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Association of Interleukin 17F with Arthritis in Punjabi Families of Pakistan

ASSOCIATION OF INTERLEUKIN 17F WITH ARTHRITIS IN PUNJABI FAMILIES OF PAKISTAN

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ABSTRACT

Arthritis is a chronic inflammatory disease that causes severe joint pain. Interleukin 17F (IL17F) is considered as a candidate gene functionally; it mediates pro-inflammatory responses, depending on the type and site of inflammation. The present study examined the polymorphism of IL17F (rs763780 and rs2397084) among the families affected by arthritis. Demographic data and blood samples were collected from the families with at least one affected offspring with arthritis. Analysis of the IL17F gene polymorphism was performed by the digestion of DNA with NlaIII and AvaII. The results showed that IL17F rs763780 (AA, AG and GG genotypes) and rs2397084 (AA, AG and GG genotypes) were associated with arthritis (OA & RA). It was evaluated that about 65 and 21 percent of the individuals mutated with homozygous mutation for wild type allele A, heterozygous mutation A/G against selected SNPs respectively. But homozygous polymorphic allele for allele G was only found against rs2397084. Mutation in rs2397084 resulted to change Lysine into Arginine, whereas mutation in rs763780 changed Histidine into Arginine. Maternal history was found as a stronger factor in transferring arthritis. The results of this study revealed an association of arthritis with IL17F among Pakistani population.

Keywords: Arthritis, Interleukin 17F, Punjab, gene polymorphism, mutation

INTRODUCTION

Arthritis is a complex, chronic disorder that affects multiple joints (Karacan et al., 2018). There are two important forms of arthritis, one is Rheumatoid arthritis (RA) and second is Osteoarthritis (OA) (Yelin et al., 2007). The prevalence of Rheumatoid arthritis is about 0.5-1% of the population worldwide and varies widely between different countries (Gibofsky, 2012; Ferucci et al., 2005). In India the prevalence of RA was found to be 0.75%. In Pakistan, southern population (Karachi) and northern population were affected by RA 0.142% and 0.55% respectively (Alam et al., 2011; Xing et al., 2018). In epidemiology, 18% of women and 9.6% of men with ages in the range of and above their fifties suffer from OA and it was assessed that the prevalence of OA can be doubled by 2020 on the account of obesity and old age (Barton et al., 2008). RA and OA are heterogeneous diseases that involve interaction of biological factors (age, sex and BMI etc.) as well as genetic factors in their pathogenicity (Deane et al., 2017). *IL-17* was defined in 1995/96, as a pro-inflammatory cytokine, made by T cells and firstly named CTLA-8 (Miossec et al., 2009). Its association with RA and other inflammatory diseases was established immediately (Zhang et al.,

2013). Two SNPs in *IL-17F* gene (rs763780 and rs2397084) are present in the coding region at positions 7488 A/G and 7383A/G respectively. Both SNPs are localized in exon 3 and cause the change in DNA sequence by substitution of adenine to guanine and lead to change of an amino acid in the sequence of protein (Awasthi and Kuchroo, 2009). Arthritis is emerging rapidly in Pakistani population due to the genetic variations and positive family history. Cousin marriages lead to narrowing of the genetic pool so that defected genes transfer generation after generation. This study is imperative to finding the inheritance pattern of arthritis in families. There is a need to discover probable genetic factors contributing to this disease. On genetic grounds, first degree relatives of a proband can be expected to be more susceptible to arthritis than the cousins and the latter to have more susceptibility than unrelated persons. Up to our knowledge it is the first study in Pakistan to report *IL17F* susceptibility for arthritis in families.

MATERIALS AND METHODS

Ethical approval for the current study was obtained by the appropriate ethical committees and Board of Advance Research, department of Zoology, G.C.U. Lahore. A pre-informed consent was signed by all the subjects, enrolled in the study. Samples for case-control study were collected from the families, carrying arthritis. Some of the families were excluded due to lack of clinical assessment data. For collection of informative data from the participants a consent form was designed. All the families in this study containing at least one or two individuals affected with arthritis. The patients were clinically diagnosed with arthritis by the physician in accordance with World Health Organization (WHO) criteria and American College of Rheumatology criteria of 1987. Demographic variables were also recorded by using a questionnaire that included; age, sex, weight, height, type of arthritis (RA, OA or others), gender (male/female), BMI (above or below normal value), family history (mother, father, siblings, grandparents, first degree relatives or other). A total of four families with arthritis were included in this study. The reported data was tabulated to assess the demographic features of the individuals.

The proband families were selected for blood sampling. DNA was extracted by using blood samples of the patient and control families. For this purpose, phenol/chloroform manual method was used. All the DNA samples were quantified (concentration) and qualified (purity) on Nano-drop (thermos scientific 2000). For genotyping, concentration of DNA was more than 50 ng in µl and purity was more than 1.8 (260/280). SNPs in *IL17F* gene were identified by using literature searches as well as public SNP databases. For genotyping, *IL17F* PCR-RFLP method was selected by using thermo cycler instrument. The total reaction mixture of 12 µl with 1 µl of DNA sample, 0.75 µl of forward primer and 0.75 µl of reverse primer, 4 µl of PCR Master mix and 5 µl of DEPC. We used a set of primer sense: 5′GTGTAGGAACTTGGGCTGCATCAAT -3′, antisense primer: 5′AGCTGGGAATGCAAACAAAC-3′ was used. The conditions for PCR cycles were as follows: after heating lid, Initial denaturation was set for 5 minutes at 95°C, 35 cycles were set for denaturation step for 45 seconds at 95°C, gradient or annealing temperature for these primers was 57.5°C for 45 seconds, at 72°C elongation was set for 30 seconds, then final elongation was set for 10 minutes at 72°C and finally storage at 4°C. The amplified PCR product was digested by *NlaIII* and *AvaII* restriction endonucleases

(thermo-scientific). *NlaIII* yielded fragments of 288 bp, 130 bp and 52 bp for allele A, whereas 418 bp and 52 bp for allele G. *AvaII* yielded fragments of 470 bp for allele A whereas 395 bp and 75 bp for allele G. Online progeny tool was used to draw family pedigrees. For amino acid sequence analysis MEGA6 software was used.

RESULTS

Samples of seven families were collected from the area of Lahore and Okara, Punjab, Pakistan. All families were found with positive family history of arthritis. Among these families four were recruited for blood sampling. Sixteen individuals; six parents, eight siblings, one first degree relative and one second degree relative belonging to the families with this disease were studied. There were ten females and six males participating in this study. The ratio of females was higher compared to males. The patients of the family were diagnosed with rheumatoid and osteoarthritis. The mean age of proband was ± 46.75, whereas mean BMI of the proband was ± 27.45. It was found that the disease diagnosis age of the patients was greater than 40 years. Each of the proband was with high BMI. The clinical characteristics of the families were presented in Table 1.

Table 1. Clinical characteristics and genotyping Analysis of families

Sr No.	Code	Gender (M/F)	Age (years)	BMI (kg/m²)	Category	Disease diagnosed
Arthritis Family AF_1						
1.	M_1	F	54	30.4	Ob	RA
2.	M_2	F	31	31.3	Ob	RA
3.	M_3	M	28	26.3	Ow	Healthy
4.	M_4	F	20	26.4	Ow	Healthy
Arthritis Family AF_2						
5.	M_1	F	50	25.2	Ow	Healthy
6.	M_2	M	29	26.0	Ow	OA
7.	M_3	F	25	23.4	N	Healthy
8.	M_4	M	25	21.5	N	Healthy
Arthritis Family AF_3						
9.	M_1	F	65	26.1	Ow	OA
10.	M_2	M	31	25.7	Ow	Healthy
11.	M_3	F	35	26.6	Ow	Healthy
12.	M_4	M	1.5	19	N	Healthy
Arthritis Family AF_4						
13.	M_1	M	44	26.8	Ow	Healthy
14.	M_2	F	39	27.3	Ow	RA
15.	M_3	F	16	24.3	N	Healthy
16.	M_4	F	10	32.5	Ob	Healthy

F: Female, M: male, Ob: Obese, Ow: Overweight, N: Normal

Phenotypic Analysis of families

The family AF_1 consisted of eleven members; blood sample of four members with descriptive data of all the members was collected. The proband woman was diagnosed with RA at the age of 44 and found it to be more complicated after five years of diagnosis. Examination revealed rheumatoid arthritis affecting wrist, elbows, knees and sometimes back pain. The BMI of proband was measured 30.4 kg/m² and categorized as obese. One of the daughters had rheumatoid arthritis with severe symptoms. Rheumatic nodule on the wrist was found and fingers, knees and back were also affected. The daughter was initially diagnosed with RA at the age of 29 years. She was categorized as obese with the BMI of 31.3 kg/m². Proband mother and one sister had arthritis in family history.

The family AF_2 consisted of seven members. A blood sample of four members was collected for genotype analysis. The proband man had osteoarthritis at the age of 27 years with normal weight. Examination found that he was suffering with pain in pelvic bone. Ankles were also affected and uric acid level was high. Only the proband mother had complications of back and pelvic pain but not properly diagnosed with any form of arthritis.

Family AF_3 consisted of six members. The proband woman had osteoarthritis at the age of 65 years with high BMI and suffered from severe back and knee pain. There was no positive history of the proband with arthritis.

The family AF_4 consisted of eight members. Four members were included in genotype analysis. The proband woman was diagnosed with RA at the age of 39 with severe back pain. Proband mother had rheumatoid arthritis. Phenotypic pedigrees of four families ae represented in Figure 1.

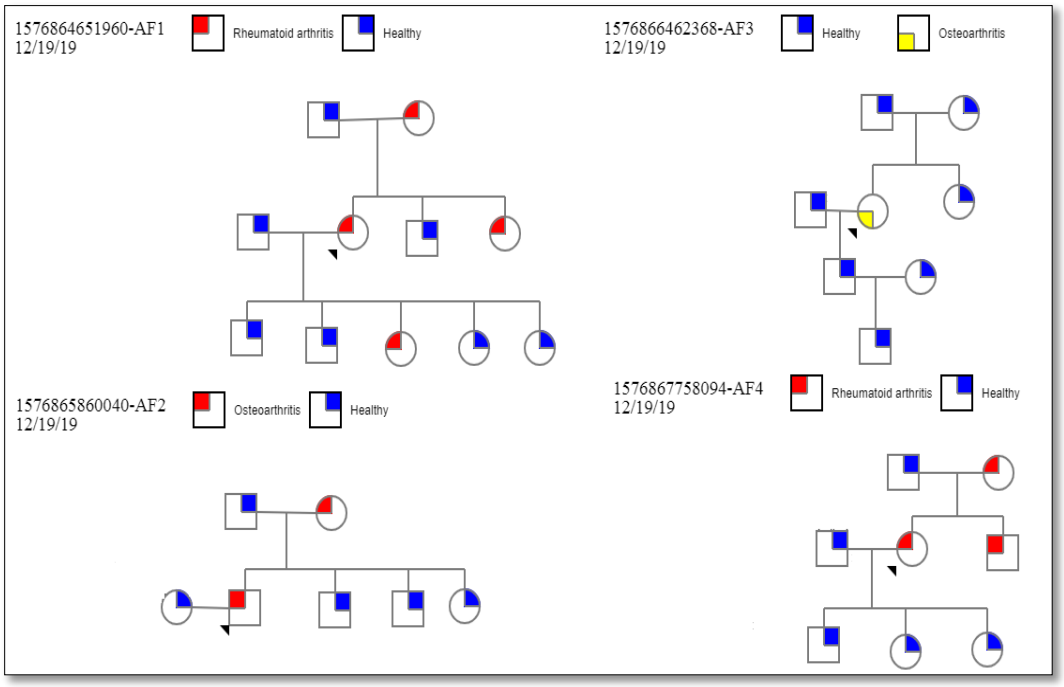

Figure 1. Phenotypic pedigrees of four families of arthritis showing how arthritis transfers from parents to siblings.

Genetic analysis for RFLP (NlaIII (HinIH) and AvaII (Eco 47I) and family pedigree

The selected *IL17F* gene was analyzed for its association with arthritis for SNPs (rs763780 and rs2397084). DNA was isolated from each blood sample and the mean concentration of isolated DNA was up to 50 ng in 1 µl and purity value was 1.8 for each sample. For optimization of primers gradient PCR was used. The annealing temperature, 57.5°C was found same for both rs 763780 and rs 2397084. PCR amplification was performed on DNA samples of all the diseased families for both SNPs (rs763780 and rs2397084). The PCR products of 470 bp of both rs763780 and rs2397084 were obtained by using gradient PCR. The amplicons were confirmed by running 1.2-2% agarose gel electrophoresis. The PCR product of rs763780 and rs2397084 were digested by restriction enzymes *NlaIII* (*HinIH*) and *AvaII* (*Eco 47I*) respectively (Figure 2).

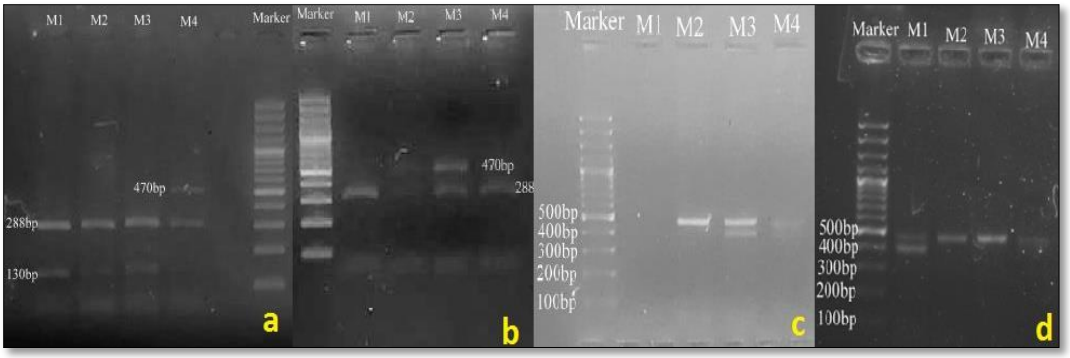

Figure 2. Identification of *IL17F* rs763780 and rs2397084 genotypes; (a) & (b) show mutation type in the families' one and four for rs763780 by the digestion of RFLP *NlaIII*, respectively. The figures (c) & (d) show mutation type in the families' one and four for rs2397084 by the digestion of RFLP *AvaII* respectively.

Genotyping of all selected families and pedigree analysis

Family AF_1 two members M_1 and M_2 were mutant with AA for both SNPs whereas M_3 showed homozygous mutation for wild type allele A for rs763780 and heterozygous mutation for rs2397084. M_4 was heterozygous mutant for both respective SNPs. All the members of family AF_2 were mutant with homozygous wild type allele A for rs763780 and rs2397084. For rs763780, M_1 of family AF_3 showed heterozygous (A/G) mutation. M_2, M_3 and M_4 was mutant with homozygous wild type allele for A. For rs2397084 M_1 and M_3 showed homozygous mutation for polymorphic allele G whereas M_2 and M_4 show homozygous mutation for wild type allele A. Among four, three of the members of the family AF_4 showed homozygous mutation for wild type allele A as well as heterozygous mutation (A/G) for both SNPs. The member M_4 showed homozygous mutation for wild type allele A for rs763780 and homozygous mutation for polymorphic allele G for rs2397084 (Figure 2).

Figure 3 represented the pedigrees of families with respect to the type of mutations in families. Family Pedigrees for

AF_1 and AF_4 families for rs76378 (a): the proband of AF_1 and two of the siblings showed homozygous (7488A/A) mutation for wild type sequence of *IL17F* gene. Whereas one of the siblings shows heterozygous mutation for the exon 3 of the gene *IL17F* (7488 A/G). The proband of AF_4 and one of the siblings showed homozygous mutation for wild type of *IL17F* (7488A/A). Whereas first degree relative and one of the siblings showed heterozygous mutation for exon 3 of *IL17F* (7488A/G). Representation of Family Pedigrees of family AF_1 & AF_4 for rs2397084 (b): the proband and one of the siblings of AF_1 showed homozygous mutation for *IL17F* (7383 A/A) and two of the siblings showed heterozygous mutation of *IL17F* (7383A/G). The proband of family AF_4 showed heterozygous mutation for *IL17F* (7383A/G). One of the siblings showed homozygous mutation for *IL17F* (7383 G/G) and two of the siblings showed homozygous mutation for *IL17F* (7383 A/A).

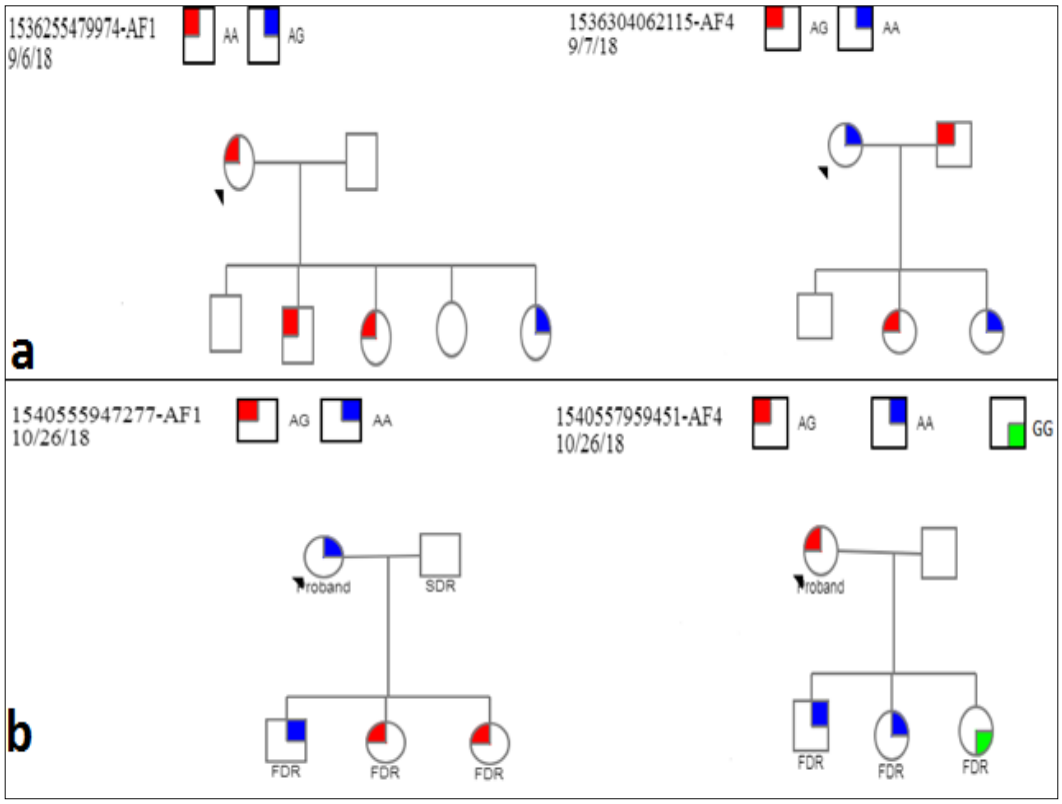

Figure 3. Representation of (a) Family Pedigrees for AF_1 and AF_4 families for rs763780 and (b) Family Pedigrees of family AF_1 & AF_4 for rs2397084.

Nucleotide sequence was aligned to analyze the change in amino acid sequence that may alter the protein structure. Mutation in rs2397084 resulted in change of Lysine into Arginine. Whereas mutation in rs763780 changed Histidine into Arginine.

DISCUSSION

Arthritis is a heterogeneous disease caused by both biological as well as genetic factors. In the current study, the mean BMI of all the probands was observed to be ± 27.45 (kg/m^2). The individuals with high

BMI were also evaluated, as they are at a high risk of various forms of arthritis (Sultana et al., 2020). The mean age for the development of arthritis was found to be 48±13 years (Hannawi et al., 2020). This study found the age for the onset of arthritis to be above the fourth decade of life. The current study revealed that females are highly associated with arthritis. As this study demonstrated, strong maternal history was involved for transferring this disease in families. Previous studies, implicate *IL17F* gene to the pathogenicity of RA and OA.

Here we investigated the role of *IL17F* gene polymorphism with susceptibility of arthritis for the first time in Pakistani population. We studied two SNPs in *IL17F* gene at location 7488A/G (rs763780) and 7383 A/G (rs2397084). Both these SNPs reside at exon 3, causing substitution of adenine into guanine (AG), which change amino acids at 126 position in the sequence of protein. The first SNP 763780 changed Lysine into Arginine and the second SNP 2397084 changed Histidine into Arginine. The results of the current study showed association of both SNPs of *IL17F* gene with OA and RA.

Four families were included for the genotyping of SNP 763780. First family (AF_1) shown three members including proband with 7488A/A homozygous mutation for wild type allele while one of the siblings showed heterozygous mutation for exon 3 7488 A/G. All the members of second family (AF_2) were mutant with wild type homozygous mutation 7488A/A. Third family showed proband with heterozygous mutation for *IL17F* (7488A/G) whereas, two siblings showed homozygous 7488A/A wild type mutation. Analysis for the fourth family showed that the proband and one of the siblings were mutant with wild type allele whereas, two of the siblings were with heterozygous mutation for exon 3 of *IL17F* (7488A/G).

The genotyping of rs2397084 was analyzed in the same families of arthritis. Three out of four families showed association with the describing SNP. Among family AF_1, two of the siblings showed heterozygous mutation for exon 3 of *IL17F* (7383A/G) whereas two of members were not mutant. For the second family (AF_2), all members showed homozygous mutation for wild type allele A.

In third family (AF_3), Proband and one of the siblings showed homozygous mutation with polymorphic allele for *IL17F* (7383G/G) whereas, the father and one of the siblings showed homozygous mutation for wild type allele A. Amongst the four families, one family (AF_4) gave different results as proband was associated with this SNP and showed heterozygous mutation for exon 3 (7383A/G), one member with homozygous polymorphic allele G and two with homozygous wild type for allele A.

CONCLUSION

Such family studies can be applied for personal diagnosis prior to onset of disease. A powerful way to study the association between a disease and genetic marker, is to show a linkage in families. The results provided evidence that *IL17F* gene (763780, 2397084) is related with the occurrence and severity of arthritis (RA and OA). These family studies suggested that the risk of arthritis among relatives (proband and siblings) is influenced by the sharing of genetic factors.

CONFLICT OF INTEREST

All the authors do not have any conflict of interest regarding the submission of this manuscript.

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