

**The *ACE* gene is associated with dementia and major depression in a population-based cohort of older individuals followed over twelve years.**

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## **Abstract**

Depression and dementia disorders have been suggested to share similar risk factors and pathogenetic background, such as cardiovascular risk factors, the apolipoprotein E (*APOE*)  $\epsilon$ 4 allele, and white matter lesions. The renin-angiotensin system (RAS) may be involved in the pathogenesis of both dementia and depression due to its role in cardiovascular and metabolic homeostasis. In a previous study we reported an association between the well-known insertion/deletion polymorphism, rs1799752, in the gene encoding angiotensin-converting enzyme (*ACE*) and dementia at baseline in a population-based Swedish cohort of older individuals. In the present study, we extended our analyses of RAS-related gene variants to also include the SNP rs5186 in the angiotensin II type 1 receptor (*AGTRI*) gene and the phenotype late life depression. The purpose of the study was to examine the influence of the *AGTRI* SNP, as well as the *ACE* I/D polymorphism, on both dementia and late-life depression in a population-based sample followed over twelve years. Like rs1799752 in *ACE*, rs5186 in *AGTRI* was associated with dementia at baseline. None of the polymorphisms were associated with dementia during follow-up, but for rs1799752 this could be explained by a significant association with onset age. When the total number of individuals who developed major depression up to year 2009 was analyzed, significant associations, remaining after exclusion of dementia up to 2012, with rs1799752 in *ACE* were found. Overall, in the investigated population-based sample of older individuals, genetic variation in *ACE*, seems to be of importance for both dementia and major depression. Still, the results have to be interpreted with caution because of the small sample size, and further studies of the importance of these genes for dementia and late life depression in population based samples are warranted.

## **Introduction**

Several studies suggest that depression and dementia disorders may share similar risk factors and pathogenetic background, including cardiovascular risk factors, the *APOE-ε4* allele, and white matter lesions (Skoog et al. 1996; Skoog 2011; Gudmundsson et al. 2015; Skoog et al. 2015). The renin-angiotensin system (RAS) may be involved in the pathogenesis of both dementia and depression due to its role in cardiovascular and metabolic homeostasis. Almost all organs, including the central nervous system, have their own local RAS with specific actions (Kehoe et al. 2009). RAS is thought to be of importance in dementia disorders since it regulates blood pressure (including the cerebral blood flow), but also due to its effects on amyloid metabolism, memory and learning (Mogi et al. 2012). It may be important in depression due to its role in the hypothalamic-pituitary-adrenocortical (HPA) axis (Aguilera et al. 1995) and immune responses (Miller et al. 2009), which is of relevance also for dementia (Heppner et al. 2015; Martocchia et al. 2015). In addition, angiotensin II receptors (AGTR1) are found in regions crucial for mood regulation ((Tsutsumi and Saavedra 1991; Jöhren and Saavedra 1996).

In RAS, angiotensinogen is converted to angiotensin I, which is converted to angiotensin II by the enzyme angiotensin converting enzyme (ACE). Angiotensin II exerts its effects through binding to different types of angiotensin II receptors, AGTR1 and AGTR2, where AGTR1 is the primary one (Skultetyova et al. 2007; Taylor et al. 2012). Genetic studies of the RAS-system in relation to dementia have primarily focused on the *ACE* gene. The widely studied *ACE* insertion/deletion (I/D) polymorphism (rs1799752) is related to the activity level of the

enzyme in the periphery (Rigat et al. 1990). We have previously reported an association between this polymorphism and dementia in a population-based Swedish cohort (Gustafson et al. 2010). However, results from meta-analyses are inconsistent (Lehmann et al. 2005; Liu et al. 2009; Belbin et al. 2011).

The recent International Genomics of Alzheimer's Project provides suggestive evidence of associations between *ACE* single nucleotide polymorphisms (SNPs) and AD in a meta-analysis of GWAS results (Lambert et al. 2013). In addition, another study using GWAS data in an attempt to replicate top findings from candidate gene studies in AD, reported variations in the *ACE* gene to be significantly associated with AD (Webster et al. 2010). Furthermore, the importance of the *ACE* gene in AD was confirmed in a recent study, primarily investigating associations between AD-related proteins and SNPs on a genome-wide basis, including a proxy-SNP (rs4343) in very high linkage disequilibrium with the I/D SNP rs1799752 (Kauwe et al. 2014).

Another RAS-related gene is the angiotensin receptor II, type 1 (*AGTRI*) gene, which has been related to cardiovascular disorders, such as hypertension (Niu and Qi 2010) and myocardial infarction (Feng et al. 2014). To date, only one study has specifically investigated the relationship between *AGTRI* and dementia, and this was a negative clinical study from South Korea on VaD (Kim et al. 2006).

Previous studies of RAS-related genes in depression have also mainly focused on the *ACE* I/D variation rs1799752, but results are inconsistent (Lopez-Leon et al. 2008; Wu et al. 2012). Despite possible etiological differences between early and late onset depression (Blazer 2003; Otte et al. 2005; Kendler et al. 2009; Taylor et al. 2013), only one study have focused on the

*ACE* gene in late-life depression. This study showed associations with several different *ACE* SNPs, e.g. the rs1799752 proxy rs4343 (Ancelin et al. 2013).

Studies on the *AGTR1*-gene in depression are mainly based on clinical samples. One study report an association between the SNP rs5186 (A1166C), a variation of probable functional importance (Sethupathy et al. 2007), and major depression in a sample of mixed ages (Saab et al. 2007), while another found no association with late-life depression (Taylor et al. 2010). In addition, other variations in the *AGTR1*-gene were reported to be associated with late-life depression (Taylor et al. 2012).

In the present study, we extended our analyses of RAS-related gene variants in psychiatric disorders in the elderly to also include the SNP rs5186 in the angiotensin II type 1 receptor (*AGTR1*) gene and the phenotype late life depression. The main aim of the study was to examine the influence of the *AGTR1* SNP, as well as the *ACE* I/D polymorphism, on dementia and late-life depression in a population-based sample followed over twelve years.

## **Material and Methods**

### ***Participants***

Participants originate from two epidemiological studies in Gothenburg, Sweden, the Prospective Population Study of Women (PPSW) and the Gerontological and Geriatric Population Studies (H70), both which have been described in detail previously (Steen and Djurfeldt 1993; Bengtsson et al. 1997; Skoog 2004; Karlsson et al. 2009). The participants were sampled from the Swedish Population Register on the basis of their birth date and were born in 1908, 1914, 1918, 1922 and 1930. Both persons living in private households and in residential care were included. In total, there were 1495 eligible individuals in 2000-2001, and

1051 agreed to participate (response rate 70.3%). Among these, 900 (86%) consented to donate their blood for genetic analyses. Due to the nature of the studies, the women (n=679) were aged 70-92 years and the men (n=221) aged 70 years. Follow-up psychiatric examinations were conducted in 2005-2006 and 2009-2010. There were 686 participants followed-up in 2005-06 (response rate among survivors 87 %), and 504 in 2009-10 (response rate among survivors 78 %). Characteristics of the study sample are presented in Table 1.

The study was approved by the Ethics Committee for Medical Research at the University of Gothenburg, and informed consent was obtained from all participants and/ or their relatives in cases of dementia.

### *Neuropsychiatric examinations and interviews*

The clinical examinations were conducted at an outpatient department or in the participant's home and included comprehensive social, functional, physical, neuropsychiatric and neuropsychological examinations, as well as close informant interviews.

Semi-structured neuropsychiatric examinations were performed by trained psychiatric research nurses. These examinations included ratings of the past month's psychiatric symptoms and signs according to the Comprehensive Psychopathological Rating Scale (CPRS),(Asberg et al. 1978), which is valid and reliable in older populations (van der Laan et al. 2005), Mini-International Neuropsychiatric Interview (Sheehan et al. 1998), self-reported history of depression, and assessment of current medications. Ratings of common symptoms and signs of dementia were performed (e.g. assessments of memory, orientation, general knowledge, apraxia, visuospatial function, understanding proverbs, following commands, naming ability and language) and has been described in detail previously (Skoog et al. 1993; Guo et al. 2007). Cognitive function was also measured with the Mini Mental State Examination (MMSE) (Folstein et al. 1975).

The psychiatric nurses who performed the examinations were supervised and trained by psychiatrists. Inter-rater reliability between psychiatrists and nurses was studied in 50 individuals who had dual ratings by either psychiatric research nurses or psychiatrists. Kappa values for the presence versus absence of symptoms and signs necessary to diagnose depression were between 0.62 and 1.00 indicating “good” (reference range kappa=0.61-0.80) or “excellent” (kappa=0.81-1.00) agreement. Inter-rater agreement for the symptoms and signs used to diagnose dementia was between good and excellent (kappa values between 0.74 and 1.00) (Wancata et al. 2007). Close informant interviews were performed in 2000, 2005, and 2009. The interviews were semi-structured and comprised questions about changes in behaviour and intellectual function, psychiatric symptoms, activities of daily living, and, in cases of dementia, age of onset and disease course.

### *Diagnoses*

Dementia was diagnosed by geriatric psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-III-R) (APA 1987), based on symptoms rated during the neuropsychiatric examinations and information from the close informant interviews, as described previously (Skoog et al. 1993; Guo et al. 2007; Skoog et al. 2015). Incident cases of dementia up to year 2012 were also based on information from the Swedish Hospital Discharge Register (ICD-10: F00.1, F01.8, F01.9, F03.9, G30.9).

Major and minor depression were diagnosed according to DSM-IV research criteria (Skoog et al. 1993; APA 1994) [ENREF 44](#), except that the use of the bereavement criterion was not applied, which makes the criteria the same as DSM-V (Maj 2012). Depression symptom

burden was measured with the Montgomery-Åsberg Depression Scale (MADRS)(Asberg et al. 1978).

### ***Genotyping***

DNA was extracted from blood samples according to standard procedures. Genotyping of *ACE* rs1799752 and *AGTRI* rs5186 was conducted according to Olsson et al (2004). Both polymorphisms were found to be in Hardy Weinberg equilibrium. *APOE* (gene map locus 19q13.2) genotyping was performed by mini-sequencing as previously described in detail (Blennow et al. 2000). Genotypes were obtained for the two SNPs (rs7412 and rs429358), which are used to unambiguously define  $\epsilon$ 2,  $\epsilon$ 3, and  $\epsilon$ 4 alleles.

### ***Statistics***

Differences in distributions and mean values of sample characteristics (Table 1) were analysed using Fisher's exact test or t-test. Associations between genotypes and dementia at baseline or depression were investigated by logistic regression models, while associations between genotypes and dementia during follow-up were investigated by Cox regression models. Three different models were tested; a crude model including only genotype data and data about diagnosis (model 1), a second model also including age and sex (when analyzing the total sample) as covariates (model 2), and a final model including *APOE*  $\epsilon$ 4 status as an additional covariate (model 3). When investigating depression, all analyses were performed both with and without exclusion of dementia up to year 2012. Associations with mean age at onset, as well as analyses of mean blood pressures at baseline versus *ACE* and *AGTRI* genotypes, were done using linear regression. The analyses were performed in SPSS 22, R 2.2 or STATA v.13.



## Results

Like previously reported for the II-genotype of *ACE* rs1799752 (Gustafson et al. 2010), the CC-genotype of *AGTRI* rs5186 were associated with dementia at baseline (Table 2). There were no interactions with sex. There was no relation between the polymorphisms and the development of dementia during follow-up (Table 3). In order to explore possible reasons for the diminished effect of the angiotensin-related polymorphisms during follow-up, we investigated the relation with age at onset of dementia. A significant association with onset age was seen only for rs1799752 in *ACE* ( $p=0.018$ ). Individuals carrying the II-genotype have approximately 2 year earlier onset than individuals with the other two genotypes (mean age at onset 79.5 years for II and 81.7 years for ID+DD). There were no interactions with sex in any of these analyses.

Regarding depression, we included all individuals who had major depression on at least one occasion during the study period, as this is a periodic disorder, not a chronic disorder as dementia. When the total number of individuals who developed depression up to year 2009 was analyzed, significant associations with the II-genotype of *ACE* rs1799752, and a trend for an association with the CC-genotype of rs5186 in *AGTRI* were found (Table 4). The significant associations with rs1799752 remained after exclusion of all individuals with dementia up to 2012. There were no interactions with sex.

Since the investigated polymorphisms previously have been associated with hypertension, although with inconsistent results, we investigated the relation with mean systolic and diastolic blood pressure in the present sample at baseline. However, no associations could be found with either *ACE* rs1799752 or *AGTRI* rs5186.

## Discussion

In a previous study in this population, our research group reported an association between the *ACE* rs1799752 and dementia (Gustafson et al. 2010). In the present study, we report a similar association between another angiotensin-related SNP, rs5186 in *AGTRI*, and dementia at baseline. These two polymorphisms were not associated with dementia during follow-up. One possible explanation is the relation with age at onset of dementia, although this finding was significant only for rs1799752 in *ACE*. Interestingly, this result is in line with previous investigations of *ACE* in AD, showing an association between the rs1799752 proxy SNP rs4343 and age at onset (Kehoe et al. 2004). These findings emphasize the importance of considering age at onset and age of samples when evaluating the effect of different genetic variations.

A significant association was found between carriers of the II-genotype rs1799752 in *ACE* and the cumulative prevalence and incidence of major depression during year 2000-2009. A trend in the same direction was seen for the CC-genotype of the *AGTRI*-SNP. This illustrates the importance of several follow-up examinations in genetic studies of depression, in order to detect more individuals with depression and to make the control group cleaner. Due to the high life-time prevalence of depression (Andrews et al. 2005; Moffitt et al. 2010), a large portion of persons in the control group will be misclassified in cross-sectional studies. The fact that we only had 9 years follow-up indicates that we probably missed some cases with life-time history of depression. The associations with depression remained also after exclusion of dementia up to year 2012, indicating that the result was not merely due to prodromal symptoms of dementia.

There is a possibility that SNPs in angiotensin-related genes, such as *ACE* and *AGTRI* interact in their effect on dementia and depression, since this type of epistasis have been shown in other disorders (Ye et al. 2003). Still no synergism between the high-risk genotypes could be observed in this study, probably because we were underpowered to detect such effects (only 17 individuals carry both high-risk genotypes).

The result of the present study suggests common mechanisms for dementia and late life depression. This is further supported by studies from our research group, reporting that white matter lesions and temporal lobe atrophy in 70-year-olds increased risk of both dementia and depression during 10-year follow-up (Gudmundsson et al. 2015), and that the *APOE* $\epsilon$ 4-allele was a risk factor for both disorders (Skoog et al. 2015). To our knowledge, this is the first study analyzing genotype-data of *ACE* rs1799752 and *AGTRI* rs5186 versus both dementia and late life depression in a population based Caucasian sample. The *ACE* gene has been studied in the British MRC CFAS study, analysing both prevalent and incident dementia, reporting no associations (Yip et al. 2002; Keage et al. 2010). However, mean age at baseline was higher compared to the present study, and no analyses of possible associations with age at onset were performed. The only study specifically investigating *AGTRI* in dementia was performed in a clinical population from Korea (Kim et al. 2006). In that study also *ACE* was included, but no associations were reported. In addition, the Asian study only examined the subtype VaD, while our results are based on a population-based study including all types of dementia. Also, as a result of different ethnicity, there is a deviation in allele frequencies, for example no individual in the Asian sample had the CC-genotype of rs5186. Interestingly, the result from the only previous population-based study of angiotensin-related genes in late life depression is in line with our study, and shows an association with a proxy-SNP of rs1799752 in the *ACE* gene (Ancelin et al. 2013).

Conflicting opinions about the role of ACE in the brain exist. The vascular hypothesis suggests that high ACE activity may increase the risk of dementia by increasing vascular risk, while the amyloid hypothesis implies that high ACE activity may decrease the risk of dementia by reducing the accumulation of amyloid  $\beta$  in the brain (Jochemsen et al. 2012). The latter is supported by the fact that the II-genotype of the *ACE* rs1799752, related to lowest enzymatic levels (Rigat et al. 1990), have been associated with an increased risk of AD (Webster et al. 2010; Lambert et al. 2013), in line with the results of our study. Furthermore, studies of white matter lesions and cortical brain atrophy in relation to ACE activity suggest both detrimental and beneficial effects of high ACE levels on the brain. Relation between the CC-genotype or the C-allele of *AGTR1* rs5186 and hypertension (Niu and Qi 2010), as well as decreased white matter integrity in the brain (Taylor et al. 2013; Salminen et al. 2014), have been shown in several studies.

The strengths with the present study are the representative population-based cohort, the comprehensive examinations performed by trained psychiatric nurses, as well as the long follow-up period and high response rate during follow-up. There are also some limitations. First, the small sample size makes some analyses hard to interpret, e.g. interactions between the investigated SNPs. Second, we were not able to classify subtypes of dementia. However, phenotypic overlap of VaD and AD is common, and these two forms of dementia may share common neuropathological mechanisms. White matter infarcts and cerebrovascular pathology are often present in individuals with AD, while senile plaques can be found in individuals with VaD (Leys et al. 1999). Furthermore, ischemic insults of the brain, such as in VaD, may stimulate formation of the pathological hallmarks of AD, i.e. neurofibrillary tangles and accumulation of amyloid precursor protein (Wen et al. 2004; Wen et al. 2004). Still, we

cannot exclude the possibility that one of the investigated polymorphisms might be of importance mainly in AD and the other in VaD, perhaps by acting through different biological mechanisms. This is further indicated by previous studies showing an established relation with *ACE* I/D in AD, but not in VaD. Third, the high age of the sample prevent inferences to other age groups. Fourth, due to the merging of two different population studies (albeit examined with identical methods during the same time), the study is unbalanced regarding gender (Skoog et al. 2015). Therefore, the group older than 70 years at baseline only comprised women. Thus, our exploratory analyses regarding interactions with sex have to be interpreted cautiously. Fifth, although we had a nine-year follow-up, some of the participants may have had depressive episodes prior to baseline and others may have had such episodes between examination waves. Thus, we might still have underestimated the number with genetic risk for depression.

Conclusively, in the investigated population-based sample of older individuals, genetic variation in angiotensin-related genes, especially in *ACE*, is associated with both dementia and major depression. Still, the results have to be interpreted with caution because of the small sample size, and further studies of these genes in population-based samples are warranted.

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**Table 1. Characteristics of the study sample.**

	Total sample, n=900 mean (sd) or n (%)
Age at baseline year 2000	74.2 (5.5)
Dementia year 2000	58 (6.4)

Dementia up to year 2012	196 (21.8)
Age at onset of dem 2000	76.2 (6.9)
Age at onset of dem 2012	81.1 (7.0)
Major dep up to year 2009	80 (8.9)
Minor dep up to year 2009	250 (27.8)
Major dep without dementia <sup>1</sup>	64 (7.1)
Minor dep without dementia <sup>1</sup>	183 (20.3)
<i>APOE</i> ε4 allele	254 (28.2)

<sup>1</sup>Depression after exclusion of dementia up to year 2012.

**Table 2. Associations between the polymorphisms rs5186 in the gene *AGTR1* at baseline year 2000.**

Gene, SNP and genotype	dementia	No dementia	crude OR (95% CI)	crude p-value	adjusted* OR (95% CI)	adjusted* p-value	adjusted** OR (95% CI)
<i>AGTR1_rs5186</i>							
<b>total sample</b>	n (%)	n (%)					
AA	26 (44.8)	459 (54.5)	---	---	---	---	---
AC	20 (34.5)	323 (38.4)	0.99 (0.52-1.82)	0.96	1.00 (0.52-1.86)	0.99	0.99 (0.52-1.86)
CC	12 (20.7)	60 (7.1)	3.45 (1.56-7.2)	<b>0.001</b>	3.25 (1.42-7.06)	<b>0.004</b>	3.19 (1.39-7.34)

\*Model including age and sex as covariates.

\*\*Model including age, sex and *APOE* ε4 status as covariates.

**Table 3. Associations between polymorphisms in the genes *AGTR1* and *ACE* and dementia during follow up.**

Gene, SNP and genotype	dementia	no dementia	crude HR (95% CI)	crude p-value	adjusted* HR (95% CI)	adjusted* p-value	adjusted** HR (95% CI)
<i>AGTR1_rs5186</i>							
<b>total sample</b>	n (%)	n (%)					
AA	68 (49.3)	391 (55.5)	---	---	---	---	---
AC	61 (44.2)	262 (37.2)	1.31 (0.93-1.86)	0.12	1.25 (0.88-1.77)	0.20	1.26 (0.89-1.77)
CC	9 (6.5)	51 (7.2)	0.99 (0.49-1.98)	0.98	0.90 (0.45-1.81)	0.78	0.88 (0.44-1.77)
<i>ACE_rs1799752</i>							
<b>total sample</b>							
DD	40 (29.2)	206 (29.3)	---	---	---	---	---
ID	66 (48.2)	326 (46.4)	1.02 (0.69-1.52)	0.90	1.02 (0.69-1.51)	0.93	0.99 (0.66-1.47)
II	31 (22.6)	171 (24.3)	0.95 (0.59-1.51)	0.82	0.92 (0.58-1.47)	0.73	0.94 (0.59-1.47)

\*Model including age at baseline and sex as covariates.

\*\*Model including age at baseline, sex and *APOE* ε4 status as covariates.

**Table 4. Associations between polymorphisms in the genes *AGTR1* and *ACE* and ever major depression up to year 2009.**

Gene, SNP and genotype	ever maj dep	no dep	crude OR (95% CI)	crude p-value	adjusted* OR (95% CI)	adjusted* p-value	adjusted** OR (95% CI)
<i>AGTR1_rs5186</i>							
<b>total sample</b>	n (%)	n (%)					

AA	40 (50.0)	445 (54.3)	---	---	---	---	---
AC	28 (35.0)	315 (38.4)	0.96 (0.57-1.59)	0.87	0.98 (0.58-1.62)	0.93	0.97 (0.57-1.59)
CC	12 (15.0)	60 (7.3)	1.94 (0.88-3.96)	0.08	1.83 (0.82-3.74)	0.12	1.79 (0.88-3.96)
<i>ACE_rs1799752</i>							
<b>total sample</b>							
DD	17 (21.3)	247 (30.2)	---	---	---	---	---
ID	37 (46.3)	372 (45.5)	1.49 (0.83-2.82)	0.20	1.53 (0.85-2.90)	0.17	1.53 (0.85-2.90)
II	26 (32.5)	199 (24.3)	2.00 (1.05-3.91)	<b>0.04</b>	2.14 (1.13-4.20)	<b>0.02</b>	2.17 (1.05-3.91)

\*Model including age at baseline and sex as covariates.

\*\*Model including age at baseline, sex and *APOE* ε4 status as covariates.