Trakia Journal of Sciences, Vol. 12, Suppl. 1, pp 191-196, 2014

Available online at:
http://www.uni-sz.bg

ISSN 1313-7050 (print)
ISSN 1313-3551 (online)

GENETIC POLYMORPHISM IN ANGIOTENSIN-CONVERTING ENZYME GENE AND CONGENITAL ANOMALIES OF THE KIDNEY AND URINARY TRACT

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ABSTRACT

The angiotensin-converting enzyme (ACE) is one of the most important members of the renin–angiotensin system that has a significant impact in the renal development. An insertion/deletion (I/D) polymorphism in ACE gene was associated with various glomerular diseases. The current study was designed to evaluate whether ACE I/D polymorphism is associated with development of congenital anomalies of the kidney and urinary tract (CAKUT) among Bulgarian children.

Genomic DNA was extracted from 119 CAKUT cases and 103 unaffected children. Genotyping of ACE I/D polymorphism was performed by polymerase chain reaction.

There were no significant differences in genotype and allelic frequency between unaffected children and CAKUT patients as hollow group as well as subdivided according to the kind of malformation. However, in the co-dominant model of inheritance, there was a trend for elevation of DI-genotype in children with renal agenesia and hypo/dysplasia (67%) and obstructive uropathies (62%) compared to controls (48%). Also, II-genotype and I-allele were overrepresented in patients with vesicoureteral reflux than controls (24% vs. 15% for II-genotype and 49% vs. 39% for I-allele), without reaching the statistical significance (p=0.1).

We could assume that I/D polymorphism of ACE gene have no significant impact on the occurrence of CAKUT among Bulgarian children.

Key word: genotype, Alu insertion, rs4340, Bulgarian children

INTRODUCTION

Congenital anomalies of the kidney and urinary tract (CAKUTs) occur in 3–4% , account for the most cases of pediatric end-stage kidney disease and predispose an individual to hypertension and cardiovascular disease throughout life (1). CAKUT comprise a wide range of renal system structural and functional malformations that occur –unilateral renal agenesia,hypoplasia, dysplasia, mulicystic renal dysplasia, hydronephrosis, megaureter, posterior urethral valves-PUV, vesicoureteral reflux-VUR and double or horseshoe kidney (2). Some of CAKUT malformations remain asymptomatic throughout life and others are manifested with recurrent urinary tract infections (UTI), abdominal-pain syndrome, acute renal failure (ARF), chronic kidney disease (CKD) and others. Such extremely variable phenotypic expression suggests the involvement of many different genetic or environmental factors that modify CAKUT manifestation.

The renin–angiotensin system (RAS) plays a major role in controlling blood pressure, fluid, and electrolyte homeostasis and has a significant impact in mammalian renal development (3). The angiotensin-converting enzyme (ACE) is one of the most important members of RAS

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which convert inactive angiotensin I to the active angiotensin II. Human ACE gene is located on the long arm of chromosome 17 (17q23) and comprises 26 exons and 25 introns. A polymorphism due to *Alu* insertion/deletion (I/D) was found in intron 16 of ACE gene. In 1990, Rigat and coworkers (4) have reported a correlation between ACE genotype and serum ACE levels. Genotype-DD was associated with approximately 2-fold higher ACE level than II-genotype. Later studies showed that this polymorphism also influence ACE levels in tissue (5-6). In addition, several studies demonstrated the significance of I/D polymorphism in ACE gene for the development and progression of renal scarring in patients with VUR and kidney hypodysplasia caused by posterior urethral valves (7-8). The DD genotype has been suggested as an independent risk factor for renal parenchymal damage in patients with congenital urologic abnormalities (9-11). However, studies of the influence of I/D polymorphism in ACE gene on Bulgarian CAKUT patients are lacking. Respectively, in our present study, we examined the association of ACE I/D polymorphism with CAKUT development in a cohort of Bulgarian children.

MATERIALS AND METHODS

Genomic DNA was extracted from venous blood from 119 CAKUT cases, 103 unaffected children by standard salting-out technique using proteinase K digestion and stored at -80°C until use.

Genotyping of insertion/deletion polymorphism in ACE (rs4340) was performed by polymerase chain reaction (PCR) amplification of the respective fragments from intron 16 of the ACE gene and size fractionation and visualization by electrophoresis. The amplification products of the D and I alleles were identified by ultraviolet trans-illumination as distinct bands; D allele: 190 bp and I allele: 490 bp. To avoid the possibility of mistyping the ID heterozygotes as DD homozygotes, because the D allele in heterozygous samples is preferentially amplified, all samples determined as DD genotype of first PCR reaction were reamplified with a second primer pair specific for the inserted sequence. The second PCR yields a 335 bp amplicon only in the presence of the I allele, and no product in samples homozygous for D allele. PCR amplification was performed in a GeneAmp PCR System 9700 (Applied Biosystems). PCR products were visualized on 2% agarose gel stained with ethidium bromide (0.5 mg/ml).

Statistical analysis was carried out using SPSS software, version 21 (IBM, Chicago, IL). Allelic and genotype frequencies were compared between groups by chi-square ($\chi 2$) test or Fisher exact test (as appropriate). The strength of the associations between genotypes of I/D polymorphism in ACE gene and CAKUT was presented as odds ratios (OR) with corresponding 95% confidence intervals (CIs) by using logistic regression method. In all cases, the limit of significance was defined as a $p<0.05$.

RESULTS

Genotype distribution of I/D polymorphism in ACE gene was determined among a cohort of 222 Bulgarian children composed of 119 CAKUT patients and 103 healthy children. The patients group was consisted of 68 (57%) girls and 51 boys (43%) on age between 20 days and 17 years. The control group was composed of 56 girls (54%) and 47 (46%) boys on age between 3 months and 18 years. The genotype distribution for the investigated polymorphism was in agreement with Hardy-Weinberg equilibrium in both groups (controls: $\chi 2=0.083$; $p=0.959$ and cases $\chi 2=1.227$; $p=0.541$). Three models of inheritance were tested in the association studies: co-dominant, dominant, and recessive model **(Table 1).**

There was no significant differences in genotype frequency between cases and controls ($\chi 2=2.656$; $p=0.265$). However, in the dominant model (DD frequency vs. DI+II frequency), there was a trend for elevation of genotypes containing *Alu* insertion (II+ID) in patients group compared to controls (73% vs. 63%; $p=0.110$). Respectively, DD genotype was in lower frequency in CAKUT patients than in controls with OR=0.629; 95% CI: 0.342-1.156 without reaching the statistical significance. According to the allelic frequency, I-allele was more frequent among CAKUT patients compared to controls (61% vs. 54%; with OR=1.331; 95%CI: 0.895-1.980), while frequency of D-allele was lower in cases than controls (39% vs. 46%) without statistical significance $p=0.139$.

Table 1. *Genotype distribution of insertion/deletion polymorphism in ACE in CAKUT cases and controls*

Model	Cases (n=119)	Controls (n=103)	p-value	OR	(95% CI)
Codominant model					
DD	32 (27%)	38 (37%)		1.00	
DI	65 (55%)	50 (48%)	0.153	1.544	(0.813-2.934)
II	22 (18%)	15 (15%)	0.176	1.742	(0.721-4.233)
Dominant model					
DD	32 (27%)	38 (37%)		1.00	
DI+II	87 (73%)	65 (63%)	0.110	1.589	(0.865-2.925)
Recessive model					
II	22 (18%)	15 (15%)		1.00	
DI+DD	97 (82%)	88 (85%)	0.434	0.752	(0.345-1.627)

Bearing in mind that CAKUT comprise a wide range of renal system structural and functional malformations, patients group was subdivided according to the kind of anomalies into four subgroups: i) hypoplasia, dysplasia and unilateral renal agenesia; ii) obstructive uropathies (hydronephrosis, megaureter, PUV), iii) vesicoureteral reflux and iv) double or horseshoe kidney. Genotype distribution and allelic frequencies of I/D polymorphism in ACE gene among four subgroups of CAKUT patients and control is presents in **Table 2**. The genotype frequencies of ACE polymorphism were not different among forth subgroups of CAKUT patients and controls analyzed by three models of inheritance ($p>0.05$). Genotype distribution of ACE polymorphism in children with double or horseshoe kidney was the most similar to that in controls ($\chi2=0.98$; $p=0.613$). However, analysis of the subgroups of patients with hypoplasia, dysplasia and unilateral renal agenesia and obstructive uropathies (hydronephrosis, megaureter) revealed that DI genotype tended to be overrepresented (67% and 62%, respectively) than controls (48%). Moreover, DD genotype was in lower frequency in both patients subgroups than in controls (22% and 24% respectively vs. 37%). Additionally, in children with vesicoureteral reflux we found elevated frequency of II genotype (24%) and decreased DD genotype (27%) compared to controls (15% for II genotype and 37% for DD genotype) with OR=2.303; 95% CI: 0.718-7.453 without reaching the statistical significance ($p=0.113$) according to the co-dominant model. Based on above results, we could accept the co-dominant model for the most suitable model for testing the association of ACE polymorphism and CAKUT pathogenesis. Also, there were no significant differences between subgroups of CAKUT patients and healthy controls with respect to allelic frequency.

DISCUSSION

The current study was designed to evaluate whether I/D polymorphism ACE gene is associated with the development and kind of malformation among Bulgarian children with CAKUT. This is the first study investigate the impact of I/D polymorphism for CAKUT development in our population. Our preliminary results demonstrated that the investigated polymorphism is not significantly associated with risk of CAKUT. According to our knowledge, there has been only one report in the literature pointing on this polymorphism in Bulgarian population of patients with different kidney disease, precisely autosome dominant polycystic kidney disease (ADPKD) (12). The authors have found 11% for II-genotype, 61% for DI-genotype and 29% for DD-genotype among 124 Bulgarian patients with ADPKD and no significant association between genotype and severity of the disease. In our cohort of 103 healthy children, the frequency of I/D genotypes were as follow: 15% for II-genotype, 48% of DI-genotype and 37% of DD-genotype. In addition, we found similarities of observed genotype distribution in our current study to other Caucasian type populations (13-16).

***Table 2**. Genotype distribution of insertion/deletion polymorphism in ACE in CAKUT cases and controls*

Cases/controls	Genotype frequency n (%)			Allelic frequency n (%)	
	DD	**DI**	**II**	**D-allele**	**I -allele**
Renal hypo/dysplasia, unilateral renal agenesia (n=27)	6 (22)	18 (67)	3 (11)	30 (56)	24 (44)
Odds Ratio (95% CI) p-value	1.00	2.280 (0.755-7.164) 0.106	0.789 (0.145-4.640) *1*	1.00	1.260 (0.658-2.409) 0.454
obstructive uropathies (n=29)	7 (24)	18 (62)	4 (14)	32 (55)	26 (45)
Odds Ratio (95% CI) p-value	1.00	1.954 (0.679-5.786) 0.171	0.691 (0.148-3.336) *0.719*	1.00	1.280 (0.682-2.4) 0.411
vesicoureteral reflux (n=41)	11 (27)	20 (49)	10 (24)	42 (51)	40 (49)
Odds Ratio (95% CI) p-value	1.00	1.382 (0.548-3.520) 0.454	2.303 (0.718-7.453) 0.113	1.00	1.5 (0.867-2.596) 0.122
double or horseshoe kidney (n=22)	8 (36)	9 (41)	5 (23)	25 (57)	19 (43)
Odds Ratio (95% CI) p-value	1.00	0.855 (0.269-2.724) 0.768	1.583 (0.373-6.601) 0.475	1.00	1.197 (0.588-2.429) 0.593
Controls (n=103)	38 (37)	50 (48)	15 (15)	126 (61)	80 (39)

The presented Odds Ratio with 95% confidence interval (95% CI) were estimated comparing the group of patients with unaffected controls with reference DD-genotype; The probability computed by Fisher exact test is presents in italic

In respect to the significance of the investigated polymorphism for CAKUT pathogenesis, our present data showed a lack of strong association between I/D genotype and CAKUT development. However, there was slightly different genotype distribution among subgroups of CAKUT patients according to the kind of malformation. In patients with hypoplasia, dysplasia and unilateral renal agenesia and obstructive uropathies (hydronephrosis, megaureter, PUV), heterozygous DI-genotype tended to be overrepresented compared to unaffected children, without reaching statistical significance. Also, there was a slight tendency of elevation of II-genotype in children with vesicoureteral reflux compared to unaffected children. Our current results are in principal agreement with other previous studies (17-20) and in contrary to others (7-8). A good explanation of this discrepancy could be a different genetic background of investigated population. As it was previously shown, ACE I/D polymorphism is quite variable among population (16, 20). In addition, we could not exclude a possible impact of ACE I/D polymorphism on renal scarring or VUR severity, as well as on the progression rate of CRF and UTI in children with congenital renal malformations. Our current data demonstrated predominantly a tendency for different genotype distribution among subgroups of CAKUT patients subdivided according to kind of malformation. Further association study according to CAKUT manifestation could reveal more precise the role of investigated polymorphism in CAKUT pathogenesis.

CONCLUSION

Our present results suggest that ACE I/D gene polymorphism have no significant impact on the occurrence of CAKUT among Bulgarian children.

ACKNOWLEDGEMENT

This work was supported by Grant no. 5/2013 from the Fund for Scientific and Mobile project from Faculty of Medicine at the Trakia University, Stara Zagora, Bulgaria.

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