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Genetic polymorphisms of renin-angiotensin system and progression of interstitial nephritis

Interstitial nephritis accounts for 20-40% of chronic renal failure and tends to progress to end-stage renal disease (ESRD). Both genetic factors and environment, affect the development of ESRD (1). The renin-angiotensin system (RAS) is one of the main pathophysiologic factors in hypertension, cardiovascular disease and renal failure. Genetic variability in RAS may predispose to the development of renal failure and affect its progression (4). Among the candidate genes of RAS, the angiotensin-converting enzyme (ACE), angiotensinogen (AGT) and angiotensin II type 1 receptor (AT1R) are of particular interest.

The ACE genotype is one of the most frequently studied genetic risk markers in cardiovascular diseases. The D allele of the insertion/deletion polymorphism is associated with atherosclerosis, stroke and renal vascular diseases. It was also found to be associated with diabetic nephropathy, IgA nephropathy and a faster progression of renal disease (12). Molecular variant of the AGT gene, M235T, is involved in progression of diabetic nephropathy (11). Known actions of angiotensin II are mediated through a stimulation of the angiotensin II type 1 receptor. A variant form of the AT1R gene has been implicated as a risk factor for hypertension and cardiovascular disease (15).

The purpose of the present study was to determine whether polymorphic variants in these three candidate genes are associated with interstitial nephritis and the progression to ESRD.

MATERIAL AND METHODS

Study subjects. The study group consisted of 90 patients with ESRD resulting from interstitial nephritis (in most cases confirmed by renal biopsy). All patients were

undergoing maintenance dialysis at the time of study. Renal disease in first-degree relatives was considered positive family history. Time to ESRD was calculated from the diagnosis of renal disease to the start of renal replacement therapy. Healthy control subjects (n=200) were recruited among hospital staff and blood bank donors. Informed consent was obtained from all subjects. The study protocol was approved by the ethics committee of Medical University of Lublin. Characteristics of studied subjects is presented in Table 1.

Table 1. Clinical characteristics of the studied subjects

	Interstitial nephritis (n=90)	Control group (n=200)
Age (yrs)	54.1 ± 12.4	48.5 ± 11.2
Sex (M / F)	47 / 43 *	119 / 81 *
Cholesterol (mg/dl)	185 ± 51.5 *	162 ± 39 *
Age at diagnosis (yrs)	43.2 ± 16.6	NA
Dialysis duration (yrs)	3.3 ± 2.8	NA
Time to ESRD (yrs)	7.7 ± 9.52	NA
Hypertension	54 (60) **	0 **
Family history of kidney disease	19 (23) **	0 **

NA = not applicable. Percentages in brackets.

Differences between groups statistically significant : * $p < 0.05$, ** $p < 0.01$

Genotyping by polymerase chain reaction (PCR). Genomic DNA was isolated from peripheral blood by a standard procedure (8). All PCR reactions were performed in 50 µl volume containing 300 ng DNA, standard buffer for Taq polymerase, 20 pM of each primer (TIB MOLBIOL), 2.5 mM of each dNTP, 1.5 mM $MgCl_2$ and one unit of Taq DNA polymerase (all reagents from MBI Fermentas).

The amplification of intron 16 of the ACE gene was performed using primers: forward 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and reverse 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3' (annealing temperature 63^0 C). Because of preferential amplification of the D allele in heterozygous samples, the second amplification was performed on DD samples with insertion-specific primers (6). The amplification products observed in 2% agarose gel were 490 bp for insertion (I) allele and 190 bp for deletion (D) allele. For M235T mutation in the AGT gene the primers were : forward 5'-CCG TTT GTG CAG GGC CTG GCT CTC T-3' and reverse 5'-CAG GGT GCT GTC CAC

ACT GGA CCC C-3' (annealing temperature 65^{0}C). The PCR product was digested with Psy I restriction enzyme, giving fragments 165 bp for the M allele and 141 bp for the T allele. The AT1R genotype was determined with primers: forward 5'-GCA GCA CTT CAC TAC CAA ATG GGC-3' and reverse 5'-CAG GAC AAA AGC AGG CTA GGG AGA-3' (annealing temperature 55^{0}C). The PCR product was digested with Bsu RI restriction enzyme, giving fragments 255 bp (A allele) and 231 bp (C allele).

Statistics. Statistical calculations were performed using Statistica PL program. Chi-square analysis was used for comparing the allele and genotype frequencies between patients and control group. Values of $p < 0.05$ were considered statistically significant.

RESULTS

We genotyped 90 patients with interstitial nephritis and 200 healthy subjects for the ACE, AGT and AT1R polymorphisms. Clinical and demographic data of both groups are outlined in Table 1. The distribution of genotype and allele frequencies was compared between patients and controls. These results are given in Table 2.

The allele and genotype frequencies for the I/D ACE polymorphism did not differ significantly between the two groups. In the M235T polymorphism of the AGT gene, the patient group had a higher frequency of the T allele than the control group ($p < 0.01$). There was also a difference in genotype frequencies. The homozygous TT genotype was

Table 2. Allele and genotype frequencies in interstitial nephritis patients and controls

	Interstitial nephritis (n=90)	Controls (n=200)	p *
ACE			
I / D allele	0.46 / 0.54	0.42 / 0.58	
Genotypes :			
II	20 (22)	41 (20.5)	
ID	42 (47)	89 (44.5)	
DD	28 (31)	70 (35)	
AGT			
M / T allele	0.46 / 0.54	0.56 / 0.44	< 0.01
Genotypes:			
MM	20 (22)	72 (36)	
MT	41 (46)	80 (40)	
TT	28 (32)	48 (24)	< 0.01
AT1R			
A / C allele	0.76 / 0.24	0.79 / 0.21	
Genotypes:			
AA	53 (59)	123 (61.5)	
AC	31 (34)	70 (35)	
CC	6 (7)	7 (3.5)	< 0.01

Percentages in brackets. p * where statistically significant

observed in 32% of patients compared to 24% in the control group ($p < 0.01$). A difference in genotype distribution was also found in the AT1R polymorphism. The higher frequency of the CC homozygotes was observed in patients than in controls (7% vs. 3.5%, $p < 0.01$).

We evaluated the correlation between the AT1R polymorphism and the progression to ESRD in the interstitial nephritis group. As only 6 subjects were homozygous for the C allele, we pooled patients with the CC and AC genotypes for comparison with the AA homozygotes. In patients carrying the C allele the average time to ESRD was significantly shorter than in individuals with the AA genotype (4.4 yrs vs. 11 yrs, $p < 0.01$).

DISCUSSION

We have analyzed three gene polymorphisms of the renin-angiotensin system for a possible relationship with interstitial nephritis and progression to ESRD. Genes of the RAS system are logical candidates since angiotensin II plays an important role in the progression of renal disease. Its action on glomerular blood pressure and promotion of mesangial hypertrophy might be responsible for a progressive loss of renal function (4, 13).

Our study group was ethnically homogenous and in most cases the renal phenotype was confirmed by a biopsy. In control group the observed allele frequencies for all three polymorphisms were as reported for the European population.

The angiotensin-converting enzyme gene polymorphism is known for its effect on renal diseases. The presence of the D allele of this polymorphism is associated with nephroangiosclerosis (9), diabetic nephropathy (5) and IgA nephropathy (13). We did not find any significant association of this allele with interstitial nephritis. The effect of the I/D polymorphism in the ACE gene might be dependent on pathophysiological changes in underlying disease.

The angiotensinogen gene polymorphism M235T was found responsible for increased risk of diabetic nephropathy (2). It is also associated with disease progression in IgA nephropathy (10,13). An involvement of the M235T polymorphism in renal disease is also observed in our study. The T allele of this polymorphism seems to be a risk factor for interstitial nephritis.

Despite a potential role of the angiotensin II type 1 receptor in renal mechanisms most of the studies did not confirm its association with renal disease progression (3, 7). In our study we found that the AT1R genotype affects the progression to end-stage renal disease in interstitial nephritis patients. The time from the onset of the disease to ESRD is shortened in the patients with the C allele of the AT1R polymorphism (4.4 yrs vs. 11 yrs in those with AA genotype). In the study of Tomino et al. (14) a relationship was found between the AT1R polymorphism and the progression of renal disease. Time to ESRD in female patients with diabetic nephropathy having AC/CC genotype was significantly shorter than in patients with the AA genotype.

The results of our study need to be confirmed in a larger patient population. Also the mechanism by which the AGT and AT1R gene polymorphisms affect the development of renal disease and progression to ESRD remains to be elucidated. In summary, our study shows the association of the AGT and AT1R gene polymorphisms with the development and progression of interstitial nephritis. The C allele of the A1166C AT1R polymorphism appears to be a risk factor for a faster progression to ESRD.

CONCLUSIONS

1. The homozygous TT genotype of the angiotensinogen gene polymorphism is associated with the development of interstitial nephritis.
2. In the angiotensin II type 1 receptor gene polymorphism, the C allele is associated with a faster progression of renal disease to the terminal stage.
3. Further studies will concentrate on elucidating the mechanism of above associations, preferably in a larger group of patients.

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SUMMARY

Genes of the renin-angiotensin system (RAS) are involved in the progression of renal failure. Among them, the angiotensin-converting enzyme (ACE), angiotensinogen (AGT) and angiotensin II type 1 receptor (AT1R) genes are of particular interest. We examined polymorphisms of these three genes for association with the development of interstitial nephritis and progression to end-stage renal failure. The allele frequency and genotype distribution were compared in 90 patients with interstitial nephritis and 200 healthy controls. DNA samples were genotyped by polymerase chain reaction (PCR). We did not find statistically significant differences between groups in the insertion/deletion polymorphism of the ACE gene. An involvement of M235T polymorphism of the AGT gene in renal disease was observed in our study. The frequency of the T allele was higher in patients than in controls (32% vs. 24%). In the A1166C AT1R polymorphism the homozygous CC genotype was also more frequent in interstitial nephritis patients (7% vs. 3.5%). In patients carrying the C allele, an average time to ESRD was significantly shorter than in subjects with the AA genotype. Our study shows the association of the AGT and AT1R gene polymorphisms with the development and progression of interstitial nephritis. The C allele of the A1166C polymorphism appears to be a risk factor for faster disease progression.

Polimorfizmy genetyczne systemu renina-angiotensyna i progresja śródmiąższowego zapalenia nerek

Geny systemu renina-angiotensyna (RAS) są zaangażowane w progresji schyłkowej niewydolności nerek. Wśród nich gen konwertazy angiotensyny (ACE), angiotensynogenu (AGT) i receptora typu 1 angiotensyny II (AT1R) są szczególnie interesujące. Badaliśmy polimorfizmy tych trzech genów w poszukiwaniu związku z rozwojem śródmiąższowego zapalenia nerek i progresją do schyłkowej niewydolności nerek. Częstość alleli i rozdział genotypów porównywano u 90 chorych ze śródmiąższowym zapaleniem nerek i 200 osób zdrowych. Próbki DNA genotypowano metodą reakcji łańcuchowej polimerazy (PCR). Nie znaleziono statystycznie istotnych różnic między grupami, badając insercyjno-delecyjny polimorfizm genu ACE. W badaniu naszym obserwowaliśmy zaangażowanie M235T polimorfizmu genu AGT w chorobie nerek. Częstość allela T była wyższa w grupie chorych niż w grupie kontrolnej (32% vs. 24%). W polimorfizmie A1166C genu AT1R homozygotyczny genotyp CC występował również znacznie częściej u chorych ze śródmiąższowym zapaleniem nerek (7% vs. 3.5%). U chorych będących nosicielami allela C średni czas do ESRD był znacząco krótszy niż u osób z genotypem AA. Nasze badania wykazały związek polimorfizmów genów AGT i AT1R z rozwojem i progresją śródmiąższowego zapalenia nerek. Allel C polimorfizmu A1166C wydaje się czynnikiem ryzyka szybszej progresji choroby nerek.