THE GG rs292001 GENOTYPE PREVAILS IN SERONEGATIVE FOR ANA AND ANTI-dsDNA ANTIBODIES PATIENTS WITH LUPUS NEPHRITIS

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ABSTRACT

Rs292001 is single nucleotide polymorphism in non-coding regions of the C1QA gene. C1q is a subcomponent of the C1 first component of the classical pathway of complement activation. Rs292001 was investigated for an association with some conventional immunological markers of lupus nephritis (LN) activity in systemic lupus erythematosus (SLE) patients – levels of C1q, C3, C4, anti-C1q, anti-nuclear (ANA) and anti-dsDNA autoantibodies.

Genomic DNA was isolated from peripheral blood of 18 patients with biopsy-proven LN. SNP genotyping for the presence of rs292001 was performed by quantitative real-time PCR method. Presence of complement C1q, C3 and C4 and anti-C1q autoantibodies was screened by ELISA. ANA and anti-dsDNA antibodies were detected by double immunodiffusion assays and indirect immunofluorescence.

We found that the GG rs292001 genotype prevailed in seronegative for ANA and anti-dsDNA antibodies LN patients (p=0.008; p<0.012). The AA rs292001 genotype showed a trend towards lower serum C1q levels.

These results reaffirm a previously established probable protective role of the G allele against the clinical activity of the SLE.

Keywords: *rs292001, ANA, anti-dsDNA Abs, C1q, Lupus nephritis*

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INTRODUCTION

Lupus nephritis (LN) is one of the most severe manifestations of systemic lupus erythematosus (SLE). The complement proteins C1q, C3 and C4 are deposited at the site of inflammation in the kidney and play an important role in the pathogenesis of LN. It is well known that hereditary C1q deficiency is strongly related to SLE, but there are several and inconsistent studies exploring single nucleotide poly-

morphisms (SNPs) of the C1q gene cluster in relation to the pathogenesis of SLE (1-10).

Genes encoding C1q chains (6A-, 6B- and 6C-) are grouped in a cluster on chromosome 1. The *C1QA* gene includes two introns and two exons. The rs292001 is SNP in the second intron of *C1QA* gene. According to several studies, the AA rs292001 genotype seems to associate with adult and juvenile SLE and LN (3,6,10).

The aim of the present study was to investigate whether rs292001 SNP, previously reported with association to SLE, correlates with some conventional immunological markers of LN activity –low levels of C1q, C3 and C4, high levels of anti-C1q, anti-nuclear (ANA) and anti-dsDNA autoantibodies.

MATERIALS AND METHODS

Study Subjects

18 serum samples from patients with SLE and biopsy-proven LN (World Health Organization Class I, II, III, IV, V and VI) were collected in the Clinics of Nephrology, University Hospital "Tzaritza Ioanna – ISUL" - Sofia, Bulgaria. The LN group included 16 (89%) women and 2 (11%) men, at an average age of 36.7±10.8 years (ranging from 23 to 58). The duration of LN lasted from 0.5 to 28 years, with an average of 9.64±7.62 years. The diagnosis of LN was based on clinical and laboratory parameters including proteinuria, urinary sediment, creatinine level, and erythrocyte sedimentation rate. The inclusion criteria were defined as proteinuria 500 mg/24 hr or higher in the last 10 days, erythrocyturia as 8 RBC per microliter or higher, renal dysfunction as any increase in creatinine value at any time of the history of the disease, and renal involvement as any of the two of the above-mentioned variables. Each patient signed a consent form at enrolment.

Genotyping for rs292001

Genomic DNA was isolated from whole blood using QIAamp DNA Blood MiniKit (QIAGEN GmbH, Hilden, Germany) and was stored at –20°C. Detection of the SNPs rs292001 was carried out using a validated TaqMan genotyping assay (Applied Biosystems, Foster City, CA). SNP genotyping was performed using an allelic discrimination assay (TaqMan® SNP Genotyping Assays, Applied Biosystems, Foster City, CA) using the 7500 Real-Time PCR System and genotypes were read using automated software (Applied Biosystems, Foster City, CA). Reactions were run in 10µl volumes using an amplification protocol of 50°C for 2min, 95°C for 10min, followed by 40 cycles of 95°C for 15s, then 60°C for 1min.

Measurement of Anti-C1q Antibodies and Complement Protein

Anti-C1q autoantibody levels were measured in human serum samples by ELISA under 0.75 M NaCl conditions as described previously (11,12). The concentrations of C1q, C4 and C3 antigens in serum were measured by means of a double-ligand ELISA (9).

Measurement of ANA and Anti-dsDNA Antibodies

The presence of ANA and anti-dsDNA antibodies were detected by double immunodiffusion assays and indirect immunofluorescence.

Statistical Analysis

Statistical analysis was carried out using software GraphPad Prism 5.01. Quantitative data were expressed as mean ±SD. The unpaired t test with Welch`s corrections for 2-group comparisons was used. Statistical significance was considered as $p<0.05$.

RESULTS

Characterization of Investigated Subjects

Eight of all 18 patients were with clinically active LN and the remaining 9 patients were in complete remission. The complete remission was defined as urinary protein excretion <0.5 g/day, normal urinary sediment (<8 RBC/µl, <8 WBC/µl, absence of casts other than hyaline), serum creatinine and albumin concentrations in reference ranges. One patient had class I, 3 – had class II, 12 – had class IV and 2 – had class V glomerulonephritis. There were no patients with LN classes III and VI. The characteristics and main pathological data of 18 LN patients at the time of renal biopsy are listed in Table 1.

Investigation for an Association Between rs292001 and the Levels of C1q, C3, C4 and Anti-C1q Autoantibodies in LN Patients

We analyzed the possible association of rs292001 with the immunological features of lupus nephritis activity – low levels of C1q, C4 and C3 and high levels of anti-C1q autoantibodies. We observed

Table 1. Patient`s characteristics

Patient`s characteristics	Value
Number	18
Sex – female, n (%)	16 (89%)
Median age (years, range)	37 (23 – 58)
Median duration of disease (years, range)	10 (0.5 – 28)
Complement C1q (mean, normal range) g/l	69 (59 – 178)
Complement C4 (mean, normal range) g/l	130 (84 – 396)
Complement C3 (mean, normal range) g/l	910 (612 – 1444)
Anti-C1q antibodies (mean, cut off)	2.029 (2.139)
Anti-nuclear antibodies (ANA) (+) № (%)	11 (61)
Anti-double stranded DNA antibodies (anti-dsDNA) (+) № (%)	8 (44)
Serum creatinine (mean, range) μmol/l	99 (56 –519)
eGFR (mean) mL/min/1.73sqm	72
Serum total protein (mean, range) g/L	63 (64 – 83)
Serum albumin (mean, range) g/L	37 (35 –50)
Proteinuria (mean, range) g/24h	1.887 (0.069 – 6.970)
Urinary sediment (+) № (%)	5/18 (28)

no significant associations between the markers of disease activity and rs292001 genotypes in the dominant model in the patient group (p>0.05 for all investigated immunological parameters, specifically GG vs. GA/AA for C1q levels – *p=0.075*; GG vs. GA/AA for C3 levels – p=0.132; GG vs. GA/AA for C4 levels – p=0.343 and GG vs. GA/AA for anti-C1q Abs levels – p=0.495). Only the carriers of A rs292001 allele showed a trend towards lower serum C1q levels.

GG rs292001 Genotype Prevailed in Seronegative for ANA and Anti-dsDNA Antibodies LN Patients

61% and 44% of LN patients were seropositive for ANA and anti-dsDNA Abs, respectively. Eight patients from the group of LN patients over 40 years of age were seropositive for ANA (8/11, 73%) and 6 – for anti-dsDNA Abs (6/11, 55%). In the group of younger patients – below 40 years of age – the rates were 57% seropositive for ANA (4/7) and 43% - seropositive for anti-dsDNA Abs patients (3/7). We examined the possible association of the rs292001 with ANA and anti-dsDNA antibodies. Genotype distribution between positive and negative for ANA and anti-dsDNA antibodies LN patients in the dominant model was compared. It was established that the GG rs292001 genotype prevailed in seronegative for ANA and anti-dsDNA antibodies LN patients (p=0.008; p<0.012) (Fig. 1A and 1B).

DISCUSSION

Genetic predisposition to SLE and LN is dependent on complex interactions of many genes and environmental and hormonal factors (13). Significant associations with the development of SLE, including LN, were established for rs292001 in studies byof Martens et al. (2009), Zervou et al. (2011) and Mosaad et al. (2015). They found that patients with A allele and AA genotype of rs292001 could be considered a susceptibility risk factor for SLE, juvenile SLE (jSLE) and LN in Dutch, Turkish and Egyptian cohorts (3,6,10). In these studies, the rs292001 was not associated with the disease severity or with a serum concentration of C1q or/and anti-C1q antibodies. In contrast, the C1q rs292001 SNP was not associated with the risk of SLE and LN in two Caucasian cohorts – Polish (14) and Bulgarian ones (9).

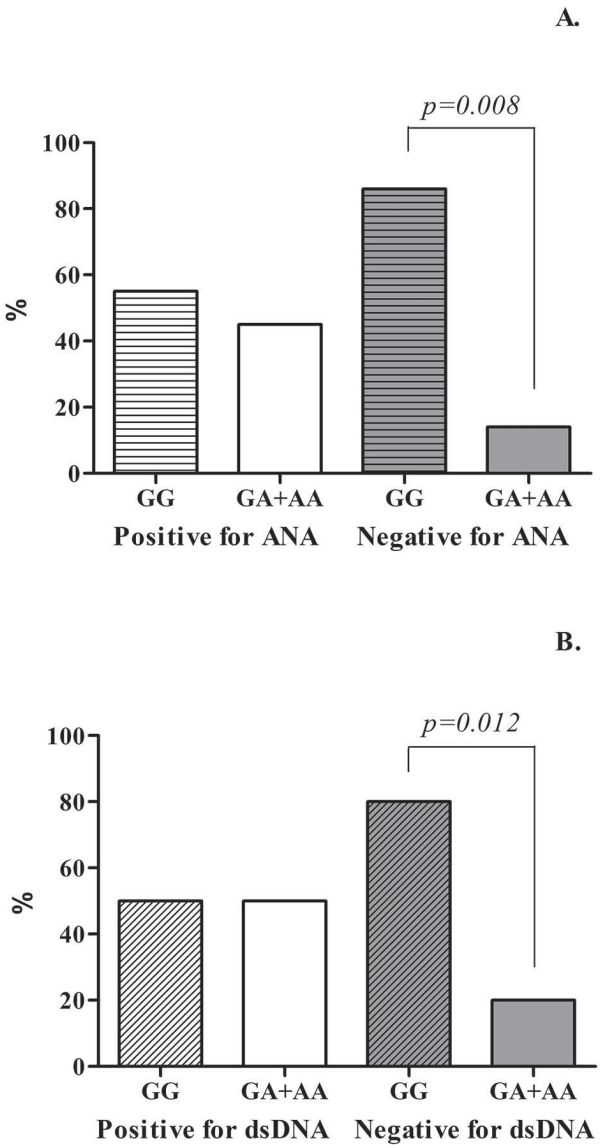

Fig. 1. Genotype distributions in the groups of seropositive and seronegative for ANA (A.) and anti-dsDNA antibodies (B.) LN patients.

In the present study, we found that the GG rs292001 genotype prevailed in LN patients seronegative for ANA and for anti-dsDNA antibodies. It is known that ANA and anti-dsDNA antibodies are considered a reliable marker for SLE activity and are implicated in the pathogenesis of LN (15). Therefore, our results are in accordance with several studies, which speculate that the GG rs292001 genotype probably provides a protective effect for juvenile systemic lupus erythematosus (jSLE) and LN (10) via an unexplained mechanism. Certainly, a limitation of our study is the relatively small sample size (only 18 LN patients). Nevertheless, the established associations add new information about the possible key role of this non-coding SNP in the pathogenesis of SLE.

The rs292001 SNP is a subject of several new studies and its role in other autoimmune and immune-mediated diseases is actively investigated. For example, the rs292001 A allele has been associated with an increased risk for type 2 diabetes (T2D) (17), for rheumatoid arthritis (16), for Behçet's disease (18) and for schizophrenia (19).

CONCLUSIONS

Our results reaffirm a previously established probable protective role of the G allele against the clinical activity of the SLE.

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