Association of *Megsin* Gene Variants With IgA Nephropathy in Northwest Chinese Population

**A STROBE-Compliant Observational Study**

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**Abstract:** *Megsin* is a mesangial cell-predominant gene that encodes a serpin family protein which is expressed in the renal mesangium. Overexpression of *megsin* has been observed in the glomeruli of patients with IgA nephropathy (IgAN). The aim of this study was to evaluate the association of *megsin* polymorphisms (rs1055901 and rs1055902) with IgAN in a Chinese population.

We examined 351 patients with histologically proven IgAN and compared them with 310 age, sex, and ethnicity-matched healthy subjects. Two single nucleotide polymorphisms (SNPs) in *megsin* were genotyped by Sequenom MassARRAY. SPSS 18.0 was used for statistical analyses, and SNP Stats to test for associations between these polymorphisms and IgAN risk. Odds ratios with 95% confidence intervals were used to assess the relationships.

We found that rs1055901 and rs1055902 SNPs were not correlated with susceptibility to IgAN in Northwest Chinese population. Analyses of the relationship between genotypes and clinical variables indicated that in patients with IgAN, rs1055901 was associated with 24-hour proteinuria, an increase in blood pressure, and Lee's grade (*P* = 0.04, 0.02, and 0.04, respectively), and rs1055902 was associated with 24-hour proteinuria and Lee's grade (*P* = 0.03 and 0.01, respectively). However, the results showed no association between these gene variants and sex of the patients.

These results indicate that *megsin* gene variants may play a role in the severity, development, and/or progression of IgAN in Northwest Chinese population.

**Abbreviations:** HWE = Hardy-Weinberg equilibrium, IgAN = IgA nephropathy, SNP = single nucleotide polymorphism.

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**INTRODUCTION**

IgA nephropathy (IgAN) is one of the most common forms of primary glomerulonephritis. The main pathological features are IgA deposits in the glomerular mesangial areas, together with complement C3, IgG, and/or IgM, and the disease is characterized by highly diverse clinical and pathological manifestations. Renal biopsy is the gold standard for diagnosis of IgAN. The prognosis is highly heterogeneous, and the therapeutic outcome is difficult to predict. Approximately 50% to 50% of patients will develop end-stage renal disease within 20 years of the initial biopsy. For these patients, kidney transplant is a treatment option, but the disease recurs in up to 50% of patients that receive a renal allograft.

IgAN is a public health problem worldwide, and in the past several years, it has become a huge burden on the Chinese health care system. IgAN is a multifactorial disease, and both genetic and environmental factors contribute to its pathogenesis. Although many gene polymorphisms have been reported to be associated with the development and/or progression of IgAN, the precise mechanism of pathogenesis has not been elucidated.

*Megsin*, the product of which is a serine protease inhibitor, clade B (ovalbumin), member 7 (SERPINB7; http://bioinfo.weizmann.ac.il; GenBank ID: AF027866), is a mesangial cell-predominant gene that belongs to the serpin superfamily. It is predominantly expressed in mesangial cells, and it is implicated in the regulation of a wide variety of processes such as matrix metabolism, cell proliferation, and apoptosis. Inagi et al.12,13 speculated that *megsin* overexpression may impact mesangial matrix degradation, leading to disposal of immune complexes and mesangial dysfunction. *Megsin* overexpression is determined in diseases that are associated with mesangial proliferation and extracellular matrix expansion in both humans and animal models. Therefore, the product of this gene is a good candidate for the potential in the pathogenesis of IgAN.

In humans, the *megsin* gene is located on chromosome 18q21.3, close to other serpin gene clusters. *Megsin* spans a 20-kb genomic region, contains 8 exons and 7 introns, and encodes a predicted peptide 380 amino acid. The transcription start site is located 391 bp upstream of the start codon. The identified regulatory regions include a promoter, located within a 4021-bp area in the 5′ region, and a protein A binding motif (CTGATT-CAC), located at −120 to −112. The 2 polymorphisms assessed in this study, rs1055901 and rs1055902, are located in the 3′-UTR, and the regulatory sequences in this region have yet to be identified. Comparison of this gene with the predicted orthologs in different species shows heterogeneity in the nucleotides equivalent to positions 2093 (rs1055901) and 2180 (rs1055902) in human *megsin*. (Orthologs are genes in different species that have evolved from a common ancestral...
gene via speciation. It often (but certainly not always) retains the same function(s) in the course of evolution. Thus, functions may be lost or gained when comparing a pair of orthologs.) Mouse, rat, cow, and horse genomes show that the nucleotide in the rs1055901 position is C, whereas in sheep it is T. In mouse, rat, and sheep genomes, the rs1055902 position is a T nucleotide, whereas in horse genomes it is an A.

Previous studies have investigated rs1055901 and/or rs1055902,16,17,22 and conflicting results were obtained. Therefore, whether these 2 megsin polymorphisms correlate with the development and/or progression of IgAN remains controversial. The purpose of this case-control study was to evaluate the association of the megsin gene polymorphisms rs1055901 and rs1055902 with IgAN in Northwest Chinese population.

METHODS

Subjects

In this study, 351 patients with IgAN (229 males and 122 females; mean age at diagnosis, 32 ± 11.9 years) treated in the First and Second Affiliated Hospitals of Xi’an Jiaotong University in Northwest China from March 2009 to April 2014 were recruited. In all cases, diagnosis of IgAN was confirmed by renal biopsy. Renal histological lesions were evaluated using Lee’s grading criteria,23 and if assessments differed, the biopsy results were reexamined simultaneously by both pathologists, in order to obtain an agreement about the grade. A total of 310 controls (186 males and 124 females; mean age, 35 ± 12.6 years) were randomly selected healthy volunteers who underwent routine health examinations in the same hospitals. All subjects were living in Xi’an city or the surroundings. Patients with comorbidities, such as lupus nephritis, purpura nephritis, hypertension, diabetes mellitus, and other secondary IgANs, were excluded. Detailed clinical information was collected, including age, sex, 24-hour proteinuria, blood pressure, serum creatinine level, and other relevant information. The Ethics Committee of Xi’an Jiaotong University approved this study and all experimental procedures, and informed consents were obtained from all study subjects at the time of recruitment.

Genotyping Methods

Whole blood samples were collected in tubes containing ethylene diamine tetraacetic acid. After centrifugation, the samples were stored at ~80°C until further analyses. DNA was isolated from the peripheral blood samples using the GoldMag DNA Purification Kit (GoldMag Co. Ltd, Xi’an City, China), and the DNA concentration was measured using an ultraviolet spectrophotometer (Nanodrop, Thermo Scientific, China), and the DNA concentration was measured using an ultraviolet spectrophotometer (Nanodrop, Thermo Scientific, Waltham, MA). Two single nucleotide polymorphisms (SNPs), rs1055901 (CC: wild-type genotype, TC: heterozygous mutant genotype, and TT: homozygous mutant genotype) and rs1055902 (TT: wild-type genotype, CT: heterozygous mutant genotype, and CC: homozygous mutant genotype), that constitute the majority of the known variations in megxin, according to the Chinese population data found in HapMap (http://www.hapmap.org), were selected for our study. Sequentom MassARRAY Assay Design 3.0 software (Sequenom Inc, San Diego, CA) was used to design the multiplexed SNP MassEXTEND assay. SNP genotyping was performed using Sequenom MassARRAY RS1000 according to the standard protocol recommended by the manufacturer. The primers used for each SNP are shown in Table 1. Sequenom Typer 3.0 software (Sequenom Inc) was used for data analyses.

Statistical Analysis

Statistical analyses were performed using SPSS 18.0 for Windows (PASW Statistics; SPSS Inc, Chicago, IL). SNP frequencies in the control subjects were tested for Hardy-Weinberg equilibrium (HWE). Student t test was used as necessary to assess the differences in age between the case and control groups. The χ2 (Pearson χ2) test was used as necessary to calculate P values and corresponding odds ratios with 95% confidence intervals. In order to determine the relationships between the alleles and genotypes and IgAN risk, and clinical variables, 3 models (codominant, dominant, and recessive) were used in this study to evaluate the association of the 2 SNPs with IgAN risk. P values <0.05 were considered statistically significant, and all statistical tests were 2-sided.

RESULTS

Characteristics of IgAN Patients and Healthy Controls

We examined a total of 661 participants, 351 patients with IgAN (229 males and 122 females; mean age at diagnosis, 32 ± 11.9 years) and 310 controls (186 males and 124 females; mean age, 35 ± 12.6 years) for further genetic association analyses. The genotype distributions of rs1055901 and rs1055902 in the case and control groups were in HWE (P = 0.91 and 0.15, respectively). The basic characteristics of the patients and controls are shown in Table 2. Hypertension was defined as systolic pressure ≥140 mm Hg and/or diastolic pressure ≥90 mm Hg on 3 occasions at diagnosis, or the use of antihypertensive medication to achieve normal blood pressure. The 24-hour proteinuria was divided into 2 groups, <3.5 g and ≥3.5 g. Lee’s grades were divided into 2 groups: I+ II + III and IV + V. No significant differences were observed between the IgAN cases and controls in terms of age distribution (P = 0.16) or sex (P = 0.45).

Association Between Megsin Gene Variants and IgAN Risk

The distributions of allele and genotype frequencies of the rs1055901 and rs1055902 variants in both IgAN cases and healthy controls are shown in Table 3. We found that the
TABLE 2. Characteristics of IgAN Patients and Control Participants

| Characteristics     | Cases   | Control  | P   |
|---------------------|---------|----------|-----|
| Number              | 351     | 310      |     |
| Age (mean ± SD)     | 32 ± 11.9 | 35 ± 12.6 | 0.45* |
| Sex                 |         |          |     |
| Male                | 229     | 186      |     |
| Female              | 122     | 124      | 0.16† |
| SCr, μmol/L (mean ± SD) | 8.2 ± 5.9 | 34.0 ± 7.9 |   |
| BUN, mmol/L (mean ± SD) | 159.5 ± 146.0 | 1.00 (reference) |       |
| ALB, g/L (mean ± SD) |         |          |     |
| Serum IgA, g/L (mean ± SD) | 2.8 ± 1.7 | 1.00 (reference) |       |
| Serum C3, g/L (mean ± SD) | 1.1 ± 0.3 | 1.00 (reference) |       |
| Cho, mmol/L (mean ± SD) | 6.1 ± 1.4 | 1.00 (reference) |       |
| Proteinuria, g/24 h  | <3.5    | 268      |     |
| Blood pressure, mm Hg| ≥140/90 | 143      |     |
| Lee’s grades        | I + II + III | 253 |     |
|                     | IV + V  | 83       |     |

ALB = serum albumin, BUN = blood urea nitrogen, Cho = cholesterol, SCr = serum creatinine.

*P values were calculated from 2-sided t tests.
†P values were calculated from Student t tests.

The distribution of the rs1055901 genotypes was as follows: CC, 57.1%; TC, 36.8%; and TT, 6.1% in the healthy controls; CC, 53.0%; TC, 39.3%; and TT, 7.7% in patients with IgAN. Assuming that the minor allele of each SNP was a risk allele when compared with the wild-type allele, we found that the frequencies of the rs1055901 C allele in the IgAN and control groups were 75.9% and 78.5%, respectively.

Association Between Megsin Gene Variants and Clinical Variables in Patients With IgAN

To investigate whether meggin variants were associated with the clinical features of IgAN, further analyses were performed. The distributions of meggin gene variants were examined for a series of clinicopathological variables, including 24-hour proteinuria, sex, blood pressure, and Lee’s grade. As shown in Table 4, in patients with IgAN, rs1055901 was associated with 24-hour proteinuria, blood pressure, and Lee’s grade (P = 0.04, 0.02, and 0.04, respectively). The investigated gene variants in the 2 SNPs (rs1055901 and rs1055902) were not associated with sex (P = 0.88 and 0.85, respectively).

DISCUSSION

Mesangial cells play an important role in the maintenance of the normal structure and function of the glomeruli by mediating extracellular matrix remodeling and immune complex disposal. Consequently, it can be hypothesized that meggin...
TABLE 4. The Associations Between the Megsin Polymorphisms and Clinical Variables of IgA Nephropathy Patients

| Variables | rs1055901 | P* | OR (95% CI) | T/T (%) | C/T + C/C (%) | P* | OR (95% CI) |
|-----------|-----------|----|-------------|---------|---------------|----|-------------|
| Proteinuria, g/24 h | C/C (%) | T/C + T/T (%) | P* | OR (95% CI) | T/T (%) | C/T + C/C (%) | P* | OR (95% CI) |
| <3.5 | 147 (54.9%) | 121 (45.1%) | 1.00 (reference) | 159 (59.3%) | 109 (40.7%) | 1.00 (reference) |
| ≥3.5 | 31 (41.3%) | 44 (58.7%) | 0.04 | 1.72 (1.03–2.90) | 34 (45.3%) | 41 (54.7%) | 0.03 | 1.76 (1.05–2.92) |
| Sex | Female | 64 (52.5%) | 58 (47.5%) | 1.00 (reference) | 69 (56.6%) | 53 (43.4%) | 1.00 (reference) |
| Male | 122 (53.3%) | 107 (46.7%) | 0.88 | 0.97 (0.62–1.50) | 132 (57.6%) | 97 (42.4%) | 0.85 | 0.96 (0.61–1.49) |
| Blood pressure, mm Hg | <140/90 | 84 (58.7%) | 59 (41.3%) | 1.00 (reference) | 82 (57.3%) | 61 (42.7%) | 1.00 (reference) |
| ≥140/90 | 63 (45.3%) | 76 (54.7%) | 0.02 | 1.72 (1.07–2.75) | 68 (48.9%) | 71 (51.1%) | 0.16 | 1.40 (0.88–2.25) |
| Lee’s grades | I + II + III | 152 (61.7%) | 101 (38.3%) | 1.00 (reference) | 156 (61.7%) | 97 (38.3%) | 1.00 (reference) |
| IV + V | 39 (47.0%) | 44 (53.0%) | 0.04 | 1.70 (1.03–2.80) | 37 (44.6%) | 46 (55.4%) | 0.01 | 1.99 (1.21–3.30) |

rs1055901, C > T denotes that a C is changed into a T by mutation; rs1055902, T > C denotes that a T is changed into a C by mutation. CI = confidence interval, OR = odds ratio.

* Two-sided \( \chi^2 \) test for the distributions of genotype frequencies.

contributes to the pathogenesis of mesangial proliferative glomerular diseases, such as IgAN. Functional characterization of this mesangium-predominant gene and elucidation of the exact pathogenic mechanisms are crucial for the effective treatment of kidney diseases.

Megsin was first cloned by a Japanese group in 1998,9 and afterward it quickly emerged as a gene that might be associated with, or contribute to, various mesangial lesions. Inagi et al24 examined the megsin gene expression profile in the human mesangial cell culture, and showed that it was specifically or mainly expressed in glomerular mesangial cells. Megsin transcripts were detected in mesangial cells from whole kidney, and protein expression was confirmed using a polyclonal antibody. Transgenic mice studies showed that megsin overexpression can lead to a progressive mesangial matrix expansion and proliferation numbers of mesangial cells, together with the increased formation of immune complexes, Ig, and complement deposition.23 Previous studies showed that megsin likely contributes to the development and/or progression of IgAN.10,17–22 We believe that these data obtained from a Northwest Chinese population support the proposed role of megsin in IgAN.

Here, we performed a case-control study in order to investigate the relationships between 2 SNPs, rs1055901 and rs1055902, and IgAN in Northwest Chinese population. However, our results do not confirm the influence of rs1055901 and rs1055902 on IgAN risk in Northwest Chinese. No obvious effects of these polymorphisms were found in the allele and genotype analyses. However, our results were consistent with the findings of previous studies, including those by Szelest et al23 in Hungarian population, Lim et al18 in population of Korean descent, and Maixnerova et al19 in a Czech population.

The genotype results were similar to those meta-analyses by Wu et al22 in 2011 suggested that the C allele of rs1055901, but not the T allele of rs1055902, was correlated with IgAN susceptibility. A recent meta-analysis by Mao et al10 found that the C allele of rs1055901 was associated with IgAN risk in the overall population and in Asians, whereas the C allele of rs1055902 was correlated with IgAN risk in Caucasians. The results obtained in this study differed from these observations.

Furthermore, we observed that the C allele frequency in rs1055901 was 75.5% among the healthy control subjects. This is significantly higher than the frequencies of 62.9% reported by Li et al17 in South China, 66.2% in a population of Korean descent,18 and 48.5% in a Hungarian population.20 It is possible that environmental and genetic differences can explain these regional and/or ethnic disparities in disease prevalence, which are paramount to the pathogenesis of IgAN, and may provide us with some evidence related to the development of IgAN.

Considering the relationship between genotypes and clinical parameters and/or the development of IgAN, Szelest et al18 examined 110 patients, and 87 of these patients were followed up for at least 3 years in a progression study. The results showed genotype differences between the initial genotypes and the progression of IgAN. A study by Xia et al3 showed that the (1055901) C-T (1055902) haplotype was present more often in patients with rapid disease progression, and it was associated with hypertension, severe proteinuria, and Lee’s grades IV and V. Consequently, it was hypothesized that megsin was associated with more severe forms of IgAN and more rapid disease progression. In Czech population, Maixnerova et al19 showed that there were no differences in the megsin genotype distributions among the groups of IgAN patients with normal renal function, progressive renal insufficiency, and the control groups. Furthermore, it was found that the TT haplotype may play a protective role in the progression of IgAN. In a population of Korean descent, Lim et al18 inferred that the CC genotype of rs1055901 and the TT genotype of rs1055902 were associated with a better renal survival. They also suggested that the (1055901) T-C (1055902) haplotype in the 3'-UTR of the megxin gene is associated with rapid disease progression in Korean IgAN patients. In a recent meta-analysis by Wu et al22 obtained results similar to those obtained in the Czech study in terms of the
haplotype analysis. Our results were similar to those in the study of patients of Korean descent, in which the CC genotype of rs1055901 and the TT genotype of rs1055902 were associated with more milder disease (24-hour proteinuria <3.5 g, blood pressure <140/90 mm Hg, and Lee’s grades I + II + III) when compared with severe disease (24-hour proteinuria ≥3.5 g, blood pressure ≥140/90 mm Hg, and Lee’s grades IV + V).

The limitations of our study include an incomplete follow-up duration and highly variable patient characteristics. In addition, the sample size in our study was small, especially the samples used for subgroup analyses and genotyping, and therefore it may not have had enough statistical power to determine real associations. Furthermore, we did not perform a haplotype analysis, which may limit further evaluation of the potential role of megisin polymorphisms in the development of IgAN.

Currently, whether the C allele of rs1055901 and the T allele of rs1055902 are associated with IgAN susceptibility remains unclear, and it was not elucidated whether it is the C/T substitutions themselves or different gene variants nearby that produced the controversial results. However, our results suggest that genetic variations in megisin that are thought to be located in the 3’-UTR do not confer susceptibility to IgAN in a Northwest Chinese population. Further analyses of the genotypes and clinical variables indicated that rs1055901 is associated with 24-hour proteinuria, blood pressure, Lee’s grade, and that rs1055902 is associated with 24-hour proteinuria, and Lee’s grade in patients with IgAN. These observations support a role for megisin in the development and/or progression of IgAN, although the precise pathogenic mechanism remains unclear.

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