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Association between hypertension and variation in the α- and β-adducin genes in a white population

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Background. The substitution of tryptophan for glycine at amino acid 460 (Gly460Trp polymorphism) of the α-subunit of the heterodimeric cytoskeleton protein adducin increases renal sodium reabsorption and may be involved in the pathophysiology of essential hypertension. In the present study, we investigated in multivariate analyses whether the risk of hypertension was associated with the C1797T polymorphism of the β-adducin gene.

Methods. A total of 1848 subjects randomly selected from a white population were genotyped. Study nurses measured blood pressure at the participants' homes.

Results. The frequencies of the α-adducin Trp and β-adducin T alleles were 0.23 and 0.11, respectively. In men ($N = 904$), the β-adducin T allele was not associated with hypertension [adjusted relative risk (RR) vs. CC homozygotes 0.94, $P = 0.77$], but T allele carriers had lower plasma renin activity (PRA) and 24-hour urinary aldosterone excretion ($P < 0.04$). In all women ($N = 944$), β-adducin T allele carriers had a higher risk of hypertension than CC homozygotes (RR 1.81, CI 1.18–2.77, $P = 0.007$), but similar PRA and 24-hour urinary aldosterone excretion ($P > 0.29$). In 345 post-menopausal women and 190 users of oral contraceptives, the RRs of hypertension were 2.47 (CI 1.34–4.64, $P = 0.003$) and 2.56 (CI 0.83–7.86, $P = 0.10$), respectively. For systolic pressure in women, there was a significant interaction ($P = 0.02$) between the α- and β-adducin polymorphisms. Only in female carriers of the mutated α-adducin Trp allele was the systolic pressure significantly higher in β-adducin T allele carriers compared with CC homozygotes (+3.8 mm Hg, $P = 0.02$). Furthermore, in the presence of the mutated α-adducin Trp allele, the RRs associated with the β-adducin T allele were 2.35 ($P = 0.01$) in all women, 2.92 ($P = 0.03$) in post-menopausal subjects, and 3.79 ($P = 0.09$) in users of oral contraceptives.

Conclusions. The 1797T allele of the β-adducin gene is associated with increased risk of hypertension in post-menopausal women and in users of oral contraceptives, particularly in the presence of the mutated α-adducin Trp allele. We hypothesize that inhibition of the renin-aldosterone system in men and absence of such a compensatory mechanism in women may explain, at least to some extent, the sexual dimorphism of the blood pressure phenotype in relation to the C1797T β-adducin polymorphism.

Key words: adducin, hypertension, blood pressure, renin, candidate gene, blood pressure, point mutation, C1797T polymorphism.

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Adducin is a ubiquitously expressed membrane-skeleton protein, which is composed of either α and β or α and γ subunits that to a large extent are similar in amino acid sequence and domain organization [1]. Adducin plays an important role in the determination of cellular morphology and motility and in the regulation of membrane ion transport [1–3]. Point mutations of the α- and β-adducin account for up to 50% of the blood pressure difference between Milan normotensive and hypertensive rat strains [4], probably via the modulation of the Na^+-K^+ ATPase activity [2, 3]. The Gly460Trp polymorphism of the human α-adducin gene is associated with sodium sensitivity [5–8]. Hypertensive carriers of the 460Trp allele of the α-adducin gene, compared with GlyGly homozygotes, show an enhanced membrane sodium transport rate in erythrocytes [8], higher proximal tubular renal reabsorption of sodium [6, 7], and larger blood pressure changes in response to sodium loading or diuretic treatment [5]. Several [5, 9, 10], though not all [11, 12], studies also found an association between blood pressure or hypertension and the Gly460Trp polymorphism of the α-adducin gene [13].

In β-adducin deficient knockout mice, the amount of α-adducin in the membrane skeleton of erythrocytes de-

creases by 30% [14] to 80% [15], whereas the incorporation of γ-adducin rises four- [15] to fivefold [14]. Furthermore, β-adducin deficient mice have higher levels of systolic and diastolic blood pressures and pulse pressure [16]. In humans, the genes encoding the α- and β-adducin subunits have been localized to chromosome 4p16 [17] and 2p13 [18], respectively. The human β-adducin gene comprises 17 exons [19]. A common single-nucleotide polymorphism (C→T) in the β-adducin gene has been identified at position 1797 in exon 15 (C1797T, starting from ATG; SNP number, rs4984; URL, *http://www.ncbi.nlm.nih.gov/SNP/*). This change is silent in terms of amino acid composition of β-adducin. However, alternative splicing including exon 15 causes a frame shift and early termination, yielding the isoform 4, which is a smaller peptide with an alternative carboxy-terminus as compared with the first described isoform 1 [19]. In the present study, we investigated whether the risk of hypertension was associated with the C1797T polymorphism of the β-adducin gene in the presence or absence of the mutated α-adducin Trp allele. The rationale for exploring the interaction between the two adducin polymorphisms was that, if present, it would be compatible with a functional role of adducin rather than with effects of other polymorphisms in linkage disequlibrium with the genes encoding the adducin subunits.

METHODS

Study population

The protocol of the Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO) was approved by the Ethics Committee of the University of Leuven. All subjects gave informed consent. From August 1985 until November 1990, a random sample of the households living in a geographically defined area of Northern Belgium [20] was investigated with the goal to recruit an equal number of participants in each of six subgroups by sex and age (20 to 39, 40 to 59, and ≥60 years). All household members with a minimum age of 20 years were invited to take part, until the quota of their sex-age group had been fulfilled. To further study the role of genetic factors, from June 1996 until December 2000, nuclear families including children who were at least 10 years old were recruited, using the former participants (1985 to 1990) as index persons.

The study population included 2272 persons. The participation rate among the subjects contacted was 66.1%. The blood pressure of 21 participants had not been measured. Blood for DNA extraction could not be obtained from 403 former participants, because they did not consent ($N = 161$) or because they had died ($N = 180$), were terminally ill ($N = 28$), or had moved out of the area ($N = 34$). Thus, 1848 subjects were included in this analysis.

Field work

Study nurses visited the participants repeatedly in their homes. The same observer measured a subject's blood pressure five times consecutively at each of two home visits four to six weeks apart. Conventional sphygmomanometry was performed according to the guidelines of the British Hypertension Society after the subjects had rested for five minutes in the sitting position. For analysis, we averaged the 10 blood pressure readings. Hypertension was diagnosed if the blood pressure was at least 140 mm Hg systolic or 90 mm Hg diastolic, or when the patients were on antihypertensive medication.

We used a standardized and validated [21] questionnaire to collect information on medical history, smoking habits, alcohol intake, use of medications and the menstrual cycle of women. In the interval between the two home visits, the participants collected a 24-hour urine sample in a wide neck plastic container for the measurement of sodium, potassium, aldosterone and creatinine. A venous blood sample for the measurement of plasma renin activity (PRA) was obtained usually within two weeks after the urine collection.

Determination of genotypes

Genomic DNA was extracted from peripheral blood. Allelic discrimination of the C1797T β-adducin polymorphism was carried out using the 5′ nuclease assay [22] on an ABI Prism 7700 apparatus (Perkin Elmer, Foster City, CA, USA). The forward and reverse primers and the 1797C and 1797T probes employed in the TaqMan assay were 5′-AGGAACGAGAGCCAGGCTCT-3′, 5′-TTCATCA AAACACACACCTACCAAT-3′, 5′-VIC-TTCTTCAG CGTTGCCCTCCACAT-TAMRA-3′ and 5′-FAM-TTC TTCAGTGTTGCCCTCCACATCTG-TAMRA-3′, respectively. Per 25 μL, the polymerase chain reaction (PCR) fluid contained 50 ng DNA, 200 nmol primers, 50 nmol FAM-probe and 100 nmol VIC-probe. The amplification conditions were 50°C for two minutes and 95°C for ten minutes, followed by 40 cycles at 95° for 15 seconds and 62°C for one minute. For determination of the Gly460Trp α-adducin gene polymorphism, PCR and subsequent genotyping were performed as described previously [23].

Statistical analysis

We used the Statistical Analysis System, version 8.1 (SAS Institute, Cary, NC, USA), for database management and statistical analysis. Continuous measurements with a skewed distribution were normalized by logarithmic transformation and represented by the geometric mean with 95% confidence interval. The Fisher exact test was used to compare genotype and allele frequencies between normotensive subjects and hypertensive patients. Analysis of covariance (ANCOVA) was performed to test genetic associations with continuous phenotypes by com-

Table 1. Characteristics of the participants

Characteristic	Men[a]	Women[a]	*P*
Number *%*	904 (48.9)	944 (51.1)	
Age *years*	42.2 ± 16.3	42.7 ± 16.2	0.54
Body mass index *kg/m²*	25.4 ± 3.9	25.0 ± 5.1	0.08
Systolic blood pressure *mm Hg*	125.5 ± 14.7	120.8 ± 16.7	<0.001
Diastolic blood pressure *mm Hg*	77.2 ± 10.5	74.1 ± 10.2	<0.001
Pulse rate *beat/min*	67.4 ± 9.2	70.8 ± 9.6	<0.001
Prevalence of hypertension	217 (24.0)	206 (21.8)	0.26
Taking antihypertensive drugs	91 (10.1)	121 (12.8)	0.06
Plasma renin activity *ng/L/sec*	0.49 (0.46–0.52)	0.47 (0.44–0.50)	0.23
Urinary volume *L/day*	1.48 ± 0.61	1.56 ± 0.70	0.02
Creatinine excretion *mmol/day*	13.7 ± 3.6	9.2 ± 2.4	<0.001
Sodium excretion *mmol/day*	202 ± 69	165 ± 58	<0.001
Potassium excretion *mmol/day*	77 ± 30	63 ± 24	<0.001
Urinary Na^+/K^+ ratio	2.84 ± 1.07	2.80 ± 1.10	0.42
Aldosterone excretion *nmol/day*	25.6 (24.3–26.9)	27.1 (25.5–28.8)	0.15

Values are arithmetic means ± SD, geometric means (95% confidence interval), or number of subjects (%).
[a] Plasma renin activity was measured in 711 men and 739 women. Urinary volume, creatinine and electrolytes were available in 889 men and 933 women, respectively. Urinary aldosterone was measured in 574 men and 598 women.

Table 2. Genotype and allele frequencies

Genes	Genotypes			*P*[a]	Alleles		*P*[a]
α-Adducin	GlyGly	GlyTrp	TrpTrp		Gly	Trp	
Men (*N* = 904)							
Normotensive	420 (61.1)	230 (33.5)	37 (5.4)		1070 (77.9)	304 (22.1)	
Hypertensive	137 (63.1)	72 (33.2)	8 (3.7)	0.63	346 (79.7)	88 (20.3)	0.46
Women (*N* = 944)							
Normotensive	423 (57.3)	268 (36.3)	47 (6.4)		1114 (75.5)	362 (24.5)	
Hypertensive	117 (56.8)	79 (38.4)	10 (4.8)	0.71	313 (76.0)	99 (24.0)	0.90
β-Adducin	CC	CT	TT		C	T	
Men (*N* = 904)							
Normotensive	549 (79.9)	131 (19.1)	7 (1.0)		1229 (89.4)	145 (10.6)	
Hypertensive	174 (80.2)	42 (19.3)	1 (0.5)	0.88	390 (89.9)	44 (10.1)	0.86
Women (*N* = 944)							
Normotensive	593 (80.4)	141 (19.1)	4 (0.5)		1327 (89.9)	149 (10.1)	
Hypertensive	152 (73.8)	51 (24.8)	3 (1.4)	0.06	355 (86.2)	57 (13.8)	0.04

[a] Fisher's exact test was used to compare hypertensive patients with normotensive subjects

paring wild-type homozygotes with carriers of mutated alleles, while controlling for covariables and confounders. Multiple logistic regression was used to estimate the adjusted relative risk of hypertension associated with the mutated alleles. To test genetic interactions, we defined dummy variables coded 0 or 1 depending on the absence or presence of the mutated alleles. Finally, our analyses were repeated using generalized estimating equations to allow for the non-independence of the phenotypes within families [24]. In the PROC GENMOD procedure of the SAS package, the intra-familial correlation matrices were defined based on our own data with adjustments applied for covariables.

RESULTS

Characteristics of the subjects

The 1848 participants included 904 men (48.9%) and 423 hypertensive patients (22.9%), of whom 212 were on antihypertensive drug treatment (Table 1). The subjects ranged in age from 10 to 84 years. Among men, 31.1% ($N = 281$) were current smokers, and 36.1% ($N = 326$) reported intake of alcohol. In women these proportions were 27.7% ($N = 262$) and 13.5% ($N = 127$), respectively. Three hundred and forty-five women (36.6%) reported natural or surgical menopause, 190 (20.1%) used oral contraceptives and 32 (3.4%) took hormonal replacement therapy.

Single-gene analyses

The frequencies of the α-adducin ($P = 0.65$) and β-adducin ($P = 0.14$) genotypes did not deviate from Hardy-Weinberg equilibrium. In unadjusted single-gene analyses, the prevalence of hypertension was not associated with the genotype or allele frequencies of the α-adducin gene, whereas the frequencies of the β-adducin CT and TT genotypes and the T allele tended to be higher in hypertensive compared with normotensive women (Table 2).

Table 3. Single-gene associations with systolic and diastolic pressures, urinary Na^+/K^+ ratio, plasma renin activity and aldosterone excretion

Variable	α-Adducin GlyGly		α-Adducin GlyTrp+TrpTrp			β-Adducin CC		β-Adducin CT+TT		
	N	Statistic	*N*	Statistic	*P*[a]	*N*	Statistic	*N*	Statistic	*P*[a]
Men										
Systolic pressure *mm Hg*	557	126.1 ± 0.6	347	124.5 ± 0.7	0.07	723	125.6 ± 0.5	181	125.2 ± 1.0	0.69
Diastolic pressure *mm Hg*	557	77.4 ± 0.4	347	76.7 ± 0.5	0.28	723	77.0 ± 0.3	181	77.9 ± 0.7	0.22
Urinary Na^+/K^+ ratio	504	2.81 ± 0.05	306	2.87 ± 0.06	0.46	651	2.84 ± 0.04	159	2.81 ± 0.08	0.68
Plasma renin activity *ng/L/sec*	393	0.50 (0.47–0.54)	250	0.47 (0.43–0.52)	0.33	513	0.51 (0.48–0.54)	130	0.44 (0.38–0.50)	0.04
Aldosterone excretion *nmol/day*	402	25.5 (23.7–27.3)	208	25.2 (23.1–27.4)	0.83	404	26.2 (24.7–27.8)	106	22.3 (19.9–25.1)	0.02
Urinary aldosterone/K^+ ratio	402	0.35 (0.32–0.37)	208	0.35 (0.32–0.38)	0.94	404	0.36 (0.33–0.38)	106	0.31 (0.27–0.35)	0.04
Women										
Systolic pressure *mm Hg*	540	121.0 ± 0.6	404	120.5 ± 0.6	0.58	745	120.6 ± 0.5	199	121.4 ± 0.9	0.46
Diastolic pressure *mm Hg*	540	74.3 ± 0.4	404	73.9 ± 0.4	0.41	745	73.8 ± 0.3	199	75.1 ± 0.6	0.08
Urinary Na^+/K^+ ratio	466	2.77 ± 0.05	355	2.83 ± 0.06	0.37	655	2.75 ± 0.04	166	2.98 ± 0.08	0.01
Plasma renin activity *ng/L/sec*	379	0.46 (0.42–0.50)	264	0.45 (0.41–0.49)	0.70	501	0.45 (0.42–0.49)	142	0.45 (0.39–0.51)	0.93
Aldosterone excretion *nmol/day*	295	27.5 (25.3–29.8)	206	26.8 (24.3–29.6)	0.72	388	27.7 (25.7–29.7)	113	25.7 (22.4–29.4)	0.34
Urinary aldosterone/K^+ ratio	295	0.45 (0.41–0.49)	206	0.46 (0.42–0.51)	0.65	388	0.45 (0.42–0.49)	113	0.45 (0.40–0.52)	0.97

Values are arithmetic means ± SE or geometric means (95% CI). Systolic and diastolic blood pressures were adjusted for age, age^2, body mass index, smoking, alcohol intake, and use of antihypertensive drugs or oral contraceptives. Plasma renin activity (PRA) and the urinary measurements were adjusted for age, body mass index and, in women, for the use of oral contraceptives. Urinary aldosterone excretion and PRA were also adjusted for 24-hour urinary sodium excretion, and for PRA, also for the time of day at which the blood had been sampled.

[a] *P* value for comparisons between GlyGly or CC homozygotes and Trp or T allele carriers, respectively

With adjustment for age, age^2, body-mass index, smoking, alcohol intake and use of antihypertensive drugs or oral contraceptives, the single-gene associations with blood pressure did not reach statistical significance (Table 3). However, with similar adjustments applied, we found for hypertension a significant interaction (P = 0.03) between the β-adducin C1797T polymorphism and gender (Fig. 1). In men, the risk of hypertension was similar across the β-adducin C1797T genotypes (P = 0.77), whereas in women it was significantly higher in T allele carriers than in CC homozygotes [relative risk (RR) 1.81, 95% confidence intervals (CI) 1.18-2.77, P = 0.007]. The latter association was driven by the results in 345 post-menopausal women (RR 2.47, 95% CI 1.34–4.64, P = 0.003) and 190 users of oral contraceptives (RR 2.56, 95% CI 0.83–7.86, P = 0.10), whereas it was not observed in the pre-menopausal subjects who did not take oral contraceptives (RR 0.85, 95% CI 0.35–2.06, P = 0.72). For α-adducin, the sex-by-genotype interaction was not significant (P = 0.14). If the non-independence of phenotypes within families also was accounted for, the above results remained unaltered.

In further analyses of PRA and the urinary measurements, we excluded participants using antihypertensive medications because of the divergent effects of different drug classes on the renin-aldosterone system. Men and women were analyzed separately. We allowed for age, body mass index (BMI), and in women also for the use of oral contraceptives. In addition, urinary aldosterone excretion and PRA were also adjusted for 24-hour urinary sodium excretion, and for PRA, also for the time of day at which the blood had been sampled. In men ($P < 0.04$), but not in women ($P > 0.34$), the adjusted values of PRA and urinary aldosterone excretion were significantly lower in β-adducin T allele carriers than in CC homozygotes (Table 3). In women, the urinary Na^+/K^+ ratio was higher in T allele carriers than in CC homozygotes (P = 0.01) and the strength of the latter association tended to increase with use of oral contraceptives (Table 4 and Fig. 2).

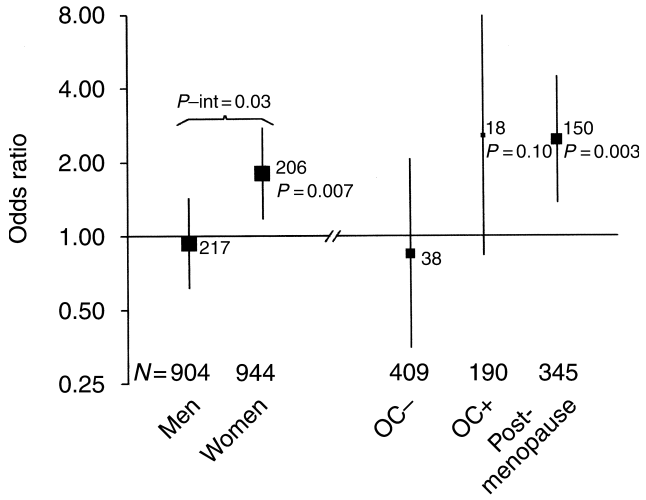

Fig. 1. Risk of hypertension in β-adducin T allele carriers versus CC homozygotes in men and women, in users (OC+) and non-users (OC−) of oral contraceptives and in post-menopausal women. Squares represent odds ratios adjusted for age, age^2, body-mass index, smoking, alcohol intake and, in all women, for the use of oral contraceptives. The size of the squares is proportional to the number of cases given alongside the symbols. Vertical lines denote 95% confidence intervals. Number of subjects (N) and significance levels for odds ratios (P) and for the sex-by-genotype interaction (P-int) are given.

Interaction between the α- and β-adducin genes

With similar adjustments as before, further analyses in women showed a significant interaction (P = 0.02)

Table 4. Single-gene associations between biochemical measurements and β-adducin polymorphism in women

Variable	CC		CT+TT		
	N	Statistic	*N*	Statistic	*P*[a]
Non-users of oral contraceptives					
Urinary Na^+/K^+ ratio	310	2.75 ± 0.07	79	3.00 ± 0.13	0.09
Plasma renin activity *ng/L/sec*	236	0.48 (0.44–0.54)	67	0.47 (0.38–0.56)	0.72
Aldosterone excretion *nmol/day*	185	28.8 (25.9–32.1)	54	26.1 (21.3–31.8)	0.38
Urinary aldosterone/K^+ ratio	185	0.48 (0.43–0.53)	54	0.48 (0.39–0.58)	0.97
Users of oral contraceptives					
Urinary Na^+/K^+ ratio	145	2.87 ± 0.09	37	3.39 ± 0.18	0.01
Plasma renin activity *ng/L/sec*	103	0.54 (0.47–0.62)	29	0.63 (0.49–0.82)[b]	0.28
Aldosterone excretion *nmol/day*	85	37.1 (31.6–43.6)[b]	28	33.7 (25.5–44.7)	0.56
Urinary aldosterone/K^+ ratio	85	0.61 (0.51–0.72)[b]	28	0.62 (0.47–0.83)[b]	0.89
Post-menopausal women					
Urinary Na^+/K^+ ratio	200	2.65 ± 0.07	50	2.68 ± 0.14	0.85
Plasma renin activity *ng/L/sec*	162	0.37 (0.32–0.42)	46	0.35 (0.27–0.45)	0.76
Aldosterone excretion *nmol/day*	118	20.6 (18.5–23.0)[b]	31	20.6 (16.7–25.5)	0.99
Urinary aldosterone/K^+ ratio	118	0.33 (0.30–0.37)[b]	31	0.33 (0.27–0.41)[b]	0.95

Values are arithmetic means ± SE or geometric means (95% CI), adjusted for age and body mass index. Urinary aldosterone excretion and plasma renin activity (PRA) were also adjusted for 24-hour urinary sodium excretion and, for PRA, also for the time of day at which the blood had been sampled.

[a] *P* value for comparisons between CC homozygotes and T allele carriers

[b] $P < 0.05$ vs. non-users of oral contraceptives

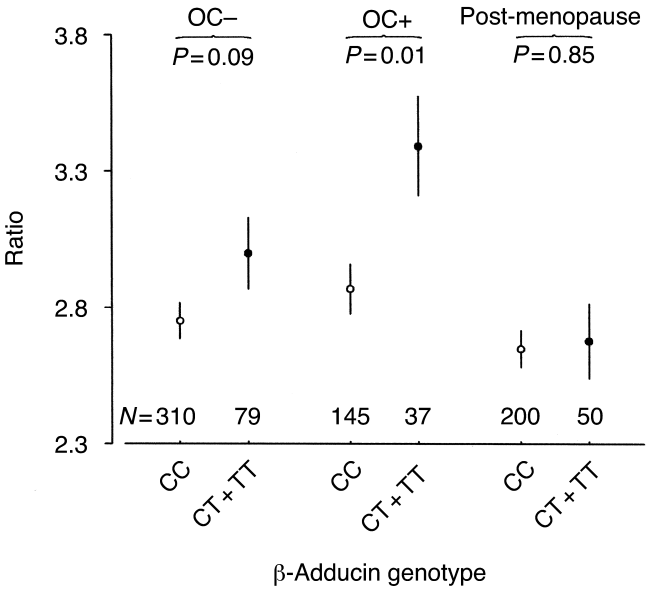

Fig. 2. Urinary Na^+/K^+ ratio by β-adducin genotype in users (OC+) and non-users (OC−) of oral contraceptives and in post-menopausal women. Circles (CC genotype) and dots (T allele carriers) represent means adjusted for age and body-mass index. Vertical lines denote SEs. Number of subjects (*N*) and significance levels (*P*) for the differences between T allele carriers and CC homozygotes are given.

between the α- and β-adducin polymorphisms in relation to systolic blood pressure. Only in the presence of the mutated α-adducin Trp allele was systolic pressure significantly higher in β-adducin T allele carriers than CC homozygotes (123.5 vs. 119.7 mm Hg, $P = 0.02$). Furthermore, in the presence of the mutated α-adducin Trp allele, the relative risks of hypertension associated with β-adducin T allele were 2.35 (95% CI 1.23–4.52, $P = 0.01$) in all women, 2.92 (95% CI 1.11–7.67, $P = 0.03$) in post-menopausal subjects and 3.79 (95% CI 0.82–17.52, $P = 0.09$) in users of oral contraceptives, respectively. Finally, in the presence of the mutated α-adducin Trp allele, PRA was lower in male β-adducin T allele carriers than their CC homozygous counterparts (geometric means 0.38 vs. 0.50, $P = 0.02$), whereas in female Trp allele carriers the opposite was observed for urinary Na^+/K^+ ratio (CT+TT vs. CC 3.12 vs. 2.77, $P = 0.02$).

DISCUSSION

In our white population, the mutated β-adducin T allele was associated with an approximately twofold increase in the risk of hypertension in post-menopausal women and in users of oral contraceptives. In contrast, the T allele of the β-adducin gene was not associated with hypertension in pre-menopausal women not using oral contraceptives and in men. In the presence of the mutated α-adducin Trp allele, the association between systolic pressure and the β-adducin T allele in all women was significantly enhanced, while similar trends were observed for the risk of hypertension in all women, users of oral contraceptives and post-menopausal subjects. The prevalence of hypertension in post-menopausal women was 43%. The positive predictive value and attributable risk associated with the β-adducin T allele therefore may be estimated to be 80.9% and 24.7%, respectively [25]. In post-menopausal α-adducin Trp allele carriers, these proportions would be 91.7% and 13.5%, respectively [25].

Several lines of evidence support a possible association between hypertension and the β-adducin mutation. First, adducin modulates several mechanisms of membrane sodium transport, which may play an important role in the pathophysiology of hypertension, such as Na^+ pump ac-

tivity, Na^+-K^+-CI^- cotransport, Li^+-Na^+ countertransport and passive Na^+ permeability [2, 3, 8]. Second, β-adducin deficiency in mice leads to higher levels of systolic and diastolic blood pressures and pulse pressure [16]. Third, point mutations of α- and β-adducin account for 50% of the blood pressure difference between Milan normotensive and hypertensive rat strains [4]. The function of the C1797T β-adducin polymorphism is still unknown. However, in the present study the mutated α-adducin Trp allele apparently potentiated the risk of hypertension associated with the β-adducin 1797T allele in women. In humans, the mutated α-adducin 460Trp allele is associated with enhanced sodium reabsorption at the proximal renal tubules [6, 7]. Therefore, we hypothesize that the β-adducin mutation also increases the risk of hypertension via increased sodium reabsorption and chronic volume expansion.

The renin-angiotensin-aldosterone system may play a role in the sexual dimorphism of blood pressure [26]. This concept may explain why the mutated β-adducin T allele was associated with hypertension in women but not in men. Indeed, inhibition of PRA and the adrenal aldosterone secretion, as exemplified by the diminished urinary aldosterone excretion, may represent a counter-regulatory mechanism in response to the chronic volume expansion induced by the mutated adducin. Thus, in men inhibition of the renin-aldosterone system probably underlies the lack of association between hypertension and the mutated β-adducin T allele. No difference was observed in the PRA or urinary aldosterone excretion of women in relation to the β-adducin genotypes.

In women, the association between hypertension and the β-adducin polymorphism was dependent on menopausal status and the intake of oral contraceptives in premenopausal subjects. Young women not using oral contraceptives may be protected against hypertension by their endogenous sex hormones [26]. The acute and long-term vasodilatory effects of estradiol are well-documented [27, 28], and this sex steroid may counteract the development of hypertension. Endogenous progesterone has natriuretic and blood pressure lowering effects, mainly due to its competition with mineralocorticoids at the type 1 corticosteroid receptor [29]. The higher urinary Na^+/K^+ ratio observed in pre-menopausal T allele carriers compared with their CC homozygous counterparts in our study may reflect competitive antagonism of aldosterone at distal renal tubule, which may compensate for the enhanced proximal tubular sodium reabsorption associated with mutated adducin.

The favorable vascular and natriuretic effects of the endogenous female sex hormones may be overruled by the use of oral contraceptives, due to the activation of renin-angiotensin-aldosterone system [29]. Exogenous estrogens, given orally and particularly in higher doses, stimulate the synthesis of hepatic proteins including angiotensinogen [30, 31]. Higher levels of plasma angiotensinogen may produce a small increase of PRA and angiotensin II [31]. A slight rise in the intrarenal angiotensin II level may be sufficient for a reduction in renal blood flow, which in turn may lead to increases in exchangeable sodium and blood pressure [31]. Furthermore, exogenously administered progestogens may induce natriuresis and lead to a compensatory activation of the renin-angiotensin aldosterone system [29]. In keeping with this working hypothesis, we found that women using oral contraceptives compared with non-users had a significantly higher PRA and urinary aldosterone excretion (Table 4). They also had an increased urinary Na^+/K^+ ratio, which may be the expression of the antagonism of aldosterone at the receptor level by exogenous progestogens. Competitive antagonism of the renal tubular aldosterone receptors may entail a further stimulation of the adrenal aldosterone secretion through potassium retention.

The significant association between hypertension and β-adducin in post-menopausal women may be explained by the disappearance of the protective effects of the endogenous female sex hormones [27, 32]. Post-menopausal subjects in the present study showed no difference in the urinary Na^+/K^+ ratio across the β-adducin genotypes (Fig. 2). In the absence of endogenous progesterone, natriuresis is not stimulated and the aldosterone-induced potassium excretion is not inhibited [33]. In the absence of hormonal replacement therapy, the elasticity of the large arteries of post-menopausal women declines and systolic blood pressure may consequently rise [32, 34, 35]. Without the vasodilatory and the natriuretic protection of female sex hormones, post-menopausal women have a diminished potential to compensate for genetic mechanisms that might increase their blood pressure through chronic volume expansion, stiffening of the large arteries or peripheral arterial vasoconstriction [32]. Furthermore, the activity of the renin-angiotensin-aldosterone axis decreases with aging [36] and hence the possibility to compensate for increased sodium retention via inhibiton of this system may be attenuated. In the absence of regulatory and counter-regulatory mechanisms, sodium fluid balance mainly depends on renal sodium handling [37]. The membrane skeleton protein adducin is an important determinant of proximal tubular sodium reabsorption [6, 7]. Based on previous findings in experimental animals [4] and hypertensive patients [5], we speculate that in post-menopausal women, chronic sodium retention and expansion of the circulating volume associated with the mutated adducin may explain the increase in blood pressure.

A limitation of our study was that DNA had not been obtained from 17.7% of our previously recruited cohort including 180 subjects (44.7%) who died before our genetic study was initiated. However, selective survival is

unlikely to have influenced our results. Indeed, the average age at death was 74 years, whereas fewer than 5% of subjects in our present cross-sectional sample were older than 70 years.

The α-adducin Gly460Trp polymorphism per se was not associated with hypertension, but enhanced the associations between hypertension and the mutated β-adducin T allele. Previous studies on the relationship between hypertension and the α-adducin Gly460Trp polymorphism produced contradictory results [38], probably because of confounding by age [13] or because other pathophysiologic mechanisms or epistatic interactions have not been taken into account [39]. There is a growing body of evidence showing that complex age-related disorders, such as hypertension, cannot be studied from an exclusive genocentric point of view, but only within their epidemiologic context [40]. Genetic effects only may become evident in aging subjects in the presence of facilitating environmental or lifestyle factors, such as high sodium intake [40]. Our research is population-based and participants were on average 43 years old. In selected cases and controls of more advanced age, Cusi el al first reported a positive association between hypertension and the α-adducin Trp allele [5]. Furthermore, two recent studies consistently showed that the α-adducin Gly460Trp polymorphism was associated with low-renin hypertension [41, 42]. A previous prospective analysis of our population sample found that the presence of the α-adducin Trp allele increased the risk of hypertension associated with the DD genotype of the angiotensin-converting enzyme from 32% to 59% [23].

In conclusion, the mutated β-adducin 1797T allele is associated with increased risk of hypertension in postmenopausal women and users of oral contraceptives, particularly in the presence of the mutated α-adducin. We hypothesize that inhibition of the renin-aldosterone system in men and absence of such a compensatory mechanism in women may explain to some extent the sexual dimorphism of the blood pressure phenotypes in relation to the C1797T β-adducin polymorphism. The significant interaction between the α- and β-adducin genes for systolic pressure in women suggests that these proteins may play a functional role in blood pressure regulation rather than other polymorphisms that are in linkage disequilibrium with the adducin genes and for which there is no proven ground for functional interaction. When confirmed by molecular, pathophysiologic or other genetic epidemiologic studies, our findings may have important implications for the management of hypertension and the prevention of cardiovascular complications. Indeed, use of oral contraceptives and menopause are associated with a higher incidence of hypertension [32] and related cardiovascular disorders [43].

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