Association of Gene Polymorphism of Polymeric Immunoglobulin Receptor and IgA Nephropathy

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Abstract

Objective In order to explore the possibility that genetic predisposition to dysfunction of mucosal immunity and the IgA processing pathway plays a role in the pathogenesis of mesangial IgA1 deposition in IgAN, we examined the possible association of the gene polymorphism of plgR in the patients with and without IgAN.

Subjects and Methods Genomic DNA of peripheral blood cells was isolated from 372 individuals including 172 histologically confirmed IgAN patients. Segments of the plgR gene were PCR amplified and restriction fragment length polymorphism was determined as A1 and A2 with and without Pvu II site, respectively.

Results The plgR genotype distribution was significantly different between the patients with IgAN and those without IgAN. Allele frequency of A2 was higher in IgAN than in other renal diseases (A1 and A2; 0.516 and 0.484 in IgAN, 0.641 and 0.359 in others, \( \chi^2 = 9.84, P = 0.0017, \) Odds ratio=1.71). Moreover, the subjects with A2A2 genotype were associated with a relatively low level of serum IgA only in the patients with IgAN but not in other renal diseases. The difference of allele frequencies was more remarkable in the patients with a serum IgA level of less than 300 mg/dl (A1 and A2; 0.439 and 0.561 in IgAN, 0.702 and 0.298 in others, \( \chi^2 = 12.44, P = 0.0004, \) Odds ratio=3.01).

Conclusion This is the first demonstration of the plgR gene polymorphisms in IgAN which are associated with its clinical phenotype. Gene polymorphisms of plgR may be candidate genetic markers of susceptibility to IgAN. (Internal Medicine 40: 867-872, 2001)

Key words: IgA nephropathy, polymeric immunoglobulin receptor, gene polymorphism

Introduction

IgA nephropathy (IgAN) is characterized by a predominance of glomerular mesangial IgA deposition (1). Elevated serum IgA levels are noted in one-third to one-half of the patients with IgAN (2). The IgA immune system in human is composed of two compartments-mucosal and systemic. The majority of IgA is polymeric IgA (plgA) synthesized in the mucosal system, which translocates into mucosal fluids via the epithelial, polymeric immunoglobulin receptor (plgR). All mucous membranes are protected by antibodies of a unique immunoglobulin (Ig) class called secretory IgA (slgA) (3). This is indeed the predominating Ig class of the body; more IgA is translocated to the gastrointestinal lumen every day than the total daily production of IgG. The capacity of the secretory component (SC), which is produced by cleavage of the plgR, to transport IgAs is remarkable (4). Proinflammatory cytokines, in particular interferon-\( \gamma \) and also tumor necrosis factor-\( \alpha \) and interleukin-4, have been reported to increase the epithelial expression of SC protein (5-7). SC has been identified as one of the immunoglobulin (Ig) super-gene family members because of sequence homology and its repeating disulfide-stabilized Ig-like domains (8). Furthermore, a restriction fragment length polymorphism (RFLP) has been found, revealing a two-allele RFLP with an autosomal codominant inheritance pattern by southern blot of genomic DNA (9).

Although, the role of plgR in the pathogenesis of IgAN is unknown, it is thought to be reasonable to hypothesize that individual variation in the mucosal immune response to common environmental antigens, such as food, bacterial, or viral antigens, may participate in determining susceptibility to the development of IgAN. It is well known that there is familial clustering of IgAN.

In order to examine the possibility that genetic predisposition to mesangial IgA1 deposition is associated with plgR gene
polymorphism, we analyzed the genotype distribution of \( \text{plgR} \) in patients with IgAN and compared it with patients histologically confirmed to have no mesangial IgA deposition.

**Materials and Methods**

**DNA samples**

The protocol of the study was approved by the ethics committee of the institution involved and informed consent for genetic studies was obtained from all participants. Genomic DNA of peripheral blood cells was isolated by an automatic DNA isolation system (NA-1000, Kurabo, Osaka) from 372 individuals including 172 histologically confirmed IgAN patients and 200 other subjects. The othersubjects consisted of 124 renal disease patients who were histologically confirmed not to have any mesangial IgA deposition, including 36 mesangial proliferative glomerulonephritis without mesangial IgA deposition, 31 membranous nephropathy, 11 minimal change nephrotic syndrome, 7 mesangioproliferative glomerulonephritis, and 39 others. In the other 76 cases, immunofluorescence examination was not available or renal biopsy was not performed. Diagnosis of IgAN was based on kidney biopsy that revealed the presence of dominant or codominant glomerular mesangial deposition of IgA by immunofluorescence examination. Serum IgA levels at the time of kidney biopsy or before the treatment were measured in all renal patients.

**Determination of genotypes**

\( \text{plgR} \) fragments were amplified by PCR using the primer pair 5'-GAG GTG AAT AGT GTG GAA GGT-3' and 5'-TTG TTC ACA TAA CCA CTG-3'. The reaction mixture contained 1×PCR buffer, 1.5 mmol/l MgCl\(_2\), 200 mmol/l deoxynucleotide triphosphates (dNTPs), 1 unit Taq DNA polymerase (Takara, Kyoto), 10 pmol of each primer, and 50–200 ng genomic DNA. The PCR amplification reaction consisted of a cycle at 94°C for 5 minutes, followed by 25 cycles of denaturation at 93°C for 30 seconds, annealing at 59°C for 1 minute, and extension at 72°C for 2 minutes. The PCR products were digested with restriction endonuclease \( \text{Pvu II} \) (Takara), and electrophoresed on 1% agarose gel. The RFLPs of the PCR products were determined as A1 and A2, by the presence and absence of the \( \text{Pvu II} \) site, respectively.

**Direct PCR sequencing**

In order to identify the polymorphic site in the \( \text{plgR} \) gene, we performed direct sequencing of the PCR products from 20 individuals of both IgAN and other renal diseases by the Taq DNA polymerase cycle sequence method as previously described (10, 11). The BigDye Terminator Cycle Sequencing FS Kit was purchased from Perkin-Elmer (Foster City, CA). The same pair of oligonucleotide primers as those for PCR-RFLP analysis were used for bidirectional sequencing. Each sequencing cycle consisted of denaturation at 96°C for 30 seconds, annealing at 59°C for 30 seconds, and extension at 60°C for 4 minutes. After 25 cycles of the reaction, alkaline-heat denatured sequences were loaded onto the sequencing gel. Automated DNA sequencer (model ABI PRISM 310, Perkin-Elmer) was used for the analysis.

**Statistical analysis**

Statview 5.0 statistical software (SAS Institute Inc. Cary, NC, USA) was used for statistical analyses on a Macintosh G3 computer. Chi-square analysis was used when comparing allele frequency between the groups. Hardy-Weinberg equilibrium was analyzed using a Chi-square test with 1 df (degrees of freedom). When comparing the IgA level in each group, an analysis of variance (ANOVA) was used. Values of \( P<0.05 \) were considered to indicate statistical significance.

**Results**

We first localized the polymorphic site in the \( \text{plgR} \) gene previously reported by Krajević et al (9) and identified its nucleotide sequence, which has not been completely determined. PCR direct sequencing revealed a single nucleotide polymorphism (SNP) of T/G transition at the position-161 nucleotide upstream from the putative origin of Exon 4 (Fig. 1). The A1 allele corresponds to T, and A2 to G at this position. We also confirmed the presence of \( \text{Pvu II} \) site in the Exon 3, which is consistent with the data reported previously (9).

Next, we performed genotyping of all subjects by PCR-RFLP with \( \text{Pvu II} \). Each genotype was clearly identified by agarose gel electrophoresis of the PCR products digested with \( \text{Pvu II} \) (Fig. 2). The absence of the original band of 1.79-kb and the presence of 0.19-kb fragment after \( \text{Pvu II} \) digestion ensured that \( \text{Pvu II} \) enzyme worked for every sample. The presence of polymorphic \( \text{Pvu II} \) site (allele A1) resulted in the cleavage of 1.59-kb fragment (allele A2) into 1.34-kb and 0.25-kb fragments (Fig. 2).

The \( \text{plgR} \) genotype distribution was compared between patients with IgAN and other renal disease patients who were confirmed histologically to have no mesangial IgA deposition, because it has been known that mesangial IgA deposition can occur even in individuals with normal urinalysis. The genotype distribution was significantly different between IgAN and others (\( \chi^2=15.11, P=0.0045, \) Table 1). An allele frequency of A2 was higher in IgAN than in other renal diseases (A1 and A2: 0.512 and 0.488 in IgAN, 0.641 and 0.359 in Others. \( \chi^2=9.84, P=0.0017 \)). The expected frequency of the \( \text{plgR} \) genotype (A1A1: A1A2: A2A2=41: 46: 13 in IgAN, and 41: 46: 13 in other renal diseases), under the assumption of Hardy-Weinberg equilibrium, did not differ from the observed genotype frequencies in our patients. The allele frequency in the control subjects in this study was consistent with that reported previously (A1: A2=0.65: 0.35) (9). We also examined the allele frequency in 62 normal subjects and found that it was the same as in other renal diseases (data not shown).

Serum IgA levels were significantly higher in IgAN (367.9±117.0 in IgAN vs. 274.8±109.5 in other renal diseases, \( P<0.0001 \)). The IgA levels were not different in each genotype of the patients without mesangial IgA deposition. However, it was significantly lower in the patients with A2A2 genotype.
Gene Polymorphism of plgR in IgAN

Figure 1. The nucleotide sequence of the polymorphic site in plgR gene. Direct sequencing of the PCR products by the Taq DNA polymerase cycle sequence method was performed as described in Materials and Methods. Box indicates Pvu II (CAG/CTG) site and the position of T/G transition is indicated by arrows. A1 allele corresponds to T, and A2 to G at this position. Twenty subjects were examined by PCR-direct sequence and representative data of A1A1 and A2A2 genotype are shown.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>N</th>
<th>PvuII</th>
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<th>PvuII</th>
<th>N</th>
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<tr>
<td>A1A1</td>
<td>1.78</td>
<td></td>
<td>1.59</td>
<td></td>
<td>1.34</td>
<td></td>
<td>0.25</td>
<td></td>
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<tr>
<td>A2A2</td>
<td>0.19</td>
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<td>0.19</td>
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Figure 2. Genotype of each subject was determined by PCR-RFLP as described in Materials and Methods. 1% agarose electrophoresis of PCR products was run with and without restriction endonuclease Pvu II treatment. The absence of the original band of 1.78-kb and the presence of 0.19-kb fragment after Pvu II digestion ensured that the enzyme worked for every sample. The presence of polymorphic Pvu II site (allele A1) resulted in the cleavage of 1.59-kb fragment (allele A2) into 1.34-kb and 0.25-kb fragments. N: non-treated PCR products, Pvu II: PCR products treated with Pvu II. Genotypes determined are indicated below.

1.78: N1A1, N1A2; 1.59: N1A1, N2A2; 1.34: N1A1, N1A2; 0.25: N1A1, N1A2; 0.19: N1A1, N1A2

compared to other genotypes only in IgAN (347.6±125.7 in A1A1 and A1A2 combined vs. 409±104.6, P<0.05, Fig. 3). The difference of allele frequencies was more remarkable in the patients with a serum IgA level of less than 300 mg/dl (A1 and A2; 0.439 and 0.561 in IgAN, 0.702 and 0.298 in others, \( \chi^2=12.44, P=0.0004, \text{Odds ratio}=3.01 \).
Table 1. Genotype and Allele Frequency of pIgR in Patients with IgAN and Controls

<table>
<thead>
<tr>
<th></th>
<th>IgAN (n=172)</th>
<th>Other renal diseases biopsy proven (n=124)</th>
<th>Other renal diseases IF not available (n=76)</th>
</tr>
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<tbody>
<tr>
<td>A1A1</td>
<td>44 (25.6)</td>
<td>51 (41.1)</td>
<td>35 (46.0)</td>
</tr>
<tr>
<td>A1A2</td>
<td>88 (51.1)</td>
<td>57 (46.0)</td>
<td>31 (40.8)</td>
</tr>
<tr>
<td>A2A2</td>
<td>40 (23.3)</td>
<td>16 (12.9)</td>
<td>10 (13.2)</td>
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$\chi^2=15.11$, $P=0.0045$. IF: Immunofluorescent microscopy.

<table>
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<th></th>
<th>IgAN</th>
<th>Other renal diseases biopsy proven</th>
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<tbody>
<tr>
<td>A1</td>
<td>0.512</td>
<td>0.641</td>
</tr>
<tr>
<td>A2</td>
<td>0.488</td>
<td>0.359</td>
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</table>

$\chi^2=9.84$, $P=0.0017$.

Figure 3. Serum levels of IgA in cases with or without A1 allele in renal disease patients without mesangial IgA deposition (A) and in IgAN patients (B). The serum IgA levels were not different in each genotype of renal patients without IgA deposition. However, it was significantly lower in cases with A2A2 genotype compared to other genotypes only in patients with IgAN. (347.6±125.7 in A1A1 and A1A2 combined vs. 409±104.6, $P<0.05$). Boxes show 25 and 75 percentiles and error bars show standard deviations.

Discussion

IgAN is characterized by the deposition of IgA1 molecule in the glomerular mesangium. The mechanisms involved in mesangial IgA1 deposition and initiation of inflammatory glomerular damage remain poorly understood. Not only infectious antigens but also food antigens could be responsible for triggering an increased production of polymeric IgA1. However, failure to identify specific antigens has led increasingly to the view that polymeric IgA may be antigen-independent (12). A variety of abnormalities of the IgA immune system are recognized in IgAN (13). However, none of these can completely account for mesangial IgA deposition.
It is well known that mesangial IgA is predominantly pIgA1 (2, 12). Dimeric and polymeric IgAs are synthesized mainly by plasma cells in mucosa-associated lymphoid tissues, and need to be transported across the epithelia in order to perform their protective role against environmental antigens.

Recurrent gross hematuria is often recognized in concomitance with acute episodes of mucosal infection, especially that of upper respiratory, gastrointestinal or urogenital tract, in a subset of patients with IgAN, suggesting that IgAN may be caused by hypersensitivity of mucosal IgA system. The clinical deterioration after tonsil stimulation is more frequent in IgAN than in other renal diseases (14). In addition, it has been reported that stimulation of the tonsils causes serum IL-6 elevation and changes in the urinary IL-6 levels in IgAN cases (15). On the other hand, many immunization studies have indicated that the response to mucosal immunization is diminished, for example using cholera sub-unit toxin in IgAN (16), although the systemic response to chronic mucosal infection (Helicobacter) shows an exaggerated circulating pIgA response (17). These findings may indicate that failure or dysregulation of the mucosal antigen exclusion system participates in the pathogenesis of increased circulating IgA1 in IgAN.

To the best of our knowledge, this is the first demonstration of the pIgR gene polymorphism, an important molecule of the mucosal immune system, in IgAN. We have shown a higher frequency of A2 allele and lower A1 in IgAN compared to patients with non-renal, as well as renal diseases without IgA deposition. Thus, this genotype distribution is thought to be specific for IgAN. Moreover, of interest, A2A2 genotype was associated with low serum IgA level only in patients with IgAN but not in others. This indicates that the pIgR gene polymorphisms may be related to the specific mechanisms that determine the susceptibility to IgAN. Although this study can not provide the functional explanation for the relationship between the A2A2 genotype and a low serum IgA, it may be a reflection of a dysregulation in mucosal immunity in IgAN, and the genotype distribution may have a linkage to the defective antigen clearance in mucosa.

Several reports have supported genetic predisposition rather than environmental exposure as a cause of familial clustering of IgAN (18, 19). Recently, it has been reported that the polymorphism in the I alpha1 promoter region, the regulatory region on class switch recombination, possibly causes enhanced IgA production in some, but not all, patients with IgAN (20). Many other gene polymorphisms have been reported to be associated with IgAN. For example, polymorphisms of specific HLA antigen encoded by the MHC locus, genes within the Ig heavy chain loci, and T-cell receptor constant alpha chain gene were reported to be associated with IgAN (21–23).

Genetic factors, which determine the susceptibility to dysregulation of the IgA immune system and to the pathogenesis of IgAN are thought to be heterogeneous. And there is a possibility that not only an abnormality in the IgA production but also in the clearance pathway of IgA play a role in the pathogenesis of IgAN. This possibility is further supported by the present study, since the majority of pIgA is transported by pIgR into the gastrointestinal lumen. Three molecules are known to participate in the clearance of IgA: pIgR, Fc alpha receptor, and asialoglycoprotein receptor (ASGPR). Certainly, Fc alpha receptor and ASGPR are also the functional candidates for a genetic study in IgAN. IgAN is a heterogeneous disease, and few reported abnormalities are consistently seen in all patients. Our finding of characteristic pIgR genotype distribution in IgAN is no exception in this respect.

Because the polymorphic restriction site is localized in the third intron, the SNP of pIgR gene reported in this study may not have a direct effect on the function of this molecule nor on the serum level of IgA1. It is possible that this SNP is in linkage disequilibrium with more significant polymorphisms that can cause amino acid substitution or alter the transcriptional activity of pIgR gene. Further study is necessary to clarify the functional relationship between pIgR gene polymorphisms and the pathogenesis of IgAN. Especially, the mechanisms in which serum IgA levels are relatively lower in A2A2 genotype only in IgAN, not in other renal diseases may help us to understand the relationship of mucosal immunity and the pathogenesis of IgAN. The possible relationship between pIgR gene polymorphism and histological injury, clinical course or renal survival also remains to be elucidated in future investigations.

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References

11) Smith DP, Johnstone EM, Little SP, Hsiung HM. Direct DNA sequencing...


