Research Article

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GROUP SPECIFIC COMPONENT (GC) PHENOTYPES IN RHEUMATOID ARTHRITIS: ABSENCE OF AN ASSOCIATION

Varun Chaithanya Gurram[1*], Sunil Kumar Polipalli[2], Vijay Kumar Karra[3], Seema Kapoor[2], Balaji Choudhury[4], Veena Manogna Poldasu[1] and Sudhakar Godi[1].

[1]Department of Human Genetics, Andhra University, Visakhapatnam, Andhrapradesh, India
[2]Pediatrics Research & Genetic Lab, Department of Pediatrics, MAMC & Associated Lok Nayak Hospital, New Delhi, India.
[3]Department of Medicine, MAMC, New Delhi, India
[4]Arthritis and Rheumatology centre, Visakhapatnam, India

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Abstract: Group Specific Component (GC) is a major vitamin D-binding protein in plasma. It mediates bone resorption by activating osteoclasts and also there is decreased expression of GC in Rheumatoid Arthritis (RA) patients. The current study tried to investigate any association between GC phenotypes and progress of RA in South Indian population. This study is conducted on 185 subjects of which 85 are RA patients and 100 age and sex matched controls. GC phenotypes were determined by using 7% polyacrylamide gels. All statistical analyses were done using SYSTAT 12 software and a probability value of <0.05 was considered statistically significant. The frequency of GC-1 allele is higher and GC-2 allele is lower in both patients and controls respectively. In both the subjects the frequency of 1-1 phenotype is high and 2-2 phenotype is low and there is equal distribution of 2-1 phenotype. GC phenotypes are also not associated with clinical parameters [Hb, ESR, TC and DC] and clinical features (Morning stiffness, Rheumatoid factor, Age of onset and Gender). There is no association between GC phenotypes and RA.

Keywords: Group Specific Component, Rheumatoid Arthritis, Osteoclasts.

INTRODUCTION

Rheumatoid Arthritis (RA) is a chronic progressive autoimmune disease, which causes functional disability and leads to significant pain as well as joint destruction [1]. This is a common disease in tropical areas [2]. The prevalence rate of 0.75% was observed in Adult Indian population [3]. Where as in industrialized countries, it affects 0.5–1.0% of adults. [4] Women are mostly affected with RA. It affects thrice as many women as men [5]. The pathogenesis of RA involves the following stages i.e. Initiation, Perpetuation and Tissue damage. Each stage involves different cell and molecular interactions [6-7]. In RA patients, there is joint and bone destruction. So, the present study aims to identify whether Group Specific Component (GC) phenotypes have any association with the pathogenesis of RA in South Indian population since it mediates bone resorption by activating osteoclasts and also it has been evident that there is decreased expression of GC in RA patients [8].

GC is a major vitamin D-binding protein in plasma. It is a glycosylated alpha-globulin with 58 kD in size. It has 458 amino acids, which are coded by 1690 nucleotides on chromosome 4 (4q11–q13) and is linked to the albumin and alpha-fetoprotein genes [9]. The sequence of this gene includes 4228 base pairs of the 5'-flanking region and 8514 base pairs of the 3' flanking region respectively [10]. The GC proteins migrate as post albumins and very close to the slow edge of albumin. Each allele produces two bands, the faster of GC-2 having the same mobility as the slower of GC-1. The heterozygous GC 2-1 posses' three bands and the homozygous produces two bands each. The variants in the protein differ in the charge. The three phenotypes are denoted GC 1-1, GC 2-2 and GC 2-1 and their patterns are a fast arc, a slow arc and a bimodal arc. The two alleles, GC-1 and GC- 2 are responsible for the differences.

MATERIALS AND METHODS

The study is carried on 185 subjects of which 85 are RA patients and 100 age and sex matched controls. All patients fulfilled the American College of Rheumatology (ACR) 1987 revised criteria for classification of RA [11] and had a disease history of minimum 3 years. 2 ml of intravenous blood samples were collected from patients and controls into an EDTA vacutainer in aseptic conditions after obtaining informed consent from subjects. The blood specimens were centrifuged for 10 minutes at 1000rpm and the supernatant plasma was separated into labeled vials and stored at -20°C.

***Corresponding Author:**
Dr. Varun Chaithanya Gurram,
Department of Human Genetics,
Andhra University, Visakhapatnam,
Andhra Pradesh, India.

GC Phenotyping: Polyacrylamide gel electrophoresis [Disc gel electrophoresis –DISC PAGE] was employed in the present study to screen for GC system [12].

Statistical Analysis: SYSTAT 12 software has been extensively used for statistical analysis to check for any possible association. The allelic and phenotypic frequencies were compared using z test for comparison of proportions. Also the allelic and phenotypic frequencies of subjects in relation to gender, age of onset and clinical features such as morning stiffness & rheumatoid factor were compared using z test for comparison of proportions. Comparison of clinical profiles in correspondence with GC phenotypes in RA patients was done using Analysis of Variance (ANOVA). In all the above statistical tests, a probability value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Allelic frequencies of GC: Allelic frequencies of GC in RA patients and controls are presented in Table 1. The frequency of GC-1 allele is higher in both patients (0.74) and controls (0.77); likewise, the frequency of GC-2 allele is lower in both patients (0.26) and controls (0.23). There is no significant difference between patients and controls with regard to allelic frequencies of GC.

Table 1: Allelic frequencies of Group Specific Component in Rheumatoid Arthritis Patients and Controls.

Allele	Control		RA		P-value	Significance
	n (200)	Frequency	n (170)	Frequency		
1	155	0.77	126	0.74	0.44	NS
2	45	0.23	44	0.26	0.44	NS

n = number of alleles, p value = probability value of the statistical test, S= significant, NS= not significant.

Phenotypic frequencies of Group Specific Component: Phenotypic frequencies of GC in RA patients and controls are shown in Table 2. Out of 85 patients 47 have 1-1 phenotype (0.55), 32 have 2-1 phenotype (0.38) and 6 have 2-2 genotype (0.07). In controls 60 have 1-1 genotype (0.60), 35 have 2-1 phenotype (0.35) and 5 have 2-2 phenotype (0.05) of total 100 individuals. In both controls and patients the frequency of 1-1 phenotype is highest and 2-2 phenotype is lowest and there is equal distribution of 2-1 phenotype. There is no significant difference between patients and controls with regard to phenotypic frequencies of GC (p = 0.51, 0.70 and 0.55 for 1-1, 2-1 and 2-2 phenotypes respectively).

Table 2: Phenotypic frequencies of Group Specific Component in Rheumatoid Arthritis Patients and Controls.

Phenotype	Control		RA		P-value	Significance
	n (100)	Frequency	n (85)	Frequency		
1-1	60	0.60	47	0.55	0.51	NS
2-1	35	0.35	32	0.38	0.70	NS
2-2	5	0.05	6	0.07	0.55	NS

n = number of individuals, p value = probability value of the statistical test, S= significant, NS= not significant.

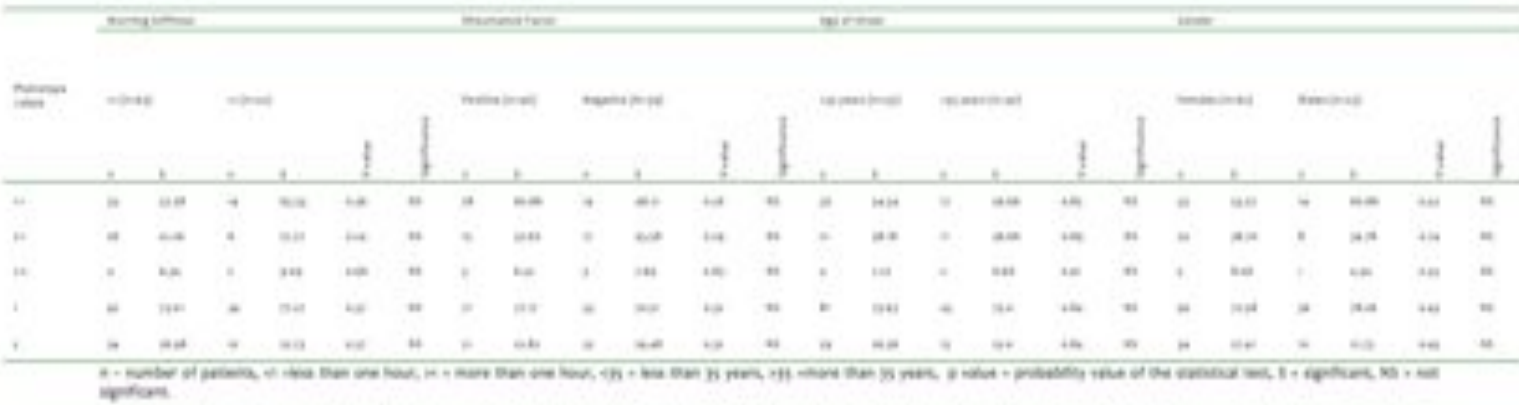
Table 3: Comparison of Clinical Profiles in correspondence with Group Specific Component phenotypes in Rheumatoid Arthritis Patients.

Parameter		1-1 (Mean±SD)	2-1 (Mean±SD)	2-2 (Mean±SD)	P-value	Significance
Hb		11±2	11±1	10±1	0.44	NS
ESR		41±22	42±20	30±13	0.42	NS
TC		9203±2419	9089±2201	8432±1825	0.74	NS
	Neutrophils	67±8	65±8	62±5	0.27	NS
	Lymphocytes	27±7	29±8	34±6	0.11	NS
DC	Eosinophils	4±2	3±2	3±1	0.14	NS
	Monocytes	2±1	2±2	2±1	0.84	NS

Hb=hemoglobin, ESR= erythrocyte sedimentation rate, TC=total count, DC=differential count, SD=standard deviation, p value=probability value of the statistical test, S= significant, NS= not significant.

Comparison of Clinical profiles in correspondence with Group Specific Component phenotypes: The clinical parameters i.e. Hb (hemoglobin), ESR (erythrocyte sedimentation rate), TC (total count) and DC (differential count) which are substantial in the progress of RA are compared between 1-1, 2-1 and 2-2 phenotypes of GC in patients to estimate the effect of these phenotypes on the clinical variables. None of these phenotypes with regard to the above said clinical parameters have any statistical significance. The p values for Hb (0.44), ESR (0.42), TC (0.74) and DC (0.27/0.11/0.14/0.84) all of which are insignificant. This analysis indicates that none of the above GC phenotypes have any effect on these clinical variables in RA patients. The values are tabulated and presented in table 3.

Table 4: Phenotype and Allele frequencies of Group Specific component in Rheumatoid Arthritis Patients in relation to Clinical features.



Phenotype and Allele frequencies of Group Specific Component polymorphism in relation to Clinical features: The phenotypic and allelic frequencies of GC are also compared with clinical features like Morning stiffness, Rheumatoid factor, that are significant in the development of RA and other important factors like Age of onset and Gender that influence the course of the disease for any possible association as shown in the Table 4.There is no Association between these clinical features, distribution of Alleles and Phenotypes of GC.

DISCUSSION

RA is a disease with sternness ranging from a modest to crippling, erosive and deforming polyarthritis with extra articular involvement. In the present study GC polymorphisms of RA patients were analyzed for any possible association in South Indian population. In the present study, the differences in allelic and phenotypic frequencies are statistically insignificant between patients and controls (Table 1 and 2). High frequencies of GC 1-1 and 2-1 phenotypes and GC-1 allele are observed in all the subjects. In the similar type of study in other RA groups contrasting results but statistically insignificant were obtained. Kahl et al., (1989)[13] reported that there is slightly high frequency of GC-2 allele and a low frequency of the GC-1S allele in patients when compared to controls but the differences are statistically insignificant. Similarly, Papiha etal., (1985) [14] reported that in RA patients GC-2 allele is 11% more when compared to controls, giving a relative risk of 1.55. This impute to the ethnic variations in GC polymorphisms. It is also observed that GC phenotypes are not associated with clinical parameters (Hb, ESR, TC and DC) and clinical features (Morning stiffness, Rheumatoid factor, Age of onset and Gender) of RA.

CONCLUSION

In the present study it is identified that GC phenotypes are not associated with RA but ethnic differences in GC polymorphisms were identified. There are few limitations of this study. The study is done on only severely affected patients and also the sample size is low. By settling these issues i.e. by including patients with mild severity and increasing

sample size more encouraging results can be obtained.

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