PETERSEN F, YU X: Meta-analysis reveals an association of STAT4 polymorphisms with systemic autoimmune disorders and anti-dsDNA antibody. *Human Immunol* 2013; 74: 986-92.
17. WATFORD WT, HISSONG BD, BREAM JH, KANNO Y, MUUL L, O'SHEA JJ: Signaling by IL-12 and IL-23 and the immunoregulatory roles of STAT4. *Immunol Rev* 2004; 202: 139-56.
18. NGO ST, STEYN FJ, MCCOMBE PA: Gender differences in autoimmune disease. Frontiers in neuroendocrinology 2014; 5: 347-69.
19. WHITACRE CC: Sex differences in autoimmune disease. *Nat Immunol* 2001; 2: 777-80.
20. CHIARONI-CLARKE RC, LI YR, MUNRO JE *et al*.: The association of PTPN22 rs2476601 with juvenile idiopathic arthritis is specific to females. *Genes Immun* 2015; 16: 495-8.
21. GOULIELMOS GN, CHIARONI-CLARKE RC, DIMOPOULOU DG *et al*.: Association of juvenile idiopathic arthritis with PTPN22 rs2476601 is specific to females in a Greek population. *Pediatr Rheumatol Online J* 2016; 14: 25.