

CLINICAL STUDY

ACE gene polymorphism in Egyptian children with idiopathic nephrotic syndrome

Fahmy ME¹, Fattouh AM², Hegazy RA², Essawi ML³*Pediatric Department, Research Institute of Ophthalmology, Cairo, Egypt. fahmymona80@hotmail.com***Abstract:** *Background:* Idiopathic nephrotic syndrome is a common renal disease in children. ACE gene insertion/deletion (I/D) polymorphism has been studied as a predictor of clinical response to steroid therapy.*Objective:* To investigate the distribution of angiotensin-converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism in children with idiopathic nephrotic syndrome (INS), as well as its relation with patient's clinical response to steroid therapy.*Methods:* The studied subjects included 50 children with INS compared to 20 unrelated healthy children. Each individual genotype was determined using PCR amplification of extract genomic DNA and allele distribution based on size of the PCR fragments.*Results:* Patients with INS had a significantly higher percentage of DD genotype ($p < 0.05$) than the control group. D allele frequency was significantly higher in INS patients than healthy controls.*Conclusion:* Our results showed that INS is associated with a higher incidence of DD genotype, especially in non-SS patients. This data suggested that DD genotype may play a role in the clinical response to steroid. Angiotensin II may be involved in part in the response to steroid treatment in children with INS (Tab. 4, Fig. 1, Ref. 20). Full Text (Free, PDF) www.bmj.sk.**Key words:** angiotensin-converting enzyme gene polymorphism, nephrotic syndrome, steroid sensitive, non-steroid sensitive.**Abbreviations:** ACE – angiotensin-converting enzyme, I/D – insertion/deletion, INS – idiopathic nephrotic syndrome, SS – steroid sensitive, Non-SS – non-steroid sensitive, SD – steroid dependent, SR – steroid resistance, MCNS – minimal change nephrotic syndrome, FSGS – focal segmental glomerulosclerosis.

Idiopathic nephrotic syndrome (INS) is a common renal disease in children. It is characterized by edema, massive proteinuria, hypoalbuminemia and hyperlipidemia. It is mainly seen in association with minimal change nephrotic syndrome (MCNS), which is usually associated with good prognosis. However, other pathologic entities may be present including mesangioproliferative glomerulonephritis and focal segmental glomerulosclerosis (FSGS) (1). In the absence of known etiology and pathogenesis, the different histological features of MCNS and FSGS are often considered to be different stages or variants in severity of a single disease; INS (2).

Eighty percent of the patients with MCNS respond positively to a standard course of prednisolone treatment, resulting in complete remission, while 40–50 % of these patients relapsed frequently. On the other hand, patients with FSGS respond poorly to steroid therapy and were more likely to progress to end stage renal failure compared to MCNS patients (3).

Angiotensin converting enzyme (ACE) is the key enzyme that converts inactive angiotensin I into a vasoactive and aldosterone-stimulating peptide angiotensin II. There are two forms of ACE in humans, encoded by a single gene located on chromosome 17 at q23; it is 21 kb in length and contains 26 exons and 25 introns. Polymorphism of the angiotensin I converting enzyme gene was identified in 1990 (4). The ACE gene carries insertion (I) and deletion (D) polymorphism, and the DD-genotype is reportedly related to an increase in the ACE protein expression (5). ACE I/D polymorphism with a DD genotype is at risk of developing renal disease (6).

However, ACE gene I/D polymorphism has been evaluated in nephrotic syndrome, especially in small groups of FSGS patients, with conflicting results on histology, treatment responses, and disease prognosis (7, 8).

Aim

The aim of this study is to investigate the distribution of ACE gene I/D polymorphism among Egyptian children with INS, as well as its relationship with patients' clinical responses to steroid therapy.

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Patients and methods

Fifty children diagnosed with INS were included in the study. These patients had been followed up at the Nephrology Clinic in Children Hospital, Cairo University. The control group consisted of 20 unrelated healthy children from 4 to 12 years of age (8.0 ± 2.6), without hypertension, renal or cardiac diseases or family history of renal diseases.

The medical record of each patient was reviewed retrospectively, and age at diagnosis, gender, consanguinity, family history for renal disease, levels of proteinuria, serum albumin, cholesterol, creatinine, complement 3 (C3), hypertension, hematuria, response to treatments, relapses with time and kidney biopsies were noted. A renal biopsy was performed in 14 patients and the histological findings were: FSGS in 10 and MCNS in 4 patients. The patients were divided into 2 groups according to their response to the steroid treatment: SS (both frequent and infrequent relapses) and non-SS, which were subdivided into SD and SR.

Steroid response was defined as the disappearance of proteinuria for at least 3 consecutive days. Frequent relapse was defined as two relapses within six months after cessation of first steroid treatment or three or more relapses in any 12 month period in an initially steroid responsive patient. Steroid dependency was defined as two consecutive relapses under steroid treatment or within 14 days after steroid withdrawal. Steroid resistance was accepted as no achievement of remission despite prednisolone treatment for four weeks.

The standard steroid therapy regimen consisted of prednisone, 60 mg/m²/day, with maximum of 80 mg/day in divided doses for 4 weeks, followed by 40 mg/m²/day with a maximum of 60 mg/day in divided doses on alternate days for four weeks, which tapered slowly over the next eight weeks. Cyclosporine A was used at a dose of 3–5 mg/kg/day for at least six months and cyclophosphamide was used at a dose of 2.5–3 mg/kg/day for 10–12 weeks.

Molecular studies for ACE genotype

Genomic DNA was extracted from peripheral blood lymphocytes of patients and controls by standard salting out methods (9). PCR amplification was carried out according to the method of (10) with some modification. The sequences of the forward and reverse primers were; sense oligo: 5' CTGGAGAC CAC-TCCCATCCTTTCT 3' and anti-sense oligo: 5'GATGTG GCCATCACATTCGTCAGAT 3' in a final volume 30 µl containing 1x buffer (FINNZYMES) containing 1.5 mM MgCl₂, 0.2 mM dNTP and 5 p moles of each primer and 1 unit Taq polymerase. PCR conditions were 94 °C initial denaturation for 10 min and then 30 cycles with denaturation for 1 min, annealing at 59 °C for 1 min and extension at 72 °C for 2 min using Biometra T Gradient thermal cycler. PCR products were analyzed on 2 % agarose gel. A PCR product of 490 bp indicated homozygous genotype for insertion (II), 190 bp indicate homozygous genotype for deletion (DD) and presence of both indicate heterozygosity (ID) (9, 10).

Statistical analysis

All continuous data were expressed as mean±SD. Commercial statistics software (SPSS 10.0) was used for data analysis. The chi-square test and Fisher's exact test (when available) was used to compare groups. $p < 0.05$ was considered statistically significant.

Results

Fifty children diagnosed with INS compared to 20 healthy children as a control group were recruited in this study. Among the 50 subjects with INS 36 (72 %) were boys and 14 (28 %) were girls. Nineteen children were SS while 31 children were non-SS (16 SD & 15 SR). The demographic, clinical and laboratory characteristic of the patients are shown in Table 1.

For the entire group of children with INS, the frequencies of II, ID and DD genotypes of the ACE gene were 12 %, 46 % and 42 % respectively. On the other hand, the frequencies for II, ID and DD genotypes in the control group were 50 %, 40 % and 10 % respectively. As shown in Table 2, the total patients group had a significantly higher percentage of DD genotype ($p < 0.05$) compared to the control group, which was attributed mainly to the non-steroid sensitive group. Also the frequency of II genotype was significantly different between the nephrotic group patients and healthy control ($p < 0.01$).

Tab. 1. The demographic, clinical and laboratory characteristics of the steroid sensitive and non-steroid sensitive nephrotic syndrome.

	SS n=19	non-SS n=31	p value
Onset age (years)	4.0±1.71	5.6±3.1	<0.05
Gender M/F	14/5	22/9	NS
Family history	0/19	3/31	NS
Consanguinity	0/19	8/31	<0.05
White blood cell count, 10 ³ /µl	8.6±1.33	8.27±2.04	NS
Creatinine (mg/dl)	0.48±0.13	0.64±0.27	<0.01
Albumin (g/dl)	3.36±0.84	2.98±1.03	NS
Cholesterol (mg/dl)	235.7±80.91	243.42±80.25	NS
Systolic blood pressure (mmHg)	91.58±20.88	96.45±24.15	NS
Diastolic blood pressure (mmHg)	61.58±15	64.52±14.10	NS

SS – steroid sensitive, non-SS – non-steroid sensitive, NS – non-significant

Tab. 2. Genotypes frequencies of ACE I/D polymorphism in children with nephrotic syndrome and control groups.

Genotypes	Patients			Control n=20
	SS n=19	non-SS n=31	Total n=50	
II	3(15.8%)	3(9.7%)	6(12%)	10(50%) ^a
ID	9(47.4%)	14(45.2%)	23(46%)	8(40%)
DD	7(36.8%)	14(45.2%)	21(42%)	2(10%) ^b

SS – steroid sensitive, non-SS – non-steroid sensitive, ^a – $p < 0.01$ total patients vs control, ^b – $p < 0.05$ total patients vs control

Tab. 3. ACE Gene Polymorphism Presentation (DD genotype) in Nephrotic Syndrome Patients and Control Group.

	Control	Nephrotic syndrome (%)				
	n=20	total n=50	SS n=19	non-SS n=31	SD n=16	SR n=15
DD	2 (10%)	21 (42%)	7 (36.8%)	14 (45.17%) ^a	8 (50%) ^a	6 (40%) ^a
non-DD	18 (90%)	29 (58%)	12 (63.2%)	17 (54.83%)	8 (50%)	9 (60%)

SS – steroid sensitive, non-SS – non-steroid sensitive, SD – steroid dependent, SR – steroid resistant, non-DD (ID, II),
^a– p<0.05 total patients vs control, non-steroid sensitive vs control, steroid dependent vs control, steroid resistant vs control

Tab. 4. ACE Gene Polymorphism Presentation (D-allele) in Nephrotic Syndrome Patients and Control Group.

	Control n=20	Total n=50	SS n=19	non-SS n=31	SD n=16	SR n=15
D-allele+	10 (50%)	44 (88%) ^a	16 (84.2%) ^b	28 (90.3%) ^a	14 (87.5)	14(93.3%) ^c
D-allele-	10 (50%)	6 (12%)	3 (15.8%)	3 (9.7%)	2 (12.5%)	1 (6.7%)

SS – steroid sensitive, non-SS – non-steroid sensitive, SD – steroid dependent, SR – steroid resistant, D-allele + (DD, ID); D-allele – (II), ^a– p<0.001 control vs total patients, control vs Non-SS, ^b– p< 0.05 control vs SS, ^c– p<0.01 control vs SR

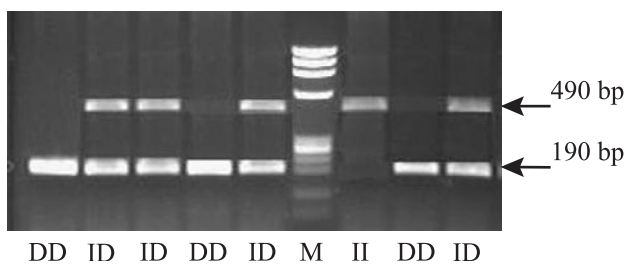


Fig. 1. Insertion/Deletion (I/D) polymorphism of ACE gene. The amplification product was electrophoresed on 3 % agarose gel and visualized by ethidium bromide staining. Lanes 1, 4 and 8: DD-genotype. Lanes 2, 3, 5 and 9: ID-genotype. Lane 7: II-genotype. Lane 6: marker of Phi X174 DNA-Hae III digest.

Table 3 demonstrates the ACE gene polymorphism presentation (DD) among nephrotic group patients and healthy control. There was a significant difference between non-SS, SD & SR versus the control group (p<0.05). There was no significant difference between the SS versus the control group.

Table 4 illustrated the presence of the D-allele, among the studied subjects and control group. There was a significant difference between the control group versus SS, SD & SR. There was no significant difference between SS versus SD &SR and SD versus SR.

Figure 1 presents the electrophoresis patterns of PCR products from the three genotypes (DD, ID, II).

Discussion

Idiopathic nephrotic syndrome represents a heterogeneous group of glomerular disorders, and patients diagnosed with it

are generally divided into SS and SR. Genetic factors may affect the renal disease phenotype in several ways: susceptibility for acquiring disease, variations in clinical or histological findings, treatment responses and disease progression (11). There is significant evidence showing that the renin-angiotensin system is involved in the pathogenesis of progressive renal disorders (12). Plasma ACE levels depend on the genotype of the ACE gene Alu-insertion/deletion polymorphism (13). ACE gene locus is on chromosome 17q32. ACE converts angiotensin I to angiotensin II in the renin-angiotensin-aldosterone system. A higher concentration of angiotensin II in DD genotype is thought to cause progressive renal disease. Acting through various growth factors, angiotensin II has been postulated to cause structural changes in renal system and alteration of renal hemodynamics (12–14). ACE gene I/D polymorphism has been largely studied in several non-renal and renal diseases such as diabetic nephropathy, myocardial infarction, and hypertension, with conflicting results. However ACE gene I/D polymorphism has been studied in a small and heterogenous group of nephrotic syndrome patients (11).

In the current study, there was a significant difference in the distribution of ACE gene polymorphism between the nephrotic syndrome patients and the control. The frequency of DD genotype was much higher in the total patient group compared to that in control, while II frequency was significantly lower in nephrotic syndrome compared to control group. The difference between nephrotic syndrome patients and controls was attributed mainly to the non-SS patients. This high frequency of DD in nephrotic syndrome patients is similar to the studies by Serdaroglu et al and Tsai et al where DD frequency was 48 % and 52 % respectively (11–15). In addition, a similar finding was reported in INS children in a Kuwaiti study (16) and in children with steroid sensitive nephrotic syndrome in a Turkish study (8).

In our study, the presence of DD genotype was significantly higher in non-SS group compared to the control group & SS group (45 % and 36 % respectively) but the latter difference did not reach the statistical significance. This finding suggests that the DD genotype is not only a risk factor for the occurrence of nephrotic syndrome but also for steroid dependence or steroid resistance in INS patients. Frishberg et al reported that they achieved remission in two thirds of children with the II genotype, but in only 24 % of children with the DD and ID genotypes (17). They concluded that ACE genotype interfered with steroid response in the patients with FSGS. Lee et al reported a steroid response in 1/8 patients with the DD genotype and 5/18 patients with the other genotypes (18). However, the result of our study is in contrast to Hori et al who reported identical ACE gene genotype distributions in SS and SR (19). Our findings disagree with that of Sasse et al, who demonstrated that the ACE gene polymorphism is irrelevant to clinical outcome, steroid responsiveness or morphology in Swiss children with INS (20).

Whether the presence of the D allele is a risk factor for the development of end-stage renal disease is a matter of concern. Lee et al reported that 42 % of those with the DD genotype, 28 % of those with the ID genotype and 25 % of those with the II genotype were associated with ESRD (18). Frishberg et al defined renal functional failure as a doubling of the serum creatinine level, and reported that this end-point had been achieved in 50 % of those with the DD or ID genotypes and in none of those with the II genotype (17). Luther et al followed the progression of nephrotic syndrome with reciprocal serum creatinine and reported rapid progression in 2/21 patients with the II genotype and 31/50 patients with the ID or DD genotypes (7). In our study, we did not perform a longitudinal study to follow the serum creatinine of patients, however, patients with non-SS nephrotic syndrome (who have a higher D-frequency) showed a significantly higher serum creatinine levels at the time of the study.

In conclusion, our results showed that INS is associated with a higher incidence of DD genotype, especially in non-SS patients. These data suggest that DD genotype may play a role in the clinical response to steroid. Angiotensin II may be involved in part in the response to steroid treatment in children with INS.

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