Original Research Article

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Transmembrane Protein 187 (TMEM187) and Interleukin 1 Receptor Associated Kinase (IRAK1) gene polymorphism association with rheumatoid arthritis susceptibility in Egyptian patients

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Abstract

Rheumatoid arthritis (RA) is a chronic auto-inflammatory disease of connective tissue with progressive joint damage and systemic disorders. RA is considered a multifactorial disease triggered by a genetic predisposition and environmental factors. Polymorphisms have been identified in the Xq28 locus as risk loci for RA. The aim of study was to assess the association between two polymorphisms in the Xq28 region containing Transmembrane Protein 187 (TMEM187) gene (rs13397) and interleukin1 receptor associated kinase (IRAK1) gene (rs1059703) with the disease susceptibility and activity in Egyptian RA patients. This study was conducted on 100 RA patients and 100 age and sex matched normal controls, together were recruited from the Rheumatology Department, Beni-Suef University Hospital. We detected TMEM187 (rs13397) and IRAK1 (rs1059703) gene polymorphisms using the real time PCR TaqMan allelic discrimination assays. We found that the frequency of the major genotypes (GG) of TMEM 187 gene was higher in RA group (54%) compared to controls (50%); while the minor genotypes (AA) was higher in the control group (22%) compared to the diseased one (18%), but such differences did not reach statistical significance (p=0.599). Regarding the IRAK1 gene, we revealed that the frequency of the major genotypes (AA) of the rs1059703 was slightly higher in RA group (48%) compared to controls (46%); while the minor genotypes (GG) was the same in both groups (26%). However, there was higher incidence of minor genotype in the TMEM187 and IRAK1 genes in males; with a statistical significance (p=0.004 and 0.015, respectively). We concluded that the major allele G of TMEM187(rs 13397) could be considered as a risk genetic allele for RA in Egyptian populations.

Keywords: RA, TMEM187, IRAK1, RT-PCR, SNP.

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Introduction

Rheumatoid arthritis (RA) is one of the systemic autoimmune disorders that is characterized by chronic destructive inflammation involving the synovial joints.[1] It affects approximately 0.5 – 1 percent of adults in the developed countries. The prevalence of RA is 3 times more common in females when compared to males.[2]

Disease Activity Score of 28 joints (DAS 28) is broadly used to denote RA activity as well as response to therapy. The joints included in DAS 28 are (bilaterally): proximal interphalangeal joints (ten joints), metacarpophalangeal joints (ten), wrists (two), elbows (two), shoulders (two) and knees (two). On examining the joints, both the number of joints with tenderness upon touching (TEN 28) and swelling (SW 28) are counted.

The role of genetics in RA has been suggested to reach nearly 50%-60%, taking into consideration that the HLA class II molecules are the most detected genetic factor playing a role in RA.[3] Genes for the predilection to RA could be conditionally classified into groups of cytokines in conjunction with their receptors, chemokines in conjunction with their receptors, essential elements of intracellular signaling pathways, as well as co-stimulating factors.[4]

A study by Eyre et al., 2012, documented that single nucleotide polymorphism (SNP) rs13397 is accompanied by RA risk among cases of northern European origin. Such polymorphism is set within the transmembrane (TMEM) protein gene (TMEM 187).[5] The TMEM proteins must contain ≥ 1 putative transmembrane segment that may span totally (integral polytopic) or partly (integral monotopic) via biological membrane.[6]

It has been proposed that there is a linkage disequilibrium block of TMEM187- interleukin1 receptor associated kinase (IRAK1) within the Xq28 locus that is accompanied by RA vulnerability and might has an independent role of the pivotal genetic risk locus for RA.[7] The IRAK1 gene is located on the X-chromosome. IRAK1 polymorphisms were postulated to be accompanied by the risk of various autoimmune disorders including RA, systemic lupus erythematosus, systemic sclerosis, and autoimmune thyroid diseases.[8]

The aim of the current research was to study the TMEM187 (rs 13397) and the IRAK1 (rs 1059703) gene polymorphism in the Xq28 region using the real-time polymerase chain reaction (RT-PCR) TaqMan allelic discrimination assays and evaluate their association with RA vulnerability and severity.

Subjects and Methods

The current study was carried out on 100 RA patients (22 males and 78 females) with an age ranged between 24 and 70 years. Patients were recruited from Rheumatology Outpatient Clinic and Department of Rheumatology in Beni-Suef University Hospital. Diagnosis of RA was made based upon the 2010 ACR/EULAR classification criteria.[9] As for the control group, 100 age and gender matched, apparently healthy subjects were included.

Patients were subjected to full history taking, clinical examination and evaluation of disease activity using the DAS 28 joints. Also, the erythrocyte sedimentation rate (ESR) was measured. In addition, patients made a subjective assessment (SA) of disease activity during the preceding seven days on a scale between zero and one hundred, where zero was "no activity" and one hundred the "highest activity possible". With these parameters, DAS 28 was estimated according to the method described by Villaverde et al., 2000 [10]:

$$\text{DAS 28} = 0.56 \times \sqrt{TEN28} + 0.28 \times \sqrt{SW28} + 0.70 \times ln\, ln\,(ESR) + 0.014 \times SA$$

The study protocol was reviewed and approved by the Research Ethical Committee of the Faculty of Medicine, Beni-Suef University (approval dated January 2019). All participants signed an informed consent before being included in the study.

Collection of blood samples

From each participant a venous blood sample (5-7 ml) was withdrawn via sterile venipuncture then divided into two parts. The first portion was collected in a plain vacutainer for serum

separation and the assessment of C-reactive protein (CRP), and rheumatoid factor (RF) by latex agglutination test for the qualitative and semi-quantitative detection of CRP and RF in human serum using the commercially available reagent (AMS, U.K). The anti-cyclic citrullinated peptide (Anti-CCP) was assessed by a quantitative immunoassay (Elecsys Anti-CCP, on cobas e 411 analyzer, Roch Diagnostics, Hitachi High-Technologies Corporation, Tokyo Japan), according to the manufacturer's instructions.

Genotyping for the detection of TMEM187 SNP (rs 13397) and IRAK1 SNPs (rs 1059703)

The second portion was collected as EDTA anticoagulated blood, used for DNA extraction by commercial kits (Catalog No. 51104, QIAamp® DNA Blood Mini Kits, Qiagen, USA), according to the manufacturer's instructions. The extracted DNA was used in the RT-PCR method to determine TMEM187 SNP (rs 13397) and IRAK1 SNPs (rs 1059703), through SNP genotyping assay based on Applied Biosystems™ TaqMan probes.

For SNP TMEM rs 13397, the TaqMan® minor groove binder (MGB) probes/extension primers (VIC TCCACTTTGCGTTTTTGTTTCTGAC) and (FAM CATTTCAACACTCACCCAAGATTCC) were used for the detection of sequencing of alleles 1 and 2, respectively, whereas for SNP IRAK1 rs 1059703, (VIC AGGGGGGATGCAGCTGG CGGCCTCC) and (FAM AATGCCCGGGCACCCCC GCCACCAC) used for the detection of sequencing of allele 1 and 2, respectively. VIC and FAM fluorescent dye primers (Applied Biosystems, Singapore) were used according to the manufacturer's protocol (Custom TaqMan® Genomic Assays Protocol: Submission Guidelines).

Each SNP was performed in a 25µl reaction volume, contained 5µl DNA, 12.5µl 2xTaqMan Universal PCR Master Mix (cat no. 4324018, Applied Biosystems, Singapore), 1.25µl 20x SNP assay mix, and the final volume of 25 adjusted with nuclease-free water. The reaction was performed though the use of the StepOne™ RT-PCR machine (Applied Biosystems, Singapore). The cycling Life Technologies real-time instrument software (USA) was used to plot the results of the allelic discrimination data as a scatter plot of allele 1 (VIC® dye) versus allele 2 (FAM™ dye). Each well of the 96 wells was represented as a separate point in the plot. The results of ten percent of the amplification reactions were repeated twice to confirm results and it was proved to be identical.

Statistical analysis

Data were managed using the statistical package of social science (SPSS program, SPSS Inc., Chicago, IL, USA) version 25 for Microsoft Windows. Descriptive statistics: data were summarized using mean, ± standard deviation (±SD), median, minimum, and maximum for quantitative variables and frequencies (number of cases) and relative frequencies (%) for categorical variables. Analytic Statistics: Comparing groups was done by Chi-square-test: to compare qualitative data and Fisher exact test to compare qualitative data < 5 in frequency. Odds ratios (ORs) with 95% confidence intervals (CI) were calculated whenever applicable, to test association between genotype and the disease. The significance of the OR was estimated using 2 by 2 contingency tables. The Mann Whitney U test was used to detect the significance in the difference between 2 nonparametric variables. The level of significance was taken at p-value of <0.05 with confidence level 95%. The results were represented in tables and graphs [11].

Results

This study was conducted on 100 RA, 78 (78%) were females and 22 (22%) were males, with age ranged between 24 to 70 years. The control group included 100 normal adults from the same geographical area, their age ranged from 31 to 65 years, and 64% were females. The descriptive data and laboratory investigations of the patients are represented in Table 1.

Table 1. Clinical and laboratory data of the 100 RA patients.

Studied parameters	Observed data
Disease Duration	6.04±4.49
No. of tender joints	64.30±33.36
ESR (mean ±SD)	10.68±6.91
DAS score (mean ±SD)	5.65± 0.97
Min.-max.	3.19-7.49
CRP:	Number (%)
Positive	74 (74)
Negative	26 (26)
Anti-CCP:	
Positive	62 (62)
Negative	38 (38)
RF:	
Positive	74 (74)
Negative	26 (26)

As regards the distribution of genotypes frequency of TMEM 187 rs 13397 the GG genotype (wild type) was found in 54% of cases and 50% of controls. Whereas AG genotype (heterozygous mutant) was determined in 28% of the cases and controls. Additionally, AA genotype (homozygous mutant) was detected in 18% of the cases and 22% of the controls.

As regards the IRAK1 rs 1059703 the AA genotype (wild type) was detected in 48% of the cases and 46% of the controls. The AG genotype (heterozygous mutant) was determined in 26% of the cases and 28% of controls. Finally, the GG genotype (homozygous mutant) was detected in 26% of the cases and controls (Table 2).

Table 2. Distribution of genotypes frequency in TMEM 187 rs 13397 and IRAK1 rs 1059703 in the 100 RA patients and 100 controls.

Genotypes	Cases No. (%)	Controls No. (%)	Odds ratio (Confidence interval)	*p* value
rs13397Genotype:				
GG	54 (54.0%)	50 (50.0%)	Reference	
AA	18 (18.0%)	22 (22.0%)	OR=0.76 (0.24-2.40)	NS
AG	28 (28.0%)	28 (28.0%)	OR=0.93 (0.33-2.56)	
Allele Frequency:	N 200	N 200		
A	64 (32.0%)	72 (36.0%)	OR=0.84	NS
G	136 (68.0%)	128 (64.0%)	(0.45-1.57)	
rs1095703Genotype:				
AA	48 (48.0%)	46 (46%)	Reference	
GG	26 (26.0%)	13 (26.0%)	OR=0.96(0.33-2.78)	NS
AG	26 (26.0%)	14 (28.0%)	OR=0.89(0.31-2.55)	
Allele Frequency:	N 200	N 200		
G	78 (39.0%)	80 (40.0%)	OR=1.04	NS
A	122 (61.0%)	120 (60.0%)	(0.57- 1.91)	

P > 0.05 is not significant (NS).

We attempted to estimate the risk of RA between females and males based on the results of gene polymorphisms and the different laboratory investigations. Concerning TMEM 187 rs 13397 gene polymorphism, there was statistically significant difference in GG, AG, AA genotypes among gender as males were (22.2%, 0.0%, 55.6%), while females were 77.8%, 100%, 44.4%, respectively (*p*= 0.004) (Table 3).

Table 3. Comparison between the sex of patients and their laboratory investigations as regard the TMEM187 gene polymorphism.

rs13397Genotype	Genotype			*p* value
	AA 18 (%)	GG 54 (%)	AG 28 (%)	
Sex:				
- Male	10 (55.6%)	12 (22.2%)	0 (0.0%)	0.004
- Female	8 (44.4%)	42 (77.8%)	28 (100%)	
CRP:				
- positive	14 (77.8%)	36 (66.7%)	24 (85.7%)	NS
- negative	4 (22.2%)	18 (33.3%)	4 (14.3%)	
Anti-CPP:				
- positive	16 (88.9%)	30 (55.6%)	16 (57.1%)	NS
- negative	2 (11.1%)	24 (44.4%)	12 (42.9%)	
RF:				
- positive	16 (88.9%)	40 (74.1%)	18 (64.3%)	NS
- negative	2 (11.1%)	14 (25.9%)	10 (35.7%)	

P > 0.05 is not significant (NS).

As regards the IRAK1 1059703, there were statistically significant differences on comparing AA, AG, GG genotypes between genders, as males were 45.5%, 0%, 54.5%, and females 48.7%, 33.3%, 17.9%, respectively (p= 0.015) (Table 4).

Table 4. Comparison between the sex of patients and their laboratory investigations as regard the IRAK1 gene polymorphism.

rs1095703Genotype	Genotype			*p* value
	AA 48 (%)	GG 26 (%)	AG 26 (%)	
Sex:				
- Male	10 (20.8%)	12 (46.2%)	0 (0.0%)	0.015
- Female	38 (79.2%)	14 (53.8%)	26 (100%)	
CRP:				
- positive	36 (75.0%)	18 (69.2%)	20 (76.9%)	NS
- negative	12 (25.0%)	8 (30.8%)	6 (23.1%)	
Anti-CPP:				
- positive	28 (58.3%)	22 (84.6%)	12 (46.2%)	NS
- negative	20 (41.7%)	4 (15.4%)	14 (53.8%)	
RF:				
- positive	38 (79.2%)	20 (76.9%)	16 (61.5%)	NS
- negative	10 (20.8%)	6 (23.1%)	10 (38.5%)	

P > 0.05 is not significant (NS).

Furthermore, the patients included in this study were divided into 2 groups according to the disease activity. The first group included 32 patients in remission [DAS <2.6, n=0 (0%)], low activity [DAS≥ 2.6-<3.2, n=2 (2%)] and moderate activity [DAS ≥ 3.2-≤ 5.1, n=30 (30%)]. The second group included 68 (68.0%) patients with high activity (DAS >5.1) [12]. Table 5 shows the distribution of DAS 28 disease activity scores by gender.

Table 5. Distribution of DAS 28 score by gender in the 100 RA patients.

DAS 28 score	Study patients		
	Total	Male	Female
High	68 (68%)	14 (20.6%)	54 (79.4%)
Remission /low/Moderate	32 (32%)	8 (25%)	24 (75%)

A comparison between the 2 groups of RA patients (Remission/ low/ moderate and severe) and gene polymorphism was carried out and there was no statistically insignificant difference between the two groups (Table 6).

Table 6. Comparison between the 2 groups of RA patients (Remission/ low/ moderate and severe) and TMEM 187 and IRAK 1 gene polymorphism.

		DAS groups		*p*-value
		High	Remission low moderate	
		N=68 (%)	N=32 (%)	
TMEM 187 rs 13397	GG	36 (52.9%)	18 (56.3%)	NS
	AG	18 (26.5%)	10 (31.3%)	
	AA	14 (20.6%)	4 (12.5%)	
IRAK1 rs 1059703	AA	34 (50%)	14 (43.8%)	NS
	AG	16 (23.5%)	10 (31.3%)	
	GG	18 (26.5%)	8 (25%)	

P > 0.05 is not significant (NS).

Discussion

The present study was carried out to investigate the association between TMEM187 gene polymorphism and RA susceptibility and activity between men and women. In addition, we studied the IRAK1gene polymorphism in the Xq28 region as both TMEM 187 and IRAK1 genes are physically near each other, and the potential linkage disequilibrium in this site resulted in a controversial regarding which gene could account for disease propensity.

RA is one of the chronic, autoimmune, inflammatory diseases that might result in progressive joint destruction and deformity, causing functional disability. The chronic inflammatory status of RA is accompanied by extra-articular problems and increased mortality as well.[2] Considering the pivotal socioeconomic burden of RA resulted from lost productivity and elevated rates of using the healthcare resources; early diagnosis of RA, the treat-to-target hypothesis, and disease-modifying antirheumatic drugs that could stop or decrease progression of structural joint destruction and reducing mortality risk, caused a significant improvement in RA management.[13] In general, females are more commonly influenced by rheumatic disorders in comparison with males, and such predominance is elevated in two of the commonest diseases, RA, and systemic lupus erythematosus, with

women-to-men ratio in general reaching 3:1 and 11:1, respectively.[14]

Despite genome-wide association studies have determined > 100 loci associated with RA risk, only 20% of such loci was proved to have coding variants, whereas the other variants are found in non-coding sites.[15] The role of genetics in RA was postulated to reach approximately 50%-60% taking into consideration that the HLA class II molecules are the most detected genetic factor playing a genetic role.[3] Various studies have discussed the linkage between polymorphisms and gene expression of complex diseases as autoimmune diseases, but few studies have discussed genes located on the X-chromosome.[16,17,18]

A study by Khalifa et al., 2017, determined TMEM187 gene polymorphism (SNP rs13397) as a new risk gene for RA in Tunisian as well as French female populations.[7] TMEM187 gene is located in the Xq28 region, a highly complex region including many genes (TMEM187, IRAK1, MECP2, LICAM), that have been involved in a lot of autoimmune diseases such as systemic lupus erythematosus and RA.[19] Moreover, Pascual et al., 2016, reported abnormal expression of TMEM187 gene in children as well as adults suffering celiac disease, indicating its role in autoimmunity pathogenesis.[20] Along with being considered as proapoptotic gene, it is composed of 2 exons that encode a multi-pass membrane protein expressed in various tissues. This is a gene coding a 261 AA protein with six transmembrane helical domains. It is related to a group of genes that entertain microRNA genes in their introns or exons.[21]

It has been demonstrated that SNPs for TMEM187 were in linkage disequilibrium with SNPs of other accompanied by autoimmune disorders such as IRAK1, MECP2, SYTL4.[16] IRAK1 is an essential signal regulator having an important role in regulating the nuclear factor of kappa beta (NF-κβ) pathway and incorporated in toll-like receptor signaling that consequently promotes interleukin 6 (IL-6) expression, tumor necrosis factor alpha (TNF-α), and IL-8.[22]

The present case-control study included 100 RA patients in comparison with 100 age and gender matched healthy unrelated controls for analysis of TMEM187 (rs13397) and IRAK1 (rs1059703) gene polymorphism using RT-PCR TaqMan allelic discrimination assay. In the current work, the frequency of the major homozygous genotypes (GG) of the TMEM 187 gene was more in RA group (54 %) in comparison with controls (50%); whereas the minor homozygous genotypes (AA) were more in the control group (22%) in comparison with the diseased group (18%). However, this increase did not reach a statistical significance (p=0.599).

In contrary of our results, the study by Khalifa et al., 2017, revealed a statistically significant elevation in the major homozygous genotypes (GG) of the TMEM 187gene in Tunisian as well as French apparently healthy controls (70 % and 65%, respectively) compared to Tunisian and French RA patients (53 % and 45 %, respectively) (p<0.05).[7]

As regards allele frequency in our study, the major allele G of the TMEM187 was more in RA cases (68 %) in comparison with control subjects (64%). Nevertheless, this elevation did not reach a statistical significance (p=0.55). Again, our results opposed those of a study by Khalifa et al., 2017, as they documented that the rs 13397 minor allele A was the risk allele with RA propensity for both populations.[7]

Regarding the IRAK1 gene, the present study showed that the frequency of the major homozygous genotypes (AA) of the rs 1059703 was slightly higher in RA group (48%) compared to controls (46%); while the minor homozygous genotypes (GG) was the same in both groups (26%). In contrast to our results, the study by Khalifa et al., 2017 suggested a statistically significant elevation in the major homozygous genotypes of the IRAK1 gene in Tunisian as well as French control subjects (68% and 67%, respectively) in comparison with Tunisian as well as French RA patients (50% and 42%, respectively) (p<0.05).[7]

Concerning allele frequency in our study, the major allele of the IRAK1 was somewhat equivalent in both RA cases and controls (61% and 60%, respectively). Once more, the current study findings were contradictory to those of the study by Khalifa et al., 2017, as they showed that the major allele of the rs 1059703 was

increased in Tunisian as well as French control women (78% and 80%, respectively).[7] Our results might be dissimilar to the previously conducted research because of the presence of male subjects in our patients and controls groups, along with the small sample size and the different ethnic population between studies. Moreover, our study was conducted on Egyptian population that might have different genetic makeup.

When our patients were stratified according to gender, there was an increased incidence of minor genotype in the TMEM187 and IRAK1 genes in men, this elevation was statistically significant (p=0.004 and 0.015, respectively). In addition, we stratified the patients into 2 groups (remission/low/moderate activity group versus. high activity group) depending upon the DAS 28 classification score. We observed a statistically significant differences between the TMEM187 genotypes of men and women within the remission/low/moderate activity group.

To the best of our knowledge, there are no previous studies concerning gender distribution in RA patients in conjunction with the TMEM 187 and IRAK1 genes. Nevertheless, our study may throw a beam of light on the mechanism that the risk alleles can escape X-chromosome inactivation and could have an increased dosage in women in comparison with men.

Additionally, the skewed X-chromosome inactivation results when inactivation of one X-chromosome is favored over the other. By such mechanism, the risk allele might be expressed in > 50% of the female cells, participating in an elevated dosage in women.[19] Interestingly, X-chromosome in humans has one of the highest concentration of miRNAs of all chromosomes (118 miRNAs, based on miRbase), but the Y-chromosome has 4 miRNAs in humans only.[23] Ultimately, some X-linked miRNAs are encoded by genes or genomic sites that entail SNPs accompanied by rheumatic disorders or by genes that could escape XCI that might influence their expression.[24] Thus, TMEM187 might be an emerging gene for RA since it belongs to a group of genes which host microRNA in their sequence.

In conclusion, the present study illustrated the involvement of TMEM187 (rs 13397) and IRAK1 (rs 1059703) polymorphisms within the Xq28 locus in RA susceptibility and disease activity in Egyptian populations. Our findings support the notion that the two polymorphisms may be essential in the susceptibility for RA, particularly in females.

Author Contributions

HMZ, SSA, and RAMK performed the laboratory work, HMZ made the statistical analysis, EAA and HMA examined the patients and collected the samples. All authors participated in writing and reviewing the manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was reviewed and approved by the Research Ethical Committee of the Faculty of Medicine, Beni-Suef University (approval dated January 2019).

Informed consent

All participants signed an informed consent before being included in the study.

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