Brief Report

No association between a genetic variant of the p22\textsuperscript{phox} component of NAD(P)H oxidase and the incidence and progression of IgA nephropathy

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Abstract

Background. The course of glomerulonephritis varies even within the same histological entity, which suggests that genetic factors determine the progression of inflammatory renal diseases. We studied a potential relationship between the C242T gene polymorphism of p22\textsuperscript{phox}, a subunit of the NAD(P)H oxidase, and frequency as well as progression of immunoglobulin A (IgA) nephropathy. Patients with lupus nephritis were also investigated. The distribution of the C242T gene variation of p22\textsuperscript{phox} has not been previously studied in patients with renal disease.

Methods. Patients with IgA nephropathy were from a homogenous ethnic group of patients living in Northern Germany (n = 127). Patients with active lupus nephritis WHO classes III/IV (n = 46) were also studied. All diagnoses were confirmed by renal biopsy. Healthy blood donors (n = 151) exhibited a genotype distribution similar to previously reported values for Caucasians (CC, 41.2%; CT, 45%; TT, 13.8%). However, C242T genotype distribution was not significantly different (\(\chi^2\) test) in patients with IgA nephropathy (CC, 44.9%; CT, 48%; TT, 7.1%) or in active lupus nephritis (CC, 54.3%; CT, 34.7%; TT, 11%). Grouping of IgA nephropathy patients as those with mild renal impairment at the time of biopsy (serum creatinine < 1.3 mg/dl) and those with more severe renal failure (serum creatinine > 1.3 mg/dl) also failed to show a relationship with p22\textsuperscript{phox} polymorphism. Log-rank analysis for up to 15 years in selected cases of IgA nephropathy did not show a significant difference in renal survival rate among the three genotypes.

Conclusions. It appears that the C242T polymorphism is not associated with IgA nephropathy or active lupus nephritis and may not affect the progressive deterioration of renal function in patients with IgA nephropathy. However, whether the C242T polymorphism plays a role in other renal diseases remains to be studied.

Keywords: angiotensin II; genetics; immunoglobulin A (IgA) NAD(P)H oxidase; nephropathy; oxygen radicals; p22\textsuperscript{phox}; progression of chronic renal disease

Introduction

Immunoglobulin A (IgA) nephropathy is the most common primary glomerulonephritis in the world and is characterized by immune complex deposits containing IgA [1]. Since its first description, IgA nephropathy has become known as a heterogeneous disease, ranging from histological patterns with minimal lesions to diffuse proliferative glomerulonephritis [2]. Initially believed to be a benign condition, it has emerged in recent years that many patients will progress to end-stage renal disease [1,3]. Several studies have tried to predict progression of IgA nephropathy on clinical parameters and/or the histological pattern of the biopsy [1–3]. The different incidence in various ethnic populations and the heterogeneous patterns of disease progression suggest that genetic factors may determine susceptibility to IgA nephropathy as well as the natural course of this glomerulonephritis. In fact, polymorphisms of components of the renin–angiotensin system have been widely studied in IgA nephropathy, with controversial results [4,5]. Nevertheless, angiotensin II (ANG II) plays an important role in the progression of chronic glomerulonephritis including IgA and may even exert immunostimulatory action on the kidney [6,7]. ANG II is involved in the generation of reactive oxygen species in the kidney by activating the membrane bound NAD(P)H oxidase [8,9]. This membrane-bound multi-enzyme complex is composed of several subunits including a 22-kDa \(\alpha\)-subunit called p22\textsuperscript{phox}.
Recently, a polymorphism has been described in the human p22phox gene [10,11]. The present study was undertaken to determine whether this p22phox polymorphism is associated with the incidence and progression of IgA nephropathy. In addition, patients with active lupus nephritis (WHO classes III/IV) were also studied as another inflammatory renal disease.

Subjects and methods

The study was approved by the local ethics committee. All studied subjects were Germans living in Northern Germany (city of Hamburg, states of Schleswig-Holstein and Lower Saxony). We investigated a total of 127 patients with IgA nephropathy (91 male). Diagnosis was made between the years 1985 and 2000. An additional group of 46 patients (8 male) with active lupus nephritis (WHO classes III/IV) from the same region was also investigated. All diagnoses were based on renal biopsy specimens using immunohistochemistry and electron microscopy. Most diagnoses were made by a single pathologist (Department of Pathology, University of Hamburg). Serial creatinine values were retrospectively obtained from the patients’ records. Healthy German blood donors (age 20–55 years) served as controls.

DNA was isolated from peripheral venous blood using the QIAamp® DNA blood mini-kit from Qiagen (Hilden, Germany) according to the manufacturer’s recommendation. The C to T mutation at the 242 in exon 4 of p22phox produces a Rsal digestion site. To avoid possible mistyping of the genotypes because of incomplete digestion, a second Rsal restriction site was included in the amplification products as previously described [10]. DNA fragments containing the C242T polymorphic site of p22phox were amplified from genomic DNA with the following primers: 5'CTCTGTGT-TGTCTTCAAGG3' and 5'ACTCACAGGAGATG-CAGGACG3'. The polymerase chain reactions were performed in a total volume of 20 μl containing 50 ng genomic DNA. Reactions were carried out for 40 cycles with an annealing temperature of 62°C for 90 s, and extension step at 72°C for 90 s, and a denaturation step at 92°C for 30 s. Amplification products were digested with Rsal (New England Biolabs, Beverly, MA), and run on 2% agarose gels containing ethidium bromide. The size of the uncut wild type amplification product is 509 bp [10]. Subjects with the CC genotype revealed two fragments of 396 and 113 bp after amplification product is 509 bp [10]. Those with TT are characterized by the size of uncut wild type amplification product is 509 bp. Unequivocal genotyping was possible in all studied individuals.

Results

Figure 1 shows an example of genotyping for 20 patients. This method of genotyping for the C242T polymorphism has the advantage that complete Rsal digestion is controlled for by a second restriction site. Rsal-digested amplification products of the wild type (CC) reveal two fragments of 396 and 113 bp, whereas the C242T polymorphism is associated with the incidence and progression of IgA nephropathy. In addition, patients with active lupus nephritis (WHO classes III/IV) were also studied as another inflammatory renal disease.

The distribution of the genotypes was according to the Hardy–Weinberg equilibrium. Distributions of genotypes in healthy controls was similar to those reported for a normal US population but was different from healthy Japanese [10–13]. There was no significant difference in genotypes or allele frequencies between normal controls and patients with IgA nephropathy (Table 1). No significant difference was found in the prevalence of these allelic subgroups between patients with acute lupus nephritis (WHO classes III and IV) and healthy controls (Table 1). In addition, no significant relationship with genotypes was found when patients with IgA nephropathy were grouped in those with an assumed more benign course, characterized by a serum creatinine of <1.3 mg/dl at the time of biopsy (CC, 43.7%; CT, 51.25%; TT, 5.00% genotype), and those with a predicted more malign outcome with serum creatinine >1.3 mg/dl at time of biopsy (CC, 50.00%; CT, 43.75%; TT, 6.25% genotype, not significant). Distribution of various genotypes was also equal when IgA nephropathy patients were separated into those with end-stage renal failure and those with...
pre-terminal renal failure at the time of genotyping (no end-stage renal disease: CC, 47.90; CT, 45.80; TT, 6.30; end-stage renal disease: CC, 45.70; CT, 47.10; TT, 7.20% genotype, not significant.)

Finally, patients reaching the primary end-point (doubling of serum creatinine and/or start of dialysis) were compared by univariant analysis according to the C242T genotype (Figure 2). However, no difference was observed in deterioration of renal function among the three genotypes (Figure 2).

**Discussion**

The present study is, to the best of our knowledge, the first investigating a potential relationship between the C242T polymorphism of p22phox and renal disease, in this case IgA nephropathy and active lupus nephritis. NAD(P)H oxidases are membrane-associated enzymes that catalyse electron reduction of oxygen resulting in the formation of reactive oxygen species such as superoxide anion [8]. NAD(P)H oxidase comprises several distinct subunits including gp41phox and p22phox that are both electron-transfer proteins [8]. p22phox is widely expressed in vascular tissue, but is also present in mesangial cells, podocytes, and proximal tubular cells (for review see [8]). Expression of p22phox is increased in models of ANG II-induced hypertension, and ANG II stimulates and increases in p22phox transcripts in various renal cell types *in vitro* [8,9].

The previously described C242T nucleotide transition of p22phox results in the substitution of histidine 72 with tyrosine, thereby modifying one of the two haeme-binding sites that is probably essential for the stability of the enzyme [14]. Although one might expect that the C242T mutation reduced NAD(P)H oxidase activity, and a decreased superoxide production has indeed been demonstrated in human blood vessels with the 242T allele [14], it has been alternatively claimed that this mutation leads to an increase in oxygen radical production by subtle alterations of haeme-binding [10,11].

The p22phox polymorphism has been previously investigated in patients with coronary atherosclerosis [10–13]. Not surprisingly, these investigations showed conflicting results in various ethnic populations. For example, Inoue *et al.* [10] reported that the risk of coronary disease was lower in individuals carrying the T allele in a Japanese population. In contrast, the C242T polymorphism did not confer protection from endothelial dysfunction or coronary artery disease in a large study with 2205 male Caucasians [12,13]. Interestingly, in a prospective study the C242T variant was associated with progression of coronary atherosclerosis [11].

We studied the p22phox polymorphism in patients with inflammatory renal diseases because ANG II may play an important role in the pathogenesis of these diseases. Reactive oxygen species have been shown to activate proinflammatory mediators such as chemokines, mainly by stimulating the nuclear transcription factor κB (for review see [2]). Along this line, ANG II has been recently recognized as an important pro-inflammatory mediator, and part of the protective effect of ACE inhibitors on the progression of renal disease, as exemplified in IgA nephropathy, may be mediated by suppression of chronic inflammation. However, we failed to detect an association between the frequency of the C242T polymorphism of p22phox and IgA nephropathy, and active lupus nephritis. Moreover, no association was found with progression of IgA nephropathy. The frequency of the 242T allele in the Caucasian population, including our study, is 3–4 times higher than that in the Japanese population.

**Table 1.** Genotype and gene frequencies for the C242T p22phox polymorphism in controls, patients with IgA nephropathy, and patients with active lupus nephritis

<table>
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<tr>
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<th>n</th>
<th>Genotype n (%)</th>
<th>Allele n (%)</th>
<th>P</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>CT</td>
<td>TT</td>
</tr>
<tr>
<td>Controls</td>
<td>152</td>
<td>63 (41.2%)</td>
<td>69 (45%)</td>
<td>20 (13.8 %)</td>
</tr>
<tr>
<td>IgA</td>
<td>127</td>
<td>57 (44.9%)</td>
<td>61 (48%)</td>
<td>9 (7.1%)</td>
</tr>
<tr>
<td>Lupus</td>
<td>46</td>
<td>25 (54.3%)</td>
<td>16 (34.7%)</td>
<td>5 (11%)</td>
</tr>
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IgA, IgA nephropathy; Lupus, lupus nephritis.

![Fig. 2. Log-rank analysis of renal survival up to 15 years was studied in 97 patients. There was no significant association with any of the three genotypes in patients reaching the primary end-point (start of dialysis); P = 0.6189, not significant.](image)
Therefore the positive association of the C242T mutation with coronary artery disease in the study reported by Inoue et al. [10] probably reflects spurious associations intrinsic to case-control polymorphism association studies.

The problem with ethnically different populations has been exemplified by the conflicting results that implicate the insertion/deletion polymorphism of ACE in the development and progression of IgA nephropathy [4]. However, a recent meta-analysis clearly demonstrated that IgA nephropathy is not associated with the D allele of ACE in Asian and Caucasian populations, but there may be a weak association with the homozygous DD genotype, at least in Asian patients [5]. For this and other reasons, positive association studies have been criticized, and such findings should be confirmed in several large populations as well as in family-based studies [15]. Although the number of patients with active lupus nephritis was rather small, our study had a larger number of patients with IgA nephropathy, and many investigations looking at polymorphism of other genes, including those of the RAS in this population, have used fewer patients. Nevertheless, multi-centre collaborative studies are necessary to further study potential associations of the C242T polymorphism and IgA nephropathy as well as other diseases. Newer tests that are free of the bias due to population stratification should be also applied in future studies [15]. Finally, other polymorphisms have been recently described in the human p22phox genes that remain to be studied in renal diseases [12]. Despite these potential limitations, we believe that our study provides a salient finding even if it was a negative one.

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