Polymorphisms in DNA repair genes XRCC1 and OGG1 lead to the progression of rheumatoid arthritis in Pakistani patients

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Abstract: This study points at the elucidation of a possible association of Rheumatoid arthritis (RA) with Ser326Cys in OGG1 Arg194Trp and Arg399Gln polymorphisms of XRCC1 using a sample size of 100 patients and 100 controls from a Pakistani population. This association was determined using Random Fragment Length Polymorphism Analysis as well as the DAS scoring system. In RA, oxidative damage due to free radical production leads to destructive proliferative synovitis showing cellular transformations of synoviocytes into a tumorigenic state. XRCC1 and OGG1 genes, which are part of the DNA Break Excision Repair pathway, manifest various polymorphisms which may cause a variation in the response to inflammation by changing DNA repair potential. Our results showed a significant association between the DAS28 score values as well as the genotypic state of the RA patients. It was seen that the score was significantly higher in GG genotypes thereby corroborating the role of the polymorphism XRCC1 Arg399Gln. Using a Pearson's correlation test it was found to be <0.000003. It has been shown by the results in this research that an increased risk of DNA damage exists when the polymorphic genotypes studied, exist in a RA patient.

Keywords: Rheumatoid Arthritis, XRCC1, OGG1, BER, DNA damage

INTRODUCTION

The autoimmune disease Rheumatoid Arthritis (RA) can be characterized as a chronic inflammatory disorder, which is accompanied by the pain, swelling and stiffness of joints (Feldmann *et al*., 1996). It has been seen that advanced disease states eventually lead to a functional loss of mobility as well as an increased risk of morbidity as well as mortality. It is notable that according to research in 1997, 22% of all deaths account for RA and other rheumatic diseases (Sacks *et al*., 2004). Across varying geographic regions, the disease prevalence varies. It is thought that at any given point in time that 0.5-1% of the world's population suffers from rheumatoid arthritis (Silman *et al*., 2001). The onset of the disease has said to originate between 30 to 50 years, although it may also occur at any age (Rindfleisch *et al*., 2005). Women are said to suffer more from RA, possibly due to higher body fat since obesity is associated with RA (Visser *et al*., 1999). There is also a link between the less educated and socioeconomic status of an individual and the increased risk of RA (Bengtsson *et al*., 2005).

A deregulated immune system is said to be the underlying cause of RA, characterized by elevated inflammatory cytokine levels (Sattar *et al*., 2003). Initially there is the production of rheumatoid factors followed by articular localization as well as articular synovitis, which is characteristic of the articular phase (Jimenez-Boj *et al*., 2005). The later formation of the interstitial zone, which is hypoplastic, in nature and consists of infiltrating synovial macrophages as well as fibroblasts, along with angiogenesis and hypoxic conditions are the characteristic feature of rheumatoid joints (Firestein, 2003).

Due to the immense progression in genome wide association studies (GWAS), there has been a significant progression in the evaluation of the genetic basis of RA. The evaluation of single nucleotide polymorphisms (SNPs) as well as their significant association with RA has been an avenue for study of late. This is due to the widespread prevalence of SNPs in the human genome, approximately 1 in every 1330 bases (Lewin *et al*., 2004). It has been validated that RA is multifactorial disease and the genetic basis for the clinical presentation of the disease is significantly plausible (Jirholt *et al*., 2001). Estimations into the association of genetic factors and RA have been evaluated at 60% (Macgregor *et al*., 2000). The currently studied genetic background of RA has been approximated at 16% (Svendsen *et al*., 2002), leaving 84% to be validated. Chromosomal regions evaluated as significant in RA are 6p, 1p13, 16p, 18q, 6q16, 18q and 1q41-43 (Gregersen, 2005). The Human Leukocyte Antigen (HLA) gene locus remains to be the strongest evaluated link between genetic susceptibility and RA (de Vries N *et al*., 2002). Although other non-HLA genes may determine the diseased phenotype (Orozco *et al*., 2006), genetic susceptibility by the HLA gene has been evaluated as 30-60% conclusive (Imboden *et al*., 2009).

Human DNA comes under significant stress due to environmental factors like ultraviolet radiation, ionizing

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radiation and chemical modifications like depurination, deamination, oxidative stress and alkylation (Lindahl *et al*., 1993). As a result of these modifications cellular micro-pathologies might alter the normal rate of cellular proliferation (Hoeijmakers *et al*., 2001). There are 400 SNPs that have been evaluated in 80 genes out of a total of 130 genes identified to have a potential role in DNA repair mechanisms of the human body (Mohrenweiser *et al*., 2003). In RA, localized oxidative stress in the synovial joint has been evaluated as a possible mechanism deregulating the RA synovial cell. Breakage of DNA strands observed in mononuclear cells isolated from RA patients corroborate its prodigious role in DNA repair (Bhusate *et al*., 1992). Tumor suppressor gene p53 exhibits mutations in RA synovial cells further validating the association of DNA damage and RA (Yamanishi *et al*., 2002). The initiator of BER DNA repair pathway, OGG1 located at the 3p25 chromosomal region plays its role in the removal of 7, 8-dihydro-8-oxoguanine (8-oxoG) from DNA, which results in a G-to-T transversion at the complementary DNA strand (Srivastava *et al*., 2009). X-ray Cross Complimentary Factor 1 (XRCC1) interacts with 8-Oxoguanine Glycosylase 1 (OGG1) and results in the increased activity of OGG1 by two to three fold. It has been noted that polymorphisms exist in the genes responsible for the expression of both proteins. Particularly codon 326 causing a serine to cysteine change in case of OGG1, which has shown reduced DNA repair ability (Hill *et al*., 2006). Variants in XRCC1 have also been evaluated at codon 194 in the 6th exon causing a change from arginine to tryptophan. Another change in exon 10 leads to the mutation of arginine to glutamine at codon 399 thereby reducing DNA repair potential of XRCC1 (Spitz *et al*., 2003). It is known that polymorphisms in genes responsible for DNA repair pathways results in genomic instability and hence untoward apoptosis or tumorigenesis (Caldecott *et al*., 2003).

Methodology

Patients and controls

Blood samples were collected from 100 rheumatoid arthritis patients from the Rehmat Noor Arthritis Research Center and Clinic, Rawalpindi (RNC) as well as 100 age matched controls. The patients were selected having at least 4 of the revised American College of Rheumatology (ACR) and European League Against Rheumatism (EULAR) classification criteria points for rheumatoid arthritis (Aletaha D *et al*., 2010). Clinical information collected pertaining to the age, sex, disease activity as well as duration of the disease for all patients. All patients provided a written informed consent for participating in this study. Furthermore the institutional review board for experimental ethics at ASAB NUST also approved the study.

Evaluation of DAS28 values

Patients were evaluated according to the disease activity score (DAS28), the number 28 representing the 28 joints evaluated under this scoring system. This involves a rheumatologist or nurse examining joints for signs of inflammation and then correlating it to the erythrocyte sedimentation rate as well as the C reactive protein test. The DAS28 score was then evaluated using a DAS calculator. This allowed for a measure of the degree of inflammation in an individual.

Extraction and Quantification of Genomic DNA

The organic phenol-chlorofom method was used for the isolation of genomic DNA from cells (Sambrook *et al*., 2001). The extracted DNA was purified and later precipitated. The samples were stored at 4°C for further use. The optical density (OD) was measured at 260 nm in order to obtain the concentrations of DNA using 1/100 to 50µg/ml DNA dilution in quartz cuvette (Green *et al*., 2012).

Primer Designing of XRCC1 gene for XRCC1Arg399Gln, XRCC1Arg194Trp and OGG1 Ser326Cys SNP evaluation

Pre-designed primers for human XRCC1 and OGG1 gene for RFLP were used. (Yosunkaya, *et al*., 2011). Sequence was obtained from NCBI nucleotide database. CLUSTALW tool (CLUSTAW©) was used for an appropriate sequence homology search. Primers were designed using conserved defined flanking regions on the gene. The designed primers were altered by BLAST (NCBI) and by OLIGOCALC (OLIGOCALC©). The primers designed for XRCC1Arg399Gln were, 5'-TCCTCCACCTTGTGCTTTCT-3' for the forward primer, 5'-AGTAGTCTGCTGGCTCTGGG-3' for the reverse primer. For XRCC1Arg194Trp the forward primer was designed as 5'-GCCCCGTCCCAGGTA-3' and the reverse primer was 5'-AGCCCCAAGA CCCTTTCACT-3'. For OGG1Ser326Cys the forward primer designed was 5'-ACTGTCACTAGTCTCA CCAG-3' and the reverse primer was 5'-CCTTCCGGCC CTTTGGAAAC-3'.

PCR Amplification and genotyping of XRCC1 A399G, XRCC1 A194T and OGG1 S326C:

XRCC1 A399G, XRCC1 A194T and OGG1 S326C polymorphisms was detected by using PCR-RFLP analysis using a previously published protocol (Hizawa, *et al*., 2004). For the independent amplification of human OGG1 and XRCC1 genes, genomic DNA quantity used was 200ng in a PCR reaction mixture of 12.5µl. PCR was progressed with in a SwiftTMMaxPro thermal cycler (Applied Bio systems, USA) followed by visualization of 50% of DNA products on a 2% agarose gel using an UV trans-illuminator in a Gel Documentation System (Wealtec Dolphin doc, Sparks USA) and then photographed.

Restriction fragment length polymorphism analysis of PCR-Amplified XRCC1 A399G, XRCC1 A194T and OGG1 S326C

The A to G substitution at codon 399 in X-ray Repair Cross Complementing gene- XRCC1 destroys the site for *BcnI* enzyme. The PCR products were then digested with the restriction endonuclease *BcnI* (1U/10µl) (Thermo Scientific, #ER0061) which recognizes the sequence 5'-CCSGG-3'at 37°C for 16 hours. Likewise, the C to T substitution at codon 399 encoding sequence in XRCC1 destroys the site for *MspI* enzyme and hence, only a single internal control site for *MspI* remains. PCR products were digested with the enzyme *MspI* (1U/10µl) (Thermo Scientific, #ER0541) at 37°C for a couple of hours. Finally, a single nucleotide substitution in the sequence at the 326 position that encodes for amino acid Serine causes the encoding of Cysteine instead of Serine and the sequence in turn harbors the restriction site for the *ItaI* enzyme. Digestion of the PCR products was performed using *ItaI* (1U/5µl) (Thermo Scientific), this was at 37°C for 2 hours. *ItaI* recognizes the sequence defined as 5'-GCNGC-3'. Succinctly, digestion was followed by electrophoresis analysis using a 2% agarose gel with subsequent visualization in a UV trans illuminator in a Gel Documentation System (Dolphin doc, USA) and photographed.

STATISTICAL ANALYSIS

For all statistical analysis various analytical softwares were used. SPS© ver 16.0 was used to conduct the Pearson's test as well as the chi squared test. Verification was performed using Graphpad Prism© ver 5.01. Microsoft Office Excel© was used for the construction of graphs and indices.

RESULTS

DAS28 score evaluation

Significant DAS28 scores were evaluated for all individuals included in the study. 43% of the patients had a high DAS28 score while 42% of individuals had a moderate DAS28 score. It was seen that only 7% of individuals had a low DAS28 score and only 7% of individuals were in remission (as shown in fig. 1). The mean age for the individuals in the experiment was seen to be at 44.47 years of age. Conclusively it was observed that a higher DAS28 score coinciding to a higher presence of disease was seen in the age group 45-53 years.

Polymorphisms, their genotype and DAS28 association

Statistical analysis of the polymorphism Arg399Gln in XRCC1 and RA using Chi-square test elucidates a significant association (p-value <0.0001). Likewise, Fischer's Exact Test results also demonstrated a highly significant and plausible p-value (<0.0001). A Z-test applied to validate both the genotypic and allelic findings also a showed substantial p-value (<0.0001). Furthermore, significant associations were elucidated between the recorded DAS28 score values as well as the genotypic status observed in the RA patients using a Pearson's correlation test (<0.000003). The score observed to be significantly higher in GG genotypes hence validating the role of polymorphism XRCC1 Arg3999Gln.

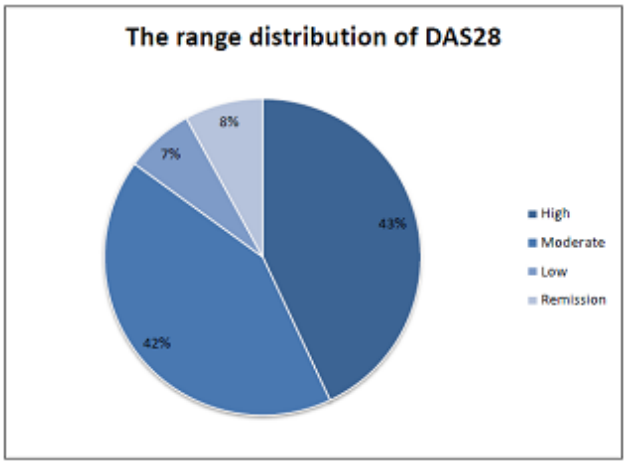

Fig. 1: Pie chart showing the range distribution of DAS28 among Rheumatoid Arthritis Patients

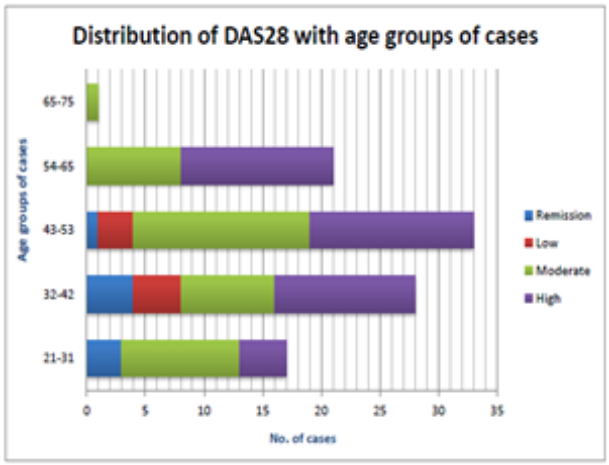

Fig. 2: Chart showing distribution of disease activity score DAS28 with relation to age groups

The Arg194Trp polymorphism in XRCC1, on the other hand, has shown to have insignificant association with RA in all the three tests. Values for Chi-square test, Fisher's Exact test and Z-test came out to be 0.3281, 0.3703 and 0.281 respectively. It was also observed that there was no significant association observed in the DAS28 score and the genotypic status of the RA patients. It was demonstrated however that the score was higher for the TT genotype. The Pearson's correlation test was seen to have a p value of (0.131) which was also non significant.

Likewise, OGG1 Ser326Cys polymorphism did not show a significant association. Chi-squared test showed an insignificant association; p value (0.249) while Fischer's

Exact Test and Z-test gave the values0.0946 and 0.074 respectively which are non-significant. Hence, there is the lack of a significant association between DAS28 score and the genotype of RA patients. The score was however found to be significantly higher in CC genotypes. Pearson's correlation test was also found to be insignificant; p value (0.22).

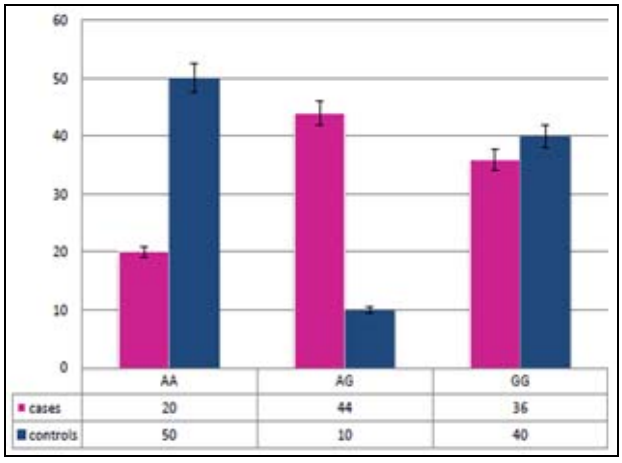

Fig. 3: Genotypic association in the XRCC1 A399G polymorphism of genetic frequencies in cases compared to control patients.

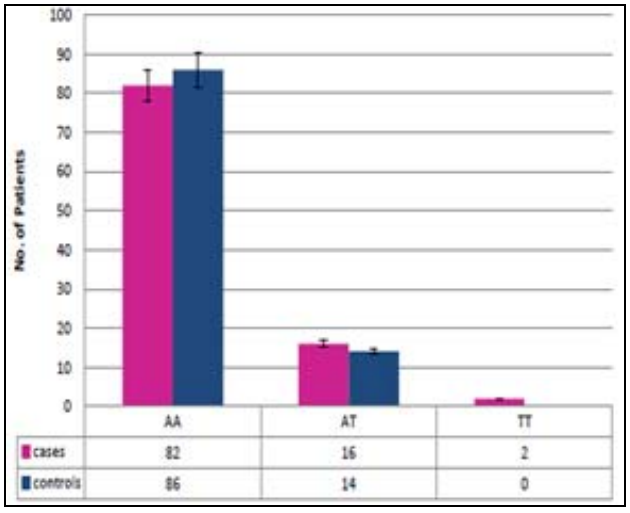

Fig. 4: Genotypic association in the XRCC1 A194T polymorphism of genetic frequencies in cases compared to control patients.

DISCUSSION

Rheumatoid arthritis (RA) is a characterized by chronic inflammation and severe deformities in joints, tendons and bones (Østergaard *et al*., 2003). Previous studies on the genetics of RA and the formation of a diseased phenotype have significantly increased over the years. The present study entails the analysis of XRCC1 Arg399Gln, OGG1 Ser326Cys and XRCC1 Arg194Trp gene polymorphisms in RA patients from a Pakistani population. It is notable that previously performed studies have indicated that the prevalence rate of RA spikes at the age of 35-40 years (Gabriel *et al*., 1999), therefore the large share of patients isolated for this study were within the age group 30-80 years. RA exhibits a gender bias with a three times higher prevalence in females than seen in males (Karlson, 2004); therefore, majority of patients involved in the study were females. The base excision repair system (BER) has been previously shown to be the main DNA repair pathway for the removal of modified nucleotide bases (Lindahl *et al*., 1999). It has been seen that the process commences with DNA glycosylase recognition of the modified base and continues until its excision (David *et al*., 2007). One of the major DNA glycosylases 8-oxoG in mammals is the oxoguanine-glycosylase (OGG1) that discriminates 8-oxoG from its normal counterpart (Barnes *et al*., 2004). For sufficient BER activity, a scaffold protein without any known enzymatic activity, known as the X-ray repair cross-complementing-1 factor (XRCC1), interacts with all of the involved enzymes in the BER pathway and then organizes their activities. The interaction of XRCC1 with OGG1 increases the glycosylase activity of OGG1 by 2 to 3 fold (Bhakat *et al*., 2006). It was the aim of this research to associate the malfunctioning of DNA damage repair genes and RA in Pakistani patients.

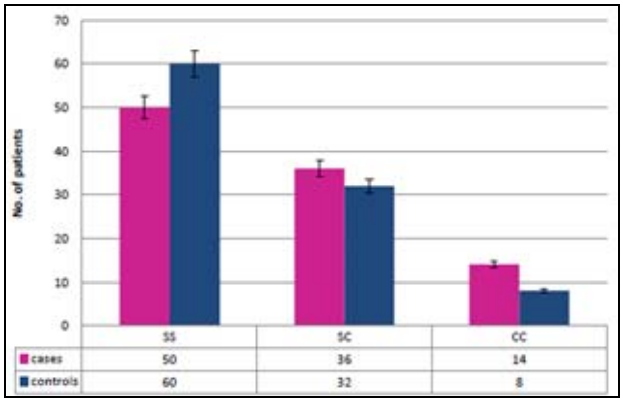

Fig. 5: Genotypic association in the OGG1 C326G polymorphism of genetic frequencies in cases compared to control patients.

It has been validated in our research that the XRCC1 Arg399Gln variant polymorphism has significant role in RA disease progression and severity our results have been supported by independent researcher who performed the same study in a Turkish population (Yosunkaya *et al*., 2012). Our results also are comparable to them as the other two variants of our study XRCC1 Arg194Trp and OGG1 Ser326Cys show negative association with RA. This data hence supports an ethnic based similarity that exists between the Turkish population as well as the Pakistani population (Huffman *et al*., 2005). These findings support the possibility of a common genetic lineage for both population groups. It is notable that in our study the frequency of the hetrozygote genotype observed for the XRCC1 Arg399Gln phenotype is in

Table 1: Genotypic Frequencies of various SNPs under study calculated through the Chi-square test, showing the P-value of genotypes.

Polymorphism Under Study	Genotype %	Cases (n=100)	Controls (n=100)	X^2 Statistic		
				X^2 Value	df*	P-value (alpha<0.05)
rs25487	AA	20	50	29.16	2	<0.0001
	AG	44	41			
	GG	36	9			
rs179978	AA	82	86	2.229	2	<0.3281
	AT	16	14			
	TT	2	0			
rs1052134	CC	50	60	2.7812	2	<0.2490
	CG	36	32			
	GG	14	8			

*Degrees of Freedom

Table 2: Allelic Frequency of various SNPs under study calculated through Fisher's exact test showing the P-value of the alleles.

Polymorphism Under Study	Allele	Cases (n=100)	Controls (n=100)	OR (95% CI)*	RR (95% CI)**	P-value
rs25487	A	84 (42%)	141 (70.5%)	0.3030 (0.2003-0.4583)	0.5632 (0.4613-0.6876)	<0.0001
	G	116 (58%)	59 (29.5%)			
rs179978	A	180 (90%)	186 (93%)	0.6774 (0.3320-1.382)	0.8361 (0.6194-1.129)	<0.3703
	T	2 (10%)	14 (7%)			
rs1052134	C	136 (68%)	152 (76%)	0.6711 (0.4321-1.042)	0.8264 (0.6755-1.011)	<0.0946
	G	48 (24%)	48 (24%)			

*Odds Ratio, **Relative Risk.

statistical concordance with results obtained from the Turkish population. Our results of cases vs. controls with respect to XRCC1 Arg399Trp polymorphisms (44% and 41%) were very similar to the Turkish populations in the aforementioned study, 41 and 40%. Another interesting association of results within this meta-analysis was seen amongst our results, which were in concordance with the Caucasian population 39 and 55% cases and controls respectively (Kocabas *et al*., 2006). It has been observed in another independent study that systemic lupus erythematosus polymorphic allele frequency of the allele G was seen to be significantly different between our results (58% and 29.5%, cases and controls respectively) and their observed frequency (68.6% and 72.6%, cases and controls respectively) (Lin *et al*., 2009). It has been observed that differences due to random variation, sample variations, genetic heterogeneity, environmental stress, genetic lineage and lifestyle may contribute to these fluctuations in statistical data. It is notable to suggest here that the genetic polymorphisms associated with OGG1 causing the variation Ser326Cys may not allow OGG1 to assume conformational changes required to modify DNA during damage, hence not showing inefficient DNA repair at damage sites. However, it is notable that the presence of the XRCC1 Arg399Gln polymorphic variation will result in a conformational change in the protein structure of XRCC1 deviating from its wild type alignment on damaged DNA sites. This equates to altered DNA damage site recognition and repair. It is also seen that the polymorphic variation XRCC1 Arg194Trp is similar in effect to the OGG1 Ser326Cys polymorphic change (Kohno *et al*., 2006). This is validated by the negative association shown in our results of the XRCC1 Arg194Trp polymorphic variation with RA. The variation and deficient function of XRCC1 due to the polymorphic Arg194Trp variation has been studied in numerous cancers but was not shown to have an effect as far as RA was concerned, possibly due to an alternate route by a downstream protein or unaltered protein binding efficiency of XRCC1 in RA patients (Kiyohara *et al*., 2006). It is possible that polymorphisms are inadvertently affecting protein expression profiles. Furthermore, we suggest a larger sample size as it is required in order to determine the locations of other polymorphisms particularly in the promoter region of the XRCC1 gene, which may have a statistically significant correlation with RA.

CONCLUSION

It is concluded by our results that we found XRCC1 Arg399Gln to be significantly associated with RA. Genetic discrepancies in the studied gene may be the cause for irregularities in its proper functioning and repair of lesions in DNA. We also found that the polymorphic variations XRCC1 Arg194Trp and OGG1 Ser326Cys

Table 3: Genotypic and allele frequency calculations of SNPs using the Z test.

Polymorphism Under Study	Genotype %	Cases (n=100)	Controls (n=100)	Z Test Statistic		
				N	Z Value	P-value (alpha<0.05)
rs25487	AA	20	50	70	5.998	<0.0001
	AG	44	41	85		
	GG	36	9	45		
rs179978	AA	82	86	168	1.077	<0.281
	AT	16	14	30		
	TT	2	0	2		
rs1052134	CC	50	60	110	1.789	<0.074
	CG	36	32	68		
	GG	14	8	22		

were not associated with RA in Pakistani patients. It is advised however that a small sample size warrants for a large scale analysis in various ethnic populations to determine the extent and accurate association of the studied polymorphisms in question with relation to RA.

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