Blood type gene locus has no influence on *ACE* association with Alzheimer's disease.

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Abstract
The *ABO* blood group locus was recently found to contribute independently as well as via interactions with *ACE* gene variation to plasma levels of angiotensin converting enzyme (ACE). Variation in *ACE* has also previously been implicated as conferring susceptibility for Alzheimer's disease (AD), but has also been proposed to confer risk via interactions with other as yet unknown genes. More recently, larger studies have not supported *ACE* as a risk factor for AD, while the role of ACE pathway in AD has come under increased levels of scrutiny with respect to various aspects of AD pathology and possible therapies. We explored the potential combined involvement of *ABO* and *ACE* variation in the genetic susceptibility of 2067 AD cases compared to 1376 non-demented elderly. Including the effects of *ABO* haplotype did not provide any evidence for the genetic association of *ACE* with AD.

1. Introduction

The renin angiotensin system (RAS) has become a biological pathway of increasing interest in the pathogenesis of Alzheimer's disease (AD) and potentially as a basis for future interventions. Previous genetic associations between *ACE,* that encodes for angiotensin converting enzyme (ACE), one of the key enzymes in the RAS, played a significant role in the growing interest of this system in AD. This was aided by the fact that the suggested *ACE* risk variant contributed to lower levels of plasma ACE while it was also shown that ACE degraded amyloid-β peptide, which is widely involved in the pathology of AD (reviewed in Kehoe and Wilcock, 2007). However, more recent haplotype studies and Genome Wide Association Studies (GWAS) have not supported the role of *ACE* as a genetic risk factor for AD. Previous meta-analyses have suggested however the possibility that *ACE* may mediate risk via epistatic interactions with other genetic risk variants (Lehmann et al., 2005).

Recently it was shown that the variants in the *ABO* blood group locus, independently and when combined with *ACE* variants, explained a greater proportion of the population variance of *ACE* in plasma than *ACE* alone (Terao et al., 2013). In particular, alleles tagging the *ABO* blood group Type A1 were associated with the lowest plasma ACE activity, while alleles tagging Type B were associated with increased ACE activity and Type A2 and O alleles were associated with intermediate activity (Terao et al., 2013). Given the previously suggested role of ACE activity in AD (Miners et al., 2008) we investigated whether variation in *ABO* when combined with *ACE* variation might be associated with increased risk of AD.

2. Methods

A combined GWAS dataset of 3448 samples from Alzheimer's Research UK (ARUK) and the Mayo Clinic genotyped on Illumina HumanHap300v1 was used. All samples were of European descent, 1487 males and 1922 females, 2069 cases with average age at death of 73.5 years (range 51-108) and 1379 controls with average age at death of 73.4 years (range 54-97 years), with *APOE* alleles: ε2 - 5.7%; ε3 - 65.9%; ε4 - 28.4%. The power of the study to detect an association with AD was calculated in QUANTO v1.2.4.

The *ABO* and *ACE* regions were imputed against 1000 Genomes Phase I haplotypes in IMPUTE2. Imputed data was further quality control checked resulting in 4599 SNPs in *ACE* for association testing. *ABO* blood group haplotypes were determined from the imputed *ABO* region and included as a covariate for the merged dataset. The imputed *ACE* region was association tested in PLINK (Purcell et al., 2007) using logistic regression corrected for sex, age at onset and *APOE* ε4 allele and including *ABO* type as an interaction term. The association test was also run including the blood group B allele and A1 allele separately as interaction terms. The power of the study to detect the interaction was calculated post-hoc using G*Power. For further details please see the supplementary materials.

3. Results

Power calculations in QUANTO v1.2.4 indicated 71% to 99% power at this sample size to detect an association with a genetic variant with MAF of 1% to 5% assuming a genetic odds ratio of 1.2 to 1.5.

ABO blood group haplotype was determined for 3396 samples, as the genotype was not available for all samples. The logistic regression model corrected for sex, age at onset and *APOE* ε4 allele dose and provided no evidence to support an *ACE* association with disease in this dataset (Suppl. Table 2, Suppl. Fig 1-3).

Additionally, the interaction terms of *ABO* blood type and *ACE* variant and the interaction terms for the presence of a B allele or A1 allele and *ACE* variants were not significantly associated with disease status and failed to improve the model containing only covariates (Suppl. Table 2, Suppl. Fig 4,5). Post-hoc power analysis showed that the effective power was 77% to 83%.

4. Discussion

In this report we have determined if *ABO* blood type can potentially modify any association of *ACE* gene variation with AD. To address this issue we have used *ABO* haplotypes (both A1 and B alleles),

modelling interactions reported for *ABO* with *ACE* on plasma ACE levels, to explore whether it had any additional effects on *ACE* SNP associations with AD (Suppl. Fig 1-5). It should be noted that the majority of these cases were pathologically confirmed as AD so the contribution to vascular AD, which is biologically plausible, remains to be determined.

In summary, we find no evidence to support a role for *ACE,* modelled with *ABO* locus genotypes, being associated with the risk of developing AD.

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Disclosure statement
The authors declare that there are no conflicts of interest. Approval was obtained from the ethics committee or institutional review board of each institution responsible for the collection of samples. All individuals who participated in this study gave written informed consent.

Appendices
Supplementary Methods and Results, Supplementary table 1, 2 and Supplementary figures 1, 2, 3, 4 and 5.

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