Deletion polymorphism of the angiotensin converting enzyme gene predicts persistent proteinuria in Henoch-Schönlein purpura nephritis

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Abstract

Objective—To study the influence of deletion/insertion polymorphism in the 16th intron of the angiotensin converting enzyme (ACE) gene on clinical manifestations of Henoch-Schönlein purpura nephritis.

Study design—Cross sectional study. ACE gene polymorphism was determined in patients (4–15 years old at onset) with Henoch-Schönlein purpura nephritis (n = 40) and compared with that in patients with IgA nephropathy (n = 79).

Main outcome measures—ACE genotypes, systemic blood pressures, urine protein excretion rate, haematuria, creatinine clearance, serum ACE activities.

Results—The initial clinical manifestations of both Henoch-Schönlein purpura nephritis and IgA nephropathy were no different among homozygotes for insertion (II) and deletion (DD), and heterozygotes (ID) for the ACE gene. In patients with Henoch-Schönlein purpura nephritis, the incidence of moderate to heavy proteinuria at four and eight years after onset was more than five times higher in the DD genotype than in the II or ID genotypes. No such trend was seen in patients with IgA nephropathy. The number of patients with Henoch-Schönlein purpura nephritis in whom proteinuria resolved at four and eight years after onset was significantly lower in the DD genotype compared with the II genotype, whereas no differences were detected among the three different genotypes in patients with IgA nephropathy. Plasma ACE activities in patients with the DD genotype were significantly higher than in those with non-DD genotypes.

Conclusions—The ACE DD genotype predicts persistent proteinuria in Henoch-Schönlein purpura nephritis. The proteinuria might be related to a defective angiotensin system which is genetically determined by the D/I polymorphism.

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Keywords: chronic glomerulonephritis; IgA nephropathy; angiotensin converting enzyme activity; Henoch-Schönlein purpura nephritis

Henoch-Schönlein purpura nephritis is a common cause of chronic glomerulonephritis in

children.¹ Although most patients follow a benign clinical course, some patients exhibit persistent urinary abnormalities and progress to terminal renal failure.²⁻⁵ The mechanisms of the renal involvement in Henoch-Schönlein purpura and the chronicity of the renal abnormality have not been elucidated.

Renal histological changes in Henoch-Schönlein purpura nephritis share some similarities with those in IgA nephropathy, another common chronic glomerulonephritis in children.⁶⁻⁹ Recent studies in adults demonstrated a genetic association between deletion (D) and insertion (I) polymorphisms of the angiotensin converting enzyme (ACE) gene and chronic progression of IgA nephropathy.¹⁰⁻¹⁴ A high incidence of deletion polymorphism in the 16th intron of the gene has been seen in adult patients with IgA nephropathy who progressed to end stage renal failure. The same polymorphism is thought to be associated with the progression of other renal (for example, diabetic nephropathy) and non-renal (for example, left ventricular hypertrophy) diseases.¹⁵⁻²¹ Although the exact mechanism of the gene polymorphism related disease progression remains unclear, a rise in ACE activity, and hence higher angiotensin II concentrations, has been implicated as a physiological consequence of the deletion.^{14 22-28}

Both experimental and clinical studies suggest that angiotensin II modulates glomerular permeability and selectivity.^{11 14 15} Therefore, it is reasonable to assume that high local angiotensin II concentrations as a result of deletion polymorphism of the ACE gene is associated with proteinuria in chronic renal diseases. In our study, we evaluated the association of the ACE D/I gene polymorphism with clinical manifestations, particularly proteinuria, in patients with Henoch-Schönlein purpura nephritis. We also examined clinical manifestations according to ACE genotypes in patients with IgA nephropathy as a reference. The onset of both diseases was well defined in the children studied; in general, Henoch-Schönlein purpura nephritis was recognised early by non-renal symptoms, and IgA nephropathy was detected early by the Japanese mass urinary screening system, which is performed every six to 12 months in all school children.^{29 30} This enabled comparisons of clinical manifestations at given periods of the disease among the three ACE genotypes in patients with Henoch-Schönlein purpura nephritis and IgA nephropathy.

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Methods

PATIENTS

A total of 186 patients with either Henoch-Schönlein purpura nephritis ($n = 59$) or IgA nephropathy ($n = 127$) attended or admitted to the department of pediatric nephrology, Tokyo Women's Medical College between 1 November 1995 and 31 October 1996 were considered for our study. Forty patients with Henoch-Schönlein purpura nephritis and 79 patients with IgA nephropathy fulfilled the entrance criteria of our study; consent was obtained from all patients or guardians. The entrance criteria required the patient to be: (1) between 4 and 15 years of age at onset of the disease; (2) followed for at least two years after onset; and (3) monitored regularly by blood pressure measurement, determination of 24 hour urine protein excretion rate, and examination of red blood cell sedimentation in first voiding urine sample. Reasons for exclusion from the study for not meeting the entrance criteria were: (1) the age of onset was < 4 or > 15 years ($n = 18$); (2) the length of follow up was less than two years ($n = 34$); or (3) the patients' medical records were incomplete because they had been referred from other institutions ($n = 17$). (There was more than one reason in some patients.) All patients with IgA nephropathy were biopsied to confirm the diagnosis. The diagnosis of Henoch-Schönlein purpura nephritis was based on clinical symptoms (cutaneous, gastrointestinal, and joint symptoms associated with haematuria/proteinuria). Thirty four patients with Henoch-Schönlein purpura nephritis underwent renal biopsy. None of the patients were treated with ACE inhibitors during or before the study. The study protocol had been approved by the institutional ethics committee for clinical investigation.

The profile of patients at onset of disease is given in table 1. The average age at onset was slightly but significantly higher in patients with IgA nephropathy ($p < 0.05$). All patients in the study presented at the renal clinic at a relatively early stage (less than one year after onset) of the disease because of the regular mass screening for urinary abnormality performed in all Japanese school children and because of the typical clinical symptoms of Henoch-Schönlein purpura nephritis. Thirty six of the patients with Henoch-Schönlein purpura nephritis and 67 of the patients with IgA nephropathy were followed for four years from onset, and 20 patients with Henoch-Schönlein

purpura nephritis and 31 patients with IgA nephropathy were followed for eight years. These subsets of patients were evaluated further to compare the time related changes of clinical presentations.

EXTRACTION OF GENOMIC DNA AND

DETERMINATION OF THE ACE GENOTYPE BY PCR

Genomic DNA was extracted from peripheral venous blood collected for routine blood tests. The genomic DNA was purified using a DNA isolation kit (Invitrogen, San Diego, California, USA), suspended in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0, and DNA concentrations were measured spectrophotometrically at 260 nm.

ACE genotypes were determined using the polymerase chain reaction (PCR) technique as reported previously.³¹ A 100 μ l reaction mixture contained 1 μ g genomic DNA, 2 μ M primers, 200 μ M each dATP, dGTP, dCTP, and dTTP, 1.5 mM $MgCl_2$, 2.5 U Taq DNA polymerase (Takara, Osaka, Japan), 50 mM KCl, and 10 mM Tris/HCl, pH 8.3. An optimised primer pair (sense: 5'-GCCCTGCAGGTGTCTGCAGGTGTCT-3', and antisense: 5'-GGATGGCTCTCCCCGCCTTGTCTC-3') was used to amplify the D and I alleles, resulting in 319 bp and 597 bp amplicons, respectively.¹⁵ The thermocycling procedure (DNA Thermal Cycler 480, Perkin Elmer, Norwalk, Connecticut, USA) consisted of one cycle at 94°C for two minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 45 seconds, and extension at 72°C for two minutes; with a final extension at 72°C for seven minutes. The PCR product was visualised with ethidium bromide staining after electrophoresis on 2% agarose gels.

Because the D allele in heterozygous samples is amplified preferentially, the ID genotype can be mistyped as the DD genotype.¹⁵ Thus, each sample found to have the DD genotype by the original primers was subjected to a repeated PCR amplification and to another PCR amplification using an insertion specific primer pair (sense: 5'-TGGGACCACAGCGCCCGCCACTAC-3' and antisense: 5'-TCGCCAGCCCTCCCATGCCATAA-3') under identical PCR conditions except for an annealing temperature of 67°C. The reaction yielded a 335 bp amplicon in the presence of an I allele, and no products in samples homozygous for DD.

MEASUREMENT OF PLASMA ACE ACTIVITY

Plasma ACE activity was determined in some patients with normal creatinine clearance (> 80 ml/minute/1.73 m^2). Blood samples were collected after at least 30 minutes of rest. Plasma was separated immediately and stored at -80°C until assayed for ACE activity. The ACE activity was determined by colorimetric measurement of the quinoneimine dye produced from the substrate p-hydroxyhippuryl-L-histidyl-L-leucine by the action of ACE as reported previously.³²

Table 1 Profile of patients at onset

	HSPN	IgA nephropathy
Number of patients	40	79
Male/female	18/22	41/38
Age at onset (years)		
Mean (SD)	8.3 (3.0)	11.7 (2.7)*
Range	4-15	4-15
Mode of initial presentation		
Screening†	0	73
Nephritic/nephrotic symptoms	40	5

* $p < 0.05$ v HSPN.

†Urinary abnormality detected at mass screening performed every 6 or 12 months in all Japanese school children. HSPN, Henoch-Schönlein purpura nephritis.

Table 2 Comparison of clinical presentations between ACE DD and non-DD genotypes in patients with Henoch-Schönlein purpura nephritis (HSPN) and IgA nephropathy

	HSPN (n = 40)			IgA nephropathy (n = 79)		
	II	ID	DD	II	ID	DD
Number of patients	17	15	8	33	35	11
Follow up (years)	7.8 (3.5)	8.6 (5.4)	7.8 (3.0)	7.0 (3.2)	6.9 (3.8)	9.1 (3.6)
Haematuria (number of patients with > 1+ by dipsticks)						
Initial	17	15	8	33	35	11
Final (number/total*)	4/17	7/13	4/7	24/33	27/34	6/11
Urine protein excretion rate (mg/m ² /day)						
Initial	1784 (2320)	1790 (1588)	1911 (1183)	563 (549)	526 (457)	723 (747)
Final	129 (332)	311 (537)	520 (436)	300 (372)	292 (524)	190 (273)
Blood pressure (mm Hg)						
Initial systolic	104 (12)	104 (6)	112 (10)	110 (11)	112 (14)	113 (16)
Initial diastolic	54 (11)	55 (8)	58 (8)	57 (12)	59 (9)	65 (8)
Final systolic	107 (15)	104 (10)	105 (9)	112 (10)	112 (10)	113 (16)
Final diastolic	53 (9)	56 (13)	52 (5)	63 (9)	60 (10)	64 (12)
Creatinine clearance (ml/min/1.73 m ²)						
Final	105 (18)	86 (40)	93 (41)	102 (22)	110 (18)	109 (10)
Degree of glomerular crescent at the first biopsy (%)†	25.3 (17.3)	29.4 (18.8)	26.2 (30.9)	ND	ND	ND
Treatment‡						
Glucocorticoids	6	5	3	14	14	3
Anticoagulants	9	12	7	28	32	10
Plasmapheresis	7	7	6	2	3	1

Values in parentheses are SD.

Kruskal-Wallis test showed significant difference of final urine protein excretion rate among HSPN. No significant differences were observed among other clinical parameters in both HSPN and IgA nephropathy patients.

*Patients progressed to chronic renal failure (creatinine clearance <50 ml/min/1.73 m²) were excluded.

†Percentage of glomeruli with crescent in a section of biopsy specimen.

‡Methods overlapped in some patients.

ND, not determined.

STATISTICS

Data are expressed as means and standard deviation (SD) unless otherwise specified. One way ANOVA followed by Bonferroni/Dunn corrections were performed for continuous data and the Kruskal-Wallis rank test was used where applicable.³³ The χ^2 test was used to compare genotype frequencies for various clinical parameters including incidence of proteinuria and treatments.³⁴ Significance was defined as $p < 0.05$.

Results

CLINICAL PRESENTATIONS OF HENOC-SCHÖNLEIN PURPURA NEPHRITIS AND IgA NEPHROPATHY IN DIFFERENT ACE GENOTYPES

Table 2 summarises the clinical presentations of patients with Henoch-Schönlein purpura nephritis and IgA nephropathy according to ACE genotype. The allele frequency of deletion was 0.388 for patients with Henoch-Schönlein purpura nephritis and 0.361 for patients with IgA nephropathy. The frequency of the DD genotype in patients with Henoch-Schönlein purpura nephritis was significantly higher than that seen patients with IgA nephropathy and the Japanese population as a whole (12% in both adults and children).^{35, 36} The age of onset (not shown in the table) and average length of follow up were not different among the three genotypes. Clinical symptoms (haematuria and blood pressure) and creatinine clearance at onset were not significantly different among the three genotypes, both for patients with Henoch-Schönlein purpura nephritis and those with IgA nephropathy. The degrees of glomerular crescent formation in the initial biopsies from patients with Henoch-Schönlein purpura nephritis were not significantly different among three genotypes. There were no differences in the distribution of treatment modalities. In patients with Henoch-

Schönlein purpura nephritis, chronic renal failure (creatinine clearance < 50 ml/minute/1.73 m²) was seen in one patient with the DD genotype and two patients with the ID genotype; it was also seen in one patient with IgA nephropathy and the ID genotype. At the final follow up, patients with Henoch-Schönlein purpura nephritis and the DD genotype but not those with IgA nephropathy had a higher degree of proteinuria. The data showed large standard deviations, which might be affected by the differences in the lengths of follow up; therefore, further analysis was performed in patients who had been followed for identical lengths of time.

Table 3 compares the incidence of proteinuria at onset, four years, and eight years after onset in patients with Henoch-Schönlein purpura nephritis and IgA nephropathy with different ACE genotypes. When patients with moderate to severe proteinuria were compared, 28 of 40 and 35 of 79 patients with Henoch-Schönlein purpura nephritis and IgA nephropathy, respectively, had urine protein excretion rate greater than 500 mg/m²/day at onset. No significant differences in the incidence of proteinuria at onset were seen among the three genotypes in the two sets of patients. In patients with Henoch-Schönlein purpura nephritis, the incidence of moderate to heavy proteinuria in the DD genotype remained high at four and eight years after onset (four of seven and three of four patients, respectively) but the incidence was significantly lower in non-DD genotypes (two of 29 and two of 16, respectively). In IgA nephropathy patients, the DD genotype was not associated with a higher incidence of moderate to severe proteinuria during the follow up period. When the incidence of patients with no proteinuria (urine protein excretion rate less than 100 mg/m²/day) at onset were compared, there were no differences

Table 3 Incidence of proteinuria in patients with Henoch-Schönlein purpura nephritis (HSPN) and IgA nephropathy with different ACE genotypes

	HSPN			IgA nephropathy		
	II	ID	DD	II	ID	DD
<i>Patients with moderate to heavy proteinuria (≥ 500 mg/m²/day)</i>						
At onset						
Number/total number (%)	9/17 (55)	12/15 (80)	7/8 (88)	15/33 (45)	15/35 (43)	5/11 (45)
p values v DD (χ^2 test)	0.073	0.651	NA	1.000	0.880	NA
4 years after onset						
Number/total number (%)	1/17 (6)	1/12 (8)	4/7 (57)	6/26 (23)	6/32 (19)	4/9 (44)
p values v DD (χ^2 test)	0.005	0.019	NA	0.221	0.113	NA
8 years after onset						
Number/total number (%)	1/9 (11)	1/7 (14)	3/4 (75)	4/12 (33)	3/12 (25)	2/7 (29)
p values v DD (χ^2 test)	0.021	0.044	NA	0.829	0.864	NA
<i>Patients with no proteinuria (< 100 mg/m²/day)</i>						
At onset						
Number/total number (%)	5/17 (35)	3/15 (20)	0/8 (0)	6/33 (18)	4/35 (11)	1/11 (0.1)
p values v DD (χ^2 test)	0.086	0.175	NA	0.784	0.400	0.784
4 years after onset						
Number/total number (%)	13/17 (76)	7/12 (58)	1/7 (14)	8/26 (31)	13/32 (41)	2/9 (22)
p values v DD (χ^2 test)	0.005	0.060	NA	0.625	0.311	NA
8 years after onset						
Number/total number (%)	7/9 (78)	4/7 (57)	0/4 (0)	5/12 (42)	6/12 (50)	3/7 (43)
p values v DD (χ^2 test)	0.009	0.058	NA	0.960	0.764	NA
<i>Mean (SD) urine protein excretion</i>						
Rate (mg/m ² /day)						
4 years after onset	129 (289)	230 (349)	743 (735)	328 (300)	251 (346)	403 (346)
p values v DD (ANOVA)	0.003	0.050	NA	0.426	0.507	NA
8 years after onset	108 (265)	168 (228)	696 (483)	551 (799)	162 (209)	263 (283)
p values v DD (ANOVA)	0.016	0.013	NA	0.216	0.163	NA

Data at onset were derived from all patients enrolled, and those at 4 and 8 years after onset were derived from patients who were followed for the respective periods.
NA, not applicable.

among the three genotypes of ACE in both patients with Henoch-Schönlein purpura nephritis and those with IgA nephropathy. In patients with Henoch-Schönlein purpura nephritis, the proportion of those who were proteinuria free increased during the follow up period in the II and ID genotypes, while the proportion in the DD genotype remained low. In the non-DD genotypes, 20 of 29 and 11 of 16 patients were proteinuria free at four and eight years after onset, respectively, whereas in the DD genotype, only one of seven and none of four were proteinuria free at four and eight years after onset, respectively ($p < 0.05$). In patients with IgA nephropathy, we observed no statistical differences in the proportion of proteinuria free patients among the three genotypes throughout the eight years of follow up. The average urine protein excretion rates at follow up are also presented in table 3 and were similar among the three genotypes. In patients with Henoch-Schönlein purpura nephritis, the urine protein excretion rates in the DD genotype remained significantly higher throughout the follow up period compared with the II or ID genotype. No such genotypic differences were observed in IgA nephropathy. Other clinical manifestations, including systolic and diastolic blood pressures, degree of haematuria, and creatinine clearance, were no different among the three genotypes for both patients with Henoch-Schönlein purpura nephritis and those with IgA nephropathy.

PLASMA ACE ACTIVITIES

Plasma ACE activity was high in children with the DD genotype (fig 1), and there was a significant difference in ACE activity between the DD and II genotypes. The average ACE activity in the ID genotype was intermediate between those for the DD and II genotypes.

Figure 1 Plasma ACE activity in patients with Henoch-Schönlein purpura nephritis and IgA nephropathy. The plasma ACE activities were determined in patients with a normal glomerular filtration rate (creatinine clearance > 80 ml/minute/1.73 m²) and not treated with ACE inhibitors. Open squares and closed circles represent Henoch-Schönlein purpura nephritis and IgA nephropathy, respectively. *Significant difference ($p < 0.05$).

Although there were outlying values in one each of the DD and the ID genotypes, we could not identify a reason for raised ACE activity in these patients.

Discussion

Our study examined the association between ACE polymorphism and clinical presentations of childhood Henoch-Schönlein purpura nephritis. Recent studies showed that D/I polymorphism of the ACE gene is associated with progression of IgA nephropathy and other renal diseases.^{10-14 16 17} Because Henoch-Schönlein purpura nephritis shares some histological similarities with IgA nephropathy, which is another common childhood chronic glomerulonephritis, we compared patients with the two conditions. A characteristic of our study was disease onset (initial appearance of urinary abnormalities) and it was well defined

in both Henoch-Schönlein purpura nephritis and IgA nephropathy. In contrast to adults with chronic glomerulonephritis, in whom onset of urinary abnormalities cannot be defined, all our patients presented within one year of onset because of the local mass screening system. This enabled us to evaluate time related changes in clinical manifestations in association with the ACE genotype of each patient.

In this study, a fifth of the patients with Henoch-Schönlein purpura nephritis had the DD genotype of the ACE gene. This frequency was higher than that seen in the general Japanese population,^{35, 36} and was also higher than that seen in our patients with IgA nephropathy. However, this might not be the true frequency in Henoch-Schönlein purpura because we studied only nephrotic/nephritic patients. Further studies are required to investigate the genotypes in all patients with Henoch-Schönlein purpura, to determine if the DD genotype is associated with nephritis.

The important finding of our study was the high incidence of persistent proteinuria in patients with Henoch-Schönlein purpura nephritis and the DD genotype. The incidence of proteinuria (urine protein excretion rate higher than 500 mg/m²/day) at four to eight years after onset in patients with Henoch-Schönlein purpura nephritis and the DD genotype was more than five times higher than that in patients with the II genotype. The incidence in the ID genotype was intermediate between those in the DD and II genotypes. Our results also showed that proteinuria resolved less frequently in patients with the DD genotype than in those with non-DD genotypes. These findings indicate that the presence of the D allele is associated with persistence, and to some extent, the severity of proteinuria in children with Henoch-Schönlein purpura nephritis.

In contrast to Henoch-Schönlein purpura nephritis, we demonstrated no association between the ACE D/I polymorphism and proteinuria in patients with IgA nephropathy. Our results in IgA nephropathy were not consistent with those of previous studies in adults, which reported a relation between ACE D/I polymorphism and severity of proteinuria.^{10-12, 14} We cannot define the exact reasons for the discrepancy between Henoch-Schönlein purpura nephritis and IgA nephropathy in our study as well as that between childhood and adult IgA nephropathy. Compared with previous studies in adults, we studied IgA nephropathy at an earlier stage of the disease (from onset to an average of approximately eight years after onset). Because the physiological effects of gene polymorphism appear to be present already at onset, other factors might mask the genetic influence of the deletion polymorphism in young patients with IgA nephropathy. In adult IgA nephropathy and diabetic nephropathy, the DD genotype predicts a greater risk of progressive renal failure.^{10-14, 16, 17} A decline in renal function takes place over 15 to 20 years in these chronic glomerular diseases.^{1-12, 37} Because most of our patients were followed for less than 10 years, conclusive data for the genomic influence on the progression of

glomerulopathy can only be obtained in the future. Our present results suggest that the effect of ACE gene polymorphism must be evaluated not only by the specific disease but also by the stage of the disease.

The modalities of treatment might influence the outcome of both Henoch-Schönlein purpura nephritis and IgA nephropathy. Although none of the patients in our study received ACE inhibitors, they were treated with other regimens, including short term glucocorticoids (initial dose of 1 mg/kg/day, then tapered to withdrawal in about one year), anticoagulants (short term intravenous heparin followed by oral warfarin and/or dipyridamole), or plasmapheresis (three times a week for two to three weeks followed by a less frequent repeat for a total of 12 to 24 times) (table 2). Chi-square analysis revealed that none of the modalities were related to the outcome of proteinuria at four and eight years after onset (data not shown). The modalities of treatment were distributed equally among the three genotypes (table 2). We cannot determine from our data whether treatment prevented end stage renal disease because the number who progressed to end stage renal disease was too small (four among 119 patients) for meaningful analysis. Thus, more studies are required to analyse the effect of treatment on clinical outcome in different genotypes, and any effect of preventing end stage renal disease.

Patients with the DD genotype showed a significant rise in serum ACE activity, which has already been demonstrated in adults^{12, 22, 25} and in children.³⁶ Thus, patients with the DD genotype might have raised ACE activity and an abnormal regulation of the renin-angiotensin system at the onset of disease. Our previous study in animals and recent studies in humans demonstrated a modulation of glomerular permeability and selectivity by the renin-angiotensin system.^{26, 38, 39} The altered local angiotensin activity superimposed on the pathophysiological conditions induced by Henoch-Schönlein purpura nephritis might have resulted in persistent proteinuria. As stated earlier, a lack of association between proteinuria and the DD genotype in IgA nephropathy does not negate a possible contribution of angiotensin in its pathophysiology. Studies in adults with IgA nephropathy have shown a greater sensitivity to the antiproteinuric effect of ACE inhibitors in patients with the DD genotype. Similar trials in children will provide more insight into angiotensin dependent proteinuria in childhood onset Henoch-Schönlein purpura nephritis and IgA nephropathy, although prognosis of the persistent proteinuria in each disease needs to be determined first to evaluate the risk/benefits of treatment.

Our study has demonstrated a strong association between persistent proteinuria in childhood Henoch-Schönlein purpura nephritis and the deletion polymorphism of the ACE gene. Our findings suggest that the physiological effect of the polymorphism is already present in childhood, and might modify the clinical manifestation of Henoch-Schönlein purpura nephritis. The long term consequences of per-

sistent proteinuria in patients with childhood Henoch-Schönlein purpura nephritis and the deletion genotype, as well as the gene effect of ACE polymorphism on childhood onset IgA nephropathy require further studies.

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