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Role of SOD1 Gene (–251A/G) Polymorphism in Changing the level of Serum Metals and Minerals in Rheumatoid Arthritis Patients

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ABSTRACT

Oxidative stress is culprit of several pathological and physiological conditions. One of them is Rheumatoid arthritis (RA). The exact cause of RA is still unknown. Reduced activity of antioxidants enzymes because of polymorphisms in antioxidant genes and variations in levels of metals and minerals creates oxidative stress may play some role in progression and pathogenesis of RA. The objective of this study was to investigate the role of Superoxide dismutase 1 (SOD1) gene polymorphism [rs2070424 (–251A/G)] in RA and to compare the levels of metals and minerals among its various genotypes in RA patients. A total of 400 individuals including age and gender matched healthy individuals (control group) and RA patients were genotyped. Detection of rs2070424 polymorphism was carried out using allele specific PCR based amplification strategy. The serum samples were analyzed for determination of metals and minerals through Atomic absorption spectrophotometer (AA 6600 Shimadzu). The statistical analysis indicated that non-significant association existed between SOD1 (rs2070424) gene polymorphism and RA ($p>0.05$). Results of HWE estimation indicate that allele frequencies were not deviant from HWE in RA group. The results of present study indicates that Pb and Cr concentration differ significantly between AA, AG and GG genotypes of SOD1 (rs2070424) gene polymorphism in RA patients as compared to control group ($p<0.05$). The present finding indicates that SOD1 (rs2070424) gene polymorphism is not associated with pathogenesis of RA; however levels of Pb and Cr may impart strong influence towards the development of RA in the presence of this polymorphism.

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Authors' Contributions
SI and AR designed the study, performed the experimental work, statistically analyzed the data and wrote the article. RB and SI helped in collection of data. MA helped in preparation of manuscript.

Key words
Rheumatoid arthritis, rs2070424, SOD1 A/G polymorphism, Metals, Minerals.

INTRODUCTION

Oxidative stress is culprit of several pathological and physiological conditions such as atherosclerosis, diabetes, aging, RA, osteoarthritis, cancer, inflammatory bowel disease, and many more (Halliwell and Gutteridge, 1985; Martinez-Cayuela, 1995; Vendemiale *et al*., 1999). "Oxidative stress" is a term introduced to illustrate the imbalance within the cells between the production of prooxidants and antioxidant defenses within the cells. It occurs either due to deficiency in the antioxidant defense systems or from excessive production of reactive nitrogen species (RNS) and reactive oxygen species (ROS) (Morel and Barouki, 1999). Free radicals are also released by activated phagocytes (*i.e.* macrophages and monocytes) during inflammation which can be a possible mechanism in development of pro-inflammatory signals (Cheeseman and Slater, 1993). Increased oxidative stress by any means contribute to the development of RA (Grabar *et al*., 2009).

Numerous mechanisms in the human body defend cellular systems from oxidative damage. These mechanisms include some intracellular enzymes like catalase (CAT), glutathione peroxidases (GPX), superoxide dismutase (SOD), thioredoxin reductase and other peroxidases. Some of the antioxidant enzymes which protects against oxidative stress are polymorphic. Polymorphism in antioxidant genes may contribute in development of RA but only a few genetic polymorphisms were found associated with RA.

Only few studies have been conducted to study the polymorphisms present in SOD1 gene in association with RA. SOD1 gene is located on ch21q22.1. The polymorphism observed in SOD1 gene is at codon 251 (rs2070424) which cause substitution of adenine (A) to Guanine (G). This polymorphism is found to be associated with reduced antioxidant capacity (Celojevic *et al*., 2013). Actually the enzyme encoded by SOD1 gene destroys free superoxide radicals formed in the body by catalyzing the dismutation of superoxide into O_2 and H_2O_2 (Borgstahl *et al*., 1996). The difference in enzymatic activity due to genotype disturbs the delicate balance between clearance of ROS and minimizing the oxidative stress. The polymorphism in SOD1 modifies ROS production and therefore may provide a mechanism for the relationship

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between inflammation and autoimmune disease like RA.

The production of ROS is not only affected by polymorphism in antioxidant genes but it is also influenced due to exposure of heavy metals. Heavy metals like lead (Pb), cadmium (Cd), chromium (Cr) and nickel (Ni) creates oxidative stress and contributes in the development of many human diseases which includes degenerative lung and heart conditions, Alzheimer disease and RA (Wester, 1987). Although the etiology of RA is still not fully understood but oxidative stress created by heavy metals may have role in development of RA.

Sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) are important major minerals in body and are crucial for attaining good health. They keep immune system healthy (Memon *et al*., 2007; Saris *et al*., 2007). Their presence is crucial to life as certain biochemical processes in human body merely depends on the availability of minerals. Trace elements which include copper (Cu), zinc (Zn), iron (Fe), manganese (Mn) and cobalt (Co) are very important for immune system, as very delicate balance exist between them and other nutrients which effect many physiological functions of body. The role of these major minerals and trace minerals in chronic inflammatory conditions such as RA is of great interest because the formation and release of cytokines and chemokines that adjust the activities of immune and other cells is also affected by trace elements status (Munoz *et al*., 2007). Trace elements interfere with the arthritis process by blocking oxidative damage and play compensatory role in response to increased oxidative stress and also enhance activities of antioxidant enzymes (Surapaneni and Venkataramana, 2007).

The SOD1 A/G polymorphism (rs2070424) is the least studied polymorphism in relation to its association with different diseases. To best of our knowledge no study has been conducted to investigate relationship between SOD1 gene polymorphism and RA. Moreover the studied genetic variation may contribute in alteration of levels of heavy metals and minerals which also has not been investigated previously. The current study was designed to analyze the role of SOD1 A/G polymorphism (rs2070424) in the development of RA. It was also aimed to compare the concentrations of selected metals and minerals among various genotypes of SOD1 A/G polymorphism (rs2070424) in RA patients and control group.

MATERIALS AND METHODS

Samples collection

All procedures were in agreement with the declaration of Helsinki. The Advance Research and Study Board, University of Sargodha has approved the protocol of the present study. Ethical Committee, University of Sargodha granted permission for the start of research work. Study comprised of two groups named as RA group consisted of RA patients and the second control group which included age and sex matched healthy individuals. A consent form was designed to keep record of name, age, gender and RF factor status related to individuals.

The samples were collected during September 2014 to February 2015 from local hospitals. 200 RA patients (confirmed by RF test) and 200 healthy volunteers were selected for study. Three mL blood from each individual was collected in EDTA coated vials (BD, USA) for genetic analysis and 2 mL for serum separation for metal and minerals analysis. Blood samples were centrifuged at 10733 g to separate serum. Serum was collected with the help of micropipette and put into eppendorf. After that these tubes were marked and stored below 4°C before further processing.

Genetic analysis

GF-1 blood DNA extraction kit (Vivantis, USA) was used for Genomic DNA isolation. Detection of rs2070424 polymorphism was carried out using PCR based amplification strategy (allele specific). DNA was detected using 0.8% agarose gel while 2% agarose gel was used for resolving PCR product.

Primers were assembled by local representative of Invitrogen, USA. One reverse and two forward primers were used. Forward primer 1 (F1) 5' TAGCTTTGT TAGCTATGCCA 3' Forward primer 2 (F2) 5' TAGCTTTGT TAGCTATGCCG 3'and reverse primer (R) 5' ATCTTTAGAA ACCGCGACTA 3' were used to amplify rs2070424. Annealing temperature of primers was 47.7°C and PCR product size was 480 base pairs. UV Transilluminator (BIOTOP Transilluminator, TU1002, China) was used to visualize genomic DNA and PCR products.

Metal and mineral analysis

The serum sample was used for metals and mineral analysis. The samples were processed by wet acid digestion method described by Memon *et al*. (2007). After wet acid digestion, the blood samples were analyzed for determination of Pb, Cd, Cr, Ni, Na, K, Ca, Mg, Cu, Zn, Fe, Mn and Co through Atomic absorption spectrophotometer (AA 6600 Shimadzu).

Statistical analysis

For analysis of Hardy Weinberg Equilibrium (HWE), gene frequencies, allele frequencies and differences in genetic and allelic frequencies, Chi square test was used. Online calculator was used to calculate Odds ratio (Bland and Altman, 2000). Chi square test and two way ANOVA

was applied to depict statistical differences between control group and RA by using SPSS Software version 16 for windows (SPAA Inc., Chicago Illinios, USA). The results were presented as Mean ± Standard deviation (SD) and a p-value of <0.05 was considered as significant.

RESULTS

The results of present study indicated no significant difference between RA and control group regarding age factor (Table I).

Table I.- Baseline characteristics of RA patients and control group.

Characteristics	RA patients (n = 200)	Control (n = 200)	Total (n = 400)	p-value
Age (Years)	45.9± 10.4[a]	42.8± 10.6[a]	44.35±10.5	0.74 [NS]

[a]Data are shown as mean ± standard deviation. Students T test was used for comparison of groups of RA and Control. [S*], Significant (P<0.05); [S**], Highly significant (P<0.01); [NS], Non-significant (P>0.05).

The genotype and allele frequencies of SOD1 [rs2070424 (–251A/G)] polymorphism along with the results for Hardy Weinberg Equilibrium (HWE) estimation is presented in Table II. The data indicates that G allele frequency was higher in control group as compared to that of A allele. When the same data was analyzed on the basis of disease presence, frequency of G allele was found to be higher than that of A allele. Results of HWE estimation indicate that allele frequencies were deviant from HWE in control group. Opposite results were observed in RA group. Results remained unchanged for the group containing all the participants of the study.

Table II.- Genotype and allele frequencies of SOD1 [rs2070424 (–251A/G)] polymorphism.

Genotype / Allele	Control (n=200)	RA (n=200)	Total (n=400)
GG	40 (20%)	57 (28.5%)	97 (24.25%)
AG	134 (67%)	116 (58%)	250 (62.5%)
AA	26 (13%)	27 (13.5)	53 (13.25%)
G	0.54	0.57	0.56
A	0.47	0.43	0.45
HWE (P)	24.03 (0.000)	6.97 (0.0083)	28.16 (0.000)

Chi square test was used to describe the association between SOD1 [rs2070424 (–251A/G)] polymorphism and RA (Table III). The analysis indicated that non-significant association existed between rs2070424 polymorphism and RA (p>0.05). GG genotype increased the risk of RA development by 1.5944 times (OR: 1.5944; 95% CI 1.0036 to 2.5331). AA genotype was found to have association with RA development but risk level was at margin (OR: 1.0445; 95% CI 0.5859 to 1.8622). AG genotype was found to have no association against the disease development (OR: 0.6802; 95% CI 0.4527 to 1.0219).

Table III.- Association of SOD1 [rs2070424 (–251A/G)] polymorphism and RA.

Genotype	Control	RA	Odds ratio	95% CI	Chi square (p- value)
GG	40	57	1.5944	1.0036 to 2.5331	4.294 (0.11 [NS])
AA	26	27	1.0445	0.5859 to 1.8622	
AG	134	116	0.6802	0.4527 to 1.0219	

[S*], Significant (P<0.05); [S**], Highly significant (P<0.01); [NS], Non significant (P>0.05).

This study was also designed to find out the concentration of different metals and minerals in various genotypes of SOD1 gene polymorphism in RA patients as compared to control. The mean concentration of Pb in AA genotype was 2.48±0.19µg/L and 6.41±0.84µg/L, in AG it was 2.17±0.11µg/L and 6.28±0.36µg/L, and in GG it was 2.07±0.12µg/L and 3.98±0.46µg/L respectively in control group and RA patient group (Fig. 1). There was significant difference between AA and AG, AG and GG genotypes in RA patients (P<0.05).

The mean concentration of Cr was found to be 0.274±0.026 µg/L, 0.269±0.016 µg/L and 0.220±0.028µg/L in AA, AG and GG genotypes in control group and in RA patients mean concentration was 3.490±0.369 µg/L, 2.923±0.113 µg/L and 0.180±0.152 µg/L in AA, AG and GG genotypes. Present finding indicated that significant difference occurred for Cr concentration between AA and AG genotypes in control group (P<0.05) while in RA patient non-significant difference occur between GG and AG, GG and AA genotypes (P>0.05). Significant difference was also present between AG, GG and AA genotypes of SOD1 gene [rs2070424 (–251A/G)] polymorphism in control group as compared to RA patients (P<0.05).

Individuals with AA, AG and GG genotypes have low concentration of Ni, K, Ca, Mg, Zn, Fe and Mn while high concentration of Cd, Na, Cu and Co was found in RA patients as compared to control group. Although there was variation in concentrations of Cd, Ni, Na, K, Ca, Mg, Cu, Zn, Fe, Mn and Co in both groups but non-significant difference was observed between AA, AG and GG genotypes of SOD1 gene [rs2070424 (–251A/G)] polymorphism in RA patients as compared to control group (P>0.05).

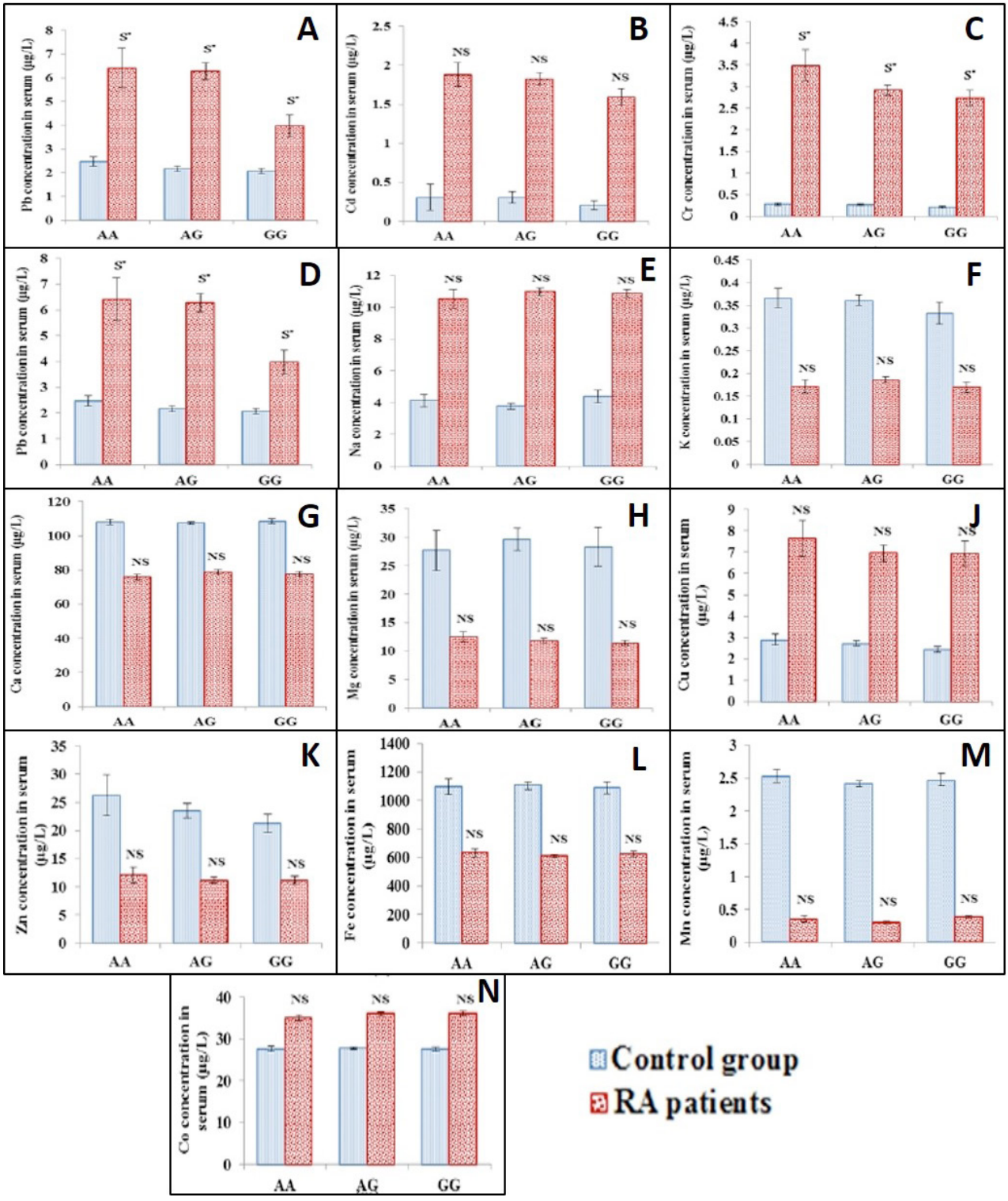

Fig. 1. Comparison of concentration of **A,** Pb; **B,** Cd; **C,** Cr; **D,** Ni; **E,** Na; **F,** K; **G,** Ca; **H,** Mg; **J,** Cu; **K,** Zn; **L,** Fe; **M,** Mn and **N,** Co in different genotype carrier of SOD1 gene [rs2070424 (-251A/G)] polymorphism in control and RA patients. ^{S*}**,** Significant ($P<0.05$); ^{S**}**,** Highly significant ($P<0.01$); ^{NS}**,** Non-significant ($P>0.05$).

DISCUSSION

The SNP rs2070424 in SOD1 gene has been focused in just few association studies. High frequency of G allele in case group as compared to A allele was found in study of association of anti-tuberculosis drug-induced Hepatitis with rs2070424 polymorphism (Kim *et al*., 2015). Zhang *et al*. (2011) observed association of rs2070424 with age related cataract and found that GG genotype was higher in patients and AA genotype has protective role towards disease development. Liu *et al*. (2009) found the protective role of AA genotype with noise induced hearing loss in Chinese workers. Results of Spisak *et al*. (2014) explains association of rs2070424 polymorphism with Alzheimer disease. They reported AG and GG genotypes as risk factor for Alzheimer disease.

So far no study has been reported for study of association between rs2070424 polymorphism and RA. The results of present study indicate frequency of G allele to be higher than that of A allele in control group as well as in RA patients. Zhang *et al*. (2011) also reported high frequency of G allele in their patients group. Our results indicate that AG genotype was found to have no association against the disease development and GG genotype increased the risk of RA development by 1.5944 times. In this study AA genotype was found to have association with RA development but risk level was at margin.

Although, few authors found association of SOD1 gene polymorphism with different diseases (Kim *et al*., 2015; Liu *et al*., 2009; Spisak *et al*., 2014; Zhang *et al*., 2011) but non-significant association has been observed between rs2070424 polymorphism and RA in our study. Young *et al*. (2006) also reported no association between rs2070424 and chronic obstructive pulmonary disease in European population. The variations in results of present study as compared to other studies can be explained in terms of disease differences as well as ethnic based differences. Further studies considering other ethnic groups and large population size were thought to be useful in considering rs2070424 as a risk of RA.

Many studies reported that exposure to heavy metals play a role in the induction or exacerbation of several autoimmune diseases like RA by creating oxidative stress (Paik *et al*., 1999). The mechanism of metal ion toxicity is partially understood but it is evident that they can produce ROS which includes nitrogen oxide (NO), superoxide ions (O_2-), hydroxyl (OH) and H_2O_2 with the help of Fenton/ Haber-Weiss reaction (Spector, 2000). No one has explored the association of SOD1 gene polymorphism with heavy metals and minerals in RA patients. Role of genetic variations in SOD1 gene in changing the heavy metals and minerals concentration is not completely known. Present study has tried to explore this aspect as well.

It was observed that Pb and Cr concentration changed due to genotypes. The concentration of Pb was significantly high in AA, AG and GG carriers in RA patients as compared to control group. The results of present finding indicates that Cr concentration differ significantly between AA and AG genotypes of SOD1 gene [rs2070424 (–251A/G)] polymorphism in RA patients. A significant difference of Cr was present between AA, AG and GG genotypes in RA patients as compared to control for SOD1 gene polymorphism. These findings suggest a complex interaction of SOD1 gene [rs2070424 (–251A/G)] polymorphism with the homeostasis of Pb and Cr concentration in human body.

In case of Cd and Ni, unlike genotypes were not significantly different in RA patients ($P>0.05$). Similarly there was non-significant difference present between AA, AG and GG genotypes for Cd and Ni concentrations in RA patients as compared to control group.

The activity of SOD1 enzyme depends upon its interaction with some trace minerals. SOD1 binds Cu and Zn ions responsible for destroying free superoxide radicals in the body. The variations in these minerals concentration in body affect the activity of SOD1 enzyme. Serum Cu and Zn concentration is directly linked to SOD1 enzyme activity as there altered concentration has been linked to depleted SOD1 enzyme activity (Endo *et al*., 2006). The lesser activity will be then responsible for less efficient control of ROS and thus controlling oxidative stress which can contribute towards development of RA (Hemdan *et al*., 2007).

It was also observed that Na, K, Ca and Mg concentration slightly changed in all genotypes carriers in control group and RA patients. The trend of change for Na, K, Ca and Mg concentration was different for each genotype but this difference was non-significant ($P>0.05$). Similarly for Cu, Zn, Fe, Mn and Co concentrations, non-significant difference was present between AA, AG and GG genotypes of SOD1 gene [rs2070424 (–251A/G)] polymorphism in RA patients as compared to control group. On the basis of present finding it is difficult to conclude the exact role of major and trace minerals and its association with SOD1 gene polymorphism.

There is a knowledge gap with respect to how metals and mineral levels have interaction with SOD1 A/G polymorphism (rs2070424) in antioxidant gene which may influence the progression and pathogenesis of RA. Presently this research field has not been explored in detail and several questions related to it still exist. Present data can help in understanding the association of this polymorphism

with RA disease. Detailed study of minerals and metals exposure, levels and their metabolism in RA patients will help in confirming the use of mineral supplements in RA patients along with traditional medication. There is a need for further investigation in order to establish the true picture of association of SOD1 A/G polymorphism (rs2070424) with development of RA disease and contribution of metals and minerals in pathogenesis and progression of the RA disease in presence of SOD1 A/G polymorphism (rs2070424).

CONCLUSION

Results of present study revealed that SOD1 (rs2070424) gene polymorphism is not associated with pathogenesis of RA. The present finding indicates that level of Pb and Cr imparts strong influence towards the progression and pathogenesis of RA in presence of this polymorphism.

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Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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Conflict of interest statement

We declare that we have no conflict of interest.

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