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ORIGINAL ARTICLE

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Polymorphism of the ACE gene in Henoch-Schönlein purpura nephritis

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Abstract Individuals with IgA nephropathy (IgAN) who are homozygous for the deletion (D) polymorphism of the gene for angiotensin converting enzyme (ACE) are reported to be at increased risk of progressive renal damage. Since IgAN and Henoch-Schönlein purpura with associated nephritis (HSPN) share a common aetiology, we have investigated this influence in 31 children with HSPN. The distribution of genotypes was as follows: II: 4, ID: 17 and DD: 10 patients. Median length of follow-up was 4.5 years (range 0.5–15.75 years). Severe onset with nephrotic oedema and crescent formation on renal biopsy was seen in 10 of 17 patients with ID genotype and 5 of 10 patients with DD genotype. In the ID group, 2 patients have undergone renal transplantation and 4 have persistent proteinuria 4, 7, 9 and 10 years after presentation. One patient in the DD group has been transplanted and 1 patient has proteinuria and a reduced glomerular filtration rate 5 years after initial presentation. All other patients have either made a complete recovery or have microscopic haematuria alone. These results do not support an association between disease severity and DD genotype in children with HSPN; however larger studies are required to confirm this.

Key words Angiotensin converting enzyme genotype · Henoch-Schönlein purpura nephritis

Introduction

Genetic determinants are likely to influence disease progression in many conditions. Insertion/deletion (I/D) polymorphisms in intron 16 of the gene encoding components of the renin-angiotensin system (RAS) are thought to determine levels of circulating angiotensin I converting enzyme [1, 2], such that individuals homozygous for the deletion (DD) polymorphism have the highest plasma concentrations, while those homozygous for the insertion polymorphism (II) have the lowest. Angiotensin II is a significant determinant of arteriolar and glomerular hypertension, and is a known cause of mesangial proliferation [3]. An association between the DD polymorphism and disease progression has therefore been sought, and confirmed, in a number of renal diseases, including diabetic nephropathy [4], focal segmental glomerulosclerosis [5] and IgA nephropathy (IgAN) [6]. More recently the DD genotype has been reported to be significantly associated with chronic renal failure [7] and proteinuria [8] in childhood Henoch-Schönlein purpura (HSP). We have investigated this influence in children with HSP nephritis (HSPN).

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Patients and methods

Ethical approval was granted prior to undertaking the study. Fifty-seven patients with HSPN were referred to our unit between 1983 and 1996. Thirty-three patients agreed to participate in the study. Two patients were excluded from the final analysis; 1 patient had co-existing reflux nephropathy; in a second patient, ACE genotyping was unsuccessful. Thirty-one children were therefore studied (18 male, 13 female). All patients fulfilled the American College of Rheumatology 1990 criteria for the classification of HSP [9]. Palpable purpura was seen in all patients, 76% had joint involvement, 60% had abdominal symptoms and 53% had macroscopic haematuria. Nephritis was defined as the presence of any haematuria or proteinuria. Renal biopsy was undertaken in patients where there was evidence of deteriorating renal function or persistent nephrosis. Immunosuppressive therapy (prednisolone/cyclophosphamide/azathioprine) was given where there was >50% crescent formation on renal biopsy, or >20% crescent formation in cases of impaired renal function.

Informed consent was obtained from parents in all cases and also from children over 12 years. Buccal scrapings were taken using a dry, sterile ‘cytotak’ brush (Medical Wire and Equipment, Bath, UK). This was subsequently immersed in sterile normal saline and agitated to remove the cells, which were then suspended in 0.05 M sodium hydroxide and boiled for 15 min to release the DNA. After neutralisation using 1 M TRIS, 10 µl of the superna-

Table 1 Patient data, angiotensin converting enzyme (*ACE*) genotype and renal outcome (*NA* not available, *PD* peritoneal dialysis, *P* prednisolone, *C* cyclophosphamide, *A* azathioprine, *MP* methylprednisolone, *PE* plasma exchange, *ACEI* ACE inhibitor, *ESRF* end-stage renal failure, *LRD Tx* living-related donor transplant, *GFR* glomerular filtration rate, *Tx* transplant)

Patient no.	ACE genotype	Age at onset (years)	Renal biopsy	Treatment	Follow-up (years)	Final outcome/urinalysis
1	II	2.5	–	–	4.5	Normal
2	II	6.2	–	–	2.7	Normal
3	II	7.5	17% crescents	–	2.9	Normal
4	II	2	–	–	0.9	Microscopic haematuria
5	ID	7	44% crescents	P/C/captopril	4.4	Proteinuria (33 mg/ mmol creatinine on ACEI)
6	ID	7.5	–	–	8.4	Normal
7	ID	12.8	–	–	3.7	Microscopic haematuria
8	ID	7.5	Mesangial proliferation	–	5.1	Normal
9	ID	4.75	–	Hydralazine	4.1	Normal
10	ID	4.9	–	–	1.4	Normal
11	ID	7.6	80% crescents	P/C/A	9.5	Proteinuria (206 mg/mmol creatinine)
12	ID	7	–	–	8.9	Normal
13	ID	5.3	9% crescents	P[a] captopril	3.25	Normal
14	ID	5.2	Failed	PE/P/C/A	13.9	ESRF/LRD Tx
15	ID	8.25	50% crescents	P/C	6.1	ESRF/Tx
16	ID	10.33	10% crescents	Captopril	6.5	Proteinuria (15 mg/mmol creatinine on ACEI)
17	ID	2	30% crescents	P/C	10.75	Proteinuria (159 mg/mmol creatinine)
					5	Hypertension
18	ID	7	Mesangial proliferation	–	4.9	Normal
19	ID	6.33	–	–	0.5	Normal
20	ID	10.5	20% crescents	P/C/captopril	2.4	Normal
21	ID	15.25	–	–	0.7	Normal
22	DD	10.2	–	–	4.8	Normal
23	DD	8.4	–	–	7.75	Normal
24	DD	6.6	100% sclerosis	Hydralazine	12.4	ESRF/Tx
25	DD	5.3	–	–	0.8	Microscopic haematuria
26	DD	10.3	80% crescents	PD/MP/A/C	5.5	Proteinuria (242 mg/mmol creatinine)/Hypertension/ GFR 50 ml/min
27	DD	7.7	–	–	1	Normal
28	DD	7.3	–	–	8.6	Normal
29	DD	3	10% crescents	–	15.7	Normal
30	DD	9.6	30% crescents	P/C/diuretics/enalapril	2	Normal
31	DD	7.9	–	–	1.2	Normal

[a] Prednisolone given for treatment of abdominal involvement

tant was used directly in a polymerase chain reaction (PCR), according to established methods [10, 11]. The 50-µl reaction mixture comprised 10× reaction buffer with 2.5 mmol/l $MgCl_2$, dNTPs at 200 µM, 10 pmol/l primers (sense 5'-CTGGAGACC-ACTC CCATCCTTTCT-3', antisense 5'-GATGTGGCCATCACA TTCGTCAGAT-3'). The PCR programme comprised 30 cycles at 94°C for 1 min, 58°C for 1 min and 72°C for 1 h. The PCR products were then run on 2% agarose gel in order to separate the 490-base pair (bp) insertion polymorphism (I) from the 190-bp deletion polymorphism (D). In some cases, where venesection was undertaken for separate reasons, DNA was extracted from whole blood in EDTA using a standard phenol/chloroform method. DD samples were repeated to confirm results. Twenty-seven children with no evidence of renal disease were recruited as healthy controls and analysed for ACE genotype as above.

Results

ACE genotype distribution was II: 4, ID: 17 and DD: 10 patients. Allele frequency was 0.4:0.6 I:D, which is the same as that reported in other European series [6, 12, 13]. Mean ages at presentation in these groups were 4.6, 7.6 and 7.6 years, respectively. Mean length of follow-up for the II, ID and DD groups was 2.8, 5.6 and 5.3 years, respectively. No patient in the II group had nephrotic oedema or impaired renal function, compared with 10 of 17 patients in the ID group and 5 of 10 patients in the DD group. Sixteen patients required renal biopsy (1 patient with II genotype, 10 patients with ID genotype and 5 patients with DD genotype). In the patient in the II group this was for confirmation of diagnosis. Eight patients in total received immunosuppressive therapy; 6 of these were ID genotype, 2 were DD genotype. Four patients in the ID group were treated with ACE inhibitors, 2 of these are still being treated with captopril for persistent proteinuria. One patient in the DD group received enalapril shortly after diagnosis for proteinuria, which has subsequently resolved. One patient in the DD group and 2 in the ID group went into end-stage renal failure 6 months to 8.4 years after initial onset. All 3 have

been successfully transplanted. A further patient in the DD group has evidence of renal insufficiency and hypertension 5 years after initial diagnosis. Four other patients in the ID group have persistent proteinuria 4–10 years after initial presentation. All remaining patients have no further urinary involvement or have microscopic haematuria alone. There was no statistically significant difference between the groups in terms of proteinuria or renal insufficiency at final follow-up (Fisher's exact test P=0.47).

Clinical and laboratory data and outcome are presented in Table 1. In the control group, genotype frequency was II: 7, ID: 14 and DD: 6. Genotype frequency in both patients and control groups were in Hardy-Weinberg equilibrium [chi-squared 0.6 (P=0.44) and 0.04 (P=0.84), respectively]. The mean age of controls with II, ID and DD genotype was 9.59, 8.52 and 7.43 years, respectively.

Discussion

Progressive renal disease, manifested histologically as advanced glomerulosclerosis, is likely to be due to a combination of immunological and haemodynamic factors. It is attractive to postulate that the RAS is implicated in the pathogenesis. The importance of the DD polymorphism as an independent risk factor for progressive renal disease in IgAN [14] and HSPN [8] has been confirmed in some studies, while others report a lack of association between DD and progressive renal disease in both IgAN [12] and HSPN [13]. We were unable to confirm this association. It is, nevertheless, interesting to hypothesise that a dominant D allele may be implicated in the progression of renal disease. This would account for the lack of difference between ID and DD polymorphisms in terms of renal function, as illustrated by the more-benign presentation and evolution in the II group. This finding must, however, be interpreted with caution, since mean age at onset was lower in this group, and length of follow-up shorter. It has been reported that disease severity is directly related to age at onset [15], while renal functional decline may occur up to 23 years after initial presentation in childhood HSPN [16].

Finally, the number of patients in this study is too small to reach significant conclusions using the power method. Since it would not be appropriate to calculate power retrospectively [17], we have calculated the number of patients required to detect similar differences as statistically significant. We estimate that a total of 672 patients would be required, in order to have 80% power of detecting similar differences in the proportion of DD and non DD patients with proteinuria and/or renal insufficiency at outcome, assuming that the ratio of non DD to DD is 2:1. Given the rarity of the condition, such a large study is unlikely to be feasible, and a highly powered meta-analysis of several, smaller studies may be required.

In summary, we were unable to confirm an association between the DD polymorphism and disease progression in childhood HSPN. However the role of the 'dominant D allele' in this condition merits further evaluation.

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