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Original Article

CD226 and CD40 gene polymorphism in Egyptian juvenile idiopathic arthritis children: Relation to disease susceptibility and activity

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ABSTRACT

Aim of the work: To study the association of CD226 rs763361 (C>T) and CD40 rs1883832 (C>T) gene polymorphism with the disease susceptibility and activity in Egyptian juvenile idiopathic arthritis (JIA) children. *Patients and methods:* 150 JIA children and 194 age and sex matched controls were included. CD226 (C>T) polymorphism was assessed using the tetra amplification refractory mutation system-polymerase chain reaction assay (ARMS-PCR) and restriction fragment length polymorphism (RFLP) for CD40 (C>T). The juvenile arthritis disease activity score (JADAS-27) was used to measure the patients' disease activity. *Results:* The mean age of the patients was 11.2 ± 1.7 years, female: male 4:1 and the disease duration was 4.8 ± 2.3 years. 16 were systemic onset, 69 polyarticular and 65 oligoarticular and their mean JADAS-27 was 5.7 ± 5.3. The CD226 TT genotype and T allele were significantly associated with JIA and more frequent than in control ($p < 0.001$). The CD226 T allele was significantly higher in patients with moderate and high activity compared to mild cases ($p = 0.004$ and $p < 0.001$, respectively). The frequency of CD40 C allele was significantly increased in patients with severe and moderate disease activity compared to those with mild ($p < 0.001$ and $p = 0.02$ respectively). *Conclusion:* There was a genetic association between the CD226 and CD40 gene polymorphism and JIA susceptibility with an impact on disease activity in an Egyptian cohort.

1. Introduction

Juvenile idiopathic arthritis (JIA) is one of the commonest rheumatic diseases with the risk of disability in children [1]. The disease symptoms vary from fever, rash and arthritis, but mainly patients present with arthritis that develops at age below 16 years and lasts for about 6 weeks in absence of other causes of arthritis. JIA is a multifactorial disease with a genetic basis which render patients more susceptible for the disease occurrence [2]. The exact cause of JIA is unknown and thought to be due to viral infection, which mostly trigger the disease occurrence in susceptible child. There are many genomic regions assumed to be contributors of JIA risk. JIA is an autoimmune disease that is characterised by elevated levels of Pro-inflammatory cytokines. These pro-inflammatory cytokines which include tumour necrosis factor-α (TNF-α) and interleukins have a major role in the disease pathogenesis [3,4]. They are known to be the cause of the release of metalloproteinases by stimulation of synovial fibroblasts, osteoclasts, and chondrocytes [5]. In addition, the both cytokines also play another role in the inhibition of down regulation of joint damage through decreasing the release of tissue inhibitors of metalloproteinases by the synovial fibroblasts; this dual action leads to joint destruction [6]. Therefore, early initiation of efficient treatment may achieve a good control of inflammation and prevent long-term harms and disability. Interestingly, treatment options including biologics have also increased for JIA children [7].

Oligo-articular JIA is the commonest subtype and those with poly-articular onset are further classified according to the rheumatoid factor positivity. Systemic JIA subtype is a chronic arthritis that is preceded by systemic features like fever, erythematous rash, lymphadenopathy and hepato-splenomegaly. Other subtypes include enthesitis-related, psoriatic subtype and undifferentiated arthritis [8].

CD226 is considered as a glycoprotein present on many cell surfaces like (natural killer) NK, T cell subsets, platelets and monocytes. It is one of the members of the Ig-family. Its main function is to mediate cellular adhesion of cells where it is expressed, like platelets adhesion to vascular endothelial cell [9]. Further, it produces activation of T and NK cells through a co-stimulatory signal

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[10]. Recently, it has been reported that CD226 single nucleotide gene polymorphisms (SNPs) associated with many autoimmune rheumatic diseases [11,12]. CD40 is a co-stimulatory molecule expressed mainly on antigen presenting cells (APCs) such as macrophages, B cells and dendritic cells. Moreover, CD40 can be expressed on another group of cells like, endothelial, smooth muscle cells and fibroblasts. It is one of the members of the Tumor Necrosis Factor Receptor (TNFR) superfamily. It mediates many immune response pathways as well as many inflammatory reactions through co- reaction with CD154, the specific CD40 ligand [13].

Although many SNPs in CD226 and CD40 genes have been reported previously, their roles in JIA susceptibility in Egyptian children and their relation to disease activity remain unknown. Therefore, the aim of the present work was to investigate the contributions of both CD226 and CD40 SNPs to JIA disease susceptibility and activity in Egyptian children.

2. Patients and methods

The study included 150 patients recruited from the Pediatric Rheumatology outpatient clinic of Mansoura Pediatric University Hospital, Internal Medicine Specialized Hospital and from Mansoura International Hospital during the year 2015. While the control consisted of 194 age and sex matched healthy children that were unrelated to JIA patients. The study conforms to the 1995 Helsinki declaration and we got an approval by the ethical committee of Mansoura University Hospital. An informed consent was available from all patients' guardians prior to their inclusion.

Patients were classified according to the International League of Associations for Rheumatology (ILAR) classification system [8]. Disease activity was measured using the Juvenile Arthritis Disease Activity Score based on 27 joints (JADAS-27) [14]. The score ranges from 0 to 57 and the cut-off score $\leq$2.7 is considered for low and $\geq$6 for high disease activity [15]. Scores > 2.7 and <6 were considered as moderate disease activity.

Routine investigations were done for all patients in the form of complete blood count (CBC), acute phase reactant [erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP)], liver function tests, serum creatinine, rheumatoid factor, anti-nuclear antibody (ANA) and anti-cyclic citrullinated peptide (anti-CCP) by the commercially available kits. Blood samples were collected from all subjects and preserved in a vacutainer EDTA tube and their storage was at −20 till the time of genotyping. Genomic DNA extraction was done using Fermentas, USA, #K0781 (Thermo Scientific GeneJET Whole Blood Genomic DNA Purification Mini Kit).

For CD226, we used tetra amplification refractory mutation system – polymerase chain reaction assay (ARMS-PCR) according to the procedure of *Ye* et al. [16] for detection of rs763361 (C>T). The PCR was performed by using primers: FO; TTGCATAAAGATCCAT GCATGAGTAC, RO; GATTTCTGTTGCATCTCAGTCAAGAA, F1(T allele); CATGGAT TGATTGGTAGGTTGCCT, R1(C allele); CCAATAAC TATAGAAGTCCCATCTCTAACG We used personal thermo cycler, Biometra, analytical Jena Company. After DNA extraction, there were several steps to taken: addition of; 1 μL of template DNA, 1 μL of each primer (10 pmol/μL), and 5 μL of the DNase-free water to 15 μL PCR master mix. Amplification was performed through several steps of denaturation; the initial step at 95 °C for 5 min, after that 30 cycles of 30 s each at 95 °C, then for 23 s at 60 °C 25 s at 72 °C and finally, the last step at 72 °C for 10 min. The products of PCR were let to migrate on agarose gel 2% containing 0.5 μg/ml ethidium bromides.

For CD40 we used restriction fragment length polymorphism (RFLP) for detection of rs1883832 (C>T) polymorphism. We used 5–CCTCTTCCCCGAAGTCTTCC 3 – (forward primer) and 5–GAAACTCCTGCGCGGTGAAT 3 – (reverse primer). After extraction, 5 μL of template DNA, 0.5 μL of each primer (25 pmol), 6.5 μL DNase – free water were added to 12.5 μL PCR Master Mix. The obtaining of amplification was performed by several denaturation steps: the initial one at 94 °C for 5 min after that, 35 cycles of 94 °C for 30 s, 61 °C for 45 s, and 72 °C for 45 s and the final step at 72 °C for 10 min. The digestion of amplified products were done by adding 5 U of the restriction enzyme Nco I (Fermentas, Burlington, Ont., Canada). Then the digestion products were analyzed on 2.5% agarose gels containing 0.5 μg/ml ethidium bromides.

2.1. Statistical analysis

SPSS version 16.0 software (SPSS Inc. Chicago, IL, USA) was used to perform statistical analysis. Results were calculated as mean ± SD. The sample with group representation was checked using the Hardy-Weinberg balance. The Allelic frequency and the differences in genotypes were studied between different groups using the Fisher's exact test or the $\chi 2$ test as appropriate. The odds ratio (OR) was calculated together with its 95% confidence interval (CI). Significance was considered at $p < 0.05$.

3. Results

The study included 150 patients (120 females and 30 males; F: M 4:1) with mean age of 11.2 ± 1.7 years and disease duration 4.8 ± 2.3 years (1–9 years). 194 control of matched age (12.3 ± 1.2 years; p = 0.22) and gender (143 female and 51 males; 2.8:1, p = 0.17) were included. Patients were 16 systemic onset, 69 polyarticular and 65 oligoarticular. The mean JADAS-27 was 5.7 ± 5.3. Patient characteristics, laboratory investigations and disease activity were shown in Table 1.

The distribution of CD226 and CD40 polymorphism in studied JIA patients and controls are shown in Table 2. When comparing the patients with control, significant higher frequency of the CD226 TT genotype and T allele in JIA patients ($p < 0.001$). Also, significant higher frequency of CD40 CC genotype and C allele was noticed in patients ($p < 0.001$). There were significant differences

Table 1
Demographic characteristics, laboratory investigations and disease activity of JIA patients.

Variable n (%)	JIA patients (n = 150)
Female:Male	120:30
Family history	113 (75.3)
Onset subtypes:	
Systemic	16(10.7)
Polyartricular RF positive	53 (35.3)
Polyartricular RF negative	16 (10.7)
Oligoarticular	65 (43.3)
Deformity:	
No	15 (10)
Wrist	14 (9.3)
Ankle	8 (5.3)
Knee	17 (11.3)
Elbow	20 (13.3)
>2 joints	64 (42.7)
Spine	12 (8)
ANA positivity	5 (3.3)
RF positivity	108 (72)
Anti–CCP positivity	122 (81.3)
Disease activity:	
Mild	50 (33.3)
Moderate	57 (38)
Severe	43 (28.7)

JIA: juvenile idiopathic arthritis, ANA: antinuclear antibodies, RF: rheumatoid factor, anti-CCP: anti-cyclic citrullinated peptide.

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