Association of the C−344T aldosterone synthase gene variant with essential hypertension: a meta-analysis

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Background **The CYP11B2 gene (*CYP11B2*) encoding aldosterone synthase has been associated with essential hypertension and some, but not all, studies have reported that the C−344T variant may influence the risk of the disease.**

Objective **We performed a systematic review of the literature by means of a meta-analysis to evaluate the influence of the C−344T *CYP11B2* polymorphism on arterial hypertension and intermediate phenotypes.**

Methods **From 485 reports, we included 42 observational studies, case–control and cohort at baseline. Fixed and random effect models were used to pool data from individual studies.**

Results **From 19 heterogeneous studies including 5343 essential hypertensive and 5882 control subjects, we found a significant association between hypertension and the C−344T variant in fixed but not in random effect models [for homozygous CC: odds ratio (OR), 0.834; 95% confidence interval (CI), 0.760–0.914; $P<0.0001$, $n=11\,225$]. Besides, homozygous CC subjects had lower plasma renin activity (*D*, −0.161; 95% CI, −0.279 to −0.043; $P<0.01$, $n=1428$) but no difference in plasma aldosterone levels (*D*, −0.006; 95% CI, −0.081 to 0.07; $P=0.88$, $n=2872$). Limiting the quantitative analysis of blood pressure to 13 studies including only untreated individuals, no significant association was found for systolic arterial blood pressure (*D*, 0.042; 95% CI, −0.057 to 0.141; $P=0.41$, $n=1775$) and diastolic arterial blood pressure (*D*, 0.026; 95% CI, −0.073 to 0.125; $P=0.61$, $n=1775$).**

Conclusion **Homozygous individuals for the −344C *CYP11B2* allele are at 17% lower risk of hypertension with respect to homozygous TT subjects. *J Hypertens* 25:5–13**

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Keywords: aldosterone, aldosterone synthase, C−344T, CYP11B2 gene, genetics, hypertension, meta-analysis, plasma renin activity, polymorphism

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See editorial commentary on page 37

Introduction

Essential hypertension, like other common diseases, is under polygenic control; thus, the combination of small quantitative changes in the expression of many genes, together with environmental factors, combine to influence the risk of the disease. As a consequence, a considerable number of gene variants have been assessed as candidate determinants of the risk of hypertension.

The renin–angiotensin–aldosterone system is an important regulator of blood pressure, and molecular variants in genes that encode components of this system have been associated with several cardiovascular diseases, such as essential hypertension, myocardial infarction and hypertrophic cardiomyopathy. Among them the CYP11B2 gene (*CYP11B2*) encodes a key enzyme of the aldosterone biosynthesis – aldosterone synthase, a P450 mitochondrial oxidase located mainly within the zona glomerulosa of the adrenal cortex [1].

At the molecular level, the role of the *CYP11B2* locus in hypertension and cardiovascular disease has been extensively evaluated, paying particular attention to the C−344T single nucleotide polymorphism in the 5′ distal promoter region of the gene. This biallelic polymorphism affects a putative steroidogenic factor-1 (SF1) binding site which is involved in the expression of steroid biosynthetic enzymes in the adrenal cortex [2], the C allele being five times more avidly bound to the SF1 than the T allele *in vitro* [3].

Some, but not all, studies have reported that the −344T allele is associated with an increased risk of hypertension [4–6]. By contrast, other groups found an association

0263-6352

between the −344C allele and hypertension [7], but not with plasma aldosterone levels [8], whereas others reported that the −344C allele of *CYP11B2* may be a genetic marker for low-renin hypertension [9]. Finally, no evidence of replication was reported by other authors [10–13].

As meta-analysis is a reliable way to address discrepancies in genetic association studies, we decided to evaluate the influence of the *CYP11B2* C−344T polymorphism on the occurrence of hypertension and related phenotypes by using this systematic approach.

Methods

Data sources and study selection

For the electronic searches, published studies were found through PubMed at the National Library of Medicine (website: http://ncbi.nlm.nih.gov/entrez/query) and in Medline databases for the query '(CYP11B2 or aldosterone synthase) and (gene or variants or polymorphism or alleles) and (*Hae*III or C−344T)'. Reference lists in relevant publications were also examined. In addition, more than 673 abstract citations on CYP11B2 from PubMed were revised using the program RefViz (Thomson, ISI Research Soft, Stamford, Connecticut, USA) by searching for the above-mentioned additional keywords in the abstract text. The literature search was done on studies up to February 2006 and according to the availability of an English-language abstract or paper for review. There were no country restrictions.

We evaluated 485 citations, identifying 42 studies that met the selection criteria: population-based or hospital-based case–control, cross-sectional studies concerning the relationship between C−344T *CYP11B2* variant and hypertension and related phenotypes such as plasma renin activity, plasma aldosterone, systolic blood pressure and diastolic blood pressure, in which information about number of subjects in each category, sufficient data to calculate outcomes, and genotyping performed with a validated molecular method could be extracted. In the case of cohorts, we included variables before any intervention. Data on seven further studies were unavailable because in the reports the investigators did not disclose the raw data and our attempts to contact the authors were unsuccessful [14–20]. Data from four studies that fulfilled the eligibility criteria were included after personal contact with the investigators [21–24].

An evaluation of study quality of the reviewed articles using the median impact factor of the journals in which they had been published was included [25].

Data collection

All odds ratios (OR) were calculated against healthy control subjects. For each study, information was collected concerning the following characteristics of the subjects: demographic information (age, sex, ethnicity) and clinical features [systolic arterial blood pressure (SABP), diastolic arterial blood pressure (DABP) and hypertension defined as SABP $>$ 140 mmHg or DABP $>$90 mmHg or treatment with antihypertensive medication]. Biochemical determinations such as plasma aldosterone (ALD) and plasma renin activity (PRA) using any standard laboratory method were also analysed, paying particular attention to the sample collection (fasting blood samples obtained in the morning after rest in a supine position). We analysed the data after converting it to an uniform unit.

All quantitative variables had to be expressed as mean $\pm$ standard deviation (SD); standard error (SE) or 95% confidence intervals (CI) were converted to SD.

Because in some of the outcomes the variation seemed to follow a co-dominant model of inheritance, to avoid choosing any *a priori* model, we decided to compare only the extreme genotypes; namely, homozygous TT versus homozygous CC, a comparison made before in a study included in this meta-analysis [26].

Statistical analysis

For quantitative variables, effect stands for standardized difference (*D*), which is defined as the mean difference (between CC and TT groups) divided by the common within-group SD; and, for dichotomic variables, effect stands for ORs with respect to the homozygous TT as a reference group unless indicated. Summary OR and corresponding 95% CI were estimated by both fixed and random effects meta-analysis. A fixed effect model using the Mantel–Haenszel method was used to summarize results, obtaining the corresponding pooled OR. We assessed heterogeneity by using Q statistics. For *D*, the Cohen test was used to summarize the results and heterogeneity was evaluated with Q statistic and the I2 statistic, a transformation of Q that estimates the percentage of the variation in effect sizes that is due to heterogeneity. All calculations were performed using the Comprehensive Meta-Analysis computer program (Biostat, Englewood, New Jersey, USA).

In the case of heterogeneity, we identified study characteristics that stratify the studies into subsets with homogeneous effects. We considered possible sources of heterogeneity and stratified the studies by ethnicity, age and gender, and repeated the analysis separately for each group. If heterogeneity continued, we ranked the studies according to their individual chi-squared, removed the studies with the higher chi-squared and repeated the process until homogeneity was achieved. If the association became homogeneous after stratification or after removing the outlier studies, we recalculated the overall effect and 95% CI, and no further action was taken. Although the studies removed in this way cannot be considered outliers, removing studies that contribute

most to heterogeneity is an unbiased way of achieving the homogeneity required for meta-analysis [27].

Sensitivity of the findings was examined by recalculating the pooled association sizes and joint values of *P* in homogeneous subgroups, as well as after excluding studies one by one. Although the random effect model may exacerbate the effect of the bias by awarding relatively more weight to smaller studies (Cochrane Database of Systematic Reviews, website: http://www.cochrane.org), as mentioned above, we included the data using a random effects model.

To check for publication bias, a funnel plot was drawn. A *P* value lower than 0.05 was considered to be statistically significant.

Results

Study characteristics

Results from 42 studies were included in this meta-analysis. Twenty-four studies were population-based [6,8,12,13,22,26,28–45] and 18 were hospital-based studies [4,7,9,10,21,23,24,46–56].

Twenty-seven studies included Caucasian subjects [4,6,8,12,21,23,26,28–30,33,34,38,40–42,44–46,48,50–52,54–57], two studies included Finnish population [36,47], two studies included Chinese subjects [31,43], nine involved Japanese individuals [7,9,10,13,22,24,35,37,49], one study included African American and White-Latino subjects [32], one study included Himalayan subjects [39] and one study included African or Afro-Caribbean people [45].

Genotyping for the C−344T polymorphism was carried out across studies using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) followed by digestion with the restriction enzyme *Hae*III in 31 studies [5–10,12,13,21–24,26,28–31,33,34,36–41,47–49,51,54,57], in six studies genotyping was performed by allele-specific oligonucleotides [4,42,44,50,52,56], in four studies by Taqman assay [32,35,43,55] and in one study by single-strand conformational polymorphism [52]. This polymorphism corresponds to the single nucleotide polymorphism (SNP) rs1799998 (chr.10, pos. 143996602; website: www.ensembl.org).Taking into account only the subjects of the control groups, genotypes were in Hardy–Weinberg equilibrium in all ethnicity groups except for the Japanese, if the study of Matsubara *et al.* [22] was included.

Considering also heterozygous TC individuals, the overall frequencies of the T/C alleles in the control groups for the C−344T *CYP11B2* variant were 0.72/0.28 in African Americans, 0.69/0.31 in Japanese (not including the Matsubara *et al.* study [22]), 0.53/0.47 in Caucasians and 0.59/0.41 in White-Latinos, indicating a significant ($P < 0.001$) variation of the polymorphism frequency across ethnic groups. In fact, after considering only the homozygous TT versus CC, the proportion of TT homozygous subjects over the CC ones was significantly different ($P < 0.001$) among populations of different ancestry (Caucasians 1.5 and White-Latinos 1.8, versus African American 7.9 or Asian 3.0).

Hypertension, systolic arterial blood pressure and diastolic arterial blood pressure

We evaluated 19 studies [4,5,7–10,13,22,29–32,38,39,42,45,49,54,57] comprising 11 225 individuals (5343 patients with essential hypertension and 5882 controls) and found a significant association between arterial hypertension and the C−344T *CYP11B2* variant in fixed but not in random models (see combined effects, Fig. 1, Table 1). As there was significant heterogeneity ($P < 0.0002$), subjects were stratified by ethnicity. The difference remains significant in 4173 Caucasians and a trend was observed in 4929 Japanese individuals (Table 1). However, the heterogeneity remains, particularly in the Japanese group ($P < 0.05$) and in Caucasians ($P < 0.02$). It is important to note, however, that in Caucasian groups both fixed and random effects models give significant results (0.0001 and 0.03, respectively, Fig. 1). By subtracting three reports [7,9,49] including Japanese people and one report including female Caucasian individuals [8] that appear to be outliers, the heterogeneity was removed in each ethnicity group, improving the statistical significance in the remaining Japanese individuals (Table 1). Although in the combined analysis the heterogeneity still remains ($P < 0.05$) owing to differences among ethnic groups, both fixed and random effects were significant (Table 1).

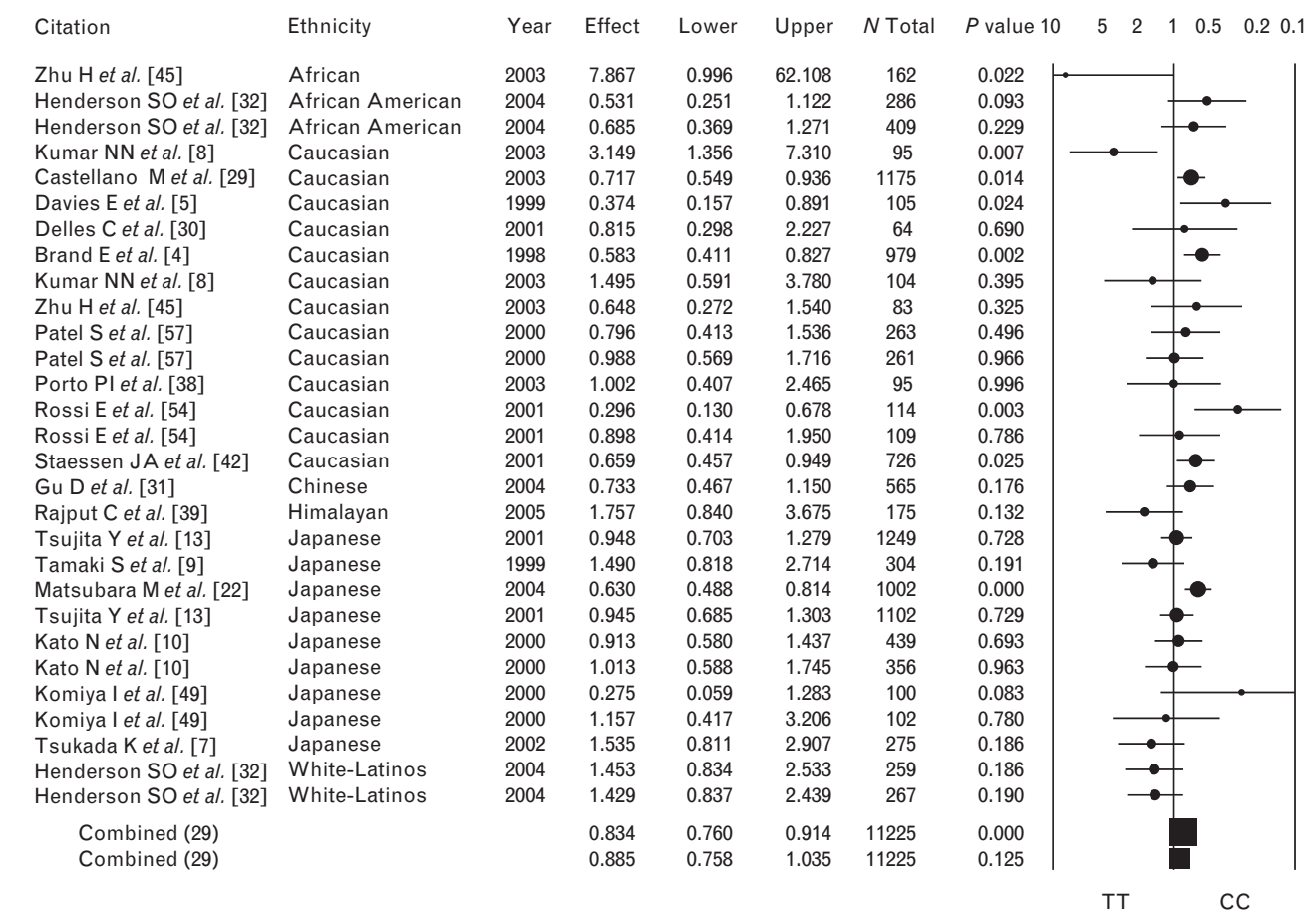
We used an additional strategy to handle persistent heterogeneous associations, estimating the effect by grouping only those large association studies with an arbitrary cutoff of 500 individuals already used by others [58,59]. Then, by fixed and random effects calculations, we observed a significant association between hypertension and the C−344T variant in nine homogeneous studies [4,10,13,22,29,31,32,42,53], encompassing 9338 individuals (for homozygous CC: OR, 0.790; 95% CI, 0.713–0.875; $P < 0.0001$ in the fixed model and OR, 0.810; 95% CI, 0.706–0.929; $P < 0.003$ in the random model).

From the funnel plot shown in Fig. 2, it seems that the there was no publication bias.

We further examined whether the association of the C−344T *CYP11B2* variant with hypertension depended on age of the subjects; no difference was observed (data not shown).

For the quantitative analysis of blood pressure, we considered only the reports in which the authors clearly specified that they included only untreated individuals or patients during the wash-out period of any antihypertensive medication for at least 3 weeks. Twelve studies were excluded since the use of medication may be a

Fig. 1



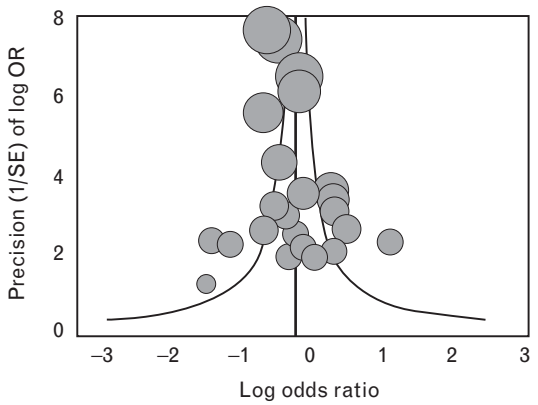
Summary estimates for odds ratios (effect), the corresponding 95% confidence interval (CI) limits (lower and upper) and significance (*P* value) were calculated by fixed and random effects meta-regression analysis for hypertension between the two groups according to the C−344T *CYP11B2* variant (homozygous TT and CC). The total number of combined studies is indicated between parentheses, the first row for fixed and the second one for random effects, respectively. The first author of the study is indicated under 'Citation'. The first author of the study appears twice in the figure when the data were regarded separately either by age or gender. In the graph, numbers indicate OR in a log scale, filled circles stand for the effect of individual studies and filled squares express fixed combined effects and random combined effects. The symbol size is proportional to the number of individuals involved in each study.

Table 1 Summary estimates for odds ratios (effect), the corresponding 95% confidence interval (CI) limits (lower and upper) and significance (*P* value) were estimated by fixed and random effects meta-regression analysis for hypertension between the two groups according to the C−344T *CYP11B2* variant (homozygous TT and CC) according to ethnicity

				Fixed effect				Random effect			
Ethnicity	*N* total	CC	TT	Effect	Lower	Upper	*P* value	Effect	Lower	Upper	*P* value
African (1)	162	13	149	7.867	0.996	62.108	0.05	7.867	0.996	62.108	0.05
African American (1)	695	78	617	0.618	0.384	0.995	0.05	0.618	0.384	0.994	0.05
Caucasian (10)	4173	1683	2490	0.726	0.627	0.841	0.00	0.765	0.601	0.975	0.03
Caucasian (9)	4078	1640	2438	0.691	0.595	0.802	0.00	0.695	0.585	0.826	0.00
Chinese (1)	565	92	473	0.733	0.467	1.150	0.18	0.733	0.467	1.150	0.18
Himalayan (1)	175	37	138	1.757	0.840	3.675	0.13	1.757	0.840	3.675	0.13
Japanese (6)	4929	1204	3725	0.871	0.758	1.002	0.05	0.935	0.750	1.166	0.55
Japanese (3)	4148	1050	3098	0.824	0.709	0.957	0.01	0.845	0.692	1.032	0.10
White-Latinos (2)	526	188	338	1.441	0.980	2.118	0.06	1.441	0.980	2.118	0.06
Combined (19)	11 225	3295	7930	0.834	0.760	0.914	0.00	0.885	0.758	1.035	0.13
Combined (16)	10 174	3061	7113	0.787	0.714	0.867	0.00	0.802	0.696	0.924	0.00

Total number of combined studies is indicated between parentheses. The second row for Caucasians and Japanese subjects studies correspond to homogeneous studies (after removing heterogeneity for sensitivity analysis).

Fig. 2



Funnel plots of precision by effect size for essential hypertension. OR, odds ratio; SE, standard error.

confounding variable in the analysis [6,12,13,22,33,35, 37,42–44,51,56]. Consequently, data about SABP were available in 13 studies encompassing 1775 individuals [9,21,24,28,30,36,39–41,47,48,50,55] and no association with the *CYP11B2* C−344T variant was found by either fixed or random models (Table 2). There was significant heterogeneity amongst the reports, $P < 0.005$, particularly in Caucasian populations. Although heterogeneity disappeared after removing one outlier study [21], the fixed effect was still not significant (*D*, 0.062; 95% CI, −0.038 to 0.162; $P = 0.22$, $n = 1732$).

In the same 13 studies where data about DABP were available in the same conditions described above [9,21, 24,28,30,36,39–41,47,48,50,55] no significant association with the variant was found (Table 3). Besides, there was significant heterogeneity ($P < 0.01$), mainly between studies including Caucasians. Despite the fact that heterogeneity disappeared after removing the outlier studies [21,30], the fixed effect was still not significant (*D*, 0.057; 95% CI, −0.044 to 0.158; $P = 0.27$, $n = 1706$).

Plasma aldosterone and plasma renin activity

ALD was not significantly associated with the C−344T *CYP11B2* polymorphism in 14 heterogeneous studies [6,7,12,23,26,28,30,34,38–40,48,52,55] including 2872 individuals (*D*, −0.006; 95% CI, −0.081 to 0.07; $P = 0.881$). Heterogeneity was removed after excluding two reports [7,39] and the effect was again not significant (*D*, 0.017; 95% CI, −0.059 to 0.094; $P = 0.655$; $n = 2708$).

Regarding PRA, there was information from eight heterogeneous studies [6,7,22,28,39,48,51,55] with a total of 1428 individuals. A significantly lower PRA level was found in the CC group, mainly in 562 Caucasian individuals, by both fixed and random models (Table 4).

Overall study quality

The median impact factor for all the included studies for hypertension was 4.87 (range 0.99–9.13), 4.87 for SABP (range 1.07–12.56), 4.87 for DABP (range 1.07–12.56), 4.87 for plasma aldosterone (range 0.99–12.56)

Table 2 Summary estimates for the standardized difference in systolic arterial blood pressure between the two groups according to the C−344T *CYP11B2* variant (homozygous CC versus TT)

						Number of individuals			Effect			
	Ethnicity	Age	Sex	Citation	Year	Total	CC	TT	*D*	Lower	Upper	*P*
	Finnish	20–50	Male	Hautanena *et al.* [47]	1998	31	12	19	0.313	−0.446	1.072	0.39
	Finnish	20–50	Mixed	Kupari *et al.* [36]	1998	42	20	22	0.392	−0.239	1.023	0.20
Fixed	Finnish (2)					73	32	41	0.359	−0.117	0.835	0.14
Random	Finnish (2)					73	32	41	0.359	−0.117	0.835	0.14
	Himalayan	51–65	Male	Rajput *et al.* [39]	2005	70	20	50	0.726	0.183	1.269	0.01
Fixed	Himalayan (1)					70	20	50	0.726	0.183	1.269	0.01
Random	Himalayan (1)					70	20	50	0.726	0.183	1.269	0.01
	Japanese	51–65	Male	Isaji *et al.* [24]	2005	393	99	294	−0.080	−0.308	0.149	0.49
	Japanese	51–65	Mixed	Tamaki *et al.* [9]	1999	64	9	55	0.175	−0.545	0.894	0.62
Fixed	Japanese (2)					457	108	349	−0.056	−0.273	0.162	0.62
Random	Japanese (2)					457	108	349	−0.056	−0.273	0.162	0.62
	Caucasian	20–50	Male	Brand *et al.* [28]	1999	92	55	37	0.269	−0.155	0.694	0.20
	Caucasian	20–50	Male	Delles *et al.* [30]	2001	38	18	20	0.089	−0.570	0.748	0.78
	Caucasian	20–50	Male	Delles *et al.* [30]	2001	26	11	15	−0.775	−1.629	0.079	0.06
	Caucasian	20–50	Male	Heller *et al.* [48]	2004	51	25	26	−0.188	−0.752	0.377	0.50
	Caucasian	20–50	Mixed	Lajemi *et al.* [50]	2001	221	80	141	0.180	−0.097	0.456	0.20
	Caucasian	20–50	Mixed	Poch *et al.* [21]	2001	43	12	31	−0.967	−1.688	−0.245	0.01
	Caucasian	51–65	Male	Russo *et al.* [40]	2002	394	182	212	0.012	−0.186	0.211	0.90
	Caucasian	20–50	Female	Sarzani *et al.* [41]	2003	99	34	65	0.008	−0.412	0.428	0.97
	Caucasian	20–50	Male	Sarzani *et al.* [41]	2003	98	37	61	0.378	−0.039	0.796	0.07
	Caucasian	20–50	Mixed	Stella *et al.* [55]	2004	113	48	65	−0.239	−0.617	0.140	0.21
Fixed	Caucasian (10)					1175	502	673	0.017	−0.100	0.135	0.77
Random	Caucasian (10)					1175	502	673	−0.027	−0.219	0.166	0.79
Fixed	Combined (15)					1775	662	1113	0.042	−0.057	0.141	0.41
Random	Combined (15)					1775	662	1113	0.051	−0.109	0.211	0.54

D stands for standardized difference. Corresponding 95% confidence interval (CI) limits (lower and upper effect) were estimated by fixed and random effect meta-analysis. The first author of the reference is indicated in the citation column. The studies were divided according to ethnicity, and the total number of combined studies is indicated in parentheses.

Table 3 Summary estimates for the standardized difference in diastolic arterial blood pressure between the two groups according to the C−344T *CYP11B2* variant (homozygous CC versus TT)

						Number of individuals			Effect			
	Ethnicity	Age	Sex	Citation	Year	Total	CC	TT	*D*	Lower	Upper	*P*
	Finnish	20–50	Male	Hautanena *et al.* [47]	1998	31	12	19	0.347	−0.413	1.107	0.34
	Finnish	20–50	Mixed	Kupari *et al.* [36]	1998	42	20	22	0.310	−0.318	0.939	0.31
Fixed	Finnish (2)					73	32	41	0.325	−0.150	0.801	0.18
Random	Finnish (2)					73	32	41	0.325	−0.150	0.801	0.18
	Himalayan	51–65	Male	Rajput *et al.* [39]	2005	70	20	50	0.651	0.111	1.191	0.02
Fixed	Himalayan (1)					70	20	50	0.651	0.111	1.191	0.02
Random	Himalayan (1)					70	20	50	0.651	0.111	1.191	0.02
	Japanese	51–65	Male	Isaji *et al.* [24]	2005	393	99	294	0.057	−0.171	0.286	0.62
	Japanese	51–65	Mixed	Tamaki *et al.* [9]	1999	64	9	55	0.442	−0.281	1.165	0.22
Fixed	Japanese (2)					457	108	349	0.093	−0.124	0.311	0.40
Random	Japanese (2)					457	108	349	0.097	−0.134	0.328	0.41
	Caucasian	20–50	Male	Brand *et al.* [28]	1999	92	55	37	0.000	−0.422	0.422	1.00
	Caucasian	20–50	Male	Delles *et al.* [30]	2001	38	18	20	−0.410	−1.077	0.256	0.21
	Caucasian	20–50	Male	Delles *et al.* [30]	2001	26	11	15	−1.117	−2.006	−0.227	0.01
	Caucasian	20–50	Male	Heller *et al.* [48]	2004	51	25	26	−0.392	−0.961	0.176	0.16
	Caucasian	20–50	Mixed	Lajemi *et al.* [50]	2001	221	80	141	0.000	−0.276	0.276	1.00
	Caucasian	20–50	Mixed	Poch *et al.* [21]	2001	43	12	31	−0.942	−1.661	−0.222	0.01
	Caucasian	51–65	Male	Russo *et al.* [40]	2002	394	182	212	−0.021	−0.220	0.178	0.84
	Caucasian	20–50	Female	Sarzani *et al.* [41]	2003	99	34	65	0.088	−0.333	0.508	0.68
	Caucasian	20–50	Male	Sarzani *et al.* [41]	2003	98	37	61	0.474	0.055	0.893	0.02
	Caucasian	20–50	Mixed	Stella *et al.* [55]	2004	113	48	65	−0.021	−0.398	0.356	0.91
Fixed	Caucasian (10)					1175	502	673	−0.044	−0.161	0.074	0.47
Random	Caucasian (10)					1175	502	673	−0.111	−0.317	0.095	0.29
Fixed	Combined (15)					1775	662	1113	0.026	−0.073	0.125	0.61
Random	Combined (15)					1775	662	1113	0.016	−0.152	0.183	0.85

D stands for standardized difference. Corresponding 95% confidence interval (CI) limits (lower and upper effect) were estimated by fixed and random effect meta-analysis. The first author of the reference is indicated in the citation column. The studies were divided according to ethnicity, and the total number of combined studies is indicated in parentheses.

and 4.87 for PRA (range 1.07–9.13). In conclusion, we observed that the data we have included in the analysis were published in leading journals with a high impact factor.

Discussion

It is well known that association studies represent a powerful approach to identification of genetic variants that influence susceptibility to common diseases [27]. However, agreement exists about the fact that epidemiological evidence for gene–disease association requires replication, validation and synthesis [60].

Some, but not all, studies have reported that the *CYP11B2* C−344T polymorphism is associated with the risk of hypertension. Both allelic distribution in groups with different ethnic backgrounds and the disparity between the studies regarding selection criteria of the population,

Table 4 Summary estimates for the standardized difference in plasma renin activity between the two groups according to the C−344T *CYP11B2* variant (homozygous CC versus TT)

						Number of individuals			Effect			
	Ethnicity	Age	Sex	Citation	Year	Total	CC	TT	*D*	Lower	Upper	*P*
	Himalayan	51–65	Male	Rajput *et al.* [39]	2005	70	20	50	0.099	−0.429	0.627	0.71
Fixed	Himalayan (1)					70	20	50	0.099	−0.429	0.627	0.71
Random	Himalayan (1)					70	20	50	0.099	−0.429	0.627	0.71
	Japanese	51–65	Mixed	Matsubara *et al.* [22]	2004	702	168	534	0.000	−0.174	0.174	1.00
	Japanese	51–65	Mixed	Tsukada *et al.* [7]	2002	94	13	81	−2.480	−3.177	−1.782	0.00
Fixed	Japanese (2)					796	181	615	−0.148	−0.316	0.021	0.09
Random	Japanese (2)					796	181	615	−1.216	−3.650	1.217	0.33
	Caucasian	20–50	Male	Brand *et al.* [28]	1999	92	55	37	−0.562	−0.992	−0.131	0.01
	Caucasian	>65	Mixed	Casiglia *et al.* [6]	2005	214	67	147	−0.290	−0.582	0.002	0.05
	Caucasian	20–50	Male	Heller *et al.* [48]	2004	51	25	26	−0.616	−1.194	−0.039	0.03
	Caucasian	51–65	Mixed	Mulatero *et al.* [51]	2002	92	29	63	−0.297	−0.746	0.151	0.18
	Caucasian	20–50	Mixed	Stella *et al.* [55]	2004	113	48	65	0.473	0.091	0.856	0.01
Fixed	Caucasian (5)					562	224	338	−0.205	−0.381	−0.030	0.02
Random	Caucasian (5)					562	224	338	−0.240	−0.622	0.142	0.22
Fixed	Combined (8)					1428	425	1003	−0.161	−0.279	−0.043	0.01
Random	Combined (8)					1428	425	1003	−0.402	−0.808	0.004	0.05

D stands for standardized difference. Corresponding 95% confidence interval (CI) limits (lower and upper effect) were estimated by fixed and random effect meta-analysis. The first author of the reference is indicated in the citation column. The studies were divided according to ethnicity, and the total number of combined studies is indicated in parentheses.

small sample size and biased selection in several parameters may contribute to these arguable results.

We performed a systematic review of the literature by means of a meta-analysis on the relationship of the variant with essential hypertension, and showed that the −344C allele was associated with a decreased risk of arterial hypertension (odds ratio 0.834, $P < 0.0001$). This conclusion arises from a total of 11 225 individuals recruited from 19 heterogeneous studies, without evidence of publication bias for the outcome.

However, a note of caution should be added because heterogeneity may potentially restrict the interpretation of the pooled risk estimates. Heterogeneity in a meta-analysis is mostly produced by differences in study design and background characteristics of the subjects, and the extent of heterogeneity might influence the conclusions. In our study, in the analysis of sensitivity, heterogeneity disappeared in each majority ethnic group after removing outlier studies, as was explained before. However, when analysing the combined studies, heterogeneity still remains, although we could not identify any clear methodological discrepancy that could account for heterogeneity (data not shown), indicating that the major component of the heterogeneity is due to differences among the ethnic groups. Nevertheless, the consequent sensitivity analysis revealed that across the majority ethnic groups the pooled risk estimates reflected similar tendencies. In addition, a random effect model, where heterogeneity is no longer an issue, provided a significant result in Caucasians.

As the largest studies may have yielded more conservative results that the smaller ones, we used an additional strategy to handle persistent heterogeneity. We estimated the effect by grouping only those large association studies with an arbitrary cutoff of 500 individuals already used by others [58,59]. Then, in nine homogeneous studies [4,10,13,22,29,31,32,42,53], we observed a significant association between hypertension and the C−344T variant by both fixed and random effects calculations.

Ethnicity may have acted as an important variable in determining association risk with hypertension, since Caucasians and Japanese homozygous for the C allele had reduced risk of hypertension (OR, 0.691, $P < 0.0001$ and OR, 0.824, $P < 0.011$, respectively). In other words, homozygosity for the −344T allele confers a 1.45 higher risk of hypertension in Caucasians and a 1.21 higher risk of hypertension in the Japanese. Even with a smaller number of subjects analysed ($n = 695$) in only one study, an important risk of hypertension (OR, 1.62; $P < 0.05$) was associated with homozygosity for the −344T variant in a study of African Americans [32].

PRA has been used to classify essential hypertensive patients into low, medium and high renin subgroups; despite different plasma renin activity, aldosterone excretion was similar between the subgroups. Therefore, patients in the high renin subgroup are characterized by signs of volume contraction and by a relative unresponsiveness of the adrenal gland to angiotensin II-induced aldosterone secretion [61]. It is therefore important to consider our findings in relation to PRA and the C−344T *CYP11B2* variant. In this regard, a significant 7% higher PRA in homozygous TT was observed in a total of 1876 individuals, particularly in 562 Caucasians. In contrast, there was no significant association of *CYP11B2* C−344T genotypes with plasma aldosterone levels.

The single nucleotide substitution from C to T at the −344 position disrupts a putative transcription binding site for steroidogenic factor 1 (SF1) and the in-vitro affinity of the T allele for SF1 is five times lower than that of the C-allele, but the polymorphism may have no impact on the transcriptional regulation of *CYP11B2* [3]. However, in a normal Caucasian population, the T allele was associated with higher excretion rates of tetrahydroaldosterone [5,52]. Interestingly, we observed that the C allele seems to be the ancestral allele since it is conserved not only in our closer relative, the chimpanzee (*Pan troglodytes*), but also in the rat, mouse and dog (website: genome.cse.ucsc.edu). It is to be noted that the C−344T variant (SNP: rs1799998) is located closely downstream to a very conserved DNA sequence of 30 bp that contains the recognition site for the transcription factor c/EBPalpha, and that other polymorphisms yet unrecognized may be the causative factors for the association we found.

Furthermore, in the absence of a significant effect of the T allele on plasma aldosterone, it is worth noting that we found that Caucasian TT homozygous individuals had 64.3% higher PRA. This elevated PRA may be adaptive to a lack of adequate response of the *CYP11B2* promoter bearing the T allele to the angiotensin II-mediated stimulus, and therefore the putative elevated plasma angiotensin II may make TT homozygous subjects more prone to hypertension.

It is also worth mentioning that we did not find any significant difference among genotypes and SABP and DABP despite efforts to remove heterogeneity and to group the studies by ethnicity, sex and age (data not shown). At first glance, it seems contradictory that the T allele be associated with hypertension as a dichotomous variable but not with arterial blood pressure (ABP), the quantitative trait behind hypertension. We wish to note that we limited the quantitative analysis of blood pressure to 13 studies including only untreated individuals, although analysing all studies with available data gave similar results (data not shown). The fact that the number of subjects analysed in studies reporting ABP was rather smaller than in hypertension studies may explain

why we found a significant association of the C−344T variant with hypertension but not with blood pressure.

To summarize, to the best of our knowledge, although there was a very small systematic review of the literature [31], this report represents the first meta-analysis including all available evidence to date indicating that subjects homozygous for the −344T allele of the *CYP11B2* gene have, at least, a 17% greater risk of essential hypertension compared with those homozygous for the C−344 allele.

Our findings may have an important impact on public health, since we observed that the TT genotype was five times more frequent than the homozygous CC genotype in African American people and two times more frequent in Asian individuals, in comparison with Caucasians. Even though these differences can be attributed to bias in the proportion of cases and controls included in the evaluated studies, considering the frequencies of C and T alleles in the control groups, there was a clearly higher proportion of allele T in African American and Japanese subjects over that in Caucasian ones, indicating that the influence of this genetic factor may be greater in the former groups. Despite the considerable controversy regarding the existence and importance of ethnic differences in genetic effects for complex diseases [62], it seems evident that genetic markers for proposed gene–disease associations vary in frequency across populations.

Finally, having faced the difficulties of doing a systematic review in this topic, and in agreement with Lohmueller *et al*. [27], we strongly encourage the inclusion in genetic association studies of all the data in standard format, either in print or in supplementary electronic material, to facilitate further meta-analysis with more robust estimates of the genetic effect; for instance complete records of all of the phenotypes available by genotypes and genotype counts for cases and controls, even though some of these data were not the primary issues of the publication.

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