Brief Report

The deletion polymorphism of the ACE gene is not an independent risk factor for renal scarring in children with vesico-ureteric reflux

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Abstract

Background. The deletion (D) polymorphism of the gene encoding angiotensin-I converting enzyme has been implicated as a risk factor for progressive renal disease in several conditions. This study was designed to evaluate the association between homozygosity for the D allele and susceptibility to renal scarring in children with vesico-ureteric reflux (VUR).

Methods. Two-hundred-and-six children with VUR (all grades) were recruited into the study. Patients were stratified into two groups according to the presence or absence of renal scarring. One-hundred-and-twelve patients (group 1) had evidence of renal scarring. Ninety-four children had no evidence of renal scarring (group 2). ACE genotypes were determined by polymerase chain reaction (PCR) amplification of genomic DNA samples.

Results. There was no association between the DD polymorphism and the presence of renal scarring. Genotype frequencies in group 1 were: II, 29; ID, 56; and DD, 27; and in group 2 were: II, 12; ID, 52; DD, 30 (P=0.21). Neither was there evidence supporting a ‘dominant’ D allele. There was no association between the DD genotype and the presence of proteinuria or reduced renal function (P>0.05). Hypertension was seen more frequently in those individuals with the DD genotype, compared with the other two genotypes (P=0.012).

Conclusion. We cannot confirm previous reports that children with vesico-ureteric reflux who are homozygous for the deletion polymorphism of the ACE gene are more susceptible to renal scarring than heterozygotes and II homozygotes.

Keywords: ACE gene polymorphisms; renal scarring; vesico-ureteric reflux

Introduction

Reflux nephropathy (RN) defines the association of vesico-ureteric reflux (VUR) and renal scars. RN accounts for up to 25% of cases of end-stage renal failure (ESRF) in adolescence and adulthood [1]. The condition is manifest histologically by focal or global glomerulosclerosis [2,3]. The renin-angiotensin system (RAS) plays a major role in the regulation of glomerular haemodynamic and sclerotic processes. The gene for angiotensin 1 converting enzyme (ACE) is located on chromosome 17. Insertion/deletion polymorphisms of this gene are denoted by the presence (I) or absence (D) of a 287 bp fragment on intron 16. These polymorphisms are reported to determine circulating and tissue ACE levels, such that individuals homozygous for the D (deletion) allele have higher tissue and plasma ACE concentrations than heterozygotes and II homozygotes [4,5]. It has recently been reported that children with VUR who are homozygous for the D allele are at increased risk of renal scarring [6]. We have investigated this influence in our study population.

Subjects and methods

Ethical approval was granted prior to undertaking the study. British caucasian children with known primary VUR, diagnosed between 1987 and 1998, were identified from a database of patients followed up in a paediatric nephrology clinic. All consenting families were recruited into the study, which was undertaken between April 1999 and April 2000. Renal scarring was denoted by areas of hypo-echogenicity on (99mTc)-dimercaptosuccinic acid scan (DMSA), and patients were allocated to group 1 or group 2, according to the presence or absence of renal scarring.

Informed consent was obtained from parents in all cases and additionally from children over 12 years. Buccal scrapes were taken using a dry, sterile ‘cytotak’ brush (Medical Wire and Equipment Co., Bath, UK). We have previously shown this to be an effective and painless method of DNA extraction in children [7]. The brush was subsequently immersed in sterile normal saline and agitated to remove the cells, which

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were then suspended in 0.05 M NaOH and boiled for 15 min to release the DNA. After neutralization using 1 M Tris, 10 μl of the supernatant was used directly in a PCR reaction. The 50 μl reaction mixture comprised 10× reaction buffer with 2.5 mmol/1 MgCl₂, dNTPs at 200 μM and 10 pmol/l primers (sense 5’-CTGGAGACCATCCTCCCATTCTTTTCT-3’, anti-sense 5’-GATGTTGCCATCACATTGTCAGAT-3’). The PCR programme comprised 30 cycles at 94°C for 1 min, 58°C for 1 min and 72°C for 2 min. The PCR products were then run on 2% agarose gel in order to separate the 490 bp insertion polymorphism (I) from the 190 bp deletion polymorphism (D). DD samples were reanalysed in a separate PCR reaction using a 335 insertion-specific primer to prevent mistyping.

Data analysis

Pearson’s chi-squared test or Fisher’s exact test, as appropriate, was used to compare genotype frequencies between patients with and without renal scarring, and to assess the association between genotype and risk factors for disease progression. The power of the study was calculated prospectively. Assuming the proportions with the DD allele to be 0.5 and 0.25 in the scarred and non-scarred groups, respectively, we estimated that 154 patients would need to be recruited into the study in order to achieve 90% power for detecting a difference at the 5% level of statistical significance when analysed using a $\chi^2$ test.

Results

Two-hundred-and-six children with primary VUR (all grades) were recruited into the study. There were 106 boys and 100 girls. One-hundred-and-twelve patients (54%) had evidence of renal scarring on 99mTc-DMSA scintigraphy (group 1), and 94 children (46%) had no evidence of renal scar formation (group 2). The male : female ratio was 1.15 : 1 in group 1, and 0.96 : 1 in group 2. Median age at presentation was 18 months in group 1 (range 0.1–13 years) and 4 months in group 2 (range 0.1–10 years). Clinical details at presentation are given in Table 1. Median length of follow-up at the time of the study was 3 years (group 1, range 0.5–16 years; group 2, range 0.5–8 years).

The distribution of ACE genotypes in group 1 was: II, 29; ID, 56; DD, 27; and in group 2 was: II, 12; ID, 52; and DD, 30. There was no association between the DD polymorphism and the presence of renal scarring ($\chi^2 = 0.56$, $P = 0.21$; difference in proportion of patients with DD allele (between groups 1 and 2) $= 0.08$, 95% CI $= -0.20 – 0.05$). I: D allele frequencies were 0.49: 0.51 in group 1, and 0.4: 0.6 in group 2 (Table 2). Genotype and allele frequencies were similar to those reported in European controls [8]. Hardy–Weinberg equilibrium was attained in both groups: $\chi^2 = 0.001$ ($P = 0.97$) and $\chi^2 = 2.10$ ($P = 0.15$) in groups 1 and 2, respectively. In patients with grade III-V VUR, genotype frequencies were (group 1): II, 11 (24%); ID, 25 (54%); DD, 10 (22%); and (group 2): II, 7 (21%); ID, 19 (58%); and DD, 7 (21%).

Fifteen patients in group I had proteinuria >1 g/l at the time of the study. Twenty patients in group 1 had evidence of reduced renal function, based on calculated glomerular filtration rate (GFR) (Table 3). Neither proteinuria nor reduced renal function was associated with the DD genotype ($P = 0.76$ and $P = 0.39$, respectively). Hypertension, defined as blood pressure >95th percentile for age, was present in 10 patients in group 1 (Table 3). Hypertension was associated with the DD genotype ($P = 0.012$). No patient in group 2 had proteinuria, reduced renal function or hypertension.

Discussion

The deletion (D) polymorphism has attracted considerable interest over the last few years as a risk factor for progressive renal disease in several conditions. However, data are conflicting. We are unable to confirm an association between the deletion polymorphism and susceptibility to renal scarring in children with VUR reported by Ozen et al. [6]. One possible reason for this difference is the severity of VUR. Ozen et al. excluded children with grades I and II VUR from their study. In our study we included all grades of VUR, since 24% of children with grade I, and 34% of children with grade II VUR had evidence of renal scarring on 99mTc-DMSA scintigraphy and susceptibility to renal scarring in children with VUR.

| Table 1. Clinical details at presentation |
|-----------------|-------|-------|-------|-------|
|                  | UTI   | ANHN  | Familial | CRF   | Other |
| Group 1 (scar)   | 85    | 10    | 6       | 9     | 2     | 112   |
| Group 2 (no scar)| 42    | 24    | 24      | 0     | 4     | 94    |

UTI, urinary tract infection; ANHN, antenatal hydronephrosis; Familial, investigation of family member with VUR; RN, CRF, chronic renal failure; Other, incidental finding during course of investigations.
isms in the TGF-

In conclusion, we are unable to confirm previous reports that the deletion polymorphism of the ACE gene is an independent risk factor for renal scarring in children with VUR. However, it is possible that in association with other genetic polymorphisms, this may contribute to increased susceptibility to scar formation and progressive parenchymal damage.

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References


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DMSA. ACE genotype frequencies in children with grades III–V did not differ from those with grades I and II VUR. Another possible explanation could be the severity of renal disease; 18/53 (34%) of patients reported by Ozen had evidence of deteriorating function, compared with 20/112 (18%) in our population. Neither study has demonstrated an association between the DD genotype and decline in renal function, although the numbers are too small to be meaningful and this would be better evaluated in a large series of adolescents and adults with RN with stable and declining renal function. Hypertension and proteinuria are the major risk factors for disease progression in RN. The DD genotype did not correlate with the presence of proteinuria. There was an association between hypertension and the DD genotype; however, this finding would need to be evaluated in a much larger series of patients with hypertension before any conclusions could be drawn.

It is not possible to differentiate accurately congenital from acquired renal scarring in these patients; however, the congenital or acquired nature of the scarring process is unlikely to affect the final common pathway to parenchymal damage, which is that of glomerulosclerosis. This process is mediated by angiotensin II, a significant determinant of glomerular and systemic hypertension, promoting increased intraglomerular pressures and hyperfiltration with resulting sclerosis. Angiotensin II increases extracellular matrix production and may promote sclerosis in a manner independent of its haemodynamic regulatory function. This fibrogenic potential of angiotensin II probably relates to its interaction with many cytokines and growth factors including transforming growth factor beta (TGF-β1), endothelin 1, platelet derived growth factor-BB (PDGF-BB) and plasminogen activator inhibitor-1 (PAI-1) [9,10]. Ozen et al. evaluated the role of PAI-I as a risk factor for renal scarring in children with VUR, but were unable to confirm an association [6]. Indeed, none of the other genetic polymorphisms of the RAS have been shown to be associated with progressive renal disease. However, there are preliminary data showing that polymorphisms in the TGF-β1 gene may influence individual susceptibility to renal scarring. This requires further evaluation.

In conclusion, we are unable to confirm previous reports that the deletion polymorphism of the ACE