Angiotensin-converting enzyme gene insertion/deletion polymorphism in newly diagnosed primary chronic glomerulonephritis*

Polimorfizm insercyjno-delecyjny genu kodującego konwertazę angiotensyny w nowo rozpoznanych pierwotnych przewlekłych kłębuszkowych zapaleniach nerek

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Abstract

Background. There are many controversies whether angiotensin-converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism is associated with susceptibility to renal disease.

Objectives. The aim of this study was to evaluate an association between I/D polymorphism of the ACE gene and early stage of primary chronic glomerulonephritis (GN).

Material and Methods. Fifty untreated patients with newly diagnosed, biopsy proven primary chronic GN and serum creatinine levels <115 µmol/L participated in the study. Control group consisted of 100 healthy subjects. The technique used was a PCR-RLFP with identification of DD, ID and II genotypes.

Results. The distribution of the genotypes (DD 5, ID 32, II 13) and alleles (I 58%, D 42%) in chronic GN was significantly different from healthy controls (DD 35, ID 45, II 20, I 42.5%, D 57.5%), p < 0.05 and p < 0.02, respectively.

Conclusion. These preliminary results suggest that I allele of ACE gene, or an unknown DNA sequence difference linked with it, may contribute to increased susceptibility to early stage of the primary chronic GN (Adv. Clin. Exp. Med. 2003, 12, 2, 161–165).

Key words: angiotensin-converting enzyme gene insertion/deletion polymorphism, primary glomerulonephritis.

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An insertion/deletion (I/D) polymorphism of the angiotensin converting enzyme (ACE) gene has been identified in humans [1, 2]. The ACE gene consist of 26 exons and spans 21 Kb on chromosome 17 [3], and I/D polymorphism is characterized by the presence or absence of a 287 base pair fragment in intron 16 resulting in three genotypes (DD, ID and II). The physiological importance of the I/D polymorphism relies on the fact that the DD genotype is associated with increased circulating and tissue ACE levels [2, 4]. Several lines of evidence have favored a contribution of a local renin-angiotensin system in the regulation of kidney function and the differences in the intrarenal and plasma compartments for angiotensin I and angiotensin II, compatible with different patterns of angiotensin II generation in the kidney, were demonstrated in the experimental study [5]. Recently it was reported that renal ACE gene expression is associated with the ACE I/D genotype in healthy Japanese subjects [6]. The in situ hybridization studies showed that both tubular and glomerular ACE mRNA expressions were week in subjects with II genotype, intermediate in subjects with ID genotype, and strong in subjects with DD genotype [6]. It was suspected, although not directly proven as yet, that increased intrarenal activity of ACE in persons with DD genotype of ACE gene may be associated with higher local angiotensin II levels. Angiotensin II is a multifunctional factor exhibiting such diverse actions as influencing renal haemodynamics and tubular transport, acting as a growth factor and a profibrogenic cytokine, and even having inflammatory properties [7]. A large number of studies have been performed to investigate the possibility that I/D polymorphism of ACE gene may be associated with susceptibility to renal disease. Whereas many studies have suggested and association between the presence of the D-allele and renal disease, this has not been an universal finding [8]. Several factors may account for these disparate findings, including differences in study design, genetic backgrounds of patients, diagnostic criteria for renal disease, environmental factors, differences in time from the onset of the disease and the influence of treatment on the course of the disease. There are no data in the literature about the possible role of I/D polymorphism of the ACE gene in the early stage of the biopsy proven primary chronic glomerulonephritis in Caucasian patients. Therefore, we investigated whether the ACE gene I/D polymorphism is associated with early stage of primary chronic glomerulonephritis.

**Material and methods**

Local Ethics Committee approved the study and all patients gave their written informed consent. The patients were recruited from the Department of Nephrology, Transplantology and Internal Diseases, University of Medicine Sciences in Poznan and as a control groups 100 healthy subjects from the hospital personnel were analysed. All persons studied were Caucasians. For the patients the following inclusion criteria were applied: biopsy proven primary chronic glomerulonephritis (GN), appearance of the earliest discrete symptoms of kidney disease or changes in urine examination not earlier than 3 months before the kidney biopsy, absence of hypertension before the onset of the disease, serum creatinine concentration below 115 µmol/L (1.3 mg/dL), no treatment, absence of diabetes mellitus and any other concomitant disease, no family history of kidney disease.

Fifty patients, 13 women and 37 men, aged 42±13 years fulfilled the inclusion criteria. The same pathologist examined renal biopsies and the following diagnoses were established: mesangial GN in 27 patients, focal segmental glomerulosclerosis in 10 patients, idiopathic membranous GN in 7 patients and membranoproliferative GN in 6 patients. Renal function was evaluated by measuring plasma creatinine concentration, glomerular filtration rate (GFR) by dynamic scintigraphy performed with gamma camera (Toshiba) after administration of technetium labelled DTPA and by creatinine clearance. Values were corrected for calculated body surface. The mean value of GFR was 100±29 mL/min/1.73 m² and the mean value of the creatinine clearance was 96±35 mL/min/1.73 m², which confirmed mild impairment of kidney function.

DNA was extracted from peripheral blood leukocytes [9]. A 25 ng genomic DNA was amplified by polymerase chain reaction (PCR; Perkin-Elmer 9600, Norwalk, CT, USA) in a 25 µL mixture of 1.5 mmol/L MgCl₂, 50 mmol/L KCl (pH 8.3), 0.001% gelatin, 200 mmol/L deoxynucleotide triphosphates, 0.48 µmol/L primers, and 1 U Taq polymerase (Ampli-Taq; Perkin-Elmer). The forward and reverse primers were, respectively, 5′-CTGGAGACCTCCTCCCCTTCTTTT-3′ and 5′-GATGTGGCCATCACATTGTCAAGAT-3′. Thirty cycles of thermocycling with one minute of denaturation at 94°C, one minute of annealing at 63°C, and two minutes of extension at 72°C, followed by five minutes of final extension at 72°C were performed. The final products, two fragments, one of 490 bp with insertion (I allele) and one of 190 bp without (D allele), were detected on 1.5% agarose gel.
The D allele was preferentially amplified. To avoid the possibility of mistyping the ID heterozygotes as DD homozygotes, a second primer pair specific for the inserted I sequence reamplified all DD samples. PCR was performed in a 25 µL mixture of 1.5 mmol/L MgCl$_2$, 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.8), 0.1% Triton X-100, 200 mmol/L deoxynucleotide triphosphates, 0.4 µmol/L primers, 3 U Taq polymerase, and 100 ng genomic DNA. The primers for the inserted sequence were 5'-TGGGACCACAGCGCCCGCACTAC-3' and 5'-TCGCCAGCCCTCCCATGCCCATATAA-3'. Forty cycles of thermocycling with one minute of denaturation at 94°C, followed by one minute of annealing-extension at 78°C, and a 10-minute final extension at 72°C were performed. Only I allele produced a 300 bp fragment, whereas no products were detected with the DD genotype, which assured the absence of mistyping.

Genotype distribution and allele frequencies in the patients and healthy controls were compared using $\chi^2$. A p value of less than 0.05 was considered significant.

**Results**

The distribution of the ACE genotype in patients with early stage of the primary chronic GN was: 5 (10%) patients with DD genotype, 32 (64%) patients with ID genotype and 13 (26%) patients with II genotype (Tab. 1). This distribution was different to the one in the control group, where the ACE genotype distribution was: 35 DD (35%), 45 ID (45%) and 20 II (20%; $p < 0.05$).

The patients with early stage of primary chronic GN were also characterized by an excess in the frequency of I allele (58%) while in the healthy controls it was only present in 42.5% ($p < 0.02$, Tab. 2).

**Discussion**

The present study shows a significant difference in the distribution of the ACE genotype in patients with early stage of the primary chronic GN and in healthy controls that has not been reported before. The results suggest that I allele of ACE gene may contribute to increased susceptibility to primary chronic glomerulonephritis. This suggestion seems surprising and due to low number of patients included in the study should be considered with caution. There are, however, some data indicating that I allele of the ACE gene, may contribute to increased susceptibility to other chronic glomerular disease, namely diabetic nephropathy. The group of Krolewski [10] used the transmission disequilibrium test (TDT) approach to examine the role of I and D alleles of ACE gene in the development of diabetic nephropathy. These authors provided strong evidence that I allele (or an unknown DNA sequence difference linked with it) contributes to increased susceptibility to diabetic nephropathy, not the D allele, as was shown in some of the case-control studies. There is also a prospective study suggesting that insertion was the risk allele for development of microalbuminuria in patients with type 1 diabetes mellitus [11]. In contrast, several studies that have compared IgA nephropathy (the most prevalent form of chronic glomerulonephritis) patients with normal population controls have found no difference in the I/D genotype frequency, indicating that ACE genotype is not a risk factor for developing IgA nephropathy [12–15]. It should be pointed out, however, that all these studies included patients with different period of time from the beginning of the disease, some of them with chronic renal insufficiency, two of the studies were performed in Japanese populations [14, 15] with the similar small number of patients as included in our study, and one of the remaining study was retrospective [12].
these factors may, at least partly, explain the difference between our results and the results of the cited studies [12–15].

The answer to the question why the possession of I allele of the ACE gene increases susceptibility to chronic GN remains speculative. It was recently demonstrated that angiotensin II infusion ameliorated the early phase of a mesangio proliferative glomerulonephritis in rats [16]. In the experimental model of glomerulonephritis induced by the anti-thymocyte antibody, the infusion of the angiotensin II 200 ng/min by osmotic minipump reduced glomerular monocyte infiltration, proliferation and matrix expansion. The authors concluded that angiotensin II-mediated induction of cyclin kinase inhibitors and transforming growth factor β (TGF-β) may contribute to the protection of the glomerulus from inflammatory injury by inducing cell cycle arrest and attenuating activation of local and recruited cells. Alternatively, angiotensin II might protect the kidney at least in part by less inflow of disease activators due to reduction of renal blood flow [16]. On the basis of this observation, it may be hypothesized that higher intrarenal concentrations of angiotensin II, attributed to subjects with DD genotype of the ACE gene, may have similar protective effects in an early phase of primary chronic glomerulonephritis, while the subjects possessing I allele of the ACE gene are more susceptible to the development of the glomerular injury due to suggested lower intrarenal levels of angiotensin II. This hypothesis in highly speculative and should be verified in further studies.

Our preliminary results, which suggest that I allele of the ACE gene, or an unknown DNA sequence difference linked with it, may contribute to increased susceptibility to primary chronic GN, need confirmation.

References


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