Role of the C-344T aldosterone synthase gene variant in left ventricular mass and left ventricular structure-related phenotypes

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ABSTRACT

Aim: We performed a systematic review of the literature by means of a meta-analysis to evaluate the influence of the aldosterone synthase gene (*CYP11B2*) C-344T polymorphism on left ventricular mass (LVM) and related phenotypes.

Design: From 485 reports, we included 14 studies about the association between the C-344T variant and left ventricular mass and left ventricular structure-related phenotypes, from which information about number of subjects in each category, outcomes data and genotyping performed with a validated molecular method could be extracted. Fixed and random effect models were used to pool data from individual studies, and the results in the abstract show the extreme genotype comparison, homozygous TT vs homozygous CC.

Results: From a total of 2157 subjects, we found no significant association between LVM and the C-344T variant (D: 0.049, 95% CI: 0.091 to 0.179, p = 0.462). Similarly, no significant association was found for interventricular septal-wall thickness (D: 0.027, 95% CI: −0.090 to 0.143, p = 0.654, n: 2105). However, homozygous TT hypertensive subjects had increased LVM (D: 0.251, 95% CI: 0.020 to 0.481, p = 0.04, n: 332). Lastly, in 10 homogeneous studies posterior wall thickness (PWT) was lower in homozygous CC individuals (D: 0.142, 95% CI: 0.016 to 0.268, p = 0.028, n = 1994).

Conclusion: Independently of hypertension, homozygous individuals for the –344T allele may have 2.4% higher PWT compared to homozygous subjects for the C-344 allele. Besides, homozygous hypertensive TT subjects show a 6.9% increase in LVM compared to CC homozygous subjects.

It has been postulated that left ventricular growth and structure result from the complex interaction among genetic, environmental and lifestyle factors.[1] Among the gene polymorphisms that have been assessed as candidate determinants of the risk of left ventricular hypertrophy (LVH), the most studied are molecular variants in genes that encode components of the renin-angiotensin-aldosterone system.[2] Among them, the CYP11B2 gene (*CYP11B2*) encodes a key enzyme of aldosterone biosynthesis, aldosterone synthase, a P450 mitochondrial oxidase located mainly within the zona glomerulosa of the adrenal cortex.[3]

At the molecular level, the role of the *CYP11B2* locus has been extensively evaluated in cardiovascular disease, with particular attention on 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 that is involved in the expression of steroid biosynthetic enzymes in the adrenal cortex,[4] with the C allele four times more avidly bound to the SF1 binding site than the T allele in vitro.[5]

It was shown that genetic variation in the *CYP11B2* affects left ventricular size and mass in young adults free of clinical heart disease, and that this polymorphism may also influence the response of the left ventricle to increases in dietary salt.[6 7]

However, the association of the C-344T variant with cardiac hypertrophy remains controversial because while some studies report that the CC genotype in *CYP11B2* may be a risk factor for sodium-sensitive cardiac hypertrophy,[8–10] other studies conclude that the TT genotype is associated with higher values of the left ventricular mass (LVM).[7 11–15] Finally, some other reports have not found any association between LVM and the *CYP11B2* variant.[16]

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 LVM in addition to some intermediate phenotypes related to left ventricular structure such as posterior wall thickness (PWT) and interventricular septal wall thickness (ISWT).

METHODS

Data sources and study selection

For the electronic searches, published studies were found through Pubmed at the National Library of Medicine (http://ncbi.nlm.nih.gov/entrez/query) and in Medline databases for the keywords "CYP11B2" or "aldosterone synthase" and "gene" or "variants" or "polymorphism" or "alleles" and "*HaeIII*" 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 RefViz program (Thomson, ISI Research Soft, Stamford, CT, USA), searching for the above-mentioned additional keywords in the abstract text. The literature search was done on studies up to December 2006 and with the availability of an English-language abstract or paper for review. There were not country restrictions.

We evaluated 485 citations identifying 14 studies that met the selection criteria: population-based or hospital-based case-control, cross-sectional studies on the relation between C-344T *CYP11B2* variant and left ventricular end-diastolic diameter (LVEDD) and left ventricular end-systolic diameter

(LVESD), PWT, ISWT and LVM in which information about number of subjects in each category, sufficient data to calculate left ventricular structure phenotypes and genotyping performed with a validated molecular method could be extracted. In the case of cohorts, we included variables before any intervention. Data from one study that fulfilled the eligibility criteria were included after personal contact with the investigators (see online figure).[16]

Note that data obtained from family-based studies are not included in this meta-analysis, as there was only one study including this design.[17]

An evaluation of the quality of the reviewed articles has been conducted using the seven methodological standards for assessing clinical epidemiological quality in molecular genetic research according to Bogardus *et al*.[18]

Whenever possible, as an additional quality criterion, an arbitrary cut-off in the number of individuals included in each study was used in order to avoid studies that enrolled a limited number of subjects.

Data collection

For each study, information was collected about the following characteristics of the subjects: demographic information (age, sex, ethnicity) and LVM evaluated by Mode-M echocardiography and calculated according to the Devereux modified formula.[19] Because LVM was expressed in some studies as absolute LVM (g) or indexed for body surface area (g/m^2 or $g/m^{2.7}$) and we had used the standardised difference between groups that makes the estimate independent of the measurement unit, we pooled the data. Other M-mode-guided calculations included in the analysis were: ISWT (mm), PWT (mm), LVEDD (mm) and LVESD (mm), which in all the cases were measured according to the guidelines of the American Society of Echocardiography as previously reported.[20] All variables had to be expressed as mean (SD); SE or 95% CI were converted to SD.

Because the variation for some left ventricular structure phenotypes seemed to follow a co-dominant model of inheritance, and in order to avoid choosing any a priori model, we decided to compare first the extreme genotypes—namely, homozygous TT vs homozygous CC, a comparison that we have recently made,[21] and we subsequently compared TC vs CC and combined the effects of the TT and TC vs CC genotype.

The number of studies including subjects of same ethnicity was enough to pool the data only in the case of people of white race.

Statistical analysis

Effect stands for standardised difference (SD), which is defined as the mean difference (between TT or TC and CC groups) divided by the common within-group standard deviation. Summary effect and corresponding 95% CI were estimated by both fixed and random effects meta-analysis. Cohen's test was used to summarise the results and heterogeneity was evaluated with φ statistic and the I2 statistic, a transformation of φ that estimates the percentage of the variation in effect sizes caused by heterogeneity.

Regarding 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 χ^2, removed the studies with the higher χ^2 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. 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 at a time.

All calculations were performed using the comprehensive meta-analysis computer program (Biostat, Englewood, NJ, USA). To check for publication bias, we used a visual inspection of funnel plots, the Begg and Mazumdar's rank correlation test[22] and the Egger regression intercept method,[23] but we only show results from the latter, as it is the most powerful approach for detecting publication bias. Finally, we did not make any multitesting correction since the different outcomes are highly correlated and the subsets of pooled data were not the same for the different outcomes. Multiple testing is not an issue when traits under study are physiologically related (in our study all the phenotypes are strongly related to each other, and each test does not represent an independent opportunity for a type I error).[24]

A p value = than 0.05 was considered to be statistically significant.

RESULTS

Study characteristics

Seven studies were population-based[6 10 12 15 16 25 26] and the other eight were hospital-based (studies)[7 8 11 13 14 27-29] (table 1). Because the two studies by Kurland *et al*[13 27] show the same LVM data, further analysis was performed using reference 13 data.

Eleven studies included white subjects,[7 10–16 25 26 28] one study included Finnish population,[6] one study included Turkish population[29] and one study involved Japanese individuals (table 1).[8]

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 11 studies.[6 8 10–12 14–16 25 26 29] In one study genotyping was performed by allele-specific oligonucleotides,[28] in another (one study) by Taqman assay[7] and in a third one (study) by solid-phase minisequencing.[13] The C-344T polymorphism corresponds to the SNP rs1799998 (chr8, pos 143996602, www.ensembl.org).

Left ventricular end-diastolic diameter

We evaluated 10 studies that provided relevant data about LVEDD.[6–8 10–12 14 15 25 29] In the TT vs CC comparison, we included 1612 individuals showing no significant association with the variant either in the fixed or the random models (fig 1). Still a highly significant heterogeneity as well as a significant publication bias was observed (table 2). Subjects were stratified by ethnicity and the heterogeneity remained, particularly in white subjects. By subtracting one report including Finnish people[6] that appeared to be outlier, and stratifying the studies by sex, the heterogeneity was removed but the effect remained not significant in 1570 individuals. In the TC vs CC as well as in the combined TT+TC vs CC comparison, we observed that there were trends towards a decreased LVEDD in TC and in the combined groups (2276 and 3230 individuals, respectively) in the random model.

Left ventricular end-systolic diameter

In the TT vs CC comparison, in eight heterogeneous studies without publication bias,[6–8 11 12 15 25 29] LVESD was not

Table 1 Characteristics of the included studies

First author (year)	Reference	Country	Ancestry	Design	Left ventricular structure phenotype
Kupari M, 1998	6	USA	Finnish	Population-based	LVEDD/PWT/ISWT/LVM
Sarzani R, 2003	10	Italy	White	Population-based	LVEDD/PWT/ISWT/LVM
Delles C, 2001	12	Germany	White	Population-based	LVEDD/LVESD PWT/ISWT/LVM
Schunkert H, 1999	15	Germany	White	Population-based	LVEDD/LVESD PWT/ISWT/LVM
Kuznetsova T, 2004	16	Belgium	White	Population-based	PWT/ISWT/LVM
Hengstenberg C, 2000	25	Germany	White	Population-based	LVEDD/LVESD/PWT/ISWT/LVM
Porto PI, 2003	26	Argentina	White	Population-based	ISWT
Stella P, 2004	7	Italy	White	Hospital-based	LVEDD/PWT/ISWT/LVM
Isaji M, 2005	8	Japan	Japanese	Hospital-based	LVEDD/LVESD/PWT/ISWT/LVM
Heller S, 2004	11	Czech Republic	White	Hospital-based	LVEDD/LVESD/ISWT/LVM
Patel R, 2000	14	USA-Canada	White	Hospital-based	LVEDD/ISWT/LVM
Kurland L, 2002	13	Sweden	White	Hospital-based	LVM
Safar ME, 2005	28	Italy	White	Hospital-based	LVM
Olcay A, 2006	29	Turkey	Turkish	Hospital-based	LVEDD/LVESD/PWT/ISWT/LVM

LVM, left ventricular mass; LVH, left ventricular hypertrophy; ISWT, interventricular septal wall thickness; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; PWT, posterior wall thickness.

associated with the variant in 1355 individuals (either in fixed or random models, fig 2, table 2). Despite the fact that heterogeneity disappeared after removing the outlier study,[6] the fixed and random effects were still not significant in 1313 individuals. Neither in the TC vs CC nor in the combined TT+TC comparison was LVESD associated with the variant in 1882 and 2673 individuals, respectively.

Posterior wall thickness

Regarding PWT, we found 10 homogeneous reports without indication of publication bias.[6–8 10 12 15 16 25 26 29] In the TT vs CC comparison including 1994 subjects, PWT was significantly lower in the CC group than the TT group (table 2) in both fixed and random models (fig 3). Some difference was also observed in the white group alone. This difference, not observed in the TC vs CC comparison (n = 2809), was also found in the combined TT+TC vs CC comparison, in which PWT remained significantly associated with the variant in both models in 4803 individuals.

Interestingly, these differences were greater when the studies with hypertensive patients alone were included in the analysis (TT vs CC comparison in both fixed and random models: D, 0.515, CI%: 0.204 to 0.827, p = 0.001, n = 170; combined vs CC comparison; fixed, D, 0.324; 95% CI: 0.122 to 0.526, p = 0.002; fixed: D, 0.323, 95% CI: 0.097 to 0.550, p = 0.005, n = 350).

Intraseptal wall thickness

Data about ISWT were available in 12 homogeneous studies without publication bias.[6–8 10–12 14–16 25 26 29] In the TT vs CC comparison of 2105 individuals, we observed no significant difference in the ISWT between the two groups (table 2) either in the fixed or random model (fig 4). No significant associations were seen in the TC vs CC and the combined TT+TC vs CC comparisons in 2996 and 4205 individuals, respectively. A similar lack of difference was observed in white subjects.

Conversely, when we stratified the studies according to the disease status of the individuals, in the combined TT+TC vs CC comparison, the ISWT mean difference was significant and

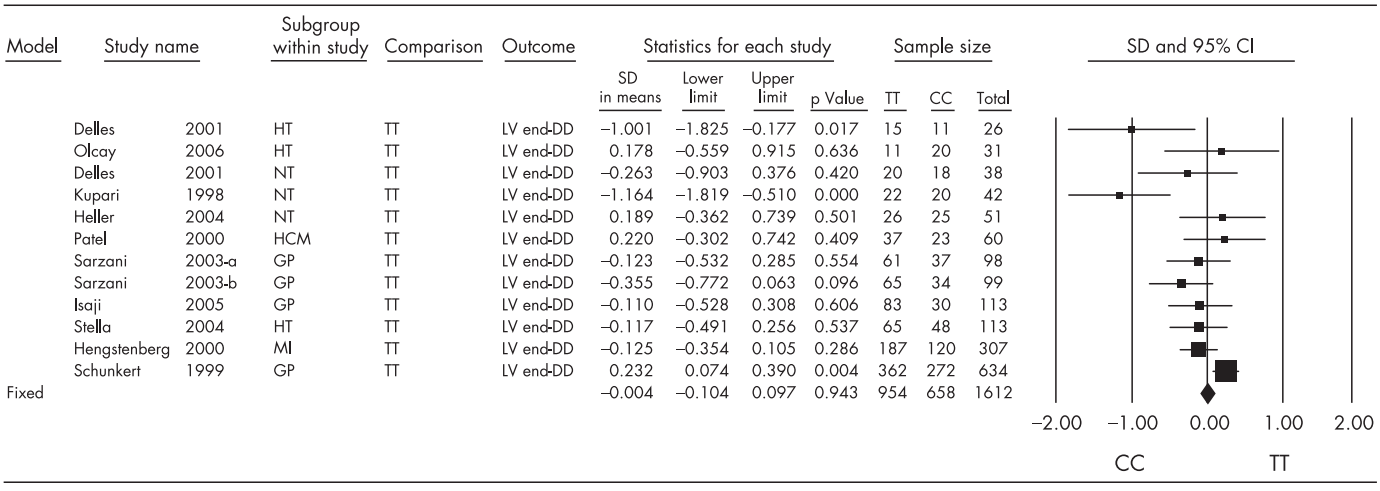

Figure 1 Summary estimates for standardised difference (SD) (effect), the corresponding 95% CI limits (lower and upper) and significance (p value) were estimated by fixed and random effects analysis for left ventricular end-diastolic diameter (LVEDD) between the two groups according to the C-344T *CYP11B2* variant (homozygous TT and CC). The first author of the study is indicated under citation (a and b indicate males and females within the study). In the graph, numbers indicate D values, solid squares stand for the effect of individual studies and solid diamond expresses combined fixed and random effects. HT, hypertensive individuals; NT, normotensive individuals; GP, general population; HCM, hypertrophic cardiomyopathy; MI, myocardial infarction.

Table 2 Summary estimates for standardised difference (SD) (effect), the corresponding 95% CI limits (lower limits: LL and upper limits: UL) and significance (p value) were estimated by fixed and random effects analysis for the outcomes, left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), posterior wall thickness (PWT), intraseptal wall thickness (ISWT) and left ventricular mass (LVM) between the extreme genotypes for the C-344T *CYP11B2* variant—namely, homozygous TT vs homozygous CC subjects (TT vs CC)

			Fixed model				Random model						Heterog	Egger
Outcome	**Comparison**	**Condition**	**SD**	**LL**	**UL**	**p Value**	**SD**	**LL**	**UL**	**p Value**	**A/B**	**Total No**	**p Value**	**p Value**
LVEDD	TT vs CC	All ethnicities	−0.004	−0.104	0.097	0.943	−0.139	−0.345	0.066	0.184	954/658	1612	0.001	0.032
		White people	0.03	−0.076	0.136	0.581	−0.75	−0.276	0.126	0.466	838/588	1426	0.01	
	TC vs CC	All ethnicities	−0.054	−0.16	0.037	0.242	−0.153	−0.312	0.007	0.061	1618/658	2276	0.01	
		White people	−0.034	−0.131	0.063	0.491	−0.119	−0.316	0.078	0.237	1414/588	2002	0.003	
	Combined	All ethnicities	−0.032	−0.099	0.036	0.36	−0.112	−0.228	0.003	0.057	2572/658	3230	0.0001	
		White people	−0.005	−0.076	0.066	0.891	−0.094	−0.227	0.04	0.169	2252/588	2840	0.0003	
LVESD	TT vs CC	All ethnicities	0.022	−0.087	0.131	0.692	−0.108	−0.338	0.123	0.361	791/564	1355	0.003	NS
		White people	0.064	−0.053	0.18	0.285	−0.022	−0.267	0.224	0.862	675/494	1169	0.02	
	TC vs CC	All ethnicities	−0.013	−0.112	0.086	0.792	−0.092	−0.26	0.077	0.287	1318/564	1882	NS	
		White people	0.011	−0.096	0.117	0.851	−0.044	−0.258	0.17	0.687	1114/494	1608	0.026	
	Combined	All ethnicities	0.003	−0.071	0.076	0.944	−0.069	−0.192	0.055	0.276	2109/564	2673	0.003	
		White people	0.034	−0.044	0.113	0.39	−0.026	−0.174	0.122	0.727	1789/494	2283	0.005	
PWT	TT vs CC	All ethnicities	0.124	0.035	0.214	**0.007**	0.142	0.016	0.268	**0.028**	1146/848	1994	NS	NS
		White people	0.132	0.038	0.225	**0.006**	0.165	0.014	0.316	**0.032**	1030/778	1808	NS	
	TC vs CC	All ethnicities	0.037	−0.044	0.118	0.368	0.041	−0.052	0.134	0.384	1961/848	2809	NS	
		White people	0.021	−0.063	0.106	0.62	0.02	−0.078	0.119	0.686	1757/778	2535	NS	
	Combined	All ethnicities	0.076	0.016	0.136	**0.013**	0.086	0.001	0.17	**0.047**	3107/848	4803	NS	
		White people	0.071	0.008	0.134	**0.027**	0.085	−0.006	0.176	0.067	2787/778	3565	NS	
ISWT	TT vs CC	All ethnicities	0.008	−0.079	0.095	0.857	0.027	−0.090	0.143	0.654	1209/896	2015	NS	NS
		White people	0.014	−0.077	0.105	0.768	0.048	−0.089	0.185	0.491	1093/826	1919	NS	
	TC vs CC	All ethnicities	0.062	−0.016	0.141	0.121	0.062	−0.016	0.141	0.121	2100/896	2996	NS	
		White people	0.049	−0.033	0.131	0.246	0.049	−0.033	0.131	0.246	1896/826	2722	NS	
	Combined	All ethnicities	0.038	−0.021	0.096	0.203	0.039	−0.031	0.11	0.274	3309/896	4205	NS	
		White people	0.033	−0.028	0.094	0.289	0.042	−0.032	0.115	0.267	2989/826	3815	NS	
LVM	TT vs CC	All ethnicities	0.058	−0.029	0.144	0.190	0.049	−0.091	0.179	0.462	1240/917	2157	NS	NS
		White people	0.076	−0.014	0.166	0.096	0.082	−0.05	0.214	0.224	1124/847	1971	NS	
	TC vs CC	All ethnicities	−0.016	−0.094	0.06	0.682	−0.016	−0.12	0.087	0.758	2166/917	3083	NS	
		White people	−0.013	−0.094	0.068	0.754	−0.01	−0.122	0.102	0.862	1962/847	2809	NS	
	Combined	All ethnicities	0.017	−0.041	0.075	0.568	0.013	−0.066	0.092	0.742	3406/917	4323	0.04	
		White people	0.076	−0.014	0.166	0.096	0.082	−0.05	0.214	0.224	1124/847	1971	0.04	

Subsequently we have compared TC vs CC subjects (TC vs CC) and the combined effects of the TT and TC vs CC genotype (Combined). In the column A/B, A represents number of subjects in groups TT or TC or combined according to the corresponding comparison and B is the number of subjects in the group CC taken as the reference group. Total N, the sum of A+B, is the total number of subjects involved in each comparison. Only in the case of white subjects, the number of studies including subjects of same ethnicity was enough to pool the data. Heterogeneity (Heterog p value) was evaluated with Q statistic and the I2 statistic and Egger's regression intercept method was used to check for publication bias (Egger's p value). A p value lower than 0.05 was considered to be statistically significant and is shown in bold for visualisation.

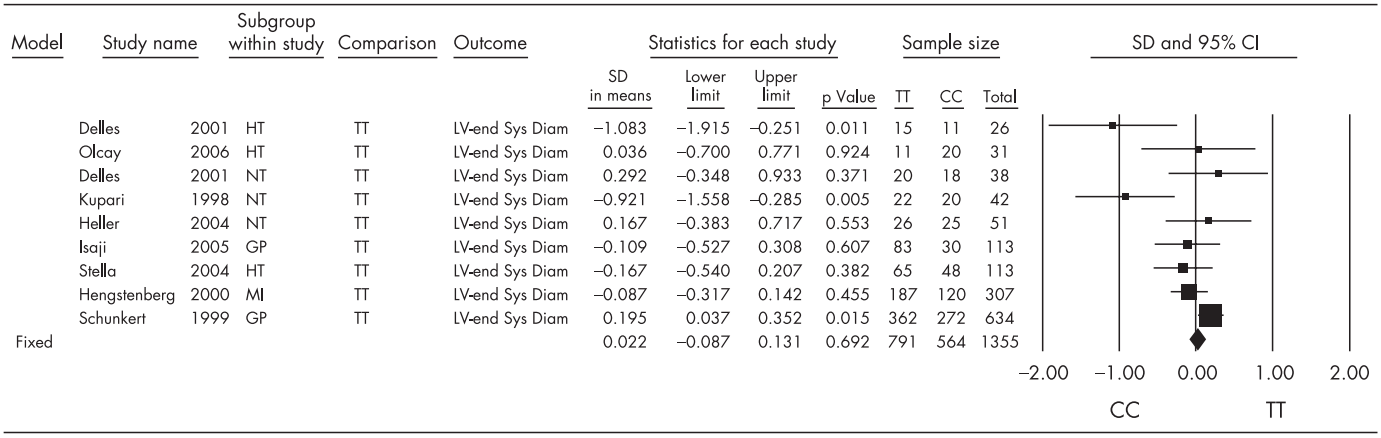

Figure 2 Summary estimates for standardised difference (SD) (effect), the corresponding 95% CI limits (lower and upper) and significance (p value) were estimated by fixed and random effects analysis for left ventricular end-systolic diameter between the two groups according to the C-344T *CYP11B2* variant (homozygous TT and CC). The first author of the study is indicated under citation. In the graph, numbers indicate D values, solid squares are the effect of individual studies and solid diamond expresses combined fixed and random effects. HT, hypertensive individuals; NT, normotensive individuals; GP, general population; MI, myocardial infarction.

identical in both fixed and random models (D: 0.264, 95% CI: 0.063 to 0.466, p = 0.01) in three studies[7 12 29] including 350 hypertensive subjects; this difference was particularly due to the white group (D: 0.384, 95% CI: 0.154 to 0.614, p = 0.001, n = 265).[7 12]

Left ventricular mass

For a difference in LVM between CC and TT homozygous subjects, we evaluated 13 studies comprising 2157 individuals who provided relevant data about LVM.[6–8 10–16 25 28 29] No statistical significance was observed by both fixed and random effects models (table 2) (fig 5). There was no significant heterogeneity between the reports and there was no publication bias. By using the TC and the combined TT+TC vs CC comparisons, no significant differences were found in LVM either with fixed or with random effect models in 3083 and 4323 individuals, respectively. In addition, when only white subjects from the study were pooled, similar results were observed.

Besides, we further analysed the data from reports whose authors clearly specified that they had included only normotensive individuals. Then, six studies including 1373 individuals were pooled[6 10–12 15 16] and, again, LVM was not associated with the *CYP11B2* C-344T variant by either fixed (D: 0.024, 95% CI: −0.083 to 0.131, p = 0.667) or random model (D: −0.057, 95% CI: −0.265 to 0.150, p = 0.89). In the combined TT+TC model, the association remains not significant in 2734 individuals (p = 0.97 and p = 0.46) in fixed and random models, respectively. Additionally, the heterogeneity (p<0.02) we observed between the pooled studies disappeared after stratification by ethnic groups.

Conversely, when we analysed the data of five homogeneous studies, including only hypertensive individuals,[7 12 13 28 29] we observed a significant and identical association between hypertension and the C-344T variant in 332 individuals (D: 0.251, 95% CI: 0.020 to 0.481, p = 0.04) in both fixed and

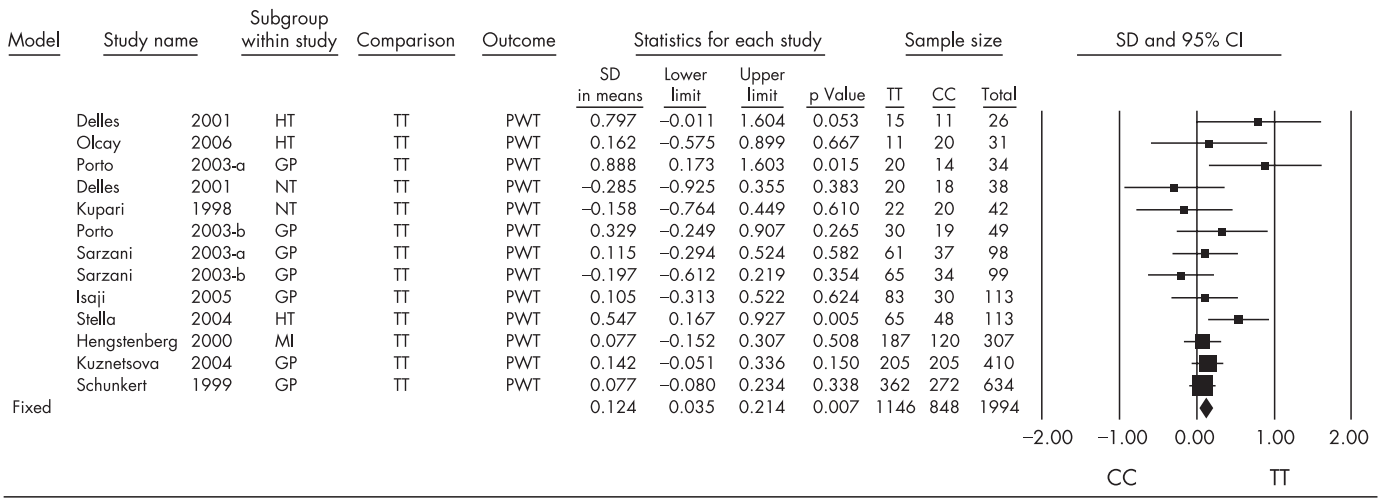

Figure 3 Summary estimates for standardised difference (SD) (effect), the corresponding 95% CI limits (lower and upper) and significance (p value) were estimated by fixed and random effects analysis for posterior wall thickness (PWT) between the two groups according to the C-344T *CYP11B2* variant (homozygous TT and CC). The first author of the study is indicated under citation (a and b indicate males and females within the study). In the graph, numbers indicate D values, solid squares are the effect of individual studies and solid diamond express combined fixed and random effects. HT, hypertensive individuals; NT, normotensive individuals; GP, general population; MI, myocardial infarction.

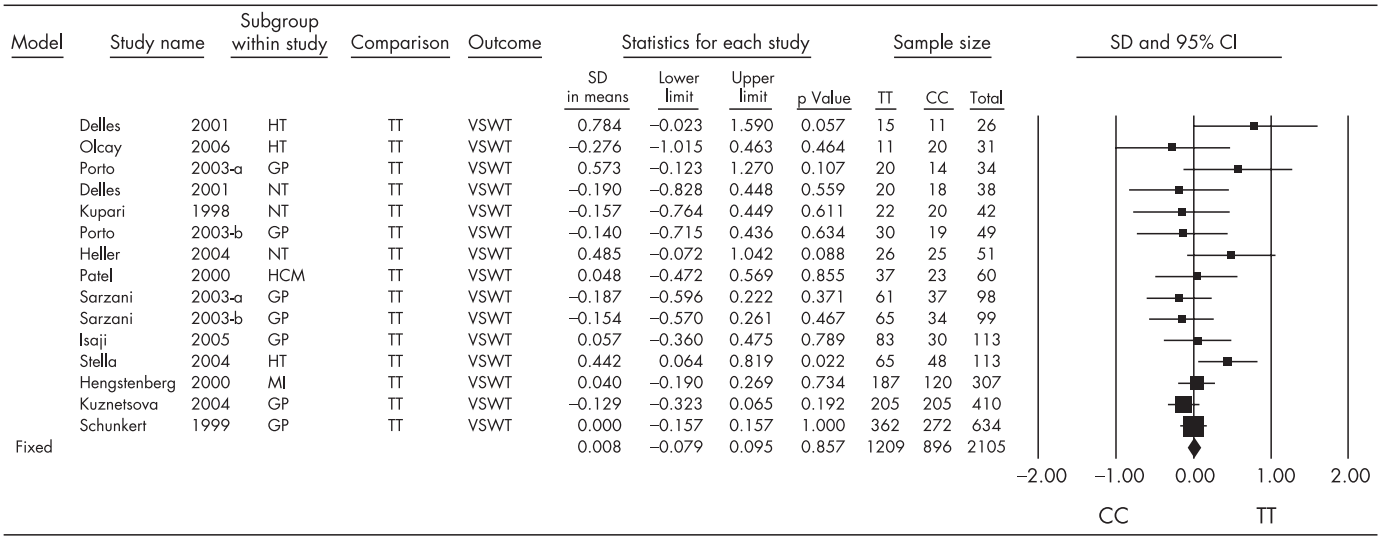

Figure 4 Summary estimates for standardised difference (SD) (effect), the corresponding 95% CI limits (lower and upper) and significance (p value) were estimated by fixed and random effects analysis for interventricular septal wall thickness (VSWT) between the two groups according to the C-344T *CYP11B2* variant (homozygous TT and CC). The first author of the study is indicated under citation (a and b indicate males and females within the study). In the graph, numbers indicate D values, solid squares are the effect of individual studies and solid diamond expresses combined fixed and random effects. HT, hypertensive individuals; NT, normotensive individuals; GP, general population; HCM, hypertrophic cardiomyopathy; MI, myocardial infarction.

random models. Although in the comparison between TC and CC groups we observed a trend toward a positive difference (data not shown), in the combined comparison in white subjects, a significant association was observed (fixed model, D: 0.182, 95% CI: 0.018 to 0.345, p = 0.03; random model, D: 0.182, 95% CI: 0.016 to 0.349, p = 0.032, n = 540).

Overall study quality

The assessment of quality of the included studies according to the use of seven methodological standards showed that all the studies performed genotyping by widely reproducible tests; however, all the studies failed to inform about either repetition or confirmation of the test with another procedure. Only one study mentioned data concerning the study blinding.[14] Furthermore, all the studies satisfactorily performed an appropriate delineation of cases and controls, adequacy of controls and appropriate quantitative analysis.

Additionally, we estimated the effects by grouping only those large association studies with an arbitrary cut-off of 100 individuals. Again, there was no statistical significance for a difference for either LVEDD, LVESD and LVM or ISWT between TT and CC homozygous subjects by both fixed and random effect models. However, for PWT, in 1577 individuals

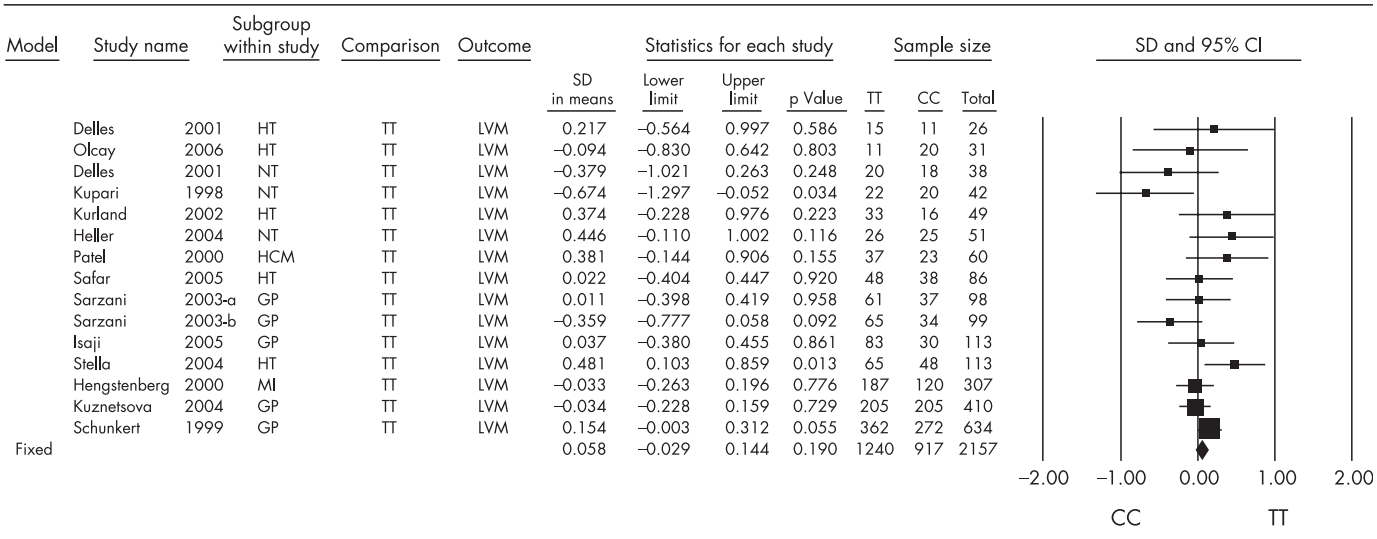

Figure 5 Summary estimates for standardised difference (SD) (effect), the corresponding 95% CI limits (lower and upper) and significance (p value) were estimated by fixed and random effects analysis for left ventricular mass (LVM) between the two groups according to the C-344T *CYP11B2* variant (homozygous TT and CC). The first author of the study is indicated under citation (a and b indicate males and females within the study). In the graph, numbers indicate D values, solid squares are the effect of individual studies and solid diamond expresses combined fixed and random effects. HT, hypertensive individuals; NT, normotensive individuals; GP, general population; HCM, hypertrophic cardiomyopathy; MI, myocardial infarction.

included in the larger studies the significant difference still remained (fixed model, D: 0.159, 95% CI: 0.029 to 0.230, p = 0.012; random model, D: 0.140, 95% CI: 0.018 to 0.263, p = 0.025).

DISCUSSION

The presence of left ventricular hypertrophy (LVH), mainly among hypertensive individuals, is an independent risk for morbidity and mortality from cardiovascular disease and is recognised as the most important predictor of chronic heart failure.[30] Moreover, recent studies evaluating the prevalence of LVH and its influence on cardiovascular risk stratification in hypertensive patients previously defined at relatively low or medium risk on the basis of routine evaluation showed that a significant number of hypertensive patients are reclassified as being at high risk after the detection of LVH.[31]

The role of genetic factors responsible for cardiac mass variance has been estimated up to 60%,[32] and different gene variants were associated with LVH and diastolic dysfunction in essential hypertension.[33]

It is well known that angiotension II and aldosterone may contribute to the secondary structural changes observed in cardiac hypertrophy and remodelling.[34] In this regard, some but not all studies have reported that aldosterone synthase gene *CYP11B2* C-344T polymorphism is associated with the increased LVM and diastolic dysfunction in hypertensive individuals with mild to moderate hypertension.

We performed a systematic review of the literature by means of a meta-analysis on the relation of the variant with the LVM and some related phenotypes associated with the left ventricular structure and showed that the *CYP11B2* -344T allele was only associated with an increased posterior wall thickness. This conclusion results from a total of 1994 individuals recruited from 10 homogeneous studies. The significance remained even after removing studies with less than 100 individuals. Besides, although there was no association in the global pooled data between the gene variant and LVM, the -344T allele was associated with increased LVM and IVWT in hypertensive individuals without evidence of publication bias.

We have recently found that in the absence of a significant effect of the T allele on plasma aldosterone white TT homozygous individuals have 64.3% higher plasma renin activity (PRA).[21] 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 makes TT homozygous subjects more prone to hypertension[21] and to its subsequent complications such as LVH.

In this regard, it should be emphasised that a higher LVM in homozygous TT individuals with respect to homozygous CC was only observed in the pool of studies including only hypertensive subjects but not in normotensive individuals included in population-based studies. However, at this time, it is not possible to ascertain whether the effect of the -344T variant on LVM is dependent or independent on hypertension, as the left ventricle of patients with arterial hypertension may be exposed to a variety of growth-regulating mechanisms, including pressure overload and humoral factor activation. Nevertheless, in the light of our findings, we might speculate that LVH represents both a manifestation of the effects of hypertension and other cardiac risk factors over time as well as a genetic predisposition leading to pathological changes in cardiac structure. Unfortunately, the influence of other determinants of cardiac size such as duration of disease, the effect of treatment, duration of hypertension, smoking, body mass index and blood pressure levels cannot be evaluated in the subgroup of hypertensive individuals since this information was not available from the studies.

We wish to note that our study only included data from unrelated people in a case-control study design. Unfortunately, no family-based reports, except the study of Mayosi *et al*[17] regarding of heart size and the aldosterone synthase gene, have been published. In this study the authors performed a thorough analysis of six polymorphisms spanning 6 kb of the *CYP11B2* in white British families, concluding that genetic polymorphisms at the *CYP11B2* make a small contribution to quantitative variation in echocardiographic measures of heart size and significant residual familial effect were present for septal wall thickness and left ventricular mass. This study also emphasises the importance of analysing the genetic variation that captures the haplotype structure of the locus and adjacent loci such as steroid 11β-hydroxylase (*CYP11B1*) in gene association studies[35] and may indicate that the functional variants could be located in the 3′ region of the *CYP11B2*.

Lastly, besides the pressure overload, aldosterone imposes volume overload on the heart and promotes fibrosis of the cardiac wall. The analysis was enriched with data about these aspects by the evaluation of the LVEDD and LVESD but we did not observe any relation with the variant.

To summarise, to the best of our knowledge, 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 show at least 2.4% higher PWT compared to those homozygous for the C-344 allele, whereas in hypertensive individuals the increase in PWT is still more dramatic, representing 11.0%. In the same way, hypertensive homozygous TT subjects show a 6.9% increase in LVM when compared to CC homozygous ones. In that context, bearing in mind that LVH is a strong predictor of adverse cardiovascular outcomes in the hypertensive population, and also an independent risk factor for coronary heart disease, sudden death, heart failure and stroke,[36] it seems reasonable to hypothesise that patients carrying the -344TT genotype are exposed to higher risk of heart disease and should be particularly monitored, as we have previously shown that these individuals have at least a 17% higher risk of essential hypertension compared to those homozygous for the C-344 allele.[21] Besides, cardiovascular disease and death rates had a 1.5-fold increase for each 50 g/m of LVM indexed by height.[37]

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REFERENCES

1. **De FU,** Prince J. Genes and environment behind ethnical differences in variations in left ventricular mass. *J Hypertens* 2004;**22**:241–3.
2. **Kuznetsova T,** Staessen JA, Thijs L, *et al*. Left ventricular mass in relation to genetic variation in angiotensin II receptors, renin system genes, and sodium excretion. *Circulation* 2004;**110**:2644–50.
3. **White PC.** Disorders of aldosterone biosynthesis and action. *N Engl J Med* 1994;**331**:250–8.
4. **White PC,** Slutsker L. Haplotype analysis of CYP11B2. *Endocr Res* 1995;**21**:437–42.
5. **Bassett MH,** Zhang Y, Clyne C, *et al*. Differential regulation of aldosterone synthase and 11beta-hydroxylase transcription by steroidogenic factor-1. *J Mol Endocrinol* 2002;**28**:125–35.

6. **Kupari M,** Hautanen A, Lankinen L, *et al*. Associations between human aldosterone synthase (CYP11B2) gene polymorphisms and left ventricular size, mass, and function. *Circulation* 1998;**97**:569–75.
7. **Stella P,** Bigatti G, Tizzoni L, *et al*. Association between aldosterone synthase (CYP11B2) polymorphism and left ventricular mass in human essential hypertension. *J Am Coll Cardiol* 2004;**43**:265–70.
8. **Isaji M,** Mune T, Takada N, *et al*. Correlation between left ventricular mass and urinary sodium excretion in specific genotypes of CYP11B2. *J Hypertens* 2005;**23**:1149–57.
9. **Rajput C,** Makhijani K, Norboo T, *et al*. CYP11B2 gene polymorphisms and hypertension in highlanders accustomed to high salt intake. *J Hypertens* 2005;**23**:79–86.
10. **Sarzani R,** Salvi F, Dessi-Fulgheri P, *et al*. Aldosterone synthase alleles and cardiovascular phenotype in young adults. *J Hum Hypertens* 2003;**17**:859–64.
11. **Heller S,** Linhart A, Jindra A, *et al*. Association of -344/T/C aldosterone synthase polymorphism (CYP11B2) with left ventricular structure and humoral parameters in young normotensive men. *Blood Press* 2004;**13**:158–63.
12. **Delles C,** Erdmann J, Jacobi J, *et al*. Aldosterone synthase (CYP11B2) -344 C/T polymorphism is associated with left ventricular structure in human arterial hypertension. *J Am Coll Cardiol* 2001;**37**:878–84.
13. **Kurland L,** Melhus H, Karlsson J, *et al*. Polymorphisms in the angiotensinogen and angiotensin II type 1 receptor gene are related to change in left ventricular mass during antihypertensive treatment: results from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation versus Atenolol (SILVHIA) trial. *J Hypertens* 2002;**20**:657–63.
14. **Patel R,** Lim DS, Reddy D, *et al*. Variants of trophic factors and expression of cardiac hypertrophy in patients with hypertrophic cardiomyopathy. *J Mol Cell Cardiol* 2000;**32**:2369–77.
15. **Schunkert H,** Hengstenberg C, Holmer SR, *et al*. Lack of association between a polymorphism of the aldosterone synthase gene and left ventricular structure. *Circulation* 1999;**99**:2255–60.
16. **Kuznetsova T,** Staessen JA, Stolarz K, *et al*. Relationship between left ventricular mass and the ACE D/I polymorphism varies according to sodium intake. *J Hypertens* 2004;**22**:287–95.
17. **Mayosi BM,** Keavney B, Watkins H, *et al*. Measured haplotype analysis of the aldosterone synthase gene and heart size. *Eur J Hum Genet* 2003;**11**:395–401.
18. **Bogardus ST Jr,** Concato J, Feinstein AR. Clinical epidemiological quality in molecular genetic research: the need for methodological standards. *JAMA* 1999;**281**:1919–26.
19. **Devereux RB,** Lutas EM, Casale PN, *et al*. Standardization of M-mode echocardiographic left ventricular anatomic measurements. *J Am Coll Cardiol* 1984;**4**:1222–30.
20. **Sahn DJ,** DeMaria A, Kisslo J, *et al*. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. *Circulation* 1978;**58**:1072–83.
21. **Sookoian S,** Gianotti TF, Gonzalez CD, *et al*. Association of the C-344T aldosterone synthase gene variant with essential hypertension: a meta-analysis. *J Hypertens* 2007;**25**:5–13.
22. **Begg CB,** Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* 1994;**50**:1088–101.
23. **Egger M,** Davey SG, Schneider M, *et al*. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997;**315**:629–34.
24. **Perneger TV.** What's wrong with Bonferroni adjustments. *BMJ* 1998;**316**:1236–8.
25. **Hengstenberg C,** Holmer SR, Mayer B, *et al*. Evaluation of the aldosterone synthase (CYP11B2) gene polymorphism in patients with myocardial infarction. *Hypertension* 2000;**35**:704–9.
26. **Porto PI,** Garcia SI, Dieuzeide G, *et al*. Renin-angiotensin-aldosterone system loci and multilocus interactions in young-onset essential hypertension. *Clin Exp Hypertens* 2003;**25**:117–30.
27. **Kurland L,** Melhus H, Karlsson J, *et al*. Aldosterone synthase (CYP11B2) -344 C/T polymorphism is related to antihypertensive response: result from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation versus Atenolol (SILVHIA) trial. *Am J Hypertens* 2002;**15**:389–93.
28. **Safar ME,** Cattan V, Lacolley P, *et al*. Aldosterone synthase gene polymorphism, stroke volume and age-related changes in aortic pulse wave velocity in subjects with hypertension. *J Hypertens* 2005;**23**:1159–66.
29. **Olcay A,** Nisanci Y, Ekmekci CG, *et al*. Aldosterone synthase -344C/T and angiotensin-converting enzyme I/D polymorphisms in Turkish hypertensive patients with normal coronary arteries. *Acta Cardiol* 2006;**61**:29–34.
30. **Gosse P.** Left ventricular hypertrophy as a predictor of cardiovascular risk. *J Hypertens Suppl* 2005;**23**:S27–S33.
31. **Cuspidi C,** Ambrosioni E, Mancia G, *et al*. Role of echocardiography and carotid ultrasonography in stratifying risk in patients with essential hypertension: the Assessment of Prognostic Risk Observational Survey. *J Hypertens* 2002;**20**:1307–14.
32. **Verhaaren HA,** Schieken RM, Mosteller M, *et al*. Bivariate genetic analysis of left ventricular mass and weight in pubertal twins (the Medical College of Virginia twin study). *Am J Cardiol* 1991;**68**:661–8.
33. **Diamond JA,** Phillips RA. Hypertensive heart disease. *Hypertens Res* 2005;**28**:191–202.
34. **Dzau VJ.** Tissue renin-angiotensin system in myocardial hypertrophy and failure. *Arch Intern Med* 1993;**153**:937–42.
35. **Barr M,** MacKenzie SM, Friel EC, *et al*. Polymorphic variation in the 11beta-hydroxylase gene associates with reduced 11-hydroxylase efficiency. *Hypertension* 2007;**49**:113–9.
36. **Gradman AH,** Alfayoumi F. From left ventricular hypertrophy to congestive heart failure: management of hypertensive heart disease. *Prog Cardiovasc Dis* 2006;**48**:326–41.
37. **Levy D,** Garrison RJ, Savage DD, *et al*. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N Engl J Med* 1990;**322**:1561–6.

Role of the C-344T aldosterone synthase gene variant in left ventricular mass and left ventricular structure-related phenotypes

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