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ACE gene polymorphism and IgA nephropathy: An ethnically homogeneous study and a meta-analysis

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ACE gene polymorphism and IgA nephropathy: An ethnically homogeneous study and a meta-analysis.

Background. Conflicting results have implicated the angiotensin-converting enzyme (ACE) D allele in the progression of renal damage in patients with IgA nephropathy (IgAN). Most of these findings have been obtained by heterogeneous studies.

Methods. We investigated the ACE insertion/deletion (I/D) gene polymorphism by polymerase chain reaction (PCR) amplification of genomic DNA in an ethnically homogenous sample size of IgAN patients from Southern Italy. The association between ACE I/D gene polymorphism and the development of the disease was examined in 247 biopsy-proven IgAN patients and 205 healthy subjects. The association with the progression of renal damage was evaluated in 136 patients with a follow-up of ≥3 years according to the slope of the creatinine clearance against time, and in 221 patients with a follow-up of ≥1 year assessing by univariate and multivariate analyses of renal survival. These associations were further estimated in a meta-analysis of seven studies retrieved in the Medline database. The meta-analysis was performed according to the Mantel-Haenszel-Peto method when homogeneity of the studies was established using the χ^2 test by Breslow-Day.

Results. No difference in the ACE I/D gene distribution between patients and controls and between patients with stable and those with deteriorating renal function was found in our study. A meta-analysis performed separately for Caucasian and Asian studies showed that the ACE I/D gene polymorphism did not contribute to the genetic susceptibility of the development of IgAN (total OR 0.93, 95% CI, 0.71 to 1.23; and 0.95, 95% CI, 0.64 to 1.42, respectively) or the progression of the renal damage (total OR 1.12, 95% CI, 0.67 to 1.88; and 2.26, 95% CI, 0.75 to 6.79, respectively) in both groups.

Conclusions. Our study and meta-analysis suggest caution in the interpretation of results from association studies enrolling heterogeneous populations. Further studies using new tests, which are free of the bias due to population stratification and ethnicity, are warranted.

Key words: angiotensin-converting enzyme gene, end-stage renal disease, RAS gene polymorphisms, progressive renal disease, primary glomerulonephritis.

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National registries of renal biopsies and epidemiological studies have shown IgA nephropathy (IgAN) as the most common form of primary glomerulonephritis in the world [1–5]. In addition, some national and international registries of end-stage renal disease (ESRD) patients receiving renal replacement therapy have shown IgAN as the most common biopsy-proven primary glomerulonephritis leading to ESRD [6–10]. Poor prognostic factors responsible for the progression of renal damage in these patients are male gender, older age, presence of hypertension, moderate or severe proteinuria, and severe renal lesions [11, 12]. It has been reported that growth factors such as platelet-derived growth factor and cytokines are responsible for the increased production of extracellular matrix contributing to the development of glomerular sclerosis [13]. Angiotensin II modulates the local production of these mediators and consequently induces mesangial cell proliferation, mesangial matrix production and progression of renal lesions [14].

Angiotensin-converting enzyme (ACE) is a carboxyl terminal dipeptidyl exopeptidase that converts angiotensin I to angiotensin II in the renin-angiotensin system. Angiotensin II is active in the circulating blood and local tissues [14]. Its production is modulated by the *ACE* gene, which consists of 26 exons, and its locus is at chromosome 17q23 [15, 16]. A 287 bp fragment identifies a polymorphism within the intron 16 of this gene and its insertion/deletion (I/D) defines the three genotypes: DD and II homozygotes and ID heterozygote [17, 18]. High values of serum ACE activity have been found in subjects with the D allele [19], and Yoshida et al were first to describe the high frequency of the DD genotype in IgAN patients with progressive deterioration of renal function [20]. Subsequent contributions have produced conflicting results [21–26]. To date, the role of ACE I/D polymorphism in the development and progression of IgAN is still controversial. Thus, we tested the ACE gene polymorphism in a large ethnically homogenous sample of Caucasian IgAN patients from South Italy, and com-

pared our data with those of other investigators. Since several studies have investigated this same question, a meta-analysis of them was performed to provide a more reliable assessment of the significance of the association.

METHODS

Study patients and controls

Two hundred and seventy IgAN Caucasian patients were diagnosed by renal biopsy in our division from January 1986 to January 1999. Of them, a cohort of 247 patients (176 males and 71 females, mean age of 30 ± 12 years) who were living in Southern Italy and had visited our out-patient facility in the last three years were included in the study. Diagnosis of IgAN was based on the presence of IgA as the sole, predominant, or codominant immunoglobulin in the mesangial area of glomeruli, without systemic disease (such as Schönlein-Henoch purpura, systemic lupus erythematosus) and liver cirrhosis. The clinical picture was characterized by relapses of macrohematuria following infections of the upper respiratory tract, and fever or persistent microhematuria with or without proteinuria. The severity of renal lesions was graded according to Lee's classification [27]: grades 1 and 2 = mild renal lesions; grade 3 = moderate lesions; grades 4 and 5 = severe renal lesions. No patient had received corticosteroid or immunosuppressive therapy in the two months prior to the study. One hundred and thirty-six patients had a follow-up of three or more years, while 221 had a follow-up of one or more years; the total mean follow-up was 5.3 ± 5.2 years.

Blood samples were obtained from all patients after appropriate informed consent. All patients had serial measurements of serum creatinine, proteinuria, and blood pressure. The serum creatinine concentration was determined by an autoanalyzer technique, using the modified Jaffe method. Creatinine clearance (C_{Cr}) was calculated by using the Cockcroft formula. Urine protein concentration was measured by the pirogallolo method. Proteinuria was classified as mild (≤1 g/day), moderate (>1 to 3 g/day) and severe (>3 g/day). The persistence of maximal level of proteinuria was considered for at least six months during the follow-up period. Proteinuric patients were considered responsive to ACE inhibitors therapy when a reduction of at least 50% of the baseline proteinuria was obtained. Hypertension was defined as systolic blood pressure higher than 140 mm Hg and diastolic blood pressure higher than 90 mm Hg. Hypertensive patients were considered responsive to antihypertensive therapy when a reduction of blood pressure below 140 to 90 mm Hg was obtained.

Two outcome measures of renal disease progression were made in our population study. First, progression of the renal damage was evaluated in 136 patients according to the slope of the creatinine clearance against the time (decrease ≥3 mL/min/year defined a deteriorating renal function). Only patients with follow-up greater than three years and normal renal function at the time of renal biopsy (serum creatinine value less than 1.5 mg/dL and/or glomerular filtration rate higher than 70 mL/min) were included in the analysis [27]. Second, renal survival was assessed in 221 patients with a follow-up of more than one year from the time of renal biopsy. The end point was ESRD treated by dialysis or renal transplantation.

Caucasian staff members and blood donors living in Southern Italy (140 males and 65 females, mean age 33 ± 11 years) without a history of renal disease or renal failure and with routinely negative urinalysis, matched for age and sex with IgAN patients, represented the control group.

Extraction of genomic DNA and genotype determinations

Genomic DNA was isolated from 2.4 mL of ethylenediaminetetraacetic acid (EDTA) whole blood with a kit (Genomix; Talent SRL, Trieste, Italy). Polymerase chain reaction (PCR) amplification to detect the ACE I/D polymorphism was carried out on 250 ng genomic DNA using the published primers: 5′-CTG GAG ACC ACT CCC ATC CTT TCT-3′ and 5′-GAT GTG GCC ATC ACA TTC GTC AGA T-3′ [17, 18]. Amplification with this primer pair produced approximately 490 and 190 bp fragments corresponding to the I and D alleles, respectively. In this reaction, a preferential amplification of the D allele and inefficiency in amplification of the I allele could generate mistyping of the ID heterozygote as a DD homozygote [29]. For this reason, all samples found to be DD after amplification with the conventional primers were reamplified using primer pair recognized insertion-specific sequences: 5′-TGG GAC CAC AGC GCC CGC CAC TAC-3′ and 5′-TCG CCA GCC CTC CCA TGC CCA TAA-3′. Only the I allele produced a 335 bp amplicon. The rate of false positives was 10% of the samples, which is similar to that found by other investigators [20, 25].

Angiotensinogen gene polymorphism (AGT M235T) was examined by a mutagenically separated PCR technique (MS-PCR) based on a three-primer one-step assay (M235T1, 5′-CCA GGG TGC TGT CCA CAC TGG CTC CGG-3′; M235T2, 5′-AAG TGG ACG TAG GTG TTG AAA GGG AGG GTG CTG TCC ACA CTG GCT TCC-3′; M235T3, 5′-TGT GGT CCT CCC ACG CTC TCT TGG-3′), as previously designed [30].

Amplification with primer M235T1 and primer M235T3 yielded a 134 bp product representing the 235T allele, whereas amplification with primer M235T2 and primer M235T3 yielded a 156 bp product, representing the M235 allele.

Statistical analysis

Allelic frequency was deduced from the genotype distribution. Analysis was performed by the χ^2 test on the

distribution of ACE D/I genotype and allele frequencies between IgAN patients and controls, and between stable renal function and deteriorating renal function in IgAN patients. Linear regression analysis was performed to generate the rate of deterioration of C_{Cr} over time. Univariate analysis of the renal survival was performed using the Kaplan-Meier technique for censored data. The influence of prognostic factors was assessed by univariate (log-rank test) and multivariate analyses (Cox's proportional hazard methods). For the statistical analysis, we used the StatView 5.0 program (1992 to 1998; SAS Institute Inc., Cary, NC, USA).

A meta-analysis of ACE genotype polymorphism studies in the development and progression of IgAN was performed using studies identified by the Medline database literature research feature from the publication of the first study [20] to May 1999. Abstracts, letters, and review articles were not considered for this analysis. All seven retrieved articles were critically assessed for their methodological quality, with particular attention to the possibility of bias in the selection of cases (biopsy-proven IgAN) and controls; clinical characteristics such as the degree of proteinuria, blood pressure control and therapy; definition of progression of renal damage; and type of statistical analyses. Each article was evaluated independently by three investigators (two of whom were blinded to the name of the journal and the authors of the article) using a scoring sheet to list those elements important for a meta-analysis [31]. The meta-analysis was performed according to the Peto method in which a 2×2 table was constructed for each candidate study [32]. This is a modification of the Mantel-Haenszel method; by a simple set of operations, it allows weighted analyses for each study based on calculation of its inverse variance. In meta-analysis graphical representation, the area of the black square indicates the amount of information contributed by each individual study and is inversely proportional to the standard error of the log of the odds ratio (OR). The main outcome measures of the meta-analysis were the development of IgAN and the deterioration of renal function, including the ESRD treated by dialysis or renal transplantation. Progression of renal damage was defined as a decrease of renal function estimated by slope of creatinine clearance against time [20, 26], slope of the reciprocal of serum creatinine [21], increase of initial serum creatinine to a mean of 4.5 ± 0.86 mg/dL [25], and occurrence of ESRD [24]. Genotype and allele frequencies were extracted, and the ORs were calculated. The statistical analyses were performed on the distribution of the DD + ID versus II genotype. To test for the stability of the results, two additional approaches were performed on the distribution of the DD versus II + ID genotypes and on allele frequency. The total OR and 95% confidence interval (CI) were calculated using Peto's odds ratio. Heterogeneity of the studies was assessed on the basis of the χ^2 test by Breslow-Day using a significance level of $P = 0.05$. Given the different allele frequencies reported for healthy controls, separate analyses were performed for Caucasians and Asians.

RESULTS

Population study

In our cohort of 247 IgAN patients, we studied the influence of the ACE genotype on the development of IgAN and the progression of renal disease. With regards to the development of disease, Table 1 shows the distribution of the ACE genotypes and alleles in our IgAN patient population at the time of renal biopsy. The ACE I/D genotype did not correlate with age ($\chi^2 = 0.63$; $P = 0.73$) or gender ($\chi^2 = 4.19$, $P = 0.12$), and did not influence the presence of hypertension ($\chi^2 = 3.29$, $P = 0.14$), the amount of daily proteinuria ($\chi^2 = 3.42$, $P = 0.49$), abnormal renal function ($\chi^2 = 1.1$, $P = 0.58$), or severity of renal lesions ($\chi^2 = 3.87$, $P = 0.42$). With regards to the progression of renal disease, we studied the distribution of the ACE genotypes and alleles in 136 IgAN patient population with a follow up $\geq$3 years. At the last follow-up, the ACE I/D genotype did not influence blood pressure ($\chi^2 = 0.10$, $P = 0.95$) and persistent proteinuria ($\chi^2 = 1.77$, $P = 0.41$). The ACE I/D genotype distribution was identical in proteinuric IgAN patients responsive and not responsive to therapy with ACE-inhibitors ($\chi^2 = 0.87$, $P = 0.65$) and in hypertensive patients responsive and not responsive to other antihypertensive drugs ($\chi^2 = 0.69$, $P = 0.71$). The ACE I/D genotype distribution was not different between 99 patients with stable renal function (average C_{Cr} slope, 0.2 ± 3.1) and 37 patients with deteriorating renal function (average C_{Cr} slope, -9.1 ± 6.2; $\chi^2 = 4.23$, $P = 0.12$).

The ten-year cumulative renal survival rate for censored data (Kaplan-Meier) in 221 patients with a follow up $\geq$1 year was 73%; 46 patients reached the end point of ESRD.

Univariate analysis of survival curves (log-rank test) showed that serum creatinine ($P < 0.0001$), daily proteinuria ($P < 0.0001$), histological grade ($P < 0.0001$), and blood pressure ($P < 0.0001$) were correlated with poor renal survival. Gender, age, and the AGT M235T and ACE genotypes did not influence the renal survival (Fig. 1). Multivariate analysis by Cox's proportional hazard model revealed that only serum creatinine ($P = 0.0007$; hazard ratio (HR) = 3.4; 95% CI, 1.7 to 7), severe proteinuria ($P < 0.0001$; HR = 8.4; 95% CI, 3.3 to 21.2), and severe histological grade ($P = 0.02$; HR = 4.2; 95% CI, 1.26 to 14.4) at the time of renal biopsy were independently predictive of poor outcome.

Table 1. Distribution of the ACE genotypes and alleles, expressed in percentages, in our IgAN patient population at the time of renal biopsy

	N of cases	Genotypes			Alleles	
		DD	ID	II	D	I
Age						
≤18 years	49	33	50	17	58	42
>18 years	198	39	44	17	61	39
Gender						
Males	176	40	41	19	61	39
Females	71	34	55	11	61	39
Normotensive	144	43	42	15	64	36
Hypertensive	103	31	50	19	56	44
Proteinuria						
≤1 g/24 h	156	39	45	16	61	39
1–3 g/24 h	72	37	42	21	58	42
>3 g/24 h	19	37	58	5	66	34
Serum creatinine						
≤1.5 mg/dL	210	38	46	16	61	39
>1.5 mg/dL	37	39	39	22	58	42
C_{Cr} (Cockcroft)						
≥70 mL/min	188	37	49	14	61	39
<70 mL/min	59	43	33	24	59	41
Renal lesions						
Mild (G1–G2)	90	35	49	16	59	41
Moderate (G3)	92	42	45	13	64	36
Severe (G4–G5)	65	38	39	23	58	42

Epidemiological analysis

The distribution of ACE I\D genotype and alleles in our study and in seven retrieved articles are reported in Table 2.

Number of patients and controls. Our study enrolled 247 IgAN patients and 205 controls, matched for age and gender. Smaller sample sizes of IgAN patients and controls were used for the study of ACE gene polymorphism by all investigators [20–23, 25], except for Schmidt et al [24] and Pei et al [26].

Race. In our study, Caucasian patients and controls were from Puglia region of South Italy, which has four million inhabitants. In four studies in which Caucasians were included, patients came from Scotland [23]; Germany, Northern Italy, Austria and Australia [24]; Alabama (USA) [25]; and Canada [26]. Three studies included Asian patients from Japan [20–22].

Gender. In our study, the number of male patients was higher than that of female patients with a ratio of 2.4 to 1, resembling the disease ratio. In three studies, the number of male patients was higher than that of female patients [20, 22, 24–26]. In Yorioka et al's study, the number of males was lower than females [21]. Three studies reported no data on control group gender [22, 23, 25].

Age. The mean age of our patients was 30 ± 12 years (range 5–66 years), and 48 of them were younger than 18 years of age. In the other studies, only adult patients were enrolled. Children, both boys and girls, were present in the studies of Tanaka et al [22] and Hunley et al [25].

Follow-up. In our study, only 26 IgAN patients had a minimal follow-up of one year; the total mean follow-up was 5.3 ± 5.2 years. In the other studies the mean follow-up ranged from 2.4 years [21] to more than ten years [20].

Methods. We used the Genomix kit (Trieste, Italy). The Quiagen kit (Germany) was used in one study [20], Genomix kit in two studies [21, 22], and the Salting out method in two studies [24, 26]. To minimize the mistyping of the ID genotype as DD, dimethyl sulfoxide (DMSO; 5%, vol:vol) was added to the PCR reaction in five studies [21–24, 26], while two PCR reactions were used in three studies [20, 25, 26]. Our study used two PCR reactions for improving the accuracy of genotyping.

Genotype frequency. The study patient populations were in Hardy-Weinberg equilibrium in all studies. However, Yoshida et al found a significant difference in the subgroup of patients with deteriorating renal function [20] and Schmidt et al found the same thing in those with ESRD [24]. In our study, the distribution of genotype frequencies was identical to that of the other Caucasian studies [23, 24, 26]. We did not find a significant difference in our patients and controls ($\chi^2 = 0.38$, $P = 0.83$). The mean frequency of DD, ID, and II genotypes in the IgAN patient population was significantly different from that of the control population in Asian patients (IgAN DD = 0.20, ID = 0.40, II = 0.40; control DD = 0.11, ID = 0.46, II = 0.43; $\chi^2 = 6.6$; $P = 0.04$) but not in Caucasians (IgAN DD = 0.37, ID = 0.44, II = 0.19; control DD = 0.34, ID = 0.48, II = 0.18; $\chi^2 = 3.87$; $P = 0.14$).

Allele frequency. In our study, the allele frequency was identical to that of other Caucasian studies, and there was no significant difference between the IgAN patients and controls ($\chi^2 = 0.27$; $P = 0.59$). The mean distribution of D and I allele frequency in IgAN patients was not significantly different from that of normal population either in Asians (D = 0.34, I = 0.66 vs. D = 0.39, I = 0.61; $\chi^2 = 2.3$; $P = 0.12$) or Caucasians (D = 0.58, I = 0.42 vs. D = 0.59, I = 0.41; $\chi^2 = 0.28$; $P = 0.6$).

Meta-analysis

The current study plus seven articles [20–26] that satisfied the specific criteria listed in the statistical analysis section of this article were chosen for the meta-analysis. The mean D allele frequency (0.34) was lower than that of the I allele (0.66) in the normal Japanese population compared with the Caucasian population (D = 0.58; I = 0.42; Table 2). Therefore, the Japanese and Caucasian population studies were analyzed separately. In total, the Asian studies comprised 198 IgAN patients and 220 controls, whereas Caucasian studies included 719 IgAN patients and 637 controls.

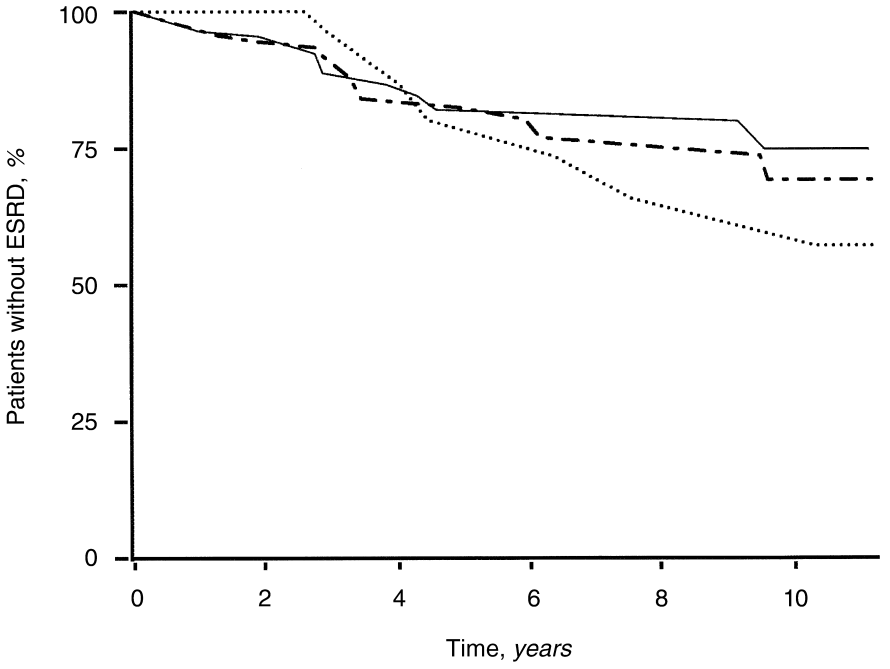

Fig. 1. Renal survival in our IgAN patients with the DD (17 events; solid line), ID (19 events; dashed line), and II (10 events; dotted line) genotypes. Log rank test, $P = 0.48$.

Table 2. Epidemiology and distribution of the ACE I/D genotype and allele in IgAN patients and controls enrolled in individual studies

		Demographics			Genotypes %						Alleles %			
	Controls		Age	Follow-up	Controls			IgAN			Controls		IgAN	
Authors [Ref]	*N*	*N*	*years*		DD	ID	II	DD	ID	II	D	I	D	I
Yoshida et al [20]	46	53	38.5 ± 2.1	>10	7	52	41	30	32	38	33	67	46	54
Yorioka et al [21]	103	48	33.5 ± 12	2.4 ± 1.5	10	46	44	17	27	56	33	67	30	70
Tanaka et al [22]	71	97	11.7 ± 0.5	ND	13	49	38	16	49	35	37	63	40	60
Harden et al [23]	98	100	14–79	ND	40	43	17	40	41	19	61	39	60	40
Schmidt et al [24]	234	204	45 ± 13.5	>5	33	50	17	38	39	22	58	42	59	41
Hunley et al [25]	ND	64	6–83	6.8 ± 0.6	ND	ND	ND	14	59	27	ND	ND	44	56
Pei et al [26]	100	168	47 ± 14	6.1 ± 4.7	30	49	21	33	48	19	60	40	57	43
Present study	205	247	30 ± 12	5.3	35	47	18	38	45	17	59	41	61	39

Ethnicity was divided into two populations: Asian studies [20–22] and Caucasian studies [23–26]. The populations were in Hardy-Weinberg equilibrium in all studies, except for those of Yoshida et al and Schmidt et al, that observed a significant difference respectively in the subgroup of patients with deteriorating renal function ($P < 0.05$) and in those with end-stage renal disease ($P = 0.04$). ND is not described.

Results of meta-analysis in the development of IgAN and in the progression of renal disease are reported in Figures 2 and 3. A pooled estimate was performed when the homogeneity of the studies was confirmed by the Breslow-Day χ^2 test (P not significant). Figure 2 shows the meta-analysis of the ACE genotype polymorphism in IgAN patients compared to controls, evaluating the ORs of the presence of ACE D allele (DD + ID versus II genotype). Only six selected studies were included [20-24, 26] because the control group data were not reported in Hunley et al's study [25]. The total OR values were 0.95 (95% CI, 0.64 to 1.42) and 0.93 (95% CI, 0.71 to 1.23), respectively.

Two additional approaches were used to investigate to what extent the D allele influenced the onset of the disease, by changing the classification of the exposure variable. Three different analyses are reported in Table 3: first, comparison of the DD + ID versus II genotype;

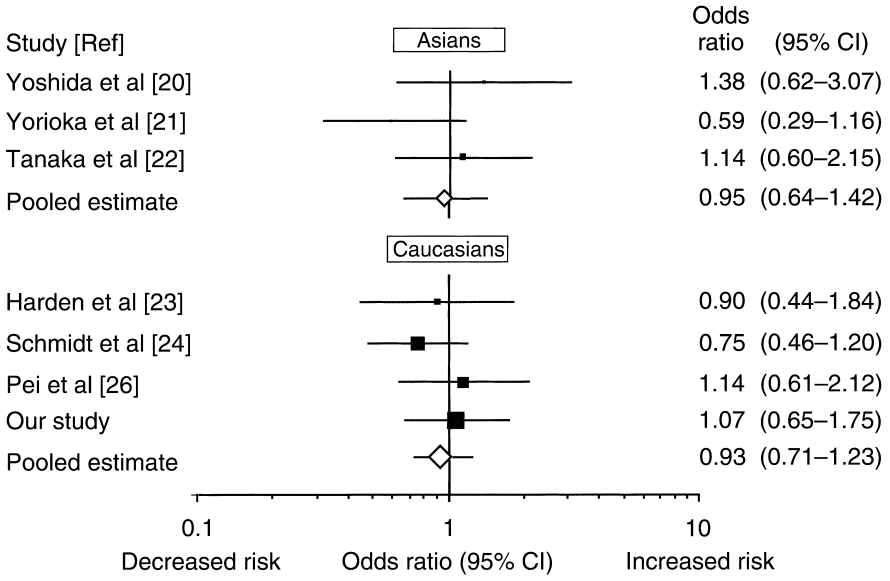

Fig. 2. Association between ACE I/D polymorphism (ACE DD + ID versus II) and IgA nephropathy. Odds ratio between IgAN patients and controls are reported. OR with 95% confidence intervals (CI) for each study together with the pooled estimate are shown. The solid vertical line shows an OR of 1.

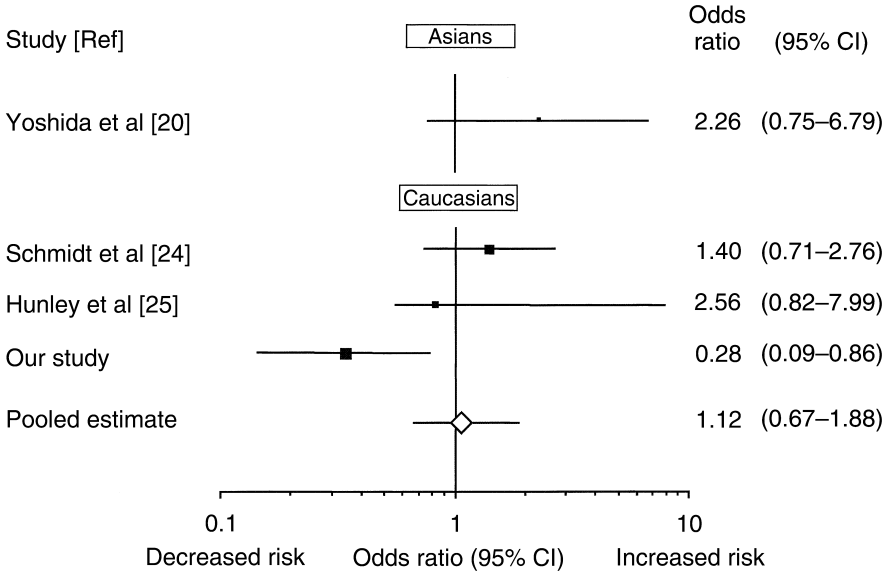

Fig. 3. Association of ACE gene polymorphisms (DD + ID vs. II) and progression of renal damage in IgAN patients. Odds ratio between IgAN patients with and without deteriorating renal function are reported.

second, comparison of the DD versus ID + II genotypes; and third, the D versus I allele frequency. It is evident that an increased risk with the homozygous DD genotype (96%) was present among Asians (OR, 1.96, 95% CI, 1.13 to 3.41). In contrast, a very small risk without statistical significance (17%) of IgAN was evident among Caucasians (OR, 1.17, 95% CI, 0.94 to 1.46).

Figure 3 shows the meta-analysis of the association between ACE D/I genotype polymorphism and deteriorating renal function in IgAN patients. The analysis in Asian population was limited to one study [20] because no data were available in the other studies. Even though the influence of the D allele in deteriorating renal function was reported in this Japanese study [20], the OR value of 2.26 did not reach a statistical significance. At the same time the significant OR value estimated in our study suggests a paradoxical protective effect of the D allele, but the meta-analysis of Caucasian population studies demonstrated a total OR value of 1.12 (95% CI, 0.67 to 1.88). This indicates that there is no relationship between the D allele and the deterioration of renal function.

DISCUSSION

Our study, which includes a cohort of patients with biopsy-proven IgAN enrolled in a restricted area with a sufficient follow-up, shows no influence of the ACE I/D gene polymorphism (genotype distribution and allele fre-

Table 3. Comparison of the association between IgAN and ACE gene polymorphisms based on three different analyses

Group	Odds ratio	95% CI	Homogeneity of results[a]
Asian IgAN patients			
DD + ID versus II	0.95	0.64–1.42	Yes
DD versus ID + II	1.96	1.13–3.14	Yes
D vs. I allele frequency	1.18	0.88–1.57	Yes
Caucasian IgAN patients			
DD + ID versus II	0.94	0.71–1.23	Yes
DD versus ID + II	1.17	0.94–1.46	Yes
D vs. I allele frequency	1.06	0.91–1.23	Yes

[a] χ^2 test by Breslow-Day

quency) on the development of the IgAN and on the progression of the renal damage.

Some investigators observed that the frequency of DD genotype was significantly higher in those patients with progressive deterioration of the renal function, and they concluded that the DD genotype was a genetic marker of poor prognosis either in Asian [20, 21] or Caucasian patients [23, 25]. Others, at least for Caucasians, did not confirm these data [24, 26]. Our study, performed in a cohort of ethnically homogeneous South Italy Caucasian patients, shows that the frequency of the DD genotype and the D allele was not significantly increased in patients with IgAN and in those with deteriorating renal function during the post-renal biopsy follow-up.

Hunley et al demonstrated that when IgAN patients were analyzed separately for their renal function, the DD genotype as well as the D allele was strictly associated with the progressive decline of the renal function. The DD patients with normal renal function, no hypertension, and proteinuria at the time of renal biopsy had a 7.5 times higher risk to develop progressive deterioration of renal function than those who did not have this genotype [25]. They concluded that the DD genotype is an independent risk factor for renal progression because D homozygous patients progressed independently of proteinuria and hypertension, which are two important risk factors responsible for poor renal prognosis. In an effort to replicate these findings, we divided our large sample of IgAN patients with normal renal function at the time of renal biopsy into two groups, nonprogressors and progressors to renal insufficiency, and found no association with DD genotype. There are several explanations for the discrepancy between our findings and the earlier observations. Previously published studies were limited by their heterogeneously selected populations [20–23, 25]. In addition, it has been pointed out that there is considerable variability in the frequency of the DD genotype among so-called controls in smaller studies and among populations [33–35]. In fact, we cannot exclude the possibility that DD genotype can influence IgAN development and progression of renal damage in Asians because the frequency of this genotype is notably low in some populations (Australian Aboriginal, Chinese, and Japanese 0.5%, 6%, and 18%, respectively), whereas it is very high in Caucasians. Most likely, stochastic or epistatic factors enhanced by small breeding population sizes, limited gene pool and geographical isolation play a major role in this population's loss of genetic polymorphism. Perhaps the I and D alleles in Japan do not bear exactly the same haplotype of the *ACE* gene as they do in Europe and are associated with some other difference in the expression of this gene. The enrollment of cases and controls of a restricted area in our study reduces the likelihood of selection bias and the effects of confounding factors. In addition, the finding of almost identical genotype and allele frequencies among a large population of controls and patients matched for number, age, and gender give us considerable confidence in the reliability of our data.

Our meta-analysis supports the notion that development of IgAN is not associated with the presence of the D allele in the Asian and Caucasian populations. Only the homozygous DD genotype could be associated with the development of IgAN (1.96 of risk) in the Asian population. The risk for the progression of renal damage was reported only in Asian patients [20]. However, given the small sample size of enrolled patients, this finding needs to be confirmed by studying a larger cohort of Asian IgAN patients. In fact, the pooled estimate in Caucasian patients does not confirm the poor prognostic role of the DD genotype and D allele. This consideration suggests that association studies, performed in a well-designed manner, should be done using large population samples of patients and controls. Recent studies documenting an increased risk of left ventricular hypertrophy in subjects with the DD genotype [36, 37] and of nephropathy in DD insulin-dependent diabetes mellitus [38–40] were not confirmed when large population studies were done or when pooled data analysis was applied [33, 41–44]. Recently, Japanese investigators reconsidered the effect of the *ACE I/D* gene in the progression of renal damage in IgAN patients and they did not find a significant correlation [45].

An important purpose of a meta-analysis is to help direct future research. Our findings suggest that additional research into the association of genetic variability of the *ACE* and other genes and IgAN is warranted in genetically homogeneous populations. For IgAN, which is a multifactorial disease with several major susceptibility loci, the sample size required to replicate a linkage or association study to a specific locus should be larger than in previous studies. For this reason, it is useful to organize a multicenter collaborative study to assemble a large cohort of patients. The number of cases associating an allele with a particular phenotype must be large enough to be convincing.

An important confounding factor recognized in association studies is ethnicity. We found a different genotype distribution in control subjects and a higher OR in the Japanese population. Therefore, whether these findings imply selection bias or a different relationship between DD genotype and IgAN in Japanese compared with Caucasian populations requires further investigation. In 1993, Spielman, McGinnis and Ewens were the first to propose a new test, the so-called transmission disequilibrium test, which is free of the bias due population stratification [46]. This test analyzes the genomic DNA of affected and nonaffected interfamilial individuals and their parents. Currently we are applying this test to study the effect of the *ACE* gene and other genes in IgAN, and consider this solution the best way to plan future studies.

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REFERENCES

1. Schena FP, the Italian Group of Renal Immunopathology: Survey of the Italian Registry of renal biopsies: Frequency of renal diseases for 7 consecutive years. *Nephrol Dial Transplant* 12:418–426, 1997
2. Espanol de Glomerulonefritis: Evolucion de la frecuencia de las glomerulonefritis primarias y secundarias en Espana en los anos 1991 y 1992. *Nefrologia* 14:228–296, 1994
3. Smith SM, Tung KS: Incidence of IgA-related nephritides in American Indians in New Mexico. *Hum Pathol* 16:181–184, 1985
4. Central Committee of the Toronto Glomerulonephritis Registry: Regional program for the study of glomerulonephritis. *Can Med J* 124:158–161, 1981
5. Research Group on Progressive Chronic Renal Disease: Nationwide and long-term survey of primary glomerulonephritis in Japan as observed in 1850 biopsied cases. *Nephron* 82:205–213, 1999
6. Valderrabano F, Berthoux FC, Jones EHP, Mehls O: Report on management of renal failure in Europe, XXV, 1994 End stage renal disease and dialysis report. *Nephrol Dial Transplant* 11(Suppl 1):S2–S21, 1996
7. United States Renal Data System 1998 Annual Data Report. Incidence and prevalence of ESRD. *Am J Kidney Dis* 32(Suppl 1):S38–S49, 1998
8. Disney APS: Some trends in chronic renal replacement therapy in Australia and New Zealand, 1997. *Nephrol Dial Transplant* 13: 854–859, 1998
9. Canadian Organ Replacement Register, Canadian Institute for Health Information, Annual Report 1996, in *Dialysis and Renal Transplantation* (vol 1). Information, Don Mills, ON, March 1996
10. Shinzato T, Nakai S, Akiba T, *et al*: Report on the annual statistical survey of the Japanese Society for Dialysis Therapy in 1996. *Kidney Int* 55:700–712, 1999
11. D'Amico G, Minetti L, Ponticelli C, *et al*: Prognostic indicators in idiopathic IgA mesangial nephropathy. *Q J Med* 59:363–378, 1986
12. Schena FP: A retrospective analysis of the natural history of primary IgA nephropathy worldwide. *Am J Med* 89:209–215, 1990
13. Schena FP, Gesualdo L, Grandaliano G, Montinaro V: Progression of renal damage in human glomerulonephritides: Is there sleight of hand in winning the game? *Kidney Int* 52:1439–1457, 1997
14. Gesualdo L, Ranieri E, Monno R, *et al*: Angiotensin IV stimulates plasminogen activator inhibitor I (PAI-1) expression in proximal tubular epithelial cells. *Kidney Int* 56:461–470, 1999
15. Mattei MG, Hubert C, Alhene GF: Angiotensin I converting enzyme gene on chromosome 17. *Cytogenet Cell Genet* 51:1041, 1989
16. Hubert C, Houot A, Corvol P, Soubrier F: Structure of the angiotensin I-converting enzyme gene. Two alternate promoters correspond to evolutionary steps of a duplicated gene. *J Biol Chem* 266:15377–15383, 1991
17. Rigat B, Hubert C, Alhenc-Gelas F, *et al*: An insertion/deletion polymorphism of the human angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 86:1343–1346, 1990
18. Rigat B, Hubert C, Soubrier F: PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidyl carboxypeptidase 1). *Nucleic Acids Res* 20:1433, 1992
19. Tiret L, Rigat B, Visvikis S, *et al*: Evidence, from combined segregation and linkage analysis, that a variant of the angiotensin I-converting enzyme (ACE) gene controls plasma ACE levels. *Am J Hum Genet* 51:197–205, 1992
20. Yoshida H, Mitarai T, Kawamura T, *et al*: Role of the deletion polymorphism of the angiotensin converting enzyme gene in the progression and therapeutic responsiveness of IgA nephropathy. *J Clin Invest* 96:2162–2169, 1995
21. Yorioka T, Suehiro T, Yasuoka N, *et al*: Polymorphism of the angiotensin converting enzyme gene and clinical aspects of IgA nephropathy. *Clin Nephrol* 44:80–85, 1995
22. Tanaka R, Lijima K, Murakami R, *et al*: ACE gene polymorphism in childhood IgA nephropathy: Association with clinicopathologic findings. *Am J Kidney Dis* 31:774–779, 1998
23. Harden PN, O'Kane KPJ, Ueda S, *et al*: Association studies, ACE polymorphism and renal disease. *Nephrol Dial Transplant* 11: 757–770, 1996
24. Schmidt S, Steir E, Hartung R, *et al*: No association of converting enzyme insertion/deletion polymorphism with immunoglobulin A glomerulonephritis. *Am J Kidney Dis* 26:727–731, 1995
25. Hunley TE, Julian BA, Phillips JA III, *et al*: Angiotensin converting enzyme gene polymorphism: Potential silencer motif and impact on progression in IgA nephropathy. *Kidney Int* 49:571–577, 1996
26. Pei Y, Scholey J, Thai K, *et al*: Association of angiotensin gene T235 variant with progression of immunoglobulin A nephropathy in Caucasian patients. *J Clin Invest* 100:814–820, 1997
27. Lee SM, Rao VM, Franklin WA, *et al*: IgA nephropathy: Morphologic predictors of progressive renal disease. *Hum Pathol* 13:314–322, 1982
28. Kobayashi Y, Hiki Y, Fujii Y, *et al*: Moderately proteinuric IgA nephropathy: Prognostic prediction of individual clinical courses and steroid therapy in progressive cases. *Nephron* 53:250–256, 1989
29. Lindpaintner K, Pfeffer MA, Kreutz R, *et al*: A prospective evaluation of an angiotensin-converting-enzyme gene polymorphism and the risk of ischemic heart disease. *N Engl J Med* 332:706–711, 1995
30. Schmidt S, Sharma AM, Zilch O, *et al*: Association of M235T variant of the angiotensinogen gene with familial hypertension of early onset. *Nephrol Dial Transplant* 10:1145–1148, 1995
31. Sacks HS, Berrier J, Reitman D, *et al*: Meta-analysis of randomised controlled trials. *N Engl J Med* 316:450–455, 1987
32. Yusuf S, Peto R, Lewis J, *et al*: Beta blockade during and after myocardial infarction: An overview of the randomized trial. *Prog Cardiovasc Dis* 27:335–371, 1985
33. Agerholm-Larsen B, Nordestgaard BG, Steffensen R, *et al*: ACE gene polymorphism: Ischemic heart disease and longevity in 10,150 individuals: A case-referent and retrospective cohort study based on the Copenhagen City Heart Study. *Circulation* 95:2358–2367, 1997
34. Liu ZH, Li LS: Polymorphism in IgA nephropathy. *Nephrology* 3:63–66, 1997
35. Lester S, Heatley S, Bardy P, *et al*: The DD genotype of the

angiotensin-converting enzyme gene occurs in very low frequency in Australian Aboriginals. *Nephrol Dial Transplant* 14:887–890, 1999
36. Schunkert H, Heuse HW, Holmer SR, *et al*: Association between a deletion polymorphism of the angiotensin converting-enzyme gene and left ventricular hypertrophy. *N Engl J Med* 330:1634–1638, 1994
37. Cambien F, Poirier O, Lecerf L, *et al*: Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. *Nature* 359:641–644, 1992
38. Marrè M, Bernadet P, Gallois Y, *et al*: Relationship between angiotensin I converting enzyme gene polymorphism, plasma levels and diabetic retinal and renal complications. *Diabetes* 43:384–388, 1994
39. Mizuiri S, Hemmi H, Inoue A, *et al*: Angiotensin-converting enzyme polymorphism and development of diabetic nephropathy in non-insulin dependent diabetes mellitus. *Nephron* 70:455–459, 1995
40. Barnas U, Schimdt A, Illievich A, *et al*: Evaluation of risk factors for the development of nephropathy in patients with IDDM: Insertion/deletion angiotensin converting enzyme gene polymorphism, hypertension and metabolic control. *Diabetologia* 40:327–331, 1997
41. Lindpaintner K, Lee M, Larson MG, *et al*: Absence of association or genetic linkage between the angiotensin-converting-enzyme gene and left ventricular mass. *N Engl J Med* 334:1023–1028, 1996
42. Parving HH, Tarnow L, Rossing P: Genetics of diabetic nephropathy. *J Am Soc Nephrol* 7:2509–2517, 1996
43. Kunz R, Bork JP, Fritsche L, *et al*: Association between the angiotensin-converting enzyme–insertion/ deletion polymorphism and diabetic nephropathy: A methodological appraisal and systematic review. *J Am Soc Nephrol* 9:1653–1663, 1998
44. Tarnow L, Glund C, Parving HH: Diabetic nephropathy and the insertion/deletion polymorphism of the angiotensin-converting enzyme gene. *Nephrol Dial Transplant* 13:1125–1130, 1998
45. Suzuki S, Suzuki Y, Kobayashi Y, *et al*: Insertion/deletion polymorphism in ACE gene is not associated with renal progression in Japanese patients with IgA nephropathy. *Am J Kidney Dis* 35: 896–903, 2000
46. Spielman RS, McGinnis RE, Ewens WJ: Transmission test for linkage disequilibrium: The insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am J Hum Genet* 52:506–516, 1993