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Correlation between Peptidyl Arginine Deiminase Type 4 Polymorphism and Disease Activity in Polyarticular Juvenile Idiopathic Arthritis in Egyptian Patients

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ABSTRACT

Background: juvenile idiopathic arthritis (JIA) is a broad term that describes a clinically heterogeneous group of arthritides of unknown cause, which begin before 16 years of age affecting one or more joints, lasting for at least 6 weeks. PADI4 is one member of PADI gene family. The PADI gene region is located at chromosome 1p36. It codes for enzymes responsible for the posttranslational conversion of arginine residues into citrulline. There are likely to be different genetic risk factors for JIA in different ethnic groups. Therefore, here we sought an influence of HLA-SE and PADI4 on JIA susceptibility in Japanese, because both HLA-SE and PADI4 were reported as significant genetic risk factors for RA independent of ethnicity. Recently, association of PADI4 gene polymorphisms with ACPA positivity and disease activity in polyarticular JIA. **Aim of the Work:** the aim of this work is to investigate PADI polymorphism rs2240340 to determine whether this polymorphism could be a marker of susceptibility to JIA in Egyptian children and adolescents and whether this single nucleotide polymorphism (SNP) is correlated with clinical parameters in JIA. **Patients and Methods:** the ethical approval was obtained from the hospital ethical research committee and each patient entering the study will sign an informed consent. Thirty patients included in this study with polyarticular types of juvenile idiopathic arthritis and all of them fulfilled ILAR classification criteria (2004). All were under the age of sixteen at time of diagnosis. They were recruited from Physical Medicine, Rheumatology and Rehabilitation Department at Al-Hussein and Sayed Galal University Hospitals during the period from January 2018 to June 2018. In this study we measure PADI4 polymorphism and correlate with disease activity in polyarticular JIA in Egyptian patients. **Results:** association of PADI4 gene polymorphisms with ACPA positivity and disease activity in polyarticular JIA and PADI4 gene polymorphism can be used as a marker of susceptibility to polyarticular JIA. **Conclusion:** PADI4 gene polymorphism became a marker of susceptibility to polyarticular JIA and gene polymorphism correlated with disease activity in ACPA positivity in polyarticular positive JIA.

Keywords: Juvenile idiopathic arthritis, Peptidyl Arginine Deiminase type 4 polymorphism.

INTRODUCTION

Juvenile idiopathic arthritis (JIA) is a broad term that describes a clinically heterogeneous group of arthritides of unknown cause, which begin before 16 years of age affecting one or more joints, lasting for at least 6 weeks. This term encompasses several disease categories, each of which has distinct methods of presentation, clinical signs, and symptoms, and, in some cases, genetic background. The cause of disease is still poorly understood but seems to be related to both genetic and environmental factors, which result in the heterogeneity of the illness [(1)]. Juvenile idiopathic arthritis is the most common autoimmune inflammatory joint disease in childhood, with prevalence of 3.43 per 100,000. Prevalence in boys was 2.58 per 100,000 and in girls 4.33 per 100,000 [(2)]. JIA is subdivided into seven clinically more homogeneous subtypes, using the International League of Associations for Rheumatology (ILAR) classification system: systemic arthritis, oligoarthritis, RF negative polyarthritis, RF-positive polyarthritis, psoriatic arthritis, enthesitis-related arthritis, and undifferentiated arthritis[(3)]. The major pathology of oligoarthritis and polyarthritis is articular inflammation and joint destruction. RF-positive polyarthritis is considered to be a counterpart of adult rheumatoid arthritis. In contrast to the above forms of JIA, the major pathology of systemic JIA is systemic inflammation, which is considered similar to adult Still's disease [(4)]. PADI4 is one member of PADI gene family. The PADI gene region is located at chromosome 1p36. It codes for enzymes responsible for the posttranslational conversion of arginine residues into citrulline. It was indicated that an RA susceptibility haplotype in PADI4 was associated with increased stability of PADI4 mRNA [(5)]. And it could lead to accumulation of PADI4 protein, with subsequent increases in citrullinated proteins and enhanced production of autoantibodies against these citrullinated peptides [(6)]. PADI4 mRNA is detected in hematological cells and pathological synovial tissues and it was reported that PADI4 significantly overexpressed in the blood cells of RA patients [(7)]. There are likely to be

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different genetic risk factors for JIA in different ethnic groups. Therefore, here we sought an influence of HLA-SE and PADI4 on JIA susceptibility in Japanese, because both HLA-SE and PADI4 were reported as significant genetic risk factors for RA independent of ethnicity [(8)].

AIM OF THE WORK

The aim of the work is to investigate PADI polymorphism rs2240340 to determine whether this polymorphism could be a marker of susceptibility to JIA in Egyptian children and adolescents and whether this single nucleotide polymorphism (SNP) is correlated with clinical parameters in JIA.

PATIENTS AND METHODS

The ethical approval was obtained from the hospital ethical research committee and each patient entering the study signed an informed consent. Thirty patients included in this study with polyarticular types of juvenile idiopathic arthritis and all of them fulfilled ILAR classification criteria (2004). All were under the age of sixteen at time of diagnosis. They were recruited from Physical Medicine, Rheumatology and Rehabilitation Department at Al-Hussein and Sayed Galal University Hospitals during the period from January 2018 to June 2018. **Patient's selection: A) Inclusion criteria (group A):** These included an established diagnosis of polyarticular juvenile idiopathic arthritis less than 16 years. RF positive Polyarticular juvenile idiopathic arthritis which defined as Arthritis affecting ≥5 joints during the first 6 months of disease, and ≥ 2 positive RF tests (as routinely defined in an accredited laboratory), at least 3 months apart during the first 6 months of disease. RF negative Polyarticular juvenile idiopathic arthritis which defined as Arthritis affecting ≥ 5 joints during the first 6 months of disease, and Test for RF is negative [(1)]. **B) Exclusion criteria:** Children older than 16 years. Infection. Malignancy. Seronegative Spondyloarthropathy. Rheumatic fever. Oher types of juvenile idiopathic arthritis. **Control group (group B):** Thirty healthy individuals, age and sex matched, were used as a control group. **Investigations:** 1) Clinical evaluation: included full history taking, clinical examination, musculoskeletal examination, disease activity by the active joint count (AJC), patient/parent global assessment of overall well-being (PT-VAS), physician global assessment of disease activity (MD-VAS), Childhood Health Assessment Questionnaire (CHAQ) and Juvenile Arthritis Disease Activity Score (JADAS 71). 2) Laboratory evaluation in the form of: CBC, ESR, CRP, RF and anti-CCP. 3) Gene analysis. **The Procedure:** Detection of (PADI4 gene rs2240340) polymorphism according to the following: Genomic DNA extraction, SNP Genotyping & Allelic Discrimination Plate Read and Analysis. The skin over the vein was sterilized by 70% alcohol and 2 ml whole venous blood sample was collected into tube containing EDTA. The samples were stored at – 20 °C until further processing. **DNA extraction**: DNA was extracted by DNA extraction kit (QIAamp®) Whole Blood Genomic DNA Purification Mini Kit (50).Catalog No.E 51104 Ge. **Single Nucleotide Polymorphism (SNP) Genotyping:** Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism technique and the primer 5`-ACC CCC CGT TCA TTC TC-3`. ***a) Prepare the PCR reaction mix volume:*** Reaction mix volume (25 µl/well) were added, consisted from: 12.5µl TaqMan® Genotyping Master Mix (2X) part No.4371353 + 1.25 µl of each primer (forward and reverse) + 11.25 µl nuclease-free water and added (1 to 10 ng of purified genomic DNA) into each well of a MicroAmp™ Optical 96-Well Reaction Plate then covered the plate with MicroAmp Optical Caps and finally Centrifuged the plate briefly for 1 min at 1000 g at room temperature (15–25°C) to spin down the contents and eliminated air bubbles from the solutions. ***b) Performing PCR:*** The reaction volume containing tubes were then gently vortexed and briefly centrifuged to collect all drops to the bottom of the tube. The samples were placed in the thermal cycler. Thermal cycling conditions were carried out in thermocycler PTC-94 (Biorad, USA) as described by **Longo et al.** [(9)] ***c) Allelic Discrimination Plate Read and Analysis:*** Restriction fragment length polymorphism (RFLP) analysis was done using FastDigest Bbv Fermentas, Germany) in 30µl total volume: 17µl water, nuclease-free + 2 µL10X FastDigest Buffer +10 µl DNA (PCR product) +1 µl (10 units) FastDigest BbvI enzyme. All components were mixed gently and spinned down. Then the mixture was incubated at 37°C in a heat block (Stuart, UK) for 3hours. The enzyme was inactivated by heating for 5 min at 65°C. ***d) Viewing Assay Results:*** The

SDS software plots the results of the allelic discrimination run on a scatter plot of Allele 1 versus Allele 2. Each well of the 96-well reaction plate is represented as an individual point on the plot. **Statistical analysis:** Data were tabulated and analyzed using the computer program SPSS (Statistical package for social science) version 16. Quantitative date were expressed as mean standard deviation (SD). Qualitative data were expressed as frequency and parentage. **The following tests were done:** Independent-samples t-test of significance: was used when comparing between two means. Chi-square test was used when comparing between non-parametric data. Receiver operating characteristics (ROC) curves: was used to detect sensitivity, specificity, cut-off point, positive predictive value (PPV) and negative predictive value (NPV). Probability (P-value) P-value <0.05 was considered significant. P-value <0.001 was considered as highly significant. P-value >0.05 was considered insignificant. Diagnostic sensitivity: It measures the incidence of true positive results in patients group. Diagnostic specificity: It measures the incidence of true negative results in a non-diseased group. Positive predictive value: It is the percent of true positive results among all positive results. Negative predictive value: It is the percent of true negative results among all negative results.

RESULTS

Table (1): Comparison between patients and control as regard age

Groups / Parameter	Patients (n=30)		Control (n=20)		p-value
	Mean	±SD	Mean	±SD	
Age (years)	10.5	3.5	9.1	3.0	0.149

This table shows no statistically significant difference (P-value > 0.05) between patients and control as regard age (years). As regard age, the mean age of patients was 10.5 years while that of control was 9.1 years.

Table (2): Comparison between patients and control as regard sex.

Groups / Parameter	Patients (n=30)	Control (n=20)	p-value
Male	11 (37%)	8 (40%)	0.3
Female	19 (63%)	12 (60%)	

This table shows no statistically significant difference (P-value > 0.05) between patients and control as regard sex. As regard sex, patients were 37% males and 63% females while control were 40% males and 60% females.

Table (3): Comparison between Polyarticular RF +ve & Polyarticular RF –ve patients as regard age.

Groups / Parameter	Polyarticular RF +ve (n= 17)		Polyarticular RF –ve (n= 13)		p-value
	Mean	±SD	Mean	±SD	
Age (years)	10.2	± 3.38	10.8	±3.97	0.658

This table shows no statistically significant difference (P-value > 0.05) between Polyarticular RF +ve group and Polyarticular RF –ve group as regard age (years). As regard age, the mean age of polyarticular RF + patients was 10.2 years while that of polyarticular RF - patients was 10.8 years.

Table (4): Comparison between Polyarticular RF +ve & Polyarticular RF –ve patients as Anti-CCP.

Groups / Parameter	Polyarticular RF +ve (n=17)	Polyarticular RF –ve (n=13)	p-value
Anti-CCP >20 (IU/ml)	10 (59%)	3 (23%)	0.04
Anti-CCP < 20 (IU/ml)	7 (41%)	10 (77%)	

This table shows statistically significant difference (P-value < 0.05) between Polyarticular RF +ve group and Polyarticular RF –ve group as Anti-CCP titer. As regard age, Polyarticular RF + patients with positive anti-CCP were 59% while that of polyarticular RF - with positive anti-CCP were 23%.

Table (5): Disease activity grading in Polyarticular RF +ve & Polyarticular RF –ve patients according to patient/parent VAS

Groups / Parameter	Low activity < 2 of 10 (n=4)	Moderate activity 2-4 of 10 (n=11)	High activity ≥ 5 of 10 (n=15)
Polyarticular RF +ve	3 (75%)	5 (45%)	11(73%)
Polyarticular RF –ve	1 (25%)	7 (55%)	4 (27%)

This table shows as regard disease activity grading in JIA patients according to patient/parent VAS, there were **Low:** Polyarticular RF +ve patients were 3 (75%) while that of Polyarticular RF –ve were 1 (25%). **Moderate:** Polyarticular RF +ve patients were 5 (45%) while that of Polyarticular RF –ve were 7 (55%). **Severe:**

Polyarticular RF +ve patients were 11 (73%) while that of Polyarticular RF –ve were 4 (27%).

Table (6): Disease activity grading in Polyarticular RF +ve & Polyarticular RF –ve patients according to JADAS71 score

Groups / Parameter	Inactive (n=15)	Low activity (n=2)	Moderate activity (n=9)	High activity (n=4)
Polyarticular RF +ve	5 (25%)	1 (50%)	6 (67%)	4 (100%)
Polyarticular RF –ve	10 (75%)	1 (50%)	3 (33%)	0 (0%)

This table shows as regard disease activity grading in JIA patients according to JADAS71, there were **Inactive:** Polyarticular RF +ve patients were 5 (25%) while that of Polyarticular RF –ve were 10 (75%). **Low:** Polyarticular RF +ve patients were 1 (50%) while that of Polyarticular RF –ve were 1 (50%). **Moderate:** Polyarticular RF +ve patients were 6 (67%) while that of Polyarticular RF –ve were 3 (33%). **Severe:** Polyarticular RF +ve patients were 4 (100%) while that of Polyarticular RF –ve were 0 (0%).

Table (7): Association between PADI4 gene polymorphisms and ACPA positivity in poly-articular JIA patients

Groups / Parameter	Anti-CCP (+) >20 (IU/ml) (n=13)	Anti-CCP (-) <20 (IU/ml) (n=17)	p-value
Allele	75	46	0.018
Recessive	26	11	0.024
Dominant	49	35	0.158

This table shows: No statistically significant difference (P-value < 0.05) between Polyarticular RF +ve group and Polyarticular RF –ve group as gene allele. No statistically significant difference (P-value > 0.05) between Polyarticular RF +ve group and Polyarticular RF –ve group as gene dominant. Statistically significant difference (P-value < 0.05) between Polyarticular RF +ve group and Polyarticular RF –ve group as gene recessive.

DISCUSSION

This study aimed to investigate PADI polymorphism rs2240340 to determine whether this polymorphism could be a marker of susceptibility to JIA in Egyptian children and adolescents and whether this single nucleotide polymorphism (SNP) is correlated clinical parameters in JIA. Thirty patients included in this study with polyarticular types of juvenile idiopathic arthritis and all of them fulfilled ILAR classification criteria (2004). All were under the age of sixteen at time of diagnosis. They were recruited from Physical Medicine, Rheumatology and Rehabilitation Department at Al-Hussein and Sayed Galal University Hospitals during the period from January 2018 to June 2018. Similar inclusion criteria were adopted by ***Consolaro et al***. [(10)], who suggested inclusion of cases, based on clinical history and symptoms

Regarding to the mean age, in the current study it was 10.5 ± 3.5 years old. ***Ringold et al***. [(11)] in their study on a retrospective of confirmed that mean age was 9.5 years old. In another study by ***Martini et al***. [(12)] found that the mean age of the studied patient group was 11.2 years old. In the present study, regarding to sex, there was female predominance (63%) and male about (27%) that was in agreement with the study done by ***Abou El-Soud et al.*** [(2)] who studied one hundred and seventy six patients with JIA with female predominance. In the present study, concerning JADAS score ranged from 1.6 to 10.3 with a mean of 5.5 ± 3.08 in the patient group. In a study by ***McErlane et al***. [(13)] found that JADAS score 5.9 ± 2.9 in the JIA patients with polyarticular RF +ve, which was near to our results. Regarding disease activity grading in JIA patients, of (polyarticular RF+ve), there were inactive in 5 (75%), low disease activity in 1 (50%), moderate disease activity in 6 (67%) and severe 4(100%) that slightly agreed with the study done by ***Bowyer et al***. [(14)] They studied fifteen patients and commented that fifteen of polyarticular RF +ve patients were classified according to JADAS71. In our study, we found an association of PADI4 gene polymorphisms with anti-CCP positivity in JIA and correlate disease activity, as was already known for RA where in Anti-CCP patients the dominant genotypes 49 and the recessive genotypes 35, that slightly agreed with the study done by ***Hisa et al.*** [(8)] who studied 185 cases and found that the dominant genotypes 214 and the recessive genotypes 70.

CONCLUSION

In our study we concluded that rs2240340 SNP polymorphism of peptidyl arginine deiminase type 4 PADI4 is significantly associated with

susceptibility JIA in our Egyptian population and correlated with Anti-CCP positivity and disease activity children. PADI4 SNP might be a useful genetic marker for polyarticular JIA.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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