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ORIGINAL ARTICLE

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Renal function in relation to three candidate genes in a Chinese population

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Abstract We recently found in a white population that the genes encoding angiotensin-converting enzyme (ACE, I/D polymorphism), α-adducin (Gly460Trp), and aldosterone synthase (−344C/T) jointly influence renal function. We therefore investigated in a Chinese population the associations between the serum concentrations of creatinine and uric acid and these three genetic polymorphisms. We genotyped 471 ethnic Han Chinese subjects from 125 nuclear families recruited in northern China via random population sampling (75%) and at specialized hypertension clinics (25%). We performed population-based and family-based association analyses using generalized estimating equations (GEE) and quantitative transmission disequilibrium test (QTDT), respectively, while controlling for covariables. The participants were 39.7 years old and included 235 women (49.9%). The blood pressure measured at the subjects' homes averaged 126/80 mmHg. Mean values were 71 μmol/l for serum creatinine, 111 ml min^{-1} 1.73 m^{-2} for calculated creatinine clearance, and 236 μmol/l for serum uric acid. With adjustment for covariables, GEE analyses of single genes demonstrated that serum uric acid, but not serum creatinine, was positively associated with the ACE D allele. Serum uric acid concentrations were 15.8 μmol/l (95% confidence interval 3.3–28.2) and 25.7 μmol/l (11.1–40.2) higher in DD homozygotes than in ID and II subjects, respectively. Further GEE analyses of the three genes combined showed that the association between serum uric acid and the ACE polymorphism was confined to carriers of the α-adducin Gly and/or aldosterone synthase C alleles. Sensitivity analyses in parents and offspring separately as well as QTDT analyses were confirmatory. Among 114 informative offspring carrying the

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α-adducin Gly allele serum uric acid was significantly and positively associated with the transmission of the ACE D allele (β=20.7 μmol/l). In conclusion, the present study extends our previous findings on the combined effects of the three candidate genes and supports the concept that these genetic polymorphisms jointly influence renal function.

Keywords α-Adducin gene · Angiotensin-converting enzyme · Aldosterone synthase · Renal function · Serum creatinine

Abbreviations *ACE:* Angiotensin-converting enzyme gene · *GEE:* Generalized estimating equations · *QTDT:* Quantitative transmission disequilibrium test

Introduction

We recently found in a cross-sectional analysis of a white population that in the presence of the mutated α-adducin Trp allele (Gly460Trp polymorphism), serum creatinine concentration, risk of mild renal dysfunction, and proteinuria were significantly higher in D allele carriers of the angiotensin-converting enzyme gene (ACE I/D polymorphism) than in ACE II homozygotes [1]. In the same population the ACE I/D, α-adducin Gly460Trp and aldosterone synthase –344C/T polymorphisms showed significant interactions in relation to the intima-media thickness of the femoral artery [2] and the incidence of hypertension [3]. The hypothesis underlying our previous studies [1, 2, 3, 4] was that these three candidate genes and their interactions lead to structural and/or functional changes in the cardiovascular-renal system via their influence on sodium reabsorption in the kidney and the circulating fluid volume.

Asians have a lower prevalence of the ACE D allele than whites [5] but higher frequencies of the α-adducin Trp and aldosterone synthase T alleles [6, 7, 8]. The ACE D allele is associated with a higher risk of diabetic nephropathy [9] in Asians, but probably not in whites [5, 9]. Such cross-racial discrepancy in phenotype-genotype associations might be due to disparity in the frequency of risk carrying alleles [10, 11, 12]. We therefore investigated in a Chinese population sample the associations between the above three candidate genes and renal function as reflected by the serum concentrations of creatinine and uric acid [13].

Methods

General design

We conducted the present study in Gaoping, a city 600 km south of Beijing, China. Its primary goal was to investigate the complex relationship between blood pressure analyzed as a continuous or binary phenotype and various candidate genes. In addition to blood pressure, several other intermediate or associated phenotypes were measured, including serum creatinine and uric acid as indexes of renal function.

The Gaoping study was set up in collaboration with the investigators of the Flemish Study on Environment, Genes and Health Outcomes [1, 3] and the European Project on Genes in Hypertension [14] with implementation of the same methods of phenotyping [15] and genotyping [12, 16]. To achieve a high degree of standardization the same study forms, coding rules, and manuals of operations were used in these studies. For the purpose of the present study these documents were translated into Chinese. In addition, the coordinators of the field work in Gaoping and the investigators involved in the construction and analysis of the database took part in the same training program as their European counterparts.

The Gaoping study was conducted according to the principles outlined in the Helsinki Declaration on investigation of human subjects. The institutional review board of Fuwai Hospital and Cardiovascular Institute approved the study protocol. All subjects gave written informed consent. Using the city registry of addresses and a computerized random number function (SAS Ranuni), nuclear families of ethnic Han Chinese were recruited from the population. To increase the number of hypertensive patients approx. 25% of the families were enrolled via specialized clinics. Nuclear families had to consist either of one parent and at least two offspring or two parents and one or more siblings. Moreover, to make repeated home visits feasible, nuclear families only qualified for participation if all family members resided within 10 km of the local study coordinating center. The age range for participation was 18–60 years.

In 2001 we enrolled 125 nuclear families with 513 family members. The participation rate among the subjects contacted was 95.9%. In 23 subjects genotyping was not complete for all genes, and 19 had incomplete anthropometric (n=6) or biochemical (n=9) measurements or other missing information (n=4). Thus the number of subjects included in the present analysis totaled 471.

Field work

All subjects were repeatedly visited in their homes. Blood pressure was measured five times at each of two home visits after 5 min rest in the sitting position. Hypertension was diagnosed if the average of the ten blood pressure readings was at least 140 mmHg systolic or 90 mmHg diastolic, or if the subject was on antihypertensive medication. Diabetes mellitus was diagnosed if the fasting plasma glucose concentration was at least 7.1 mmol/l, or if the subject was on antidiabetic medications [17]. We used a validated questionnaire [1, 3, 14, 15] to collect information on medical history, smoking habits, alcohol intake, and use of medications. Venous blood was sampled under fasting conditions for genotyping and for the measurement of serum creatinine, uric acid, glucose, total cholesterol, and triglycerides. All biochemical measurements were performed in the central laboratory of Fuwai Hospital (Beijing, China) which fulfilled the quality control criteria of the regulatory authority of Beijing. Serum creatinine and uric acid were measured using Jaffé's and uricase methods [18], respectively (Beckman Synchron LX20, Beckman Coulter).

We used published formulas to compute body surface area [19] and lean body mass [20]. Creatinine clearance was calculated using Cockroft and Gault's [21] formula and standardized to 1.73 m^2 of body surface area [19].

Characteristics of the participants

The study sample consisted of 224 parents and 247 offspring. Table 1 presents the characteristics of 109 fathers, 115 mothers, 127 sons, and 120 daughters (mean age 39.7±12.7 years; body mass index 24.1±3.4). These included 130 hypertensive patients (27.6%), 75 of whom took antihypertensive drugs (40 calcium-channel blockers; 18 ACE inhibitors; 8 diuretics; 5 β-blockers; 21 various combination tablets of low-dose hydrochlorothiazide, reserpine, and dihydralazine). Nine (1.9%) subjects were diabetic; six (1.3%)

Table 1 Characteristics of the participants

	Parents (*n*=224)		Offspring (*n*=247)	
	Fathers (*n*=109)	Mothers (*n*=115)	Sons (*n*=127)	Daughters (*n*=120)
Age (years)	53.1±4.1	51.5±3.6	28.0±4.4	28.7±5.3
Lean body mass (kg)[a]	55.5±3.6	36.8±2.0	56.1±3.5	37.1±1.8
Body-mass index	24.3±3.1	24.9±4.0	24.1±3.4	23.2±3.1
Systolic blood pressure (mmHg)[b]	137.2±19.4	135.3±20.5	121.3±11.2	111.4±10.4
Diastolic blood pressure (mmHg)[b]	85.6±11.8	82.4±10.0	79.9±11.5	72.0±8.0
Mean arterial pressure (mmHg)[c]	102.8±13.7	100.0±12.7	93.7±11.0	85.1±8.4
Taking antihypertensive drugs	33 (29.7%)	33 (28.7%)	7 (5.5%)	4 (3.3%)
Diabetic patients[d]	4 (3.7%)	4 (3.5%)	1 (0.8%)	0
Current smoking	63 (57.8%)	3 (2.6%)	78 (61.4%)	0
Alcohol intake	33 (30.3%)	0	57 (44.9%)	0
Fasting plasma glucose (mmol/l)	4.83±0.97	5.12±1.09	4.60±0.86	4.75±0.76
Serum total cholesterol (mmol/l)	4.16±0.85	4.37±1.05	4.14±1.00	4.06±1.00
Serum triglycerides (mmol/l)	1.41±0.76	1.28±0.67	1.45±1.09	1.10±0.49
Serum uric acid (μmol/l)	254.5±75.1	210.1±55.0	275.2±74.5	202.2±55.1
Serum creatinine (μmol/l)	76.4±17.6	65.8±19.6	75.4±15.8	66.3±21.4
Creatinine clearance (ml min^{-1} 1.73 m^{-2})[e]	98.0±26.8	97.0±23.0	125.9±26.8	118.6±31.0

[a] Calculated according to Kvist et al. [20]
[b] Mean of ten blood pressure readings (five at each of two separate home visits)
[c] Sum of 1/3 of systolic pressure and 2/3 of diastolic pressure
[d] Use of antidiabetic agents or a fasting plasma glucose ≥7.1 mmol/l [17]
[e] Calculated using Cockroft and Gault's [21] formula

women used oral contraceptives and none took hormonal replacement therapy. The mean serum creatinine level was 71 μmol/l (range 38–148); mean calculated creatinine clearance was 111 ml min^{-1} 1.73 m^{-2} (34–206) and serum uric acid 236 μmol/l (88–442). None of the study subjects had been diagnosed with gout or were taking uric acid-lowering agents. Four men but no women had hyperuricemia [serum uric acid ≥ 416 μmol/l (7.0 mg/dl) in men and ≥ 387 μmol/l (6.5 mg/dl) in women]. In both parents and offspring the serum creatinine and uric acid levels were higher in men than in women (Fig. 1). Serum uric acid, but not serum creatinine varied with age and sex: it decreased in men (r=–0.15, P=0.03) but tended to increase in women (r=0.12, P=0.07). Hypertensive patients had a significantly higher serum uric acid concentration than normotensive subjects (258 vs. 238 μmol/l, P=0.006). However, none of the antihypertensive drugs had a significant effect on serum uric acid (P>0.16).

Determination of genotypes

Genomic DNA was extracted from peripheral blood. The ACE I/D polymorphism was detected as described by Morgan et al. [16]. Allelic discrimination of the α-adducin Gly460Trp polymorphism was carried out as previously described [3] using a 5′nuclease assay [22] on an ABI Prism 7700 apparatus (Perkin Elmer, Foster City, Calif., USA). For determination of the –344C/T aldosterone synthase genotypes we also used the 5′ nuclease detection assay on an ABI Prism 7700 sequence detection system. The forward and reverse primers and the –344T and –344C probes were:

- 5′CTAAATCTGTGGTATAAAAATAAAGTCTATTAAAA-TAAAAGA
- 5′TTTCTCCAGGGCTGAGAGGA
- 5′FAM-CAAGGCTCCCTCTCATCTCACGATAAG-TAMRA
- 5′VIC-AAGGCCCCCTCTCATCTCACGATA-TAMRA

Per 25 μl the PCR fluid contained 50 ng DNA, 300 nmol primers, 70 nmol FAM probe and 50 nmol VIC probe. The amplification conditions were 50°C for 2 min and 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 61°C for 1 min.

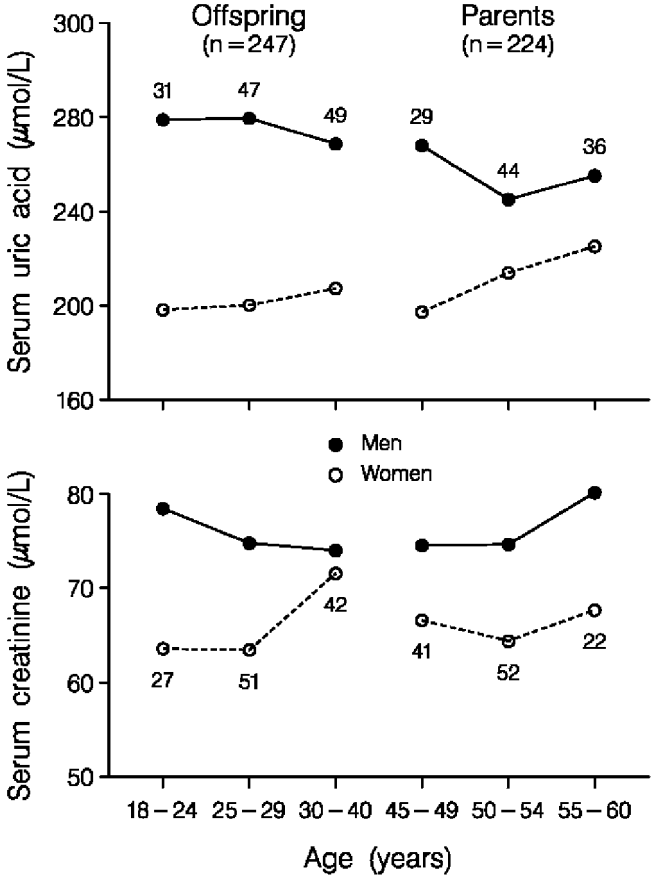

Fig. 1 Mean serum concentrations of uric acid and creatinine by sex and age in parents and offspring. For each subgroup the number of subjects is given

Statistical methods

We used SAS version 8.1 (SAS Institute, Cary, N.C., USA) for database management and statistical analysis. Comparisons of means and proportions relied on the standard normal *Z*-test and Fisher's exact test, respectively. Correlation coefficients were calculated using Pearson's method. We identified covariables by stepwise multiple regression analysis. We studied genetic associations using generalized estimating equations (GEE) to account for the nonindependence of the phenotypic measurements within families [23], while controlling for covariables and confounders. To take advantage of the family structure we also ran the quantitative transmission disequilibrium test (QTDT) program (version 2.4.2, http://www.well.ox.ac.uk/asthma/QTDT) [24]. With similar adjustments as in the other analyses, we investigated the association between the renal phenotypes and allelic transmission using the orthogonal model in a variance decomposition framework [25].

Results

Single gene association analyses

In 224 parents the frequencies of the ACE I/D (P=0.92), α-adducin Gly460Trp (P=0.18), and aldosterone synthase –344C/T (P=0.42) genotypes did not deviate from Hardy-Weinberg equilibrium. Similar genotype frequencies were observed in 247 offspring (Table 2). In GEE analyses we adjusted serum creatinine and uric acid for sex, age, age^2, body mass index, mean arterial pressure, fasting plasma glucose, serum triglycerides, current smoking (0, 1), alcohol intake (0, 1), and the use of diuretics (0, 1). With adjustment for these covariables, serum uric acid was significantly and positively associated with the ACE D (P=0.007) and α-adducin Trp alleles (P=0.03; Table 3). Serum uric acid concentration was higher in ACE DD homozygotes than in ID and II subjects by 15.7 μmol/l [95% confidence interval (CI) 3.3–28.2, P=0.01] and 25.7 μmol/l (11.1–40.2, P=0.0005), respectively; it was lower in α-adducin GlyGly homozygotes than in GlyTrp and TrpTrp subjects by 15.3 μmol/l (3.4–27.1, P=0.01) and 18.1 μmol/l (4.9–31.3, P=0.007), respectively. The aldosterone synthase TT homozygotes tended to have higher serum uric acid than the C allele carriers (P=0.09).

Table 2 Genotype frequencies in parents and offspring

Gene	Genotype		
ACE gene	II	ID	DD
Parents	86 (38.4%)	105 (46.9%)	33 (14.7%)
Offspring	93 (37.7%)	106 (42.9%)	48 (19.4%)
α-Adducin gene	GlyGly	GlyTrp	TrpTrp
Parents	50 (22.3%)	101 (45.1%)	73 (32.6%)
Offspring	61 (24.7%)	119 (48.2%)	67 (27.1%)
Aldosterone-synthase gene	CC	CT	TT
Parents	15 (6.7%)	95 (42.4%)	114 (50.9%)
Offspring	17 (6.9%)	108 (43.7%)	122 (49.4%)

Values are number of subjects (percentage of column total)

Table 3 Renal function in relation to single genes. Analyses adjusted for sex, age, body mass index, mean arterial pressure, fasting plasma glucose, serum triglycerides, smoking, alcohol intake, and use of diuretics

Gene and genotype	Serum creatinine (μmol/l)		Serum uric acid (μmol/l)	
	Mean ±SE	P[a]	Mean ±SE	P[a]
ACE		0.72		0.007
II (n=179)	68.5±2.0		228.3±4.7	
ID (n=211)	70.3±1.5		238.2±4.5	
DD (n=81)	70.2±2.1		254.0±6.2	
α-Adducin		0.25		0.03
GlyGly (n=111)	70.0±2.1		225.0±5.5	
GlyTrp (n=220)	71.1±1.5		240.3±4.9	
TrpTrp (n=140)	67.4±1.8		243.1±4.7	
Aldosterone-synthase		0.46		0.27
CC (n=32)	70.6±1.8		220.7±12.2	
CT (n=203)	71.0±1.6		236.3±5.3	
TT (n=236)	68.5±1.6		241.2±3.9*	

*P=0.09 vs. C-allele carriers
[a] Analysis of variance

Table 4 Renal function in relation to multiple genes. Analyses adjusted for sex, age, body mass index, mean arterial pressure, fasting plasma glucose, serum triglycerides, current smoking, alcohol intake, and use of diuretics

Gene and genotype	Serum creatinine (μmol/l)		Serum uric acid (μmol/l)	
	Mean ±SE	P[a]	Mean ±SE	P[a]
α-Adducin/ACE		0.26		0.09
GlyGly/II (n=55)	68.2±2.4		220.9±6.8	
Gly/Gly/ID (n=42)	70.5±3.1		222.9±7.4	
GlyGly/DD (n=14)	77.4±4.0		246.6±12.7	
GlyTrp/II (n=76)	70.7±2.0		228.2±7.0	
GlyTrp/ID (n=105)	70.6±1.8		236.6±6.5	
GlyTrp/DD (n=39)	73.3±2.4		269.8±6.5	
TrpTrp/II (n=48)	67.9±2.9		237.3±8.0	
TrpTrp/ID (n=64)	69.3±1.8		248.8±6.5	
TrpTrp/DD (n=28)	65.1±2.8		236.2±8.8	
Aldosterone-synthase/ACE		0.42		0.05
CC/II (n=12)	67.8±2.3		202.0±14.1	
CC/ID (n=14)	74.0±3.0		235.7±21.9	
CC/DD (n=6)	70.6±3.7		218.2±9.9	
CT/II (n=81)	70.8±2.0		220.8±7.0	
CT/ID (n=84)	71.9±2.6		234.3±6.9	
CT/DD (n=38)	69.6±3.1		267.2±8.0	
TT/II (n=86)	67.1±2.8		238.8±5.8	
TT/ID (n=113)	68.9±1.5		240.2±5.2	
TT/DD (n=37)	70.9±2.1		248.0±8.9	

[a] For interaction between the ACE genotype and the α-adducin or the aldosterone synthase genotype

Analyses involving multiple genes

In a further step of the GEE analyses we observed that the association of the ACE genotype with serum uric acid ($P \leq 0.09$ for interactions), but not serum creatinine, was confined to subjects who also carried the α-adducin Gly allele, the aldosterone synthase C allele (Table 4), or both alleles. Thus we performed analyses in the α-adducin

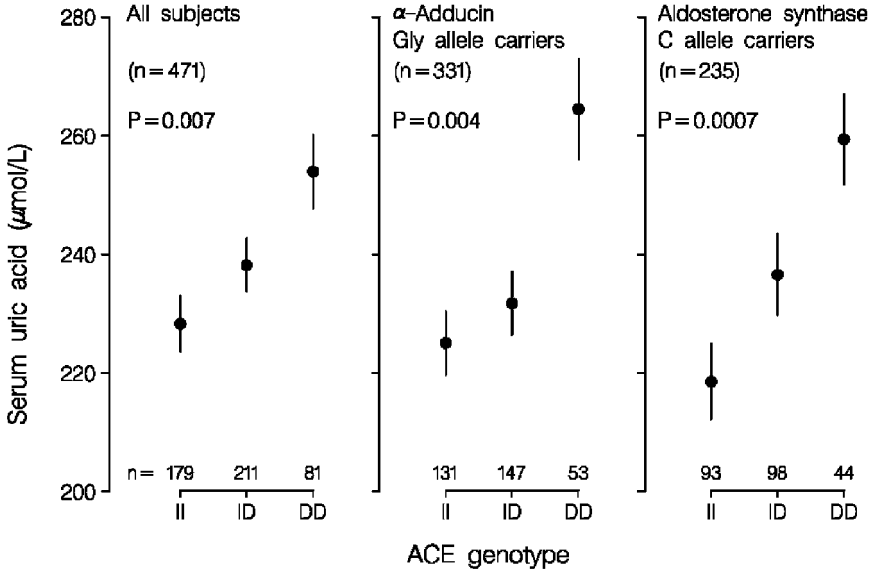

Fig. 2 Serum uric acid concentration by ACE genotype in all subjects, and in carriers of the α-adducin Gly allele, or the aldosterone-synthase C allele. Values were adjusted for sex, age, body mass index, mean arterial pressure, fasting plasma glucose, serum triglycerides, current smoking, alcohol intake, and use of diuretics. *Vertical lines* SE. *P* values were calculated using analysis of variance. For each genotype the number of subjects (*n*) is given

Table 5 Sensitivity analyses of the association between renal function and the ACE I/D polymorphism. Analyses adjusted for sex, age, body mass index, mean arterial pressure, fasting plasma glucose, serum triglycerides, current smoking, alcohol intake, and use of diuretics

Gene and genotype	Serum creatinine (μmol/l)		Serum uric acid (μmol/l)	
	Mean ±SE	P^a	Mean ±SE	P^a
Parents		0.59		0.01
II (*n*=86)	70.2±1.8		223.5±5.8	
ID (*n*=105)	71.5±2.0		233.3±6.4	
DD (*n*=33)	73.0±2.7		251.5±8.0	
Offspring		0.85		0.09
II (*n*=93)	71.0±2.7		235.6±5.7	
ID (*n*=106)	69.6±2.0		241.1±6.1	
DD (*n*=48)	70.8±2.7		258.3±8.4	

[a] Analysis of variance

GlyGly or aldosterone synthase CC homozygotes together with their corresponding heterozygotes (Fig. 2). In 331 carriers of the α-adducin Gly allele serum uric acid concentration was higher in ACE DD homozygotes than in ACE ID and II subjects by 31.4 μmol/l (95% CI 14.5–48.3, P=0.0003) and 39.3 μmol/l (20.4–58.2, P=0.0001), respectively (Fig. 2). The corresponding increases in the serum uric acid levels ($P \leq 0.009$) were 23.7 (6.0–41.5) and 40.6 μmol/l (21.6–59.7) in 235 carriers of the aldosterone synthase C allele (Fig. 2), and 41.9 (19.3–64.5) and 50.5 μmol/l (27.1–73.9) in 176 subjects who carried both the α-adducin Gly and aldosterone synthase C alleles.

Sensitivity analyses

Sensitivity analyses in parents and offspring confirmed the results in all subjects (Table 5, Fig. 3). Serum uric acid concentration was higher ($P \leq 0.02$) in ACE DD than

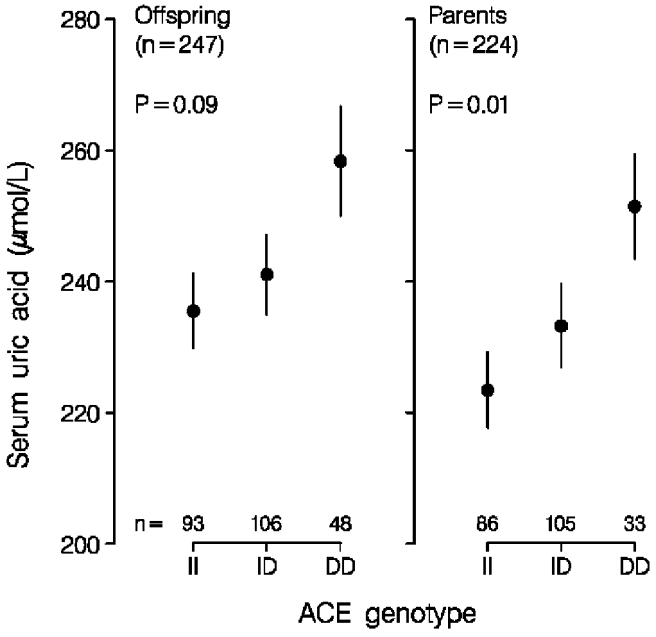

Fig. 3 Serum uric acid concentration by ACE genotype in parents and offspring. Values were adjusted for sex, age, body mass index, mean arterial pressure, fasting plasma glucose, serum triglycerides, current smoking, alcohol intake, and use of diuretics. *Vertical* lines SE. *P* values were calculated using analysis of variance. For each genotype the number of subjects (*n*) is given

II homozygotes by 28.0 μmol/l (95% CI 10.5–45.5) in all parents, and by 27.4 (3.5–51.3), 45.0 (19.2–70.9), and 48.8 μmol/l (12.9–84.7), respectively, in parents carrying the α-adducin Gly allele, the aldosterone synthase C allele, or both alleles. In offspring the corresponding increases ($P \leq 0.04$) were 22.8 (3.9–42.7), 40.3 (13.6–66.9), 28.5 (0.9–56.0), and 43.3 μmol/l (8.4–78.2), respectively.

QTDT analyses

Our study sample included 14 one-parent families with two (n=10) or more (n=4) offspring and 102 two-parent families with one (n=14), two (n=78), or more (n=10) offspring. Among the offspring we identified Mendelian inheritance errors in 18 subjects. With regard to the three genes under study the orthogonal model did not reveal significant population stratification ($0.12<P<0.99$).

In 157 informative offspring the orthogonal model only showed a slight increase in the serum uric acid concentration [regression coefficient (β)=10.7 μmol/l; P=0.19] in relation to transmission of the ACE D allele. However, in 114 informative offspring carrying the α-adducin Gly allele, this parameter increased to 20.5 μmol/l (P=0.03) whereas in 38 informative offspring homozygous for the α-adducin Trp allele the association between the serum uric acid concentration and transmission of the ACE D allele tended to be inverse (β=−17.8 μmol/l, P=0.27).

Discussion

The key finding of our study was that in Han Chinese serum uric acid was higher in the presence of the ACE D allele. This association was confined to carriers of the α-adducin Gly and/or aldosterone synthase Calleles. Overall, serum uric acid was 25.7 μmol/l (P=0.0005) higher in the ACE DD than II homozygotes. This difference increased to 39.3, 40.6, and 50.5 μmol/l in carriers of the α-adducin Gly allele, the aldosterone-synthase C allele, or both alleles (P=0.0001 for all). These associations were independent of sex, age, body mass index, blood pressure, use of antihypertensive drugs, and metabolic risk factors for hyperuricemia, such as alcohol intake, hyperglycemia, and hypertriglyceridemia. Sensitivity analyses in parents and offspring and QTDT analyses which allowed for population stratification and admixture [24] were confirmatory. Furthermore, in Han Chinese the α-adducin Trp allele was associated with higher serum uric acid concentration. This observation corroborated our previous report which showed that in untreated Italian hypertensive patients the urinary fractional excretion of uric acid was significantly lower in α-adducin Trp allele carriers than GlyGly homozygotes [11].

Previous studies did not specifically addressed the association between serum uric acid and the ACE I/D polymorphism, although several [26, 27, 28, 29, 30] have reported serum levels according to the ACE genotype. In agreement with the present findings, these studies [26, 27, 28, 29, 30] consistently showed that serum uric acid was slightly but not significantly higher in carriers of the ACE D allele. In Japanese hypertensive patients [30] serum uric acid was 0.34 mg/dl (approx. 20 μmol/l) higher in 187 ACE D allele carriers than in 131 II homozygotes. This difference was similar to the effect size observed in the present study. The mean differences in the other studies [26, 27, 28, 29] were within the 95% confidence interval of our estimate. Changes in the function of the proximal renal tubules or in the renal microcirculation might explain why serum uric acid was associated with the ACE I/D polymorphism. Indeed, the presence of the ACE D allele leads to higher systemic [31] and tissue [32] ACE levels and probably also stimulates the generation of angiotensin II [33]. In healthy Japanese subjects angiotensin II infusion deceased the fractional renal clearance of uric acid [34]. The ACE D allele is also associated with a decreased renal blood flow [35], which may stimulate the tubular reabsorption of uric acid [36].

The presence of the −344T allele in the promoter region of the aldosterone synthase gene stimulates aldosterone synthesis independent of the regulation by angiotensin II and potassium [37, 38]. The possible effect of aldosterone on the renal handling of uric acid remains to be clarified. Nonetheless, two recent randomized placebo-controlled trials [39, 40] demonstrated that long-term use of aldosterone blockers tended to reduce the incidence of hyperuricemia in patients with left ventricular dysfunction. We therefore speculate that aldosterone increases the proximal renal tubular reabsorption of uric acid in association with sodium. Thus the ACE D, α-adducin Trp, and aldosterone synthase −344T alleles likely share a common pathway for the elevation in serum uric acid, such as sodium sensitivity. Indeed, there is evidence that the ACE D, α-adducin Trp, and aldosterone synthase −344T alleles are associated with increased sodium reabsorption in the kidney [5, 41, 42]. Moreover, Asians have higher frequencies of the latter two alleles than whites [6, 7, 8], and tend to be more sodium-sensitive [42]. If all these three genetic variants affect serum uric acid via a common sodium-sensitive mechanism, the α-adducin Trp and aldosterone synthase −344T alleles may counteract the effect of the ACE D allele on serum uric acid. This might explain why in multiple gene analyses the uric acid increasing effect of the ACE D allele was strengthened in the presence of the α-adducin Gly and/or aldosterone synthase C alleles, but weakened in subjects homozygous for the α-adducin Trp or aldosterone synthase T alleles, which in fact were associated with a higher serum uric acid in single gene analyses.

The present results must be interpreted within the context of their limitations. We used single measurements of serum creatinine and uric acid. These biochemical measurements may fluctuate over time and are influenced by several factors, such as fluid balance, diet, and concomitant diseases. On the other hand, as in previous studies irrespective of race [1, 43, 44, 45, 46, 47, 48], we found that the serum concentrations of creatinine and uric acid were higher in men than in women, particularly in younger subjects. Furthermore, serum levels of creatinine and uric acid in the present study were similar to those previously observed in Chinese [8] and Japanese [27] but were lower than those in whites [1, 26, 45, 46] and blacks [47, 48]. The lower serum concentrations of creatinine and uric acid in Asians are probably due to the lower prevalence of metabolic risk factors, such as obesity, insulin resistance, hypertriglyceridemia, alcohol intake, and the use of diuretics [13]. Nonetheless, our findings in Han

Chinese remain to be confirmed in other Asian populations.

In conclusion, the present study extends our previous findings on the combined effects of three candidate genes [1, 2, 3, 4], supporting the concept that these genetic polymorphisms jointly determine a clinical entity characterized by slight deteriorations of renal function. If confirmed, our findings may have clinical implications, because in Chinese and whites the serum uric acid level predicts cardiovascular mortality and morbidity [36, 43, 49]. Furthermore, several recent experimental studies demonstrated that uric acid might play a pathogenic role in the development of hypertension and in the progression of renal disease [13].

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References

1. Wang JG, et al (2001) Renal function in relation to three candidate genes. Am J Kidney Dis 38:1158–1168
2. Balkestein EJ, et al (2002) Carotid and femoral intima–media thickness in relation to three candidate genes in a Caucasian population. J Hypertens 20:1551–1561
3. Staessen JA, et al (2001) Effects of three candidate genes on prevalence and incidence of hypertension in a Caucasian population. J Hypertens 19:1349–1358
4. Balkestein EJ, et al (2001) Carotid and femoral artery stiffness in relation to three candidate genes in a white population. Hypertension 38:1190–1197
5. Wang JG, Staessen JA (2000) Genetic polymorphisms in the renin-angiotensin system: relevance for susceptibility to cardiovascular disease. Eur J Pharmacol 410:289–302
6. Matsubara M, et al (2002) Aldosterone synthase gene (*CYP11B2*) C-334T polymorphism, ambulatory blood pressure and nocturnal decline in blood pressure in the general Japanese population: the Ohasama Study. J Hypertens 19:2179–2184
7. Sugimoto K, et al (2001) Alpha-adducin Gly460Trp polymorphism is associated with low renin hypertension in younger subjects in the Ohasama Study. J Hypertens 20:1779–1784
8. He X, et al (2001) α-Adducin gene and essential hypertension in China. Clin Exp Hypertens 23:579–589
9. Kunz R, et al (1998) Association between the angiotensin-converting enzyme insertion/deletion polymorphism and diabetic nephropathy: a methodologic appraisal and systematic review. J Am Soc Nephrol 9:1653–1663
10. Brand E, et al (1998) Structural analysis and evaluation of the aldosterone synthase gene in hypertension. Hypertension 32:198–204
11. Manunta P, et al (1999) Adducin polymorphism affects renal proximal tubule reabsorption in hypertension. Hypertension 33:694–697
12. Barlassina C, et al (2000) Synergistic effect of α-adducin and ACE genes causes blood pressure changes with body sodium and volume expansion. Kidney Int 57:1083–1090
13. Johnson RJ, et al (2003) Is there a pathogenetic role for uric acid in hypertension and cardiovascular disease? Hypertension 41:1183–1190
14. Stolarz K, et al (2003) Host and environmental determinants of heart rate and heart rate variability in four European populations. J Hypertens 21:525–535
15. Kuznetsova T, et al (2002) Quality control of the blood pressure phenotype in the European Project on Genes in Hypertension. Blood Press Monit 7:215–224
16. Morgan L, et al (1999) Angiotensin-converting enzyme insertion-deletion polymorphism in normotensive and pre-eclamptic pregnancies. J Hypertens 17:765–768
17. Alberti KGMM, et al (1998) Definition, diagnosis and classification of diabetes mellitus and its complications. I. Diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. Diabet Med 15:539–553
18. Praetorius E (1949) Enzymatic method for determination of uric acid by ultraviolet spectrophotometry. Scand J Clin Lab Invest 1:222–230
19. Gehan EA, George SL (1970) Estimation of human body surface area from height and weight. Cancer Chemother Rep 54:225–235
20. Kvist H, et al (1988) Total and visceral adipose-tissue volumes derived from measurements with computed tomography in adult men and women: predictive equations. Am J Clin Nutr 48:1351–1361
21. Cockcroft DW, Gault MH (1976) Prediction of creatinine clearance from serum creatinine. Nephron 16:31–41
22. Livak KJ, et al (1995) Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. PCR Methods Appl 4:357–362
23. Zeger SL, et al (1988) Models for longitudinal data: a generalized estimating equation approach. Biometrics 44:1049–1060
24. Abecasis GR, et al (2000) A general test of association for quantitative traits in nuclear families. Am J Hum Genet 66:279–292
25. Abecasis GR, et al (2001) Association analysis in a variance components framework. Genet Epidemiol 21 [Suppl 1]:S341–S346
26. Rossi GP, et al (2001) Exclusion of the ACE D/I gene polymorphism as a determinant of endothelial dysfunction. Hypertension 37:293–300
27. Matsubara M, et al (2002) Angiotensin-converting enzyme I/D polymorphism and hypertension: the Ohasama study. J Hypertens 20:1121–1126
28. Lee YJ, Tsai JCR (2002) ACE gene insertion/deletion polymorphism associated with 1998 World Health Organization definition of metabolic syndrome in Chinese type 2 diabetic patients. Diabetes Care 25:1002–1008
29. Yildiz A, et al (2002) The effect of angiotensin converting enzyme gene polymorphism on chronic allograft dysfunction in living donor renal transplant recipients. Clin Transplant 16:173–179
30. Kario K, et al (1998) Endothelial cell damage and angiotensin-converting enzyme insertion/deletion genotype in elderly hypertensive patients. J Am Coll Cardiol 32:444–450
31. Rigat B, et al (1990) An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 86:1343–1346
32. Danser AHJ, et al (1995) Angiotensin-converting enzyme in the human heart. Effect of the deletion/insertion polymorphism. Circulation 92:1387–1388
33. Müller DN, et al (1997) Vascular angiotensin-converting enzyme expression regulates local angiotensin II. Hypertension 29:98–104
34. Moriwaki Y, et al (2002) Effects of angiotensin II infusion on renal excretion of purine bases and oxypurinol. Metabolism 51:893–895
35. van der Kleij FGH, et al (2002) Enhanced responses of blood pressure, renal function and aldosterone to angiotensin I in DD

genotype are blunted by low sodium intake. J Am Soc Nephrol 13:1025–1033
36. Messerli FH, et al (1980) Serum uric acid in essential hypertension: an indicator of renal vascular involvement. Ann Intern Med 93:817–821
37. Curnow KM, et al (1991) The product of the CYP11B2 gene is required for aldosterone biosynthesis in the human adrenal cortex. Mol Endocrinol 5:1513–1522
38. Clyne CD, et al (1997) Angiotensin II and potassium regulate human CYP11B2 transcription through common *cis* elements. Mol Endocrinol 11:638–649
39. Pitt B, et al (1999) The effect of spironolactone on morbidity and mortality in patients with sever heart failure. N Engl J Med 341:709–717
40. Pitt B, et al (2003) Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. N Engl J Med 348:1309–1321
41. Lim PO et al. (2002) Variation in the aldosterone synthase (CYP11B2) locus contributes to hypertension in subjects with a raised aldosterone to renin ratio. J Clin Endocrinol Metab 87:4398–4402
42. Katsuya T, et al (2003) Salt sensitivity of Japanese from the viewpoint of gene polymorphism. Hypertens Res 26:521–525
43. Wang JG, et al (2001) Prognostic significance of serum creatinine and uric acid in older Chinese patients with isolated systolic hypertension. Hypertension 37:1069–1074
44. de Leeuw PW, et al (2002) Prognostic significance of renal function in elderly patients with isolated systolic hypertension: results from the Syst-Eur Trial. J Am Soc Nephrol 13:2213–2222
45. Culleton BF, et al (1999) Prevalence and correlates of elevated serum creatinine levels. The Framingham Heart Study. Arch Intern Med 159:1785–1790
46. Culleton BF, et al (1999) Serum uric acid and risk for cardiovascular disease and death: the Framingham Heart Study. Ann Intern Med 131:7–13
47. Freedman DS, et al (1995) Relation of serum uric acid to mortality and ischemic heart disease. The NHANES I Epidemiologic Follow-up Study. Am J Epidemiol 141:637–644
48. Jones CA, et al (1998) Serum creatinine levels in the US populations: third National Health and Nutrition Exmination Survey. Am J Kidney Dis 32:992–999
49. Wang JG, Staessen JA (2002) Raised concentrations of serum creatinine and uric acid and the risks of mortality and cardiovascular disease. Cardiovasc Rev Rep 23:393–397