The Influence of Three Endothelin-1 Polymorphisms on the Progression of IgA Nephropathy

(IgA nephropathy / endothelin-1 / polymorphism / renal failure)

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Abstract. The clinical course of chronic renal diseases and their progression to ESRF is highly variable. Different candidate gene polymorphisms have been advocated as possible modulators of ESRF progression. Moreover, ET-1 has been suggested as a major promoting factor in renal disease. However, limited data are available regarding an association of three ET-1 SNP K198N, T-1370G and 3A/4A with the progression of IgAN to ESRF. We examined a group of 122 pts with histologically proved IgAN (91 pts with normal renal function, 31 pts with ESRF), as a control group we used 132 genetically unrelated healthy subjects. Patients' DNAs were genotyped for three ET-1 SNP: K198N, T-1370G and 3A/4A by means of PCR. The frequencies of different genotypes and ET-1 gene haplotypes were compared among control group, IgAN pts with normal renal function and IgAN pts with ESRF. The ET-1 genotype distribution showed no differences among the groups of IgAN with normal renal function (1. K198N – 63.74% KK, 32.97% KN, 3.3% NN; 2. TT – 68.13% TT, 28.57% TG, 3.3% GG; 3. 3A/4A – 42.22% 3A/3A, 50.0% 3A/4A, 7.69% 4A/4A), IgAN with ESRF (1. K198N – 74.19% KK, 25.81% KN, 0% NN; 2. TT – 77.42% TT, 22.58% TG, 0% GG, 3. 3A/4A – 56.25% 3A/3A, 37.5% 3A/4A, 6.25% 4A/4A) and the control group (1. K198N – 66.67% KK, 31.82% KN, 1.52% NN, 2. TT – 76.51% TT, 22.72% TG, 0.76% GG, 3. 3A/4A – 43.94% 3A/3A, 44.70% 3A/4A, 11.36% 4A/4A). The analysis of haplotypes showed that the frequency of G-198, G-1370 and 4A allele combination was significantly higher in comparison with the control group (P = 0.0056). We excluded the effect of K198N, T-1370G and 3A/4A polymorphisms of the ET-1 gene in single-gene analysis on the progression of IgAN to ESRF. A significant association of the GG4A haplotype with IgAN, demonstrated by haplotype reconstruction of the ET-1 gene, could suggest a role in the pathogenesis of IgAN.

Introduction

IgA nephropathy (IgAN) is a mesangial proliferative glomerulonephritis characterized by diffuse mesangial deposition of immunoglobulin A (IgA, mainly IgA 1) or IgA-containing immune complexes (IgA-ICs). The proportion of 15–40 % patients with IgAN develop end-stage renal failure (ESRF) within twenty years.

Strong predictors of progression of IgAN have been identified and include hypertension, severe proteinuria, elevated serum creatinine level as well as histological signs of glomerular sclerosis and interstitial fibrosis. Recently, hypertriglyceridaemia, hyperuricaemia and other components of metabolic syndrome have been incriminated in the process of deterioration of renal function in IgAN. However, a variable course of IgAN progression indicates involvement of other factors as well. Recently, different candidate gene polymorphisms, affecting mainly the onset/development of arterial hypertension, have been suggested as possible modulators of the progression of IgAN towards ESRF (Syrjanen et al., 2000; Goto et al., 2002; Narita et al., 2003 a, b; Song et al, 2003). Moreover, endothelin-1 (ET-1) has been suggested as a major factor promoting renal disorders, due to its induction of renal vessel vasoconstriction and stimulatory effect on glomerular cell proliferation and extracellular matrix deposition. Single-nucleotide polymorphisms

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Abbreviations: ACE – angiotensin-converting enzyme, BP – blood pressure, ESRF – end-stage renal failure, ET-1 – endothelin-1, GFR- glomerular filtration rate, IgA – immunoglobulin A, IgAN – IgA nephropathy, IgA-CISc – IgA-containing immune complexes, PCR – polymerase chain reaction, pts – patients, RB – renal biopsy, SNP – single-nucleotide polymorphism.

(SNP), K198N causing substitution in exon 5, 3A/4A (-134delA) and T-1370G in the promoter, affecting the expression of the *ET-1* gene have been reported to be associated with elevated blood pressures (BP) and worsening glomerular filtration rate (GFR). Therefore, genetic factors may modulate ET-1 production and structure.

We investigated an association of three SNP of ET-1, i.e. K198N, T-1370G and 3A/4A, with the progression of IgAN towards ESRF, as well as with the clinical manifestation of IgAN.

Material and Methods

We examined a group of 122 pts with histologically proved IgAN (91 pts with normal renal function – e.g. normal levels of serum creatinine and normal GFR, 31 pts with ESRF); as a control group we used 132 genetically unrelated healthy subjects. Informed consent was obtained from all patients included.

Genomic DNA was isolated from peripheral blood lymphocytes by the salting-out procedure. We used the mismatch repair method for K198N and T-1370G polymorphisms. The defined primers were used for PCR. PCR conditions were the following: initial denaturation at 94° C for 5 min, followed by 30 cycles of denaturation, annealing (57° C for both alleles of K198N polymorphism, 49° C for -1370G and 57° C for the -1370T allele) and extension. The PCR products were separated on 2% agarose gel containing ethidium bromide and visualized under ultraviolet light.

The 3A/4A was detected by performing PCR amplification followed by heteroduplex analysis. The defined primers were used for PCR. PCR conditions were following: initial denaturation at 94° C for 5 min, followed by 30 cycles of denaturation, annealing (63° C) and extension. Twenty-five μl of each PCR product after addition of 0.5 μl 0.5M EDTA was denatured by heating at 95° C for 5 min and then cooled at 37° C for at least 1 h. Heteroduplex analysis was performed using Hydrolink Mutation Detection Enhancement (MDE, BMI, AT Biochem, Malvern, PA) gel solution. Eight μl of heteroduplexes and 3 μl of loading dye were electrophoresed through 1 × MDE gel with 15% urea, at 250 V for 4 h. Twenty-five-cm long gels were stained with ethidium bromide, and photographed under UV light. 3A/4A heterozygous individuals showed heteroduplexes. The homoduplexes were mixed with 3A/3A samples and heteroduplex analysis was repeated. The heteroduplexes were established as 4A/4A homozygous individuals.

Statistical analysis

Allele and genotype frequencies of the K198N, T-1370G and 3A/4A ET-1 polymorphisms were estimated by the gene counting method, and the Hardy-Weinberg equilibrium was tested. The χ^2 test was used to compare ET-1 genotype distribution in the pts and in the controls, and the frequencies of different genotypes between the IgAN groups with normal renal function and ESRF. Haplotype frequencies were estimated by the maximum likelihood method

(Haploview, version 3.2, 2005) in pts and controls. The value of P = 0.01 was considered as statistically significant.

Results

One hundred and twenty two pts with IgAN and 132 controls were genotyped for K198N, T-1370G and 3A/4A ET-1 polymorphisms (Table 1).

The pts were classified into two subgroups based on disease progression: 91 pts had normal GFR, 31 pts had ESRF. We compared the frequencies of different genotypes between the IgAN groups with normal renal function and ESRF.

The ET-1 genotype distribution showed no differences among the groups of IgAN with normal renal function (1. K198N – 63.74% KK, 32.97% KN, 3.3% NN; 2. TT – 68.13% TT, 28.57% TG, 3.3% GG; 3. 3A/4A – 42.22% 3A/3A, 50.0% 3A/4A, 7.69% 4A/4A), IgAN with ESRF (1. K198N – 74.19% KK, 25.81% KN, 0% NN; 2. TT – 77.42% TT, 22.58 % TG, 0% GG, 3. 3A/4A – 56.25% 3A/3A, 37.5% 3A/4A, 6.25% 4A/4A) and control group (1. K198N – 66.67% KK, 31.82% KN, 1.52% NN, 2. TT – 76.51% TT, 22.72% TG, 0.76% GG, 3. 3A/4A – 43.94% 3A/3A, 44.70% 3A/4A, 11.36% 4A/4A). The distribution of ET-1 genotypes did not differ among IgAN with normal renal function, IgAN with ESRF and control group (Table 2).

Haplotype analysis

We calculated the linkage disequilibrium values among the three ET-1 polymorphisms analysed, and the haplotypes were reconstructed by the maximum likelihood method (Haploview, version 3.2, 2005). The GGA haplotype (defined as G-198, G-1370 and 4A alleles) was significantly associated with IgAN (P = 0.0056) (Table 3).

Discussion

IgAN is the most common nephropathy in the world among adult patients undergoing renal biopsy (RB). However, there is a striking geographic variation (Johnson and Feehally, 2003).

It has been shown recently that mesangial IgA1 in IgAN suffers from the abnormalities of O-glycosylation (abnormal O-linked hinge-region sugars with reduced glycosylation – Hiki et al., 1999; Lejny et al., 1999; Floege and Feehally, 2000; Mestecky et al., 2002). In the absence of Gal, the terminal sugar remains N-acetylgalactosamine (GalNAc) (Tomana et al., 1997, 1999) and these sugar moieties or glycopeptides (Kokubo et al., 1999; Kokubo et al., 2000) are recognized by antibodies with anti-glycan or anti-hinge region peptide specificities (Tomana et al., 1999, 2000).

Table 1. Genotype and allele distribution for the three ET-1 polymorphisms
The genotype and allele frequencies that we observed were in line with what we expected under the Hardy-Weinberg equilibrium assumption. The allele and genotype frequencies for all three ET-1 polymorphisms in the IgAN patients did not differ significantly from those of the controls. Finally, no significant difference was observed in genotype and allele distribution stratifying cases by gender for the polymorphisms analysed

ET-1 genotype, allele	Patients with IgAN			Controls		
K198N	**men N (%)**	**women N (%)**	**all subjects N (%)**	**men N (%)**	**women N (%)**	**all subjects N (%)**
KK	58 (66..67)	23 (65.72)	81 (66.39)	44 (68.75)	44 (64.71)	88 (66.67)
KN	27 (31..03)	11 (31.43)	38 (31.15)	18 (28.13)	24 (35.29)	42 (31.82)
NN	2 (2..30)	1 (2.86)	3 (2.46)	2 (3.13)	0	2 (1.52)
G/T			(82/18.1)			(82.6/17.43)
I/D						
3A3A	45 (51..72)	11 (31.43)	56 (45.90)	31 (48.44)	27 (39.71)	58 (43.94)
3A4A	38 (43..68)	19 (54.29)	57 (46.72)	25 (39.06)	34 (50)	59 (44.70)
4A4A	4 (4.60)	5 (14.29)	9 (7.38)	8 (12.5)	7 (10.29)	15 (11.36)
3A/4A			(69.3/30.7)			(66.29/33.71)
ET-P						
TT	59 (68.61)	27 (75)	86 (70.49)	50 (78.13)	51 (75)	101 (76.51)
TG	25 (29.07)	8 (22.22)	33 (27.05)	13 (20.31)	17 (25)	30 (22.72)
GG	2 (2.33)	1 (2.78)	3 (2.46)	1 (1.56)	0	1 (0.76)
T/G			(84/16)			(87.9/12.12)

Table 2. The distribution of ET-1 genotypes among IgAN with normal renal function, IgAN with ESRF and control group

ET-1 genotype	Pts with stable renal disease (SD) N (%)	Pts with end-stage renal failure (ESRF) N (%)	Controls N (%)
K198N			
KK	58 (63.74)	23 (74.19)	88 (66.67)
KN	30 (32.97)	8 (25.81)	42 (31.82)
NN	3 (3.3)	0	2 (1.52)
I/D			
3A3A	38 (42.22)	18 (56.25)	58 (43.94)
3A4A	45 (50)	12 (37.5)	59 (44.70)
4A4A	7 (7.69)	2 (6.25)	15 (11.36)
ET-P			
TT	62 (68.13)	24 (77.42)	101 (76.51)
TG	26 (28.57)	7 (22.58)	30 (22.72)
GG	3 (3.3)	0	1 (0.76)

Table 3. Haplotype analysis

Haplotypes	Pts with IgAN	Controls	χ^2	P value
TGA	0.494	0.525	0.53	0.4664
TGC	0.241	0.295	2.029	0.1543
GTA	0.078	0.083	0.037	0.848
TTA	0.095	0.049	4.289	0.0384
GTC	0.024	0.032	0.324	0.5694
GGA	0.044	0.006	7.69	0.0056
TTC	0.023	0.01	1.497	0.2212

Subsequently, IgA-containing immune complexes (IgA-CICs) are formed (Mestecky et al., 2002). IgAN-CIC are bound to mesangial cells more efficiently than uncomplexed IgA (Novak et al., 2002, 2005). Therefore, they are likely to play a role in the pathogenesis of IgAN.

A functional abnormality of the specific glycosyltransferases responsible for the O-glycosylation of IgA1 has been widely studied in order to uncover the mechanism of altering O-glycosylation in IgAN (Mestecky et al., 1993). However, no defect in the activity of this enzyme in bone marrow B cells in IgAN has been identified so far (Buck et al., 2003). The overproduction of macromolecular aggregates of IgA1 is likely to be based within systemic immune sites such as the bone marrow, with both systemic and mucosal antigen challenges resulting in aberrant systemic immune responses (Barratt et al., 2004). Also, familial forms of IgAN (Schena et al., 2002) have been described and linkage of IgAN to chromosome 6q22-q23 has been demonstrated (Gharavi et al., 2000; Schena et al., 2002; Magistroni et al., 2003; Scolari, 2003; Suzuki et al., 2005).

The strongest predictors of an unfavourable outcome are hypertension, severe proteinuria, and elevated serum creatinine level. Among histological lesions, glomerulosclerosis and interstitial fibrosis are the most reliable prognostic markers. In addition to these risk factors, diabetes mellitus, hypertriglyceridaemia, hyperuricaemia and other components of metabolic syndrome are significantly associated with poor prognosis, while gross haematuria, smoking habits, or serum total and HDL cholesterol are not (Syrjanen et al., 2000). Although hyperuricaemia could simply be a consequence of renal disease, further studies are necessary to prove its pathogenetic role (Johnson et al., 1999). Another recent study showed that excessive body weight is associated with the progression of IgAN (Bonnet et al., 2001).

As mentioned above, different candidate gene polymorphisms, affecting mainly the onset/development of arterial hypertension, have been advocated as possible modulators of the progression of chronic nephropathies. A deletion allele in the angiotensin-converting enzyme (*ACE*) gene increases serum and tissue ACE levels and has been associated with the risk of progression of IgAN. The findings have not been confirmed in all studies, however. The link is unlikely to be specific for IgAN (Syrjanen et al., 2000; Narita et al., 2003b).

Previous studies investigating a potential effect of the *ET-1* gene on renal disease progression provided conflicting results (Pinto-Sietsma et al., 2003; Tahala et al., 2004). ET-1 stimulates glomerular cell proliferation and extracellular matrix deposition, causes vasoconstriction of renal blood vessels and likely participates in impairment of renal perfusion. The renal ischemia contributes to tubulo-interstitial injury and further loss of renal function.

Animal studies showed an independent role of endothelins for manifestation of renal disease (Pinto-Sietsma et al., 2003). However, linkage analysis in African American families failed to find an association between ET-1 and ESRF of different causes (Pinto-Sitsma et al., 2003; Tahala et al., 2004).

Three SNPs, K198N, 3A/4A (-134delA) and T-1370G in the promoter of the *ET-1* gene, have been reported to be associated with hypertension and renal insufficiency. A G to T transversion in exon 5 that causes the Lys to Asn substitution at codon 198 showed a positive association with BP in overweight people (Tiret et al., 1999; Jin et al., 2003). K/N amino acid change might affect the processing of preproendothelin and influence synthesis of ET-1. Recently, population homozygous for the N allele of K198N polymorphism and G allele of T-1370G polymorphism showed diminished glomerular filtration, lower creatinine clearance and obesity compared with the remaining genotype carriers (Pinto-Sietsma et al., 2003). The K198N polymorphism showed a positive association with BP elevation in overweight individuals (Tiret et al., 1999). Furthermore, the N198 allele was associated with higher systolic BP in pregnant women (Pinto-Sietsma et al., 2003). On the other hand, no difference in plasma ET1 level was found in respect to K198N or T-1370G polymorphisms. However, a variation of ET-1 in the kidney can be different because the majority of ET-1 is secreted at the abluminal side of the vessels and endothelin acts more as an autocrine system than a systemic peptide (Pinto-Sietsma et al., 2003).

The homozygotes with the 4A allele (insertion of adenosine) of 3A/4A polymorphism in the 5'- untranslated region in the *ET-1* gene showed significantly increased expression of the ET-1 protein due to enhanced mRNA stability (Tahala et al., 2004). Furthermore, the ET-1 plasma level was significantly higher in 3A/4A hypertensive individuals than in homozygous individuals with the 3A allele. An association between the 4A allele and increased BP has been reported (Lajemi et al., 2001). Moreover, an elevated plasma ET-1 level was observed in hypertensive subjects with the 4A allele (Tahala et al., 2004).

In our study we investigated the possible effect of the K198N, 3A/4A and T-1370G polymorphisms of the endothelin gene on the progression of IgAN in Czech patients. No effect of these polymorphisms was found in single-gene analysis.

However, the ET-1 haplotype reconstruction revealed that the GG4A haplotype (defined as G-198, G-1370 and 4A alleles) was significantly associated with the IgAN patients.

Genetically, SNP may not alter expression or function of specific proteins so strongly as to produce pathologic phenotypes in multifactorial diseases (such

as IgAN). Although the possible ET-1 pathogenetic mechanism needs further clarification, our study suggests that the haplotype reconstruction of ET-1 gene polymorphisms could be more informative than the investigation of SNP for defining the risk of progression of IgAN.

The association of the GG4A haplotype with the chronic glomerulonephritides, especially IgAN, might by explained by shared interaction of all ET-1 polymorphisms. There may also be a synergistic influence of all ET-1 polymorphisms in development of the disease. Alternatively, this GG4A haplotype could be related to other polymorphisms or mutations that are responsible for the occurrence of the disease, and this haplotype might play a role as a marker of these polymorphisms/mutations. Such linkage might be specific just for the Czech population.

However, limited data are available regarding an association of three ET-1 SNP K198N, T-1370G and 3A/4A with the progression of IgAN to ESRF. As mentioned above, our results indicated potential effect of the GG4A haplotype on the progression of IgAN. It is clear that the influence of ET-1 polymorphisms on the progression of IgAN to ESRF needs to be elucidated by further studies composed of a larger number of patients and/or performed in other countries than in the Czech Republic.

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