Angiotensin-converting enzyme (ACE) genotypes and disability in hospitalized older patients

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Abstract The association between angiotensin-converting enzyme (*ACE*) genotypes and functional decline in older adults remains controversial. To assess if *ACE* gene variations influences functional abilities at older age, the present study explored the association between the common *ACE* insertion/deletion (*I/D*) polymorphism and disability measured with activities of daily living (ADL) in hospitalized older patients. We analyzed the frequency of the *ACE* genotypes (*I/I*, *I/D*, and *D/D*) in a population of 2,128

hospitalized older patients divided according to presence or absence of ADL disability. Logistic regression analysis adjusted for possible confounding factors, identified an association between the *I/I* genotype with ADL disability (OR=1.54, 95% CI 1.04–2.29). This association was significant in men (OR=2.01, 95% CI 1.07–3.78), but not in women (OR=1.36, 95% CI 0.82–2.25). These results suggested a possible role of the *ACE* polymorphism as a genetic marker for ADL disability in hospitalized older patients.

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Introduction

The pathways of functional decline leading to disability in elderly patients are complex, involving numerous environmental and genetic factors (Stuck et al. 1993; Christensen et al. 2000). A significant association among biological, psychological and social risk factors, and development of functional limitations and disability was previously reported (Stuck et al. 1999; Wu et al. 1999; Singh 2002). In particular, a model was proposed to assess the premise that functional limitations are an intermediary stage between risk factors, pathology/impairments (e.g., musculoskeletal problems), and the onset and course of disability (Verbrugge and Jette 1994;

Lawrence and Jette 1996). Furthermore, studies carried out in elderly twins suggested that genetic factors might influence functional abilities at older ages (Frederiksen et al. 2002; Christensen et al. 2002).

The angiotensin-converting enzyme (ACE) protein is a zinc metallopeptidase that converts the inactive angiotensin I to the active octapeptide angiotensin II, which is the main active product of the rennin–angiotensin system (RAS; Erdos and Skidgel, 1987). The angiotensin I converting enzyme (peptidyl-dipeptidase A) 1 (*ACE*) gene is located on chromosome 17q23 and contains a common insertion/deletion (I/D) polymorphism which is the result of the insertion of a 287 base-pair *Alu* element in intron 16 of the gene. This insertion generates allele I (*Alu*+), compared with the normal sequence defined as allele D (*Alu*-) (Rigat et al. 1990). The presence of D allele is related to an additive effect on ACE activity, in both plasma and tissue (Reneland and Lithell 1994; Coates 2003). Mean levels of ACE activity in D/D carriers were approximately twice that found in individuals with an I/I genotype, with subjects with an I/D genotype displaying intermediate levels, indicating a codominant effect of the two alleles (Rigat et al. 1990; Costerousse et al. 1993; Danser et al. 1995). In patients with Alzheimer's disease (AD), one of the most common age-related diseases, trends towards similar patterns of genetic association were found for ACE protein concentrations (but not ACE activity) in cerebrospinal fluid (Miners et al. 2009). In contrast, no evidence of similar genetic association was identified for ACE activity in post-mortem brain tissue from AD and non-demented (Miners et al. 2009), reinforcing the suggestions that ACE function within the body is complex and whilst genetically determined, is still influenced in a tissue-specific manner (Kehoe et al. 2009).

Disability often summarizes aging-associated health/well-being deterioration (Verbrugge and Jette 1994), and the *ACE* I/D is a common polymorphism which was extensively studied for its effect on various traits including neurodegenerative disorders, cardiovascular diseases, and functional limitations (Crisan and Carr 2000; Panza et al. 2007a, b; Pilotto et al. 2009). While the majority of small-scale studies reported some associations (Crisan and Carr 2000), larger-scale studies failed to confirm them (Agerholm-Larsen et al. 2000; Keavney et al. 2000). Importantly, recent

large-scale meta-analysis of 118 studies confirmed the association between the D allele and the risk of coronary artery disease relative to the I allele (Zintzaras et al. 2008). This likely highlights the fact that the effect of the *ACE* I/D polymorphism are sensitive to specifics of subgroups in heterogeneous populations and likely other gene–gene and gene–environment interactions (Kulminski et al. 2010).

Recent studies showed that *ACE* may be involved in skeletal muscle structure and function throughout the RAS. In fact, the D allele has been associated with increased muscle strength and power, whereas the I allele has been associated with improved muscular endurance (Gayagay et al. 1998; Myerson et al. 1999; Williams et al. 2000; Charbonneau et al. 2008) and cardio-respiratory performances (Guazzi et al. 1999a, b; Payne and Montgomery, 2003; Tsianos et al. 2004; Thompson et al. 2007). However, data indicating an association between the *ACE* I/D polymorphism and functional decline or physical performance in older adults are still under debate (Frederiksen et al. 2003a, b; Kritchevsky et al. 2005; Giaccaglia et al. 2008; Yoshihara et al. 2009). Indeed, the mechanisms for the interesting findings on muscle performance and endurance remain unclear although some hypotheses have been suggested to include altered substrate delivery in subjects as a result of modulated cardiac output and muscle vascularisation (Montgomery et al. 1998), both of which *ACE* could mediate as a result of its role in the formation of the angiogenic and vasoactive angiotensin II peptide (de Resende et al. 2010). In the present study, we investigated the potential association between the *ACE* I/D polymorphism and the activities of daily living (ADL) in hospitalized older patients.

Methods

Study population

The study was conducted according to the Declaration of Helsinki and the guidelines for Good Clinical Practice, and was approved by the local Ethic Committee. Patients were recruited from the Geriatric Unit of our Institution, under a framework project investigating the incidence of genetic risk factors in the multidimensional impairment of the elderly. Written informed consent was obtained from the

patients or from relatives of critically ill patients prior to participation in the study. All patients were Caucasians and did not include people of Jewish, Eastern Europe, or Northern Africa descent, with most individuals having Central and Southern Italy ancestry. Inclusion criteria were: (a) age ≥ 65 years, (b) ability to provide an informed consent or availability of a proxy for informed consent and willingness to participate in the study, and (c) a standardized comprehensive geriatric assessment (CGA) during hospitalization. At baseline, a complete clinical evaluation was undertaken. In addition, a review of records from the patients' general practitioners was undertaken to collect data such as date of birth, gender, clinical history, current pathologies, and medication history. Particular attention was made towards data regarding recently diagnosis of cardiovascular and neurodegenerative diseases. From January 2004 to December 2007, 2,792 patients, consecutively admitted to the Geriatric Unit of the IRCCS "Casa Sollievo della Sofferenza", San Giovanni Rotondo, Italy, were screened for eligibility. From this population, 233 patients were excluded because they were younger than 65 years old, 52 patients did not give their informed consent, and 379 patients were excluded because CGA was not available. After all exclusions a cohort of 2,128 patients remained eligible for the study.

CGA and clinical diagnoses

At admission, a CGA was carried out to evaluate clinical, cognitive, functional, nutritional, and social aspects of the patients (Stuck et al. 1993), with regard to the functional status evaluated by means of ADL scale (Katz et al. 1970). Cognitive status was also evaluated with the Short Portable Mental Status Questionnaire (SPMSQ; Pfeiffer 1975) while comorbidity was assessed with the Cumulative Illness Rating Scale-Comorbidity Index (CIRS-CI; Linn et al. 1968). The nutritional status was evaluated by means of the Mini-Nutritional Assessment (MNA; Guigoz and Vellas 1999).

The ADL scale defined the level of dependence/independence on six daily personal care activities including bathing, toileting, feeding, dressing, urine and bowel continence, and transferring (in and out of bed or chair) (Katz et al. 1970). Subjects with an ADL score=6 were classified as subjects with no functional disability, subjects with an ADL score < 6 were

classified as subjects with functional disability. The ADL was assessed in relation to the conditions reported to 1 week before the admission to our Geriatric Unit. Therefore, the chronic disability defined the ADL score.

The SPMSQ is a ten-item questionnaire that reliably detects the presence of cognitive impairment by evaluating orientation, memory, attention, calculation, and language (Pfeiffer 1975). On the basis of clinical practice, subjects with a SPMSQ score ≤ 3 were classified as subjects without cognitive impairment, subjects with a SPMSQ score from 4 to 7 were classified as subjects with minor cognitive impairment, and subjects with a SPMSQ score from 8 to 10 were classified as subjects with severe cognitive impairment.

The CIRS uses five-point ordinal scales (score 1–5) to estimate the severity of pathology in each of 13 systems, including cardiac, vascular, respiratory, eye-ear-nose-throat, upper and lower gastroenteric diseases, hepatic, renal, genito-urinal, musculo-skeletal, skin disorders, nervous system, endocrine-metabolic, and psychiatric behavioral problems (Linn et al. 1968). Based on these ratings, the CIRS-CI score reflects the number of concomitant diseases.

Finally, the MNA is composed of 18 questions grouped in four categories (Guigoz and Vellas 1999): (a) an anthropometric assessment of body mass index (body weight/height²), mid-arm circumference in cm, calf circumference in cm, and weight loss; (b) a general assessment using six parameters related to lifestyle, medication and mobility; (c) a dietary assessment using eight parameters related to number of meals, food and fluid intake, and autonomy of feeding; (d) a subjective assessment, regarding the self-perception of health and nutrition. A 30-point scoring system was proposed for the MNA, and on the basis of clinical practice, subjects with an MNA score ≥ 24 were classified as well-nourished subjects; subjects with a MNA score from 17 to 23.5 were classified as subjects at risk of malnutrition; subjects with a MNA score ≤ 17 were classified as malnourished subjects (Guigoz and Vellas 1999).

The main and secondary diagnoses at discharge from the hospital, coded according to the Italian translation of the International Classification of Diseases, 9th revision, Clinical Modification (ICD-9-CM; <http://icd9cm.chrisendres.com/icd9cm/>) were also recorded in all patients. In particular, cardiovascular

diseases included the following diseases classified as ICD-9-CM code: 401–405 (essential hypertension, hypertensive heart disease, hypertensive chronic kidney disease, hypertensive chronic heart and kidney disease, and secondary hypertension), 410–414 (acute myocardial infarction, other acute and subacute forms of ischemic heart disease, old myocardial infarction, angina pectoris, and other forms of chronic ischemic heart disease), 415–429 (diseases of pulmonary circulation and other forms of heart disease including also: acute pericarditis, acute myocarditis, cardiomyopathy, cardiac dysrhythmias, and heart failure); and neurodegenerative diseases including diseases classified as ICD-9-CM code: 330–337 (also including: dementia with and without behavioral disturbance and Parkinson's disease).

Genotype analysis

Genotypes of the *ACE* I/D polymorphism (rs1799752) were determined as previously described (Seripa et al. 2003). Briefly, the forward 5'>CTG GAG ACC ACT CCC ATC CTT TCT>3' and the reverse 5'>GAT GTG GCC ATC ACA TTC GTC AGA T>3' primers were used for PCR in a total of 30 cycles at 95°C for 30 s, 60°C for 30 s, and 72°C for 45 s. Primers were synthesized by Invitrogen (Invitrogen Corporation, Carlsbad, CA, USA). Reaction buffer included 1 U of Platinum Taq DNA polymerase (Invitrogen Corporation, Carlsbad, CA, USA), 10 pmol of each primer, 100 μ M each dNTP, and 1.5 mM MgCl₂. A direct analysis of PCR product on a 1.8% agarose gel identified the three *ACE* I/D genotypes.

Statistical analysis

For continuous variables, normal distribution was verified by the Shapiro–Wilk normality test and the one-sample Kolmogorov–Smirnov test. Categorical variables were compared by the Pearson χ^2 test. Normally distributed continuous variables were compared with the Student's *t* test; otherwise continuous variables not normally distributed were compared with the Mann–Whitney test. Agreement of the observed genotype frequencies with the expected Hardy–Weinberg frequencies were verified with Arlequin Software (Schneider et al. 2000; Kocsis et al. 2004). Relative allele frequencies were estimated from genotype frequencies by direct counting

(Gerdes et al., 1992). Association between *ACE* I/D polymorphism and disability was tested using a 3×2 contingency table Fisher's exact test and reported as odds ratios (ORs) along their 95% confidence intervals (95% CI). Similarly, we investigated the association between disability and the allelic risk (i.e., I-containing and D-containing genotypes) in a 2×2 contingency table. Distribution of the *ACE* genotypes in ADL groups was investigated by means of multiple logistic regression analysis adjusted for age, gender, educational level, smoking, nutritional status (MNA), comorbidity (CIRS-CI), cognitive impairment (SPMSQ), hypertension, neurodegenerative, and cardiovascular diseases. A statistical trend was defined for a *p* value from 0.050 to 0.100. All statistical analyses were made with the SPSS statistical software, version 10.1.3 (SPSS Inc., Chicago, IL, USA). Level of significance was set at a *p* value <0.05 assuming a two-tailed model.

Results

The population study comprised 2,128 patients (992 men and 1,136 women, mean age 78.18±7.12 years, age range from 65 to 100 years). ADL disability was identified in 979 (46%) patients (389 men and 590 women, mean age 80.67±7.34 years, age range from 65 to 100 years). The remaining 1,149 patients (54%) had no disability (603 men and 546 women, mean age 76.06±6.18 years, age range from 65 to 95 years). As shown in Table 1, patients with functional disability in the ADL were more frequently females (60.3% vs. 47.5%, *p*<0.001), older (80.67±7.34 vs. 76.06±6.18; *p*<0.001), had a lower educational level (57% vs. 48% patients with years at school <5, *p*=0.001), have minor smoking habit (15% vs. 26.3%; *p*<0.001), and a minor prevalence of hypertension (55% vs. 62%; *p*=0.001) than patients with no functional disability. Disabled patients had higher comorbidity (CIRS-CI 3.32±1.77 vs. 2.47±1.42; *p*<0.001), malnutrition (32.5% vs. 5.4%; *p*<0.001), prevalence of severe cognitive impairment (16.0% vs. 0.9%; *p*<0.001), cardiovascular (57.1% vs. 49.2%; *p*<0.001) and neurodegenerative (26.0% vs. 14.3%; *p*<0.001) diseases than patients with normal functions in the ADLs.

In the overall population, the frequencies of the *ACE* genotypes were 13.20% for the I/I genotype (*n*=

Table 1 Characteristics at baseline of older hospitalized patients with functional disability (FD) and no-functional disability (NFD) evaluated by activities of daily living (ADL) score and according to the angiotensin-converting enzyme (*ACE*) genotypes

		FD	NFD	<i>p</i>	<i>ACE</i> genotype			<i>p</i>
					I/I	I/D	D/D	
Number of subjects		979	1,149		281	971	876	
Age (years)	Mean $\pm$ SD	80.67 $\pm$ 7.34	76.06 $\pm$ 6.18	<0.001	78.20 $\pm$ 6.94	78.08 $\pm$ 7.07	78.29 $\pm$ 7.24	0.82
	Range	65–100	65–95		65–99	65–100	65–100	
Sex	Male/female	389/590	603/546	<0.001	120/161	450/521	422/454	0.27
	Females (%)	60.3	47.5		42.7	46.3	48.2	
Education level (years)	>8	5	8	0.001	4.6	6.4	7.6	0.24
	=5	38	44		42.2	39.4	43.3	
	<5	57	48		53.2	54.2	49.1	
Smoke	Subjects (%)	15.6	26.3	<0.001	19.4	20.7	23.4	0.27
Nutritional Status (MNA)	Well-nourished	22.1	64.5		43.2	48.5	42.9	
	Malnutrition risk	45.3	30.1	<0.001	39.5	34.8	38.5	0.17
	Malnourished	32.5	5.4		17.3	16.7	18.6	
Cognitive Impairment (SPMSQ score)	Normal	59.4	91.2	<0.001	12.7	46	41.4	0.63
	Moderate	24.6	7.9		15.4	47.4	37.2	
	Severe	16	0.9		12.9	45.3	41.7	
Comorbidity (CIRS-CI score)	Mean $\pm$ SD	3.32 $\pm$ 1.77	2.47 $\pm$ 1.42	<0.001	2.70 $\pm$ 1.63	2.89 $\pm$ 1.65	2.87 $\pm$ 1.65	0.23
	Range	0–11	0–8		0–10	0–10	0–11	
Hypertension	Subjects (%)	55	62	0.001	37.7	41	38	0.89
Cardiovascular diseases	Subjects (%)	57.1	49.2	<0.001	57.3	50.5	54	0.09
Neurodegenerative diseases	Subjects (%)	26	14.3	<0.001	21.4	17.6	21.5	0.09

Data are showed as number (%) or mean $\pm$ standard deviation, unless otherwise indicated

MNA Mini Nutritional Assessment, SPMSQ Short Portable Mental Status Questionnaire, CIRS-CI Cumulative Illness Rating Scale-Comorbidity Index

281), 45.63% for the I/D genotype ($n=971$) and 41.17% for the D/D wild-type ($n=876$). These genotype frequencies did not differ significantly from the expected Hardy–Weinberg frequencies for one locus ($p=0.640$). Accordingly, the estimated allele frequencies were 0.36 for I allele, and 0.64 for the D allele. No significant differences in mean age, sex, educational level, nor in the prevalence of comorbidity, malnutrition, hypertension, and cardiovascular or neurodegenerative diseases were observed when patients were divided according to the three *ACE* genotypes (Table 1).

The analysis of crude estimates showed a significantly higher frequency of the *ACE* I/I genotype in patients with disability than without disability in the ADL (15.2% vs. 11.5%; $p=0.020$). No significant differences were found in I/D or D/D genotype frequencies between the two groups. As shown in Table 2, multiple logistic regression analysis adjusted

for age, gender, educational level, smoking, nutritional status (MNA), cognitive impairment (SPMSQ), comorbidity (CIRS-CI), hypertension, neurodegenerative, and cardiovascular diseases confirmed a significant association between of *ACE* I/I genotype and functional disability in ADL ($p=0.03$; OR=1.54, 95% CI 1.04–2.29). Dividing patients according to gender, a significant association between *ACE* I/I genotype and disability in the ADL was observed in men ($p=0.03$; OR=2.01, 95% CI 1.07–3.78), but not in women ($p=0.23$; OR=1.36, 95% CI 0.82–2.25). No significant associations among the other *ACE* genotypes and ADL disability were found both in men and in women (Table 2). The analysis of the estimated allele frequencies showed a statistical trend towards a higher prevalence of I allele and lower prevalence of D allele ($p=0.07$) in patients with functional disability, as compared to patients with no functional disability in the ADLs. The same analysis carried out in patients

Table 2 Estimates of angiotensin-converting enzyme (*ACE*) genotypes and allele frequencies in older hospitalized patients with functional disability (FD) as compared to patients with no-functional disability (NFD) evaluated by activities of daily living (ADL) score and according to gender

ACE genotype/allele	Men			Women			All		
	FD N (frequency)	NFD N (frequency)	<i>p</i>	OR (95% CI)	FD N (frequency)	NFD N (frequency)	<i>p</i>	OR (95% CI)	FD N (frequency)
I/I	57 (14.7%)	63 (10.4%)	0.03	2.01 (1.07–3.78)	92 (15.6%)	69 (12.6%)	0.23	1.36 (0.82–2.25)	149 (15.2%)
I/D	174 (44.7%)	276 (45.8%)	0.54	0.88 (0.57–1.34)	261 (44.2%)	260 (47.6%)	0.48	0.88 (0.63–1.26)	435 (44.4%)
D/D	158 (40.6%)	264 (43.8%)	0.41	0.83 (0.54–1.28)	237 (40.2%)	217 (39.7%)	0.84	0.96 (0.66–1.40)	395 (40.3%)
I	288 (0.370)	402 (0.333)	0.09	1.18 (0.97–1.42)	445 (0.377)	398 (0.364)	0.53	1.06 (0.89–1.25)	733 (0.374)
D	490 (0.630)	804 (0.667)		0.85 (0.70–1.03)	735 (0.623)	694 (0.636)		0.95 (0.80–1.12)	1,225 (0.626)
									1,498 (0.652)

OR odds ratio, CI confidence interval

The analyses were adjusted for age, gender (overall population), educational level, smoking, nutritional status comorbidity, cognitive impairment, hypertension, neurodegenerative, and cardiovascular diseases

stratified according to gender, demonstrated that a similar trend was again evident in men ($p=0.09$). We further investigated if the *ACE* I/I genotype was also associated with cardiovascular diseases. Multivariate logistic analyses, adjusted for age, gender, educational level, smoking status, nutritional status, comorbidity, cognitive impairment, and neurodegenerative disease showed that *ACE* I/I genotype was not associated with cardiovascular diseases ($p=0.156$; OR=1.21, 95% CI 0.93–1.58). Same results were obtained performing such analyses separately for men ($p=0.304$ OR=1.24, 95%CI 0.82–1.87) and females. ($p=0.296$; OR=1.2, 95%CI 0.85–1.71).

Discussion

In the present study, we investigated the effect of the common *ACE* I/D polymorphism on functional impairment evaluated by means of the ADL scale in a cohort of hospitalized elderly patients. Our results suggested the *ACE* I/I genotype was a possible marker for ADL disability, particularly in men.

At present, previous studies addressing the role of *ACE* genotypes in functional decline or in decline of physical performances in older people showed inconclusive results (Frederiksen et al. 2003a, b; Kritchevsky et al. 2005; Giaccaglia et al. 2008; Yoshihara et al. 2009). In a 2-year follow-up study on elderly Danish twins, *ACE* genotypes were not associated with physical abilities, determined by means of ADL score and questions about demanding activities, such as running or cognitive function (Frederiksen et al. 2003a). Moreover, a post hoc analysis showed that the training effect did not differ according to *ACE* genotypes (Frederiksen et al. 2003b). Another study which investigated the physical performance in response to exercise in well-functioning older adults, reported that elderly carriers of the *ACE* D allele had a lower risk for the development of limited mobility than people with the I/I genotype (Kritchevsky et al. 2005). Furthermore, after a 18 months of a randomized controlled exercise trial, older and obese individuals with the *ACE* D/D genotype showed greater gains in knee extensor strength compared to I/I individuals (Giaccaglia et al. 2008). The same study found that there was also a significant interaction between *ACE* I/D genotype and exercise treatment on percent change in knee strength while there was a trend towards a

greater improvement in physical disability score in D/D genotypes. However changes in 6-min walk distance were not different between genotype groups (Giaccaglia et al. 2008). Finally, a recent study on Japanese older subjects found a significant relationship between *ACE* genotype and physical function. In particular, the *ACE* D/D genotype was associated with hand-grip strength that evaluated physical function in the upper extremities (Yoshihara et al. 2009), although these findings can only be generalized cautiously and could not be applied to non-Asian populations (Sagnella et al. 1999). The suggested improved physical function and disability after exercise in older subjects linked to the presence of the *ACE* D/D genotype or D allele (Kritchevsky et al. 2005; Giaccaglia et al. 2008), and the cross-sectional association of D/D genotype with physical function in older Japanese (Yoshihara et al. 2009) confirmed the findings of the present study in which the *ACE* I/I genotype was associated to ADL disability.

Studies on physical performance of young athletes suggested that the I allele might improve endurance performance (Montgomery et al. 1998; Williams et al. 2000), possibly related to increases of type I skeletal muscle fibers (Zhang et al. 2003) or a local muscle effect consisting in an elevated levels of intramyocellular lipid used as an energy substrate (van Loon 2004). In older age, there was a significant decline in skeletal muscle strength and muscle size that may contribute to loss of mobility and to a reduction of the ADL (Foldvari et al. 2000; Bean et al. 2003). Although no specific genetic *locus* has been identified as yet, the individual response to strength training is highly variable (Folland et al. 2002), and strength gains appear to be significantly affected by inheritance (Thomis et al. 1998). The high variability in study design and methodologies among the different studies that investigated functional decline in elderly people makes difficult to extract coherent results (Kritchevsky et al. 2005; Giaccaglia et al. 2008; Charbonneau et al. 2008). Furthermore, a putative association of the I allele with endurance and ‘fatigue-resistant’ phenotypes, and the D allele with sprint or ‘power’ might account for the conflicting findings of studies investigating the association between *ACE* genotype and human physical performance (Payne and Montgomery, 2003). Collectively, these all demonstrate that the potential involvement of *ACE* genotypes in general levels of fitness and aspects of

physical function appears to be highly complex and argues the need for much greater study.

In the present study, we evaluated functional disability by using the ADL score (Katz et al. 1970). In this population of elderly hospitalized patients, there was a strong motivation to use a structured approach in evaluating disability according to the ADL, a sensitive instrument that have been validated in several settings of geriatric populations. The benefits resulting from this approach are precision and reproducibility that are enhanced by the use of a standardized and validated instrument. Furthermore, in the present study, the association between I/I genotype and disability in the ADLs, was significant in men but not in women. As best as we can determine, these observations are the first reported association between *ACE* polymorphism and disability in older age by gender. Reconciling these findings with the already complex literature surrounding the role of *ACE* in physical function and performance is by no means easy but are interesting enough to warrant further investigation. The current findings appear to contradict some previous reports of association between the I/I genotype and improved endurance or function. Yet the findings of other studies in older adults including those of response to exercise in well-functioning older adults (Kritchevsky et al. 2005), of greater gains in knee extensor strength (Giaccaglia et al. 2008), and of improved hand-grip strength (Yoshihara et al. 2009) all associated with the *ACE* D/D genotype also contradict other areas of the literature in which *ACE* has been implicated with forms of arthritis (Veale et al. 1992; Dalbeth and Haskard, 2005).

The apparent contradiction being that D/D genotype with its well-recognized association with higher plasma ACE levels would similarly be expected to result in higher levels of the pro-inflammatory angiotensin II and thus higher risk of arthritic conditions where angiotensin II may have a causative effect (Dalbeth and Haskard, 2005). On this basis, the current observed association between *ACE* genotype and disability is less likely to be related to arthritis conditions since I/I genotype should be associated with less angiotensin II. Equally, although I/I and I/D genotypes have been associated with increased risk of AD (Kehoe et al. 2009), this is unlikely to explain the observed association with disability since there were no observed associations with any of the cognitive

assessments undertaken. Interestingly, the D allele of the *ACE* I/D polymorphism is often reported to be associated with cardiovascular diseases (Crisan and Carr 2000), and the presence of cardiovascular diseases can cause disability (Verbrugge and Jette 1994). Curiously, in a recent study, the same-type relationships were observed among cardiovascular diseases, apolipoprotein E, and instrumental activities of daily living (IADL) disability, and, similarly to the present study, those effects were found to be men-specific (Kulminski et al. 2008). However, since we found a significant association between disability and *ACE* I/I genotype (i.e., a detrimental recessive model for I allele which corresponds to a protective dominant model for D allele), we investigated the same genetic model for the association with cardiovascular diseases considered as outcome, with no significant association between the *ACE* I/D polymorphism and cardiovascular diseases for all subjects and for men and females separately.

Limitations of the present study should also be considered in interpreting our findings. One such example may have been our definition of chronic disability required that the ADL was assessed in relation to the conditions reported to 1 week before the admission to our Geriatric Unit. However, a widely accepted definition of chronic disability required an impairment in a specific function (i.e., inability to perform ADL or/and IADL) existing for 90 or more days (Manton et al. 2006; Manton 2008). Since disability is a complex condition that may be influenced by functional conditions, such as malnutrition and cognitive impairment, and organic pathologies including neurodegenerative and cardiovascular diseases, we evaluated the *ACE* genotype association with ADL disability after adjustment for several functional and pathological conditions. Such adjustments were necessary to try reduce the probability that any observed associations were not spurious and caused by confounders such as the possibility that our findings could be a type I (i.e., false positive) statistical error often common in genetic association studies (Deng 2001; Koller et al. 2004), but also because as a multi-factorial condition, disability in old age is very likely related to both genetic and environmental factors (Kulminski et al. 2010) where many loci could be involved, each of which may contribute only a small effect. Indeed some environmental factors may even have masking effects while it

is well documented that an individual gene has, at best, a small effect on physical function (Williams and Folland, 2008). Finally, with regard to the *ACE* genotype distribution, it is surprising that, despite this was one of the most studied polymorphisms in case–control studies, up-to-date clear data regarding its genotype distribution in Caucasians is lacking. Notably, these data are missing on the SNP database of the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). Some insight are possible from the Rotterdam Study, in which a total of 6,153 Caucasian from Netherlands were investigated and the *ACE* genotype frequencies reported were D/D 27.8%, I/D 50.3%, and I/I 21.9% (Slegers et al. 2005). These are quite different from those reported in the present study. However, the frequency of *ACE* alleles follow a European North–South gradient in different populations and at different ages, where the frequency of the I allele decreases and the frequency of the D allele concomitantly increases (Panza et al. 2003; Panza et al. 2007a, b). This gradient may explain the proportion of *ACE* genotypes that we observed in the present study (D/D 13.2%, I/D 45.6%, and I/I 41.2%). Moreover, these frequencies did not differ from the distributions expected by Hardy–Weinberg principles. Nonetheless, we have to acknowledge that our broad definition of cardiovascular diseases as an outcome measure may also be masking genuine associations with sub-types of cardiovascular diseases which will inevitably have gene–gene and/or gene environment related etiology (Kulminski et al. 2010).

While interesting, it is clear that further studies are now needed to try and replicate these findings and if in agreement evaluate the potential relevance and mechanism by which *ACE* genotype. Indeed, most studies to date have focused on the common *ACE* indel and wider study of *ACE* variation is likely to be more informative and in turn may identify more robust variants which could serve as a predictive marker for disability in older patients. Clarification on the mechanistic basis of this association, if this is a genuine association, is also important from a clinical perspective. The potential for *ACE* genotyping to have predictive value in early diagnosis of disability is not presented and needs and could be explored. Equally the association raises questions as to whether this association has any implications or benefits in relation the use of commonly used antihypertensive

drugs, such as ACE inhibitors, which can modulate ACE activity, where it operates on a number of substrates.

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