Original Article

The Influence of Endothelin-A Receptor Gene Polymorphism on the Progression of Autosomal Dominant Polycystic Kidney Disease and IgA Nephropathy

(ADPKD / IgA nephropathy / endothelin-A receptor / polymorphism / end-stage renal disease)

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Abstract. ADPKD is the most common hereditary renal disease. IGAN is a mesangial proliferative glomerulonephritis characterized by diffuse mesangial deposition of immunoglobulin A. ET-1 has been suggested to be a major disease-promoting factor in renal diseases. The vasoconstrictor effect of ET-1 is mediated by the ET-A receptor. We have investigated the influence of C/T polymorphism in exon 8 of the *EDNRA* gene. A total number of 193 patients (87 males, 106 females) with ADPKD entered into this study. Patients were divided into three groups: 1. 47 pts with ESRD later than in 63 years (slow progressors), 2. 49 pts with ESRD before 45 (rapid progressors) and 3. 97 pts with ESRD between 45–63 years. Moreover, we examined a group of 153 pts with histologically proven IGAN (116 males, 37 females). Pts were divided into two groups: 1. 79 pts with ERSD during 5 years of the study (IGAN rapid progressors) and 2. 74 patients with normal renal function (IGAN slow progressors). As a control group we used 100 genetically unrelated healthy subjects. The distribution of C/T polymorphism did not significantly differ between rapid and slow progressors of ADPKD and IGAN. The comparison of ESRD ages showed that CC females with ADPKD failed significantly later than CT heterozygotes: CC (57.4 ± 8.1 years), CT (53.0 ± 9.1 years) and TT (54.5 ± 6.4years) (t-test, P = 0.018). To conclude, the CC genotype could be protective in ADPKD females. This genotype was described to be associated with lower pulse pressure.

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary renal disease. It is characterized by the development of multiple cysts in both kidneys. The cysts result over decades in chronic renal failure in about 50% of patients by the age of 60 years (Ravine et al., 1992). ADPKD is responsible for 8–10% cases requiring renal replacement therapy (Pirson et al., 1998).

Its clinical course is highly variable. Eighty-five % of cases are caused by the mutation of the *PKD1* gene on chromosome 16; the *PKD2* gene on chromosome 4 is mutated in about 14% cases (Ravine et al., 1992). The mean age of end-stage renal disease (ESRD) is 54 years in PKD1 patients and 73 years in PKD2 patients (Hataboer et al., 1999). Different mutations in the *PKD* genes may have different effects on the clinical course of the disease. Nevertheless, the clinical variability (interfamiliar and intrafamiliar) cannot be fully explained by different mutations in these two genes. The influence of genetic and environmental modifiers on the progression has not been clarified yet. Hypertension occurs prior to loss of renal function in about 70% of patients with ADPKD (Woo et al., 1997).

IgA nephropathy (IGAN) is a mesangial proliferative glomerulonephritis characterized by diffuse mesangial deposition of immunoglobulin A (IgA). Fifteen to forty % of patients with IGAN develop ESRD during 20 years. Some patients already have renal impairment and hypertension at presentation. The main diagnostic sign is mesangial positivity of IgA in immunofluorescence microscopy. Also familiar forms of IGAN have been described, a linkage of IgAN to chromosome 6q22-q23

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Abbreviations: ADPKD – autosomal dominant polycystic kidney disease, ET-1 – endothelin-1, *EDNRA* – endothelin-A receptor gene, ESRD – end stage renal disease, IGAN – IgA nephropathy, *PKD* gene – polycystic kidney disease gene, pts – patients.

has been demonstrated (Gharavi et al., 2000; Schena et al., 2002; Magistroni et., 2003; Scolari at al., 2003; Suzuki et al., 2005). Different candidate gene polymorphisms, affecting mainly the onset/development of arterial hypertension, have been advocated as possible modulators of the progression.

Endothelin-1 (ET-1) has been suggested to be a major disease-promoting factor in renal disease. ET-1 causes vasoconstriction of renal blood vessels and stimulates glomerular cell proliferation and extracellular matrix deposition. Alterations in the natural balance between endothelium-derived constricting and relaxing factors have been described in ADPKD and other renal diseases (Giusti et al., 1995). Enhanced formation and activity of ET-1 as a potent vasoconstrictive factor may play a role. ET-1, a 21-amino-acid peptide, is assumed to modulate vascular tone and plasma flow and promote vascular cell growth through two subtypes of receptors (ET-A and ET-B). Neoexpression of ET receptors (type ET-A) was found in glomeruli and cysts and markedly increased in medium-sized renal arteries (Ong et al., 2002). The potent vasoconstrictor effect of ET-1 is mediated by the ET-A receptor, present on smooth muscle cells. The ET-A receptor is overexpressed in the arteries of hypertensive patients. Polymorphic variants of ET-A receptor have been previously associated with migraine, pulse pressure in patients with myocardial infarction and idiopathic dilated cardiomyopathy (Charron et al., 1999; Nicaud et al., 1999; Tzourio et al., 2001).

We have investigated the influence of C1363T polymorphism in exon 8 of the ET-A receptor gene (*EDNRA*) in patients with ADPKD and IGAN.

Patients and Methods

A total number of 193 patients (pts) (87 males, 106 females) with ADPKD and ESRD entered into this study. All patients with positive family history were clearly linked to the *PKD1* gene or there was possible linkage to both *PKD* genes in the examined families. Families linked to the *PKD2* gene were not included. Blood samples were collected from 22 dialysis centres and one transplantation centre in the Czech Republic. The age of ESRD was defined as the age at starting renal replacement therapy. The diagnosis was established on the basis of bilateral cystic kidneys and in most cases on a positive family history. Patients were divided into three groups: 1. 47 pts with ESRD later than in 63 years (slow progressors), 2. 49 pts with ESRD before 45 (rapid progressors) and 3. 97 pts with ESRD between 45–63 years.

Moreover, we examined a group of 153 pts with histologically proven IGAN (116 males, 37 females). Pts with IgAN were examined for at least five years. Pts were divided into two groups: 1. 79 pts with ERSD during five years of the study or pts whose serum creatinine doubled during this period (IGAN rapid progressors) and 2. 74 patients with normal renal function or with mild decline of renal function during five years (IGAN slow progressors). Informed consent was obtained from all patients included.

As a control group we used 100 genetically unrelated healthy subjects selected among blood donors (50 males, 50 females, mean age 51.4 ± 8.2 years).

DNA samples were isolated from lymphocytes by standard procedures. All DNA samples were amplified by polymerase chain reaction (PCR) under following conditions: 1. forward primer 5′-GAAGTCTAAAACACACCTAA-3′ and 2. reverse primer 5′-CCCTTTGAAATGGTGACAAT-3′, 30 cycles (94°C – 30 s, annealing temperature 55°C – 30 s and extension 72°C – 30 s). All samples were sequenced in sequencer ABI PRISM 310.

Statistical analysis

The Hardy-Weinberg equilibrium was tested by Pearson χ^2 test. The χ^2 test was used to estimate the frequencies of genotypes among different ADPKD groups. The ages of ESRD between different groups were compared by two-tailed *t*-test.

Results

Distribution of this genotype was within Hardy-Weinberg equilibrium. The frequency of the T alelle was 0.41 and C alelle 0.59 in the control group.

ADPKD

The distribution of the C/T polymorphism did not differ between slow and rapid progressors of IGAN (Table 1). There was a non-significantly higher frequency of the C alelle in slow progressors (0.6 in comparison with 0.52 in rapid progressors).

Ninety % of pts were hypertensive or used antihypertensive drugs when they reached ESRD. Due to this fact, we did not correlate the relationship between arterial hypertension and different genotypes.

Comparing the ages of ESRD, we did not find significant differences in the ages among males (Table 2). The comparison of ESRD ages showed that CC females with ADPKD failed significantly later than CT heterozygotes (*t*-test, P = 0.018).

Table 1. Distribution of the C/T polymorphism of ET-A receptor pts with ADPKD

	CC	TC	TT
ADPKD – rapid progressors	18.4% (9/49)	65.3% (32/49)	16.3% (8/49)
ADPKD – slow progressors	31.9% (15/47)	57.4% (27/47)	10.7% (5/47)
ADPKD-ESRD 45–63 years	20.6% (20/97)	57.7% (56/97)	21.7% (21/97)
Control group	29% (29/100)	60% (60/100)	11% (11/100)

Table 2. Ages of ESRD in ADPKD pts according to different genotypes

	CC	CT	TT
Age of ESRD (in years) in all ADPKD individuals	55.4 ± 10.0	53.1 ± 9.0	53.1 ± 8.2
Age of ESRD in females	57.4 ± 8.1	53.0 ± 9.1	54.5 ± 6.4
Age of ESRD in males	52.3 ± 11.6	53.2 ± 8.9	51.4 ± 9.9

Table 3. Distribution of the C/T polymorphism of ET-A receptor pts with IGAN

	CC	TC	TT
IGAN rapid progressors	32.9% (26/79)	49.4% (39/79)	17.7% (14/79)
IGAN slow progressors	28.4% (21/74)	56.8% (42/74)	14.8% (11/74)
Control group	29% (29/100)	60% (60/100)	11% (11/100)

IGAN

The distribution of the C/T polymorphism did not differ between slow and rapid progressors of IGAN (Table 3). Seventy-five % of pts used ACEI or AT1 blockers. Due to this fact the correlation between arterial hypertension and different genotypes was not done.

Discussion

In the present study, we have investigated the possible effect of C/T polymorphism in exon 8 of *EDNRA* on the progression of ADPKD and IGAN. We demonstrated a 4-year later age of ESRD in CC females with ADPKD than in CT heterozygotes. We found no influence of this polymorphism on the progression of ADPKD in males.

IGAN is the most common nephropathy in the world among adult patients undergoing renal biopsy. Mesangial IgA1 in IGAN suffers from reduced O-glycosylation (Hiki et al., 1999; Floege and Feehally, 2000; Mestecky et al., 2002). These abnormal IgA1 are recognized by antibodies, and subsequently circulating imunocomplexes are tightly bound to mesangial cells (Kokubo et al., 2000; Mestecky et al., 2002). Strong predictors of progression of IGAN are clinical factors: hypertension, severe proteinuria, elevated serum creatinine level as well as histological signs: glomerular sclerosis and interstitial fibrosis at presentation. Recently, hypertriglyceridaemia, hyperuricaemia have been significantly associated with poor prognosis (Syrjanen et al., 2000). 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). The influence of the ET-A receptor polymorphism has not been studied yet. We supposed that the potent vasoconstrictor effect of ET-1 mediated by the ET-A receptor leading to impairment of renal perfusion and tubulointerstitial injury could contribute to loss of renal function in IGAN patients. In our study, we excluded the influence of C1363T polymorphism on the progression of IGAN.

ADPKD is a disease with huge inter- and intrafamiliar variability influenced by genetic and environmental factors. Finding new modifiers might have clinical consequences for ADPKD patients in future. Hypertension is a predictor of renal function decline. Distortion of renal vascular tree and cystic compression of surrounding tissue leads to parenchymal ischemia that contributes to the generation of hypertension and progression of the disease. An association between the T allele and higher pulse pressure was established in ECTIM study (Nicaud et al., 1999). Pulse pressure measures the pulsatile component of blood pressure and several studies have shown that it was an independent predictor of cardiovascular risk (Fang et al., 1995; Benetos et al., 1997). On the other hand, a weak association of the C allele of the C1363T variant in exon 8 of *EDNRA* with hypertension has been found (Benjafield et al., 2003). Overexpression of EDNRA in the vascular wall has been reported in hypertensive patients (Hagesawa et al., 1994). The C1363T variant is located in the 3'-UTR region. This region can contain sequences that affect the stability of mRNA. It is thus possible that the C1363T variant could alter the expression of EDNRA and the number of produced receptors. Ninety % of ADPKD pts were hypertensive or used antihypertensive drugs when they reached ESRD. Due to this fact, we were not able to establish the negative influence of hypertension according to different *EDNRA* genotypes.

We demonstrated a 4-year later age of ESRD in CC females with ADPKD than in CT heterozygotes. We found no influence of this polymorphism on the progression of ADPKD in males. The antioxidative mechanism of oestrogens and probably lower sodium uptake could improve the clinical course of most renal diseases in females. A negative effect of the T allele could probably be more expressed in females with more antioxidative mechanisms. The T allele associated with higher pulse pressure could have a negative influence on the progression of renal disease especially in females with lower cardiovascular risk. On the other hand, the results could be influenced by the limited number of homozygous patients. The oestrogen status of the examined females was not known.

To conclude, we excluded the influence of C1363T polymorphism of *EDNRA* on the progression of IGAN and ADPKD in males. The CC genotype in ADPKD females might be a predictor of a promising clinical course.

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