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Pharmacogenetic analyses of variations of measures of cardiovascular risk in Alzheimer's dementia

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***Background & objectives*: Neurodegeneration affects blood pressure variations, while renal function and cerebral perfusion are impaired by vascular risk factors. This study was aimed to estimate variations of measures of cardiovascular risk in Alzheimer's dementia by pharmacogenetic analyses of the effects of angiotensin-converting enzyme (ACE) inhibitors and statins.**

***Methods*: Consecutive patients were prospectively followed to study variations of creatinine clearance and blood pressure for one year, estimated by correlating the effects of ACE inhibitors with the *ACE Alu* I/D polymorphism and genotypes or haplotypes of rs1800764 or rs4291, and the effects of statins with *LDLR* (low-density lipoprotein receptor) genotypes or haplotypes of rs11669576 (exon 8) or rs5930 (exon 10), or genotypes of rs2695121 (liver X receptor β gene). Variations of the coronary heart disease (CHD) risk according to these cardiovascular measures were also explored.**

***Results*: All polymorphisms of the 193 patients were in Hardy-Weinberg equilibrium. Genetic determinants of cardiovascular effects affected the individual variability of the response to ACE inhibitors and statins. ACE inhibitors, but not statins, reduced blood pressure for all patients. ACE inhibitors protected carriers of alleles that supposedly decrease serum ACE levels (rs1800764-T, rs4291-A, *Alu* II) regarding creatinine clearance variations ($P<0.005$), but carriers of *Alu* DD ($P<0.02$), rs1800764-C ($P<0.05$), or rs4291-AT ($P<0.04$) showed better blood pressure lowering effects. The presence of rs2695121-T ($P=0.007$) or rs5930-A ($P=0.039$) was associated with systolic blood pressure lowering, whereas rs5930-AA was protective against decrease in creatinine clearance ($P=0.019$). Statins lowered creatinine clearance for carriers of rs2695121-CT ($P=0.026$).**

***Interpretation & conclusions*: Pharmacological response of blood pressure and creatinine clearance to ACE inhibitors and statins may be genetically mediated.**

Key words Alzheimer disease - angiotensin system - cardiovascular risk - creatinine - drug therapy - lipoprotein - pharmacogenetics - receptors - renin

The burden of vascular risk factors affects several organic mechanisms such as renal function and cerebral perfusion. Though albuminuria is a marker for subclinical cardiovascular damage, it has been described that in older people with arterial hypertension both low and high glomerular filtration rates lead to increased cardiovascular morbidity and mortality[1], possibly due to increased vascular burden or frailty, respectively. High cerebrovascular risk leads to earlier onset of Alzheimer's dementia (AD), particularly when the vascular burden is more intense during midlife[2]. In healthy older people, there is a linear negative relationship between white matter hyperintensities and late-life cognitive function that increases with age[3]; in contrast, combined cerebrovascular risk factors may be neuroprotective in late life for patients with AD, probably due to enhanced cerebral perfusion[4].

Angiotensin-converting enzyme (ACE) inhibitors such as enalapril reduce intraglomerular pressure[5] as well as the risk of incident mild cognitive impairment[6]. The large interindividual variability in blood pressure response to ACE inhibitors is probably related to genetic differences[7], while it has been demonstrated that brain-penetrating ACE inhibitors boost genetically mediated neuroprotective effects in patients with AD[8]. An *Alu* repeat insertion/deletion (I/D) polymorphism in intron 16 is the most studied functional marker of ACE, but associations of the insertion allele with AD are not consistent in all studies[9], though such associations are usually confirmed by meta-analyses[10]. The two functional variants of *ACE* with the most significant effects for higher activity of the ACE are rs1800764 and rs4291[5], affecting neuropsychiatric symptoms and risk of the amnestic phenotype of AD[11], cognitive decline[8] and urea and creatinine[5] variations: rs1800764 is located at about 0.2 kb from the transcription start site in the promoter of *ACE* in 17q23, while rs4291 is at approximately 3.8 kb from the same site[8].

An atherogenic lipid profile also increases the risk of arterial hypertension and endothelium damage[12]. The *LDLR* (low-density lipoprotein receptor) gene resides within a region linked to AD in 19p13.3, whereas rs11669576[13] and rs5930[14] are two of the most important genetic variants of the epidermal growth factor precursor homology domain of *LDLR* to be associated with variability in risk of AD[15]. The liver X receptor β (LXR-β) isoform is also expressed in the brain, regulating cholesterol homeostasis and amyloidogenesis[16], while several variants of the *LXR*-β gene close to *APOE* in chromosome 19 have been linked with variable risk of AD[17]. LXR-β modulation may also inhibit angiotensin II by suppressing the angiotensin (AT1) receptor[18].

Metabolic changes in late life lead to weaker associations between LDL-cholesterol and coronary heart disease (CHD), whereas the opposite occurs with levels of high-density lipoprotein (HDL) cholesterol[19]. Moreover, high blood pressure and atherogenic mechanisms give rise to increased arterial stiffness, which alters cerebral autoregulation and leads to cognitive decline[2], but these mechanisms may be prevented by therapy with anti-hypertensive and lipid-lowering drugs. While neurodegeneration may affect blood pressure variation in late life[20], gender differences have been reported for the associations of cerebrovascular risk factors with cognitive and functional decline[21]. This prospective study was aimed to estimate the variations in one year of systolic and diastolic blood pressure in patients with AD, as well as of creatinine clearance, by pharmacogenetic analyses of the effects of ACE inhibitors and statins, while also taking into account possible impacts of these cardiovascular measures over variations of the CHD risk.

Material & Methods

Consecutive outpatients with AD according to the National Institute on Aging - Alzheimer's Association criteria[22] were prospectively selected from November 2010 to May 2014 at the Behavioural Neurology Section of Hospital São Paulo, Federal University of São Paulo, São Paulo, Brazil. Patients were excluded if they had mild cognitive impairment, history of kidney transplant or any form of dialytic therapy or if they did not complete one year of follow up.

After diagnostic confirmation, all patients had at least three consultations in the follow up and were assessed for proxy reports regarding age, the onset of dementia and treatment with anti-hypertensive or lipid-lowering drugs, whereas weight, gender, arterial hypertension, creatinine clearance and CHD risk were objectively assessed. For statistics, only the first and the last evaluations were considered. Fasting serum creatinine levels, total cholesterol and high-density lipoprotein cholesterol were measured at the beginning and after one year of follow up. Creatinine clearance was estimated by the formula by Cockcroft and Gault[23]. Framingham projections of the 10-yr absolute CHD risk[24] were estimated for all patients. Blood pressure was measured in every evaluation, while the diagnosis

and treatment of arterial hypertension followed the Joint National Committee (JNC) 7 report[25].

This study was approved by the Institutional Ethics Committee. All patients and their legal representatives signed the written informed consent forms before the evaluation. Blood samples (5 ml) were drawn from each patient for genotyping.

Genotyping procedures: Genotyping from venous blood DNA by real-time polymerase chain reactions using TaqMan® SNP Genotyping Assays on the Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems®, USA) was undertaken only after clinical data were collected from all patients, following the standard protocols of the manufacturer. The insertion/deletion (I/D) polymorphism in intron 16 of the *ACE* gene was determined by conventional PCR[26]. The presence of the I/D polymorphism or *ACE* genotypes or haplotypes of rs1800764 and rs4291 was correlated with anti-hypertensive treatment using one of the ACE inhibitors. Presence of *LDLR* genotypes or haplotypes of rs11669576 (exon 8 of the *LDLR* gene) and rs5930 (exon 10 of the *LDLR* gene), or genotypes of rs2695121 (*LXR*-β gene), was correlated with hypocholesterolaemic therapy with a statin.

Outcome measures: The primary outcome measure was the variation in one year of creatinine clearance, systolic and diastolic blood pressure, taking the following independent variables into account: use of one of the ACE inhibitors and the *ACE* I/D polymorphism or *ACE* genotypes or haplotypes; or therapy with a statin and genotypes of the *LXR*-β gene or *LDLR* genotypes or haplotypes. In secondary analyses, the impact of changes in one year of systolic and diastolic blood pressure or creatinine clearance over variations of the CHD risk was assessed.

Statistical analysis: Paired Student's *t* test was employed for variations of weight, serum creatinine, creatinine clearance, blood pressure, total cholesterol, HDL-cholesterol and the CHD risk, taking baseline and final values after one year into account. The Hardy-Weinberg equilibrium for the I/D polymorphism and genotypes of *ACE*, *LDLR* and the *LXR*-β gene, was estimated using the Chi-square test. A general linear model with post-hoc Hochberg's GT2 was employed for variations of creatinine clearance, systolic and diastolic blood pressure, according to *ACE* genotypes or haplotypes or the I/D polymorphism, and use or no use of one of the ACE inhibitors; or else, *LDLR* genotypes or haplotypes or genotypes of the *LXR*-β gene, and use or no use of a statin. The general linear model was adjusted for gender, age, length of the dementia syndrome and weight variations in one year. Simple linear regressions were employed for correlations between variations of creatinine clearance, systolic and diastolic blood pressure and variations of the CHD risk, as well as for correlations regarding variations of creatinine clearance and systolic and diastolic blood pressure levels among themselves. All analyses were performed with IBM SPSS Statistics v20.0 (SPSS Inc., Chicago, IL, USA).

Results

Overall, 217 consecutive patients were included in this study. During follow up, 14 patients (6.5%) died, eight (3.7%) abandoned the study and two patients (0.9%) were excluded due to incomplete clinical data, resulting in a final sample of 193 patients.

Table I shows demographic and clinical profile for all patients. For blood pressure control, 81 patients (42.0%) used one anti-hypertensive drug, 53 patients (27.5%) used two and 21 (10.9%) used three anti-hypertensive drugs: 124 (64.2%) used ACE inhibitors, 22 (11.4%) used angiotensin-receptor blockers, 61 (31.6%) used diuretics, 22 (11.4%) used β-blockers, 21 (10.9%) used calcium channel blockers and only 38 (19.7%) who did not have arterial hypertension used no anti-hypertensive drug. No patient used an ACE inhibitor and an angiotensin receptor blocker at the same time, but all four patients who used ezetimibe were also treated with a statin. Systolic and diastolic blood pressure levels were significantly lowered after one year, as well as total cholesterol levels, weight, creatinine clearance and the CHD risk.

Table II shows genetic results for all patients. Minor allele frequencies were 0.497 for the *ACE* I/D polymorphism (insertion allele), 0.497 for *ACE*-rs1800764 (C), 0.345 for *ACE*-rs4291 (T), 0.078 for *LDLR*-rs11669576 (A), 0.345 for *LDLR*-rs5930 (A) and 0.381 for rs2695121 (T, *LXR*-β gene), all variants in Hardy-Weinberg equilibrium.

Table III shows the effects of genotypes of rs2695121 (*LXR*-β gene), *LDLR* and *ACE* over variations of creatinine clearance and blood pressure in one year regardless of pharmacological treatment. The presence of T alleles of rs2695121 (*LXR*-β gene) or A alleles of *LDLR*-rs5930 was associated with lower

Table I. Demographic and clinical profile of patients (n=193)

Assessed factors	n (%)	Mean±SD	*P*
Gender			
Female	128 (66.3)	-	-
Male	65 (33.7)	-	-
Age at inclusion in the study (yr)	-	78.29±6.0	-
Age at dementia onset (yr)	-	73.06±6.6	-
Length of the dementia syndrome (yr)	-	4.99±2.9	-
Weight (kg)			
Baseline	-	62.99±12.2	<0.001
Final values	-	61.36±13.0	
Yearly variation	-	−1.63±5.2	-
Serum creatinine (mg/dl)			
Baseline values	-	0.98±0.3	0.967
Final values	-	0.98±0.3	
Yearly variation	-	−0.01±0.2	-
Creatinine clearance[23] (ml/min)			
Baseline values	-	53.61±17.6	0.008
Final values	-	51.27±17.7	
Yearly variation	-	−2.34±12.0	-
Arterial hypertension	155 (80.3%)	-	-
Systolic blood pressure (mmHg)			
Baseline values	-	131.65±17.4	<0.001
Final values	-	119.89±15.3	
Yearly variation	-	−11.76±17.3	-
Diastolic blood pressure (mmHg)			
Baseline values	-	78.42±10.0	<0.001
Final values	-	73.56±9.4	
Yearly variation	-	−4.87±10.3	-
Anti-hypertensive treatment with an angiotensin-converting enzyme inhibitor (mg/day)			
Captopril	113	74.00±29.6	-
Enalapril	8	37.50±7.1	-
Perindopril	3	6.67±2.3	-
Total cholesterol (mg/dl)			
Baseline values	-	197.92±46.5	<0.001
Final values	-	181.56±38.2	
Yearly variation	-	−16.36±36.5	-
High-density lipoprotein cholesterol (mg/dl)			
Baseline values	-	53.01±14.5	0.843
Final values	-	53.15±15.1	
Yearly variation	-	0.14±9.6	-
Hypocholesterolaemic treatment with statins (mg/day)			
Atorvastatin	14	28.57±22.8	-
Rosuvastatin	2	10.00±0.0	-
Simvastatin	129	18.68±9.3	-
Use of ezetimibe	4	10.00±0.0	-
10-yr coronary heart disease risk[24] (%)			
Baseline values	-	14.42±7.4	<0.001
Final values	-	11.58±6.4	
Yearly variation	-	−2.84±5.8	-

systolic blood pressure, whereas the AA genotype of *LDLR*-rs5930 was associated with higher creatinine clearance after one year.

Table IV shows blood pressure and creatinine clearance variations in one year according to the use or no use of ACE inhibitors, and *ACE* variants. ACE inhibitors had significant systolic and diastolic blood pressure lowering effects, regardless of any *ACE* polymorphisms or haplotypes; however, these effects were more significant for carriers of *ACE Alu* DD, the C allele of *ACE*-rs1800764 or the AT genotype of *ACE*-rs4291. Carriers of *ACE Alu* II, the T allele of *ACE*-rs1800764 or the A allele of *ACE*-rs4291 who used ACE inhibitors were protected against decreased creatinine clearance variations.

Table V shows blood pressure and creatinine clearance variations in one year according to the use or no use of statins, and genotypes of rs2695121 (*LXR*-β gene), as well as *LDLR* variants. No significant impacts over variations in blood pressure or creatinine clearance were found regarding the use or no use of statin therapy.

Table II. Genetic results of studied patients (n=193)

Assessed factors	n (%)	*P*[a]
ACE I/D[b] polymorphism		
II	52 (26.9)	0.221
ID	88 (45.6)	
DD	53 (27.5)	
rs1800764 genotypes		
CC	53 (27.5)	0.131
CT	86 (44.5)	
TT	54 (28.0)	
rs4291 genotypes		
AA	89 (46.1)	0.052
AT	75 (38.9)	
TT	29 (15.0)	
ACE haplotypes		
rs1800764 CC/rs4291 AA	7 (3.6)	-
rs1800764 CC/rs4291 AT	17 (8.8)	-
rs1800764 CC/rs4291 TT	29 (15.0)	-
rs1800764 CT/rs4291 AA	28 (14.5)	-
rs1800764 CT/rs4291 AT	58 (30.1)	-
rs1800764 CT/rs4291 TT	0 (0.0)	-
rs1800764 TT/rs4291 AA	54 (28.0)	-
rs1800764 TT/rs4291 AT	0 (0.0)	-
rs1800764 TT/rs4291 TT	0 (0.0)	-
rs11669576 genotypes (*LDLR*8)		
AA	1 (0.5)	0.868
AG	28 (14.5)	
GG	164 (85.0)	
rs5930 genotypes (*LDLR*10)		
AA	24 (12.4)	0.729
AG	85 (44.1)	
GG	84 (43.5)	
LDLR haplotypes		
rs11669576 AA/rs5930 AA	0 (0.0)	-
rs11669576 AA/rs5930 AG	0 (0.0)	-
rs11669576 AA/rs5930 GG	1 (0.5)	-
rs11669576 AG/rs5930 AA	0 (0.0)	-
rs11669576 AG/rs5930 AG	10 (5.2)	-
rs11669576 AG/rs5930 GG	18 (9.3)	-
rs11669576 GG/rs5930 AA	24 (12.4)	-
rs11669576 GG/rs5930 AG	75 (38.9)	-
rs11669576 GG/rs5930 GG	65 (33.7)	-
rs2695121 genotypes (*LXR*-β gene)		
CC	74 (38.3)	0.998
CT	91 (47.2)	
TT	28 (14.5)	

[a]Hardy-Weinberg equilibrium (Chi-square test), [b]*Alu* repeat I/D polymorphism in intron 16 of the angiotensin-converting enzyme gene. *ACE*, angiotensin-converting enzyme gene; *LDLR*, low-density lipoprotein cholesterol receptor gene; I/D, insertion/deletion; *LXR*-β, liver X receptor β

Statins led to lower creatinine clearance for carriers of the CT genotype of rs2695121 (*LXR*-β gene), while carriers of the AG genotype of *LDLR*-rs5930 had lower blood pressure after one year when not using statins.

Creatinine clearance variations followed weight variations and were significantly affected by these factors in the general linear model ($P<0.001$). In spite of specific pharmacological treatment, there were no correlations between creatinine clearance and the CHD risk, neither at the beginning nor at the end of the follow up. Similarly, creatinine clearance variations were not correlated with variations of the CHD risk, but each 1 mmHg variation in systolic blood pressure led to 0.2 per cent variation in the CHD risk ($P<0.001$), and each 1 mmHg variation in diastolic blood pressure led to 0.1 per cent variation in the CHD risk ($P=0.003$). Similarly, systolic and diastolic blood pressure levels were associated at the beginning and at the end of the follow up ($P<0.001$), as well as their variations ($P<0.001$): each 1.15 mmHg variation in systolic blood pressure led to 1.00 mmHg variation in diastolic blood pressure. In contrast, creatinine clearance variations were associated neither with systolic nor with diastolic blood pressure variations.

Discussion

The burden of vascular risk factors over primary neurodegeneration in the ageing population is likely to boost the incidence of dementia in countries such as India[27] and Brazil[2]; therefore, it is important to assess treatment strategies for vascular risk in highly populated countries. Allele frequencies of the polymorphisms investigated in the present study were in agreement with previous studies. Overall, it was found that ACE inhibitors had significant systolic and diastolic blood pressure lowering effects for all patients, regardless of any *ACE* polymorphisms or haplotypes, and without

Table III. Effects of genetic variants over variations of creatinine clearance and blood pressure in one year independently of pharmacological treatment

Genetic variants	n	Systolic blood pressure variations (mmHg)		Diastolic blood pressure variations (mmHg)		Creatinine clearance variations (ml/min)	
		Mean±SD	*P*[a]	Mean±SD	*P*[a]	Mean±SD	*P*[a]
ACE Alu I/D polymorphism							
II	52	−9.96±15.9	0.915	−3.61±10.1	0.495	−4.37±12.9	0.073
ID	88	−11.75±17.0		−5.51±10.7		−0.93±12.7	
DD	53	−13.32±19.2		−4.91±10.2		−3.18±9.1	
rs1800764 genotypes (*ACE*)							
CC	53	−10.91±18.6	0.660	−4.34±9.2	0.906	−2.53±7.5	0.881
CT	86	−11.98±17.2		−5.29±11.9		−2.40±11.6	
TT	54	−12.26±16.4		−4.70±8.8		−2.05±15.8	
rs4291 genotypes (*ACE*)							
AA	89	−12.52±16.6	0.490	−4.81±9.8	0.990	−2.94±13.7	0.232
AT	75	−11.44±18.2		−5.19±11.4		−1.84±11.5	
TT	29	−10.28±17.5		−4.21±9.1		−1.79±6.7	
rs2695121 genotypes (*LXR*-β gene)							
CC	74	−7.00±16.8	0.007	−4.14±9.4	0.702	−2.00±11.7	0.485
CT	91	−15.19±17.0		−5.62±11.3		−2.69±13.5	
TT	28	−13.21±17.4		−4.36±9.5		−2.10±6.8	
rs11669576 genotypes (*LDLR*8)							
AA	1	−22.00±0.0	0.775	−18.00±0.0	0.154	12.30±0.0	0.611
AG	28	−12.21±15.0		−8.21±8.9		−4.80±16.8	
GG	164	−11.62±17.7		−4.21±10.5		−1.86±11.0	
rs5930 genotypes (*LDLR*10)							
AA	24	−12.08±17.6	0.039	−2.33±11.4	0.139	4.32±14.8	0.019
AG	85	−14.16±17.4		−5.85±10.3		−3.47±11.9	
GG	84	−9.24±17.0		−4.60±10.0		−3.10±10.8	

[a]General linear model adjusted for gender, age, length of the dementia syndrome and weight variations in one year. SD, standard deviation; I/D, insertion/deletion; *LXR*-β, liver X receptor β; *LDLR*, low-density lipoprotein cholesterol receptor gene

significant effects over creatinine clearance variations. Impacts of statin therapy were found neither for blood pressure nor for creatinine clearance variations. The presence of T alleles of rs2695121 (*LXR*-β gene) or A alleles of *LDLR*-rs5930 led to systolic blood pressure reductions. Still, the AA genotype of *LDLR*-rs5930 was protective regarding creatinine clearance variations.

Systolic and diastolic blood pressure variations were associated with variations of the CHD risk. In contrast, no associations were found between creatinine clearance and blood pressure or the CHD risk, suggesting independence between pathways that mediate effects of high blood pressure over renal function and CHD.

Variants *ACE*-rs1800764[28] and *ACE*-rs4291[5] and the deletion allele of the I/D polymorphism[10] in *ACE* have been shown to be associated with boosted serum levels of the ACE while increasing the risk of arterial hypertension[29], particularly for patients with CHD and cerebrovascular disease[7]. In our study, blood pressure lowering effects of ACE inhibitors were more significant for carriers of *ACE Alu* DD, the C allele of *ACE*-rs1800764, or the AT genotype of *ACE*-rs4291. Carriers of *ACE Alu* II, the T allele of *ACE*-rs1800764, or the A allele of *ACE*-rs4291 who used ACE inhibitors were protected regarding creatinine clearance variations. Overall, carriers of genotypes that supposedly lead to higher serum levels of the ACE led to better blood pressure response to ACE inhibitors,

Table IV. Effects of angiotensin-converting enzyme (ACE) inhibitors over variations of creatinine clearance and blood pressure in one year according to genetic variants of angiotensin-converting enzyme

Genetic variants	Mean±SD (mmHg)						Creatinine clearance variations (mean±SD in ml/min)		
	Systolic blood pressure variations			Diastolic blood pressure variations					
	Users of ACE inhibitors	Non-users of ACE inhibitors	P^a	Users of ACE inhibitors	Non-users of ACE inhibitors	P^a	Users of ACE inhibitors	Non-users of ACE inhibitors	P^a
I/D polymorphism									
II	−11.59±17.3	−7.82±13.9	0.361	−5.45±10.7	−1.18±8.8	0.119	0.97±9.6	−11.42±13.4	<0.001
ID	−12.41±17.9	−10.47±15.5	0.620	−5.66±11.4	−5.23±9.4	0.917	−0.94±13.3	−0.93±11.7	0.792
DD	−18.17±18.6	−3.06±16.7	0.003	−7.33±9.9	0.24±8.9	0.015	−4.62±9.9	−0.14±6.2	0.215
rs1800764 genotypes									
CC	−14.53±18.8	−4.42±16.7	0.038	−4.94±9.1	−3.26±9.6	0.575	−3.63±8.3	−0.58±5.5	0.381
CT	−14.37±17.7	−6.46±15.1	0.075	−7.00±12.1	−1.35±10.6	0.041	−2.47±11.3	−2.25±12.4	0.630
TT	−12.53±18.2	−11.92±14.2	0.770	−5.73±9.7	−3.42±7.6	0.321	3.38±14.9	−8.82±14.5	0.001
rs4291 genotypes									
AA	−13.39±17.4	−11.03±15.3	0.486	−6.00±10.7	−2.79±8.0	0.154	−0.16±13.2	−7.66±13.4	0.004
AT	−15.28±18.7	−5.00±15.6	0.016	−7.23±11.0	−1.75±11.5	0.037	−2.46±11.8	−0.79±11.2	0.726
TT	−12.57±18.5	−4.25±13.7	0.228	−4.00±10.4	−4.75±4.7	0.817	−2.17±7.6	−0.80±3.9	0.963
rs1800764 CC/rs4291 AA									
Yes	−4.50±25.1	−14.00±26.0	0.444	−5.00±10.0	−8.00±7.2	0.624	−4.43±2.8	2.06±10.8	0.643
No	−14.28±17.8	−7.52±15.0	0.011	−6.17±10.8	−2.35±9.3	0.021	−1.27±12.0	−4.36±12.3	0.063
rs1800764 CC/rs4291 AT									
Yes	−23.56±14.7	−1.00±17.0	0.006	−7.11±5.1	0.00±13.2	0.117	−6.68±11.1	−1.34±5.2	0.196
No	−13.22±18.1	−8.69±15.1	0.111	−6.05±11.0	−2.93±8.7	0.085	−0.96±11.8	−4.44±12.8	0.025
rs1800764 CC/rs4291 TT									
Yes	−12.57±18.5	−4.25±13.7	0.225	−4.00±10.4	−4.75±4.7	0.816	−2.17±7.6	−0.80±3.9	0.949
No	−14.25±18.0	−8.26±15.6	0.032	−6.56±10.8	−2.31±9.7	0.014	−1.21±12.6	−4.51±12.9	0.061
rs1800764 CT/rs4291 AA									
Yes	−16.18±15.0	−6.00±16.1	0.252	−6.55±12.4	2.33±8.7	0.087	−4.20±10.5	−7.86±9.0	0.241
No	−13.49±18.6	−7.97±15.4	0.042	−6.04±10.4	−3.06±9.2	0.080	−0.76±12.1	−3.72±12.5	0.108
rs1800764 CT/rs4291 AT									
Yes	−13.32±19.2	−6.60±15.2	0.206	−7.26±12.0	−2.45±11.0	0.140	−1.46±11.8	−0.57±13.0	0.847
No	−14.26±17.6	−8.29±15.5	0.045	−5.63±10.1	−2.65±8.6	0.103	−1.33±11.9	−5.51±11.7	0.052
rs1800764 TT/rs4291 AA									
Yes	−12.53±18.2	−11.92±14.2	0.772	−5.73±9.7	−3.42±7.6	0.315	3.38±14.9	−8.82±14.5	0.001
No	−14.43±18.0	−5.60±15.7	0.007	−6.26±11.1	−2.16±10.1	0.051	−2.89±10.3	−1.55±10.1	0.862

[a]General linear model adjusted for gender, age, length of the dementia syndrome and weight variations in one year. SD, standard deviation; I/D, insertion/deletion

Table V. Effects of statins over variations of creatinine clearance and blood pressure in one year according to liver X receptor β genotypes and low-density lipoprotein cholesterol receptor genotypes and respective haplotypes

Genetic variants	Mean±SD (mmHg)						Creatinine clearance variations (mean±SD in ml/min)		
	Systolic blood pressure variations			Diastolic blood pressure variations					
	Users of statins	Non-users of statins	*P*[a]	Users of statins	Non-users of statins	*P*[a]	Users of statins	Non-users of statins	*P*[a]
rs2695121 genotypes (*LXR*-β gene)									
CC	−7.68±17.6	−3.50±11.3	0.455	−3.71±9.7	−6.33±7.0	0.459	−1.51±11.3	−4.52±13.6	0.332
CT	−13.33±17.6	−20.08±14.4	0.091	−4.73±11.5	−7.96±10.8	0.170	−4.38±12.9	1.79±14.2	0.026
TT	−13.18±18.1	−13.27±17.1	0.869	−3.53±11.2	−5.64±6.4	0.449	−2.87±7.6	−0.91±5.4	0.995
rs11669576 genotypes (*LDLR*8)									
AA	−22.00±0.0	–	–	−18.00±0.0	–	–	12.30±0.0	–	–
AG	−10.44±15.4	−15.40±14.6	0.520	−7.67±9.1	−9.20±9.0	0.722	−6.65±19.8	−1.47±9.4	0.128
GG	−10.87±18.2	−14.11±16.1	0.278	−3.54±10.8	−6.45±9.0	0.111	−2.38±10.2	−0.13±13.5	0.541
rs5930 genotypes (*LDLR*10)									
AA	−12.53±19.3	−10.40±10.2	0.772	−2.21±12.7	−2.80±4.4	0.990	2.39±10.5	11.68±26.1	0.149
AG	−11.85±17.9	−22.78±12.4	0.018	−4.51±9.8	−10.83±10.9	0.020	−3.77±12.6	−2.33±8.5	0.587
GG	−9.29±17.4	−9.12±16.4	0.951	−4.37±11.1	−5.12±7.3	0.655	−3.81±10.8	−1.44±10.7	0.569
rs11669576 AA/rs5930 GG									
Yes	−22.00±0.0	–	–	−18.00±0.0	–	–	12.30±0.0	–	–
No	−10.82±17.8	−14.37±15.7	0.203	−4.06±10.7	−7.02±9.0	0.075	−2.91±11.8	−0.41±12.6	0.273
rs11669576 AG/rs5930 AG									
Yes	−14.86±19.3	−26.67±4.2	0.354	−9.14±11.0	−14.00±5.3	0.565	−6.79±24.3	−7.62±6.3	0.839
No	−10.70±17.8	−13.56±15.8	0.308	−3.90±10.6	−6.56±9.0	0.111	−2.78±10.9	0.07±12.9	0.250
rs11669576 AG/rs5930 GG									
Yes	−7.64±12.5	−10.57±15.0	0.768	−6.73±8.1	−7.14±9.7	0.901	−6.56±17.6	1.16±9.7	0.109
No	−11.16±18.2	−15.02±15.9	0.182	−3.94±10.9	−7.00±9.0	0.087	−2.68±11.2	−0.67±13.2	0.540
rs11669576 GG/rs5930 AA									
Yes	−12.53±19.3	−10.40±10.2	0.796	−2.21±12.7	−2.80±4.4	0.975	2.39±10.5	11.68±26.1	0.146
No	−10.65±17.6	−14.84±16.2	0.155	−4.44±10.4	−7.51±9.3	0.074	−3.79±11.8	−1.81±9.7	0.424
rs11669576 GG/rs5930 AG									
Yes	−11.50±17.8	−22.00±13.5	0.036	−3.97±9.6	−10.20±11.7	0.032	−3.42±10.8	−1.28±8.6	0.541
No	−10.47±17.8	−10.91±15.6	0.864	−4.28±11.4	−5.58±7.2	0.520	−2.67±12.4	−0.01±14.2	0.362
rs11669576 GG/rs5930 GG									
Yes	−9.40±18.5	−8.56±17.3	0.966	−3.53±11.6	−4.45±10.3	0.680	−2.98±8.7	−2.45±11.1	0.700
No	−11.61±17.5	−17.87±13.8	0.094	−4.33±6.2	−8.63±10.1	0.055	−2.98±13.0	0.82±13.5	0.085

[a]General linear model adjusted for gender, age, length of the dementia syndrome and weight variations in one year. SD, standard deviation; *LDLR*, low-density lipoprotein cholesterol receptor gene; *LXR*-β, liver X receptor β

whereas lower serum levels had protective effects over renal function[30].

The statin therapy did not affect blood pressure or creatinine clearance variations in our study: since our patients were old people, most of them probably had accumulated diffuse atherosclerotic plaques throughout life that would not be susceptible to lipid-lowering therapy; and statin therapy being initiated in late life for most of them was possibly too late to revert the effects of atherogenesis over the endothelium. Similarly, conflicting associations have been reported between statin therapy and cognitive decline in late life[31]. It remains to be seen whether longer-term lipid-lowering therapy might have benefits over cardiovascular measures. A meta-analysis in patients with chronic renal failure not requiring dialysis reported that the effects of statins on stroke and renal function were uncertain, while adverse effects were incompletely understood; however, statins consistently lowered death and major cardiovascular events in these patients[32]. While the AA genotype of *LDLR*-rs5930 led to increased creatinine clearance, and the AG genotype was associated with lower blood pressure after one year for patients who did not use statins, the A allele was associated with lower risk of AD when combined with other *LDLR* polymorphisms in an earlier study[14]. Another study reported the GG genotype of *LDLR*-rs11669576 associated with a higher risk of AD when in combination with APOE4+ haplotypes and the CC genotype of *LDLR*-rs5925[15]. Hypertriglyceridaemia has also been found to be associated with AD, regardless of any genetic variants[33]. It may be possible that the same *LDLR* genotypes that reduce cardiovascular risk tend to confer neuroprotection, suggesting intertwined mechanisms, but since the assessment of the risk of AD was not an outcome measure of this study, these findings should be studied with greater depth in other populations.

Regarding the *LXR*-β gene, statins led to lower creatinine clearance for carriers of the CT genotype of rs2695121, whereas the presence of T alleles of rs2695121 was associated with systolic blood pressure lowering; in an earlier study, the C allele was associated with late-onset AD when in combination with other genotypes in sibpairs[16]. Conflicting results have been found for the association of rs2695121 with the metabolic syndrome[18], even though the T allele has also been shown to be associated with obesity in women[34]. The T allele of rs2695121 may be associated with reduced risk of late-onset AD by lowering systolic blood pressure, while earlier studies found that late-life increases in body mass index may be neuroprotective for patients with AD[4], but these findings should be confirmed when other polymorphisms of the *LXR*-β gene are concurrently analyzed.

More than 80 per cent of our patients had arterial hypertension according to the JNC 7 report[25], confirming the burden of this cardiovascular risk factor in older people. People with dementia tend to experience greater late-life decrease in blood pressure as a feature of neurodegeneration[20]. This could explain the significant disparities of blood pressure variations in our findings, though our close follow up with aggressive anti-hypertensive therapy might also have affected these results.

Limitations of this study included the fact that it was conducted in a single centre, with a short follow up and no randomization, and lacking measurements of serum levels of the ACE, as well as an objective evaluation of sarcopenia. Furthermore, it was not known whether the pharmacogenetic effects of ACE inhibitors and statins were either dose-dependent or more significant when starting treatment or at any time during therapy, because many patients were already under treatment when they were included in the study. We tried to minimize these limitations by keeping observers blinded to genetic data during the evaluations. Physical activity could also be a confounding factor for our results, but most patients were sedentary; thus, the effects of exercise for our sample, if any, were probably very small. Individual variability in drug response depends on the interactions of several complex factors, including genetic and environmental issues, and must be studied in different populations.

In conclusion, our study showed that genetic determinants of cardiovascular effects affected the individual variability of the response to ACE inhibitors and statins. ACE inhibitors, but not statins, were involved in blood pressure variations in older people, regardless of any genetic variants; and lipid metabolism alleles were involved in blood pressure and creatinine clearance variability, probably due to atherogenic mechanisms. Future studies should confirm these findings in other populations, particularly when starting therapy with statins or ACE inhibitors and hence the personalization of therapeutic decisions can be achieved.

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