Supplementary data.

Suppl. Methods

All participants included in the study gave informed consent and the study was approved by the local Ethics Committee. The ARUK cohort (1197 AD patients and 886 controls) comprised samples from six Alzheimer's Research UK network centres across the EU, Belfast, Bonn, Nottingham, Manchester, Oxford and Southampton. The Mayo samples (872 AD patients and 493 controls) were obtained from the Mayo Clinic, Jacksonville and the Mayo Clinic, Rochester, USA. The combined GWAS dataset of 3448 samples was quality control checked in PLINK following standard protocol with 302297 SNPs passing quality control (see Shi et al., 2012).

To assess the ability of the study sample size to detect an association with AD, power calculations were performed in QUANTO v1.2.4 assuming the genetic variant has a minor allele frequency (MAF) between 1% and 5% and an odds ratio of 1.2 and 1.5 with a gene-environment interaction model and a log additive genetic effect. The odds ratio was selected to represent the range of odds ratios of the susceptibility loci recently found in the I-GAP (International Genomics of Alzheimer's project) meta-analysis of AD (Lambert et al., 2013).

This study aimed to assess the impact of *ABO* blood type locus on possible *ACE* association with AD. The *ABO* and *ACE* regions and a 500 000bp buffer region (*ABO* chr9:13562788-136650617; *ACE* chr17:61054422-62099205) was imputed against 1000 Genomes Phase I haplotypes in IMPUTE2 (Howie et al., 2009). Imputed data was further quality control checked (imputation R^2 >0.4 and MAF >0.01), resulting in 4599 SNPs in the *ACE* region for association testing.

ABO blood group haplotypes were determined from SNPs rs505922 (T allele indicating blood type O, C allele indicating blood type A or B) and rs8176746 (G allele indicating blood type O or A and T allele indicating blood type B) as previously described (Wolpin et al., 2010). Blood type A was further categorised into A1 and A2 blood types using rs8176704 (Wolpin et al., 2010). *ABO* blood type was included as an interaction variable for the merged dataset and the imputed *ACE* region was association tested using logistic regression in PLINK (Purcell et al., 2007) controlling for the following covariates: age, sex, *APOE* ε allele genotype and including *ABO* type as an interaction variable.

As the B allele has been found to account for a 36% increase in ACE levels and the A1 allele with a 36% decrease in ACE levels (Terao et al., 2013), the association test was run again with the same covariates as above, but including the interaction of the B or A1 alleles with *ACE* genotype instead of *ABO* blood typing. To determine the power to detect a significant interaction effect in the dataset, post-hoc power analysis was performed using G*Power (Faul et al., 2009).

Suppl. Results

Imputation for the two gene regions completed well with a concordance of >98% for both. In particular, the SNPs of interest in both gene regions imputed with good info scores much greater than the recommended cut-off of 0.4 (Suppl. Table 1).

ABO blood group haplotype was determined for 3396 samples, as the genotype was not available for both SNPs for 52 samples. For cases, 46.5% were Type O, 38.9% Type A, 11.1% Type B and 3.5% Type AB, while for controls, 45.1% were Type O, 40.1% Type A, 11.1% Type B and 3.7% Type AB.

Following association testing including the covariates sex, age-at-onset and *APOE* ε4 allele, no SNPs in *ACE* achieved genome wide significance (p-value < $5x10^{-8}$). Including the *ABO* haplotype and the additive genetic effect of each *ACE* variant tested as an interaction term in the regression provided no additional effect on the *ACE* SNP association with AD (Suppl. Table 2, Suppl. Fig 1-3). While including an interaction term of the presence of a B allele or an A1 allele alone with the additive genetic effect of each *ACE* variant also failed to improve a model containing only covariates (Suppl. Table 2, Suppl. Fig 4, 5). Post-hoc power analysis showed that there was 77% to 83% power to detect an interaction effect for each of the three interaction terms tested.

References

Faul, F., Erdfelder, E., Buchner, A., Lang, A.-G., 2009. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. Behav. Res. Methods 41, 1149–60. doi:10.3758/BRM.41.4.1149

Howie, B.N., Donnelly, P., Marchini, J., 2009. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. PLoS Genet. 5, e1000529. doi:10.1371/journal.pgen.1000529

Lambert, J.-C., Ibrahim-Verbaas, C.A., Harold, D., Naj, A.C., Sims, R., Bellenguez, C., Jun, G., Destefano, A.L., Bis, J.C., Beecham, G.W., Grenier-Boley, B., Russo, G., Thornton-Wells, T.A., Jones, N., Smith, A. V, Chouraki, V., Thomas, C., Ikram, M.A., Zelenika, D., Vardarajan, B.N., Kamatani, Y., Lin, C.-F., Gerrish, A., Schmidt, H., Kunkle, B., Dunstan, M.L., Ruiz, A., Bihoreau, M.-T., Choi, S.-H., Reitz, C., Pasquier, F., Hollingworth, P., Ramirez, A., Hanon, O., Fitzpatrick, A.L., Buxbaum, J.D., Campion, D., Crane, P.K., Baldwin, C., Becker, T., Gudnason, V.,

Cruchaga, C., Craig, D., Amin, N., Berr, C., Lopez, O.L., De Jager, P.L., Deramecourt, V., Johnston, J.A., Evans, D., Lovestone, S., Letenneur, L., Morón, F.J., Rubinsztein, D.C., Eiriksdottir, G., Sleegers, K., Goate, A.M., Fiévet, N., Huentelman, M.J., Gill, M., Brown, K., Kamboh, M.I., Keller, L., Barberger-Gateau, P., McGuinness, B., Larson, E.B., Green, R., Myers, A.J., Dufouil, C., Todd, S., Wallon, D., Love, S., Rogaeva, E., Gallacher, J., St George-Hyslop, P., Clarimon, J., Lleo, A., Bayer, A., Tsuang, D.W., Yu, L., Tsolaki, M., Bossù, P., Spalletta, G., Proitsi, P., Collinge, J., Sorbi, S., Sanchez-Garcia, F., Fox, N.C., Hardy, J., Naranjo, M.C.D., Bosco, P., Clarke, R., Brayne, C., Galimberti, D., Mancuso, M., Matthews, F., Moebus, S., Mecocci, P., Del Zompo, M., Maier, W., Hampel, H., Pilotto, A., Bullido, M., Panza, F., Caffarra, P., Nacmias, B., Gilbert, J.R., Mayhaus, M., Lannfelt, L., Hakonarson, H., Pichler, S., Carrasquillo, M.M., Ingelsson, M., Beekly, D., Alvarez, V., Zou, F., Valladares, O., Younkin, S.G., Coto, E., Hamilton-Nelson, K.L., Gu, W., Razquin, C., Pastor, P., Mateo, I., Owen, M.J., Faber, K.M., Jonsson, P. V, Combarros, O., O'Donovan, M.C., Cantwell, L.B., Soininen, H., Blacker, D., Mead, S., Mosley, T.H., Bennett, D.A., Harris, T.B., Fratiglioni, L., Holmes, C., de Bruijn, R.F.A.G., Passmore, P., Montine, T.J., Bettens, K., Rotter, J.I., Brice, A., Morgan, K., Foroud, T.M., Kukull, W.A., Hannequin, D., Powell, J.F., Nalls, M.A., Ritchie, K., Lunetta, K.L., Kauwe, J.S.K., Boerwinkle, E., Riemenschneider, M., Boada, M., Hiltunen, M., Martin, E.R., Schmidt, R., Rujescu, D., Wang, L.-S., Dartigues, J.-F., Mayeux, R., Tzourio, C., Hofman, A., Nöthen, M.M., Graff, C., Psaty, B.M., Jones, L., Haines, J.L., Holmans, P.A., Lathrop, M., Pericak-Vance, M.A., Launer, L.J., Farrer, L.A., van Duijn, C.M., Van Broeckhoven, C., Moskvina, V., Seshadri, S., Williams, J., Schellenberg, G.D., Amouyel, P., 2013. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. Nat. Genet. advance on. doi:10.1038/ng.2802

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., de Bakker, P.I.W., Daly, M.J., Sham, P.C., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81, 559–75. doi:10.1086/519795

Shi, H., Belbin, O., Medway, C., Brown, K., Kalsheker, N., Carrasquillo, M., Proitsi, P., Powell, J., Lovestone, S., Goate, A., Younkin, S., Passmore, P., Morgan, K., 2012. Genetic variants influencing human aging from late-onset Alzheimer's disease (LOAD) genome-wide association studies (GWAS). Neurobiol. Aging 33, 1849.e5–18. doi:10.1016/j.neurobiolaging.2012.02.014

Terao, C., Bayoumi, N., McKenzie, C.A., Zelenika, D., Muro, S., Mishima, M., Connell, J.M.C., Vickers, M.A., Lathrop, G.M., Farrall, M., Matsuda, F., Keavney, B.D., 2013. Quantitative Variation in Plasma Angiotensin-I Converting Enzyme Activity Shows Allelic Heterogeneity in the ABO Blood Group Locus. Ann. Hum. Genet. doi:10.1111/ahg.12034

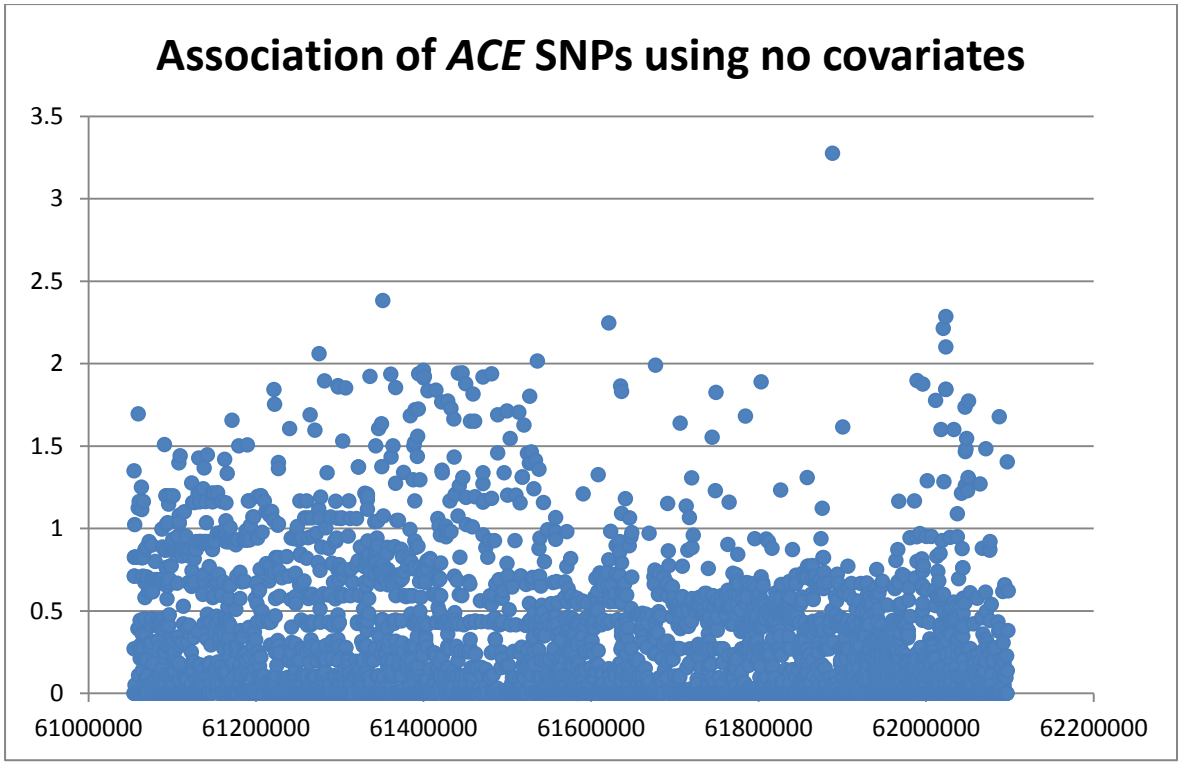
Wolpin, B.M., Kraft, P., Gross, M., Helzlsouer, K., Bueno-de-Mesquita, H.B., Steplowski, E., Stolzenberg-Solomon, R.Z., Arslan, A.A., Jacobs, E.J., Lacroix, A., Petersen, G., Zheng, W., Albanes, D., Allen, N.E., Amundadottir, L., Anderson, G., Boutron-Ruault, M.-C., Buring, J.E., Canzian, F., Chanock, S.J., Clipp, S., Gaziano, J.M., Giovannucci, E.L., Hallmans, G., Hankinson, S.E., Hoover, R.N., Hunter, D.J., Hutchinson, A., Jacobs, K., Kooperberg, C., Lynch, S.M., Mendelsohn, J.B., Michaud, D.S., Overvad, K., Patel, A. V, Rajkovic, A., Sanchéz, M.-J., Shu, X.-O., Slimani, N., Thomas, G., Tobias, G.S., Trichopoulos, D., Vineis, P., Virtamo, J., Wactawski-Wende, J., Yu, K., Zeleniuch-Jacquotte, A., Hartge, P., Fuchs, C.S., 2010. Pancreatic cancer risk and ABO blood group alleles: results from the pancreatic cancer cohort consortium. Cancer Res. 70, 1015–23. doi:10.1158/0008-5472.CAN-09-2993

Suppl. Table 1. Imputation results for the *ACE* SNPs previously associated with AD (AlzGene, http://www.alzgene.org, accessed 16/05/2014) and those shown to tag the activity of the enzyme (from Terao et al. 2013). SNPs rs4343 and rs4362 were not imputed as these genotypes were available directly from the GWAS dataset.

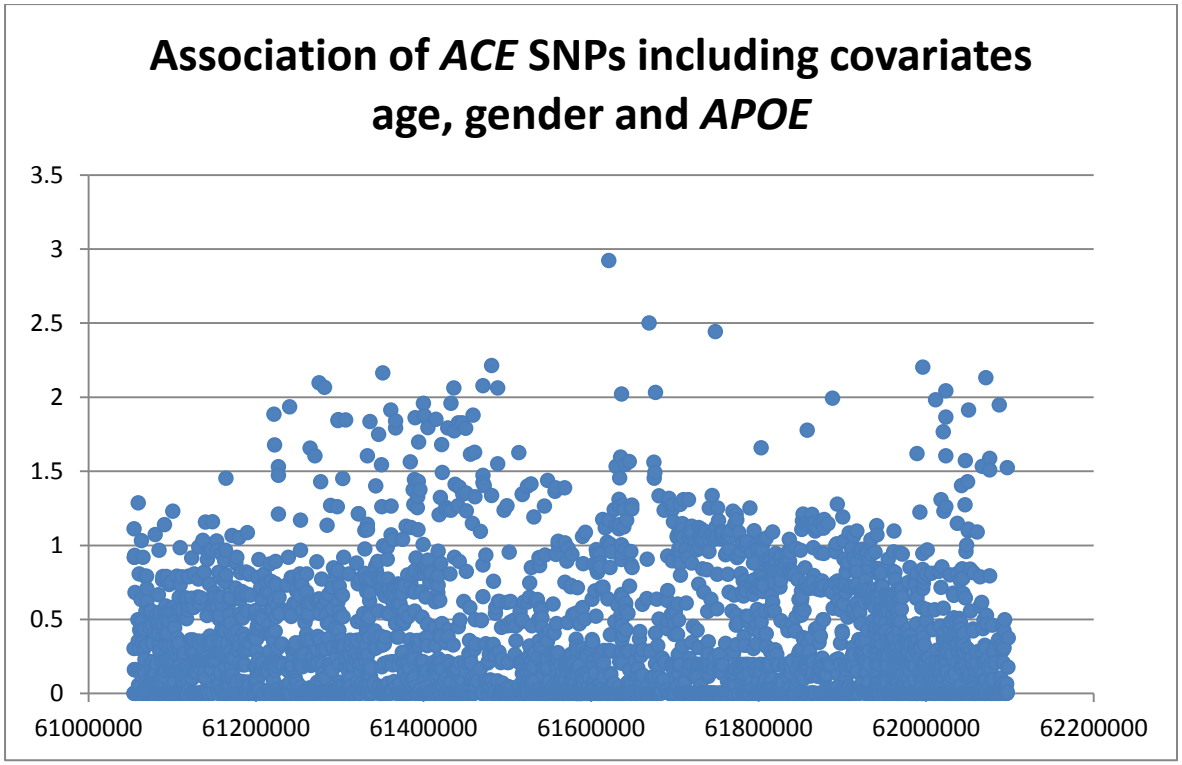
Chr	Position	RSID	Allele1	Allele2	Imputed (info score)
chr17	61550529	rs1800764	C	T	Y (0.93)
chr17	61554194	rs4291	T	A	Y (0.93)
chr17	61556298	rs4295	G	C	Y (0.94)
chr17	61566031	rs4343	G	A	N
chr17	61573761	rs4362	T	C	N

Suppl. Table 2. Association results of the selected *ACE* SNPs previously associated with AD (AlzGene, http://www.alzgene.org, accessed 16/05/2014) and those shown to tag the activity of the enzyme (from Terao et al. 2013) after including only covariates, or with the addition of the interaction variable of the *ACE* SNP and *ABO* haplotype or B allele or A1 allele alone. No significant association with AD was found.

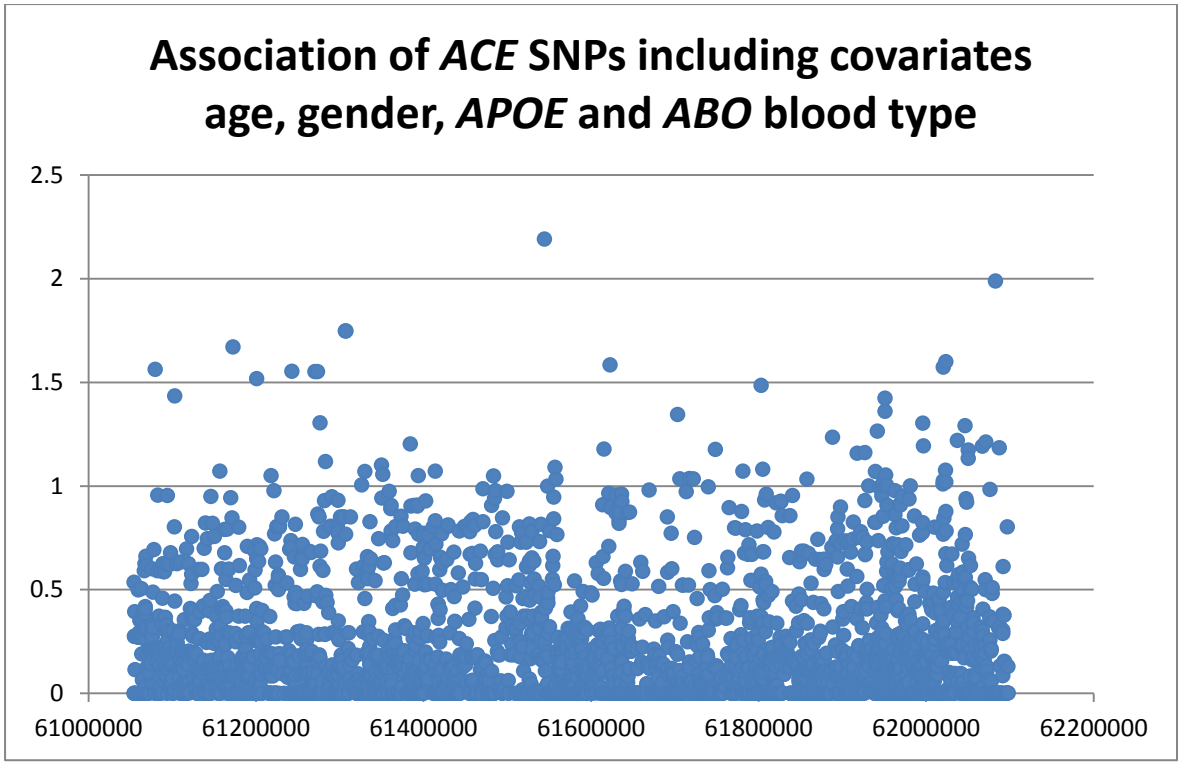
RSID	p-value (sex, age, *APOE*)	p-value (+ *ABO*)	p-value (interaction term ADDxABO)	p-value (+ B allele)	p-value (interaction term ADDxB)	p-value (+ A1 allele)	p-value (interaction term ADDxA1)
rs1800764	0.38	0.16	0.27	0.43	0.82	0.83	0.11
rs4291	0.95	0.28	0.22	0.97	0.93	0.55	0.09
rs4295	0.89	0.29	0.20	0.91	0.95	0.44	0.10
rs4343	0.80	0.71	0.54	0.66	0.38	0.45	0.16
rs4362	0.92	0.92	0.95	0.83	0.17	0.77	0.29



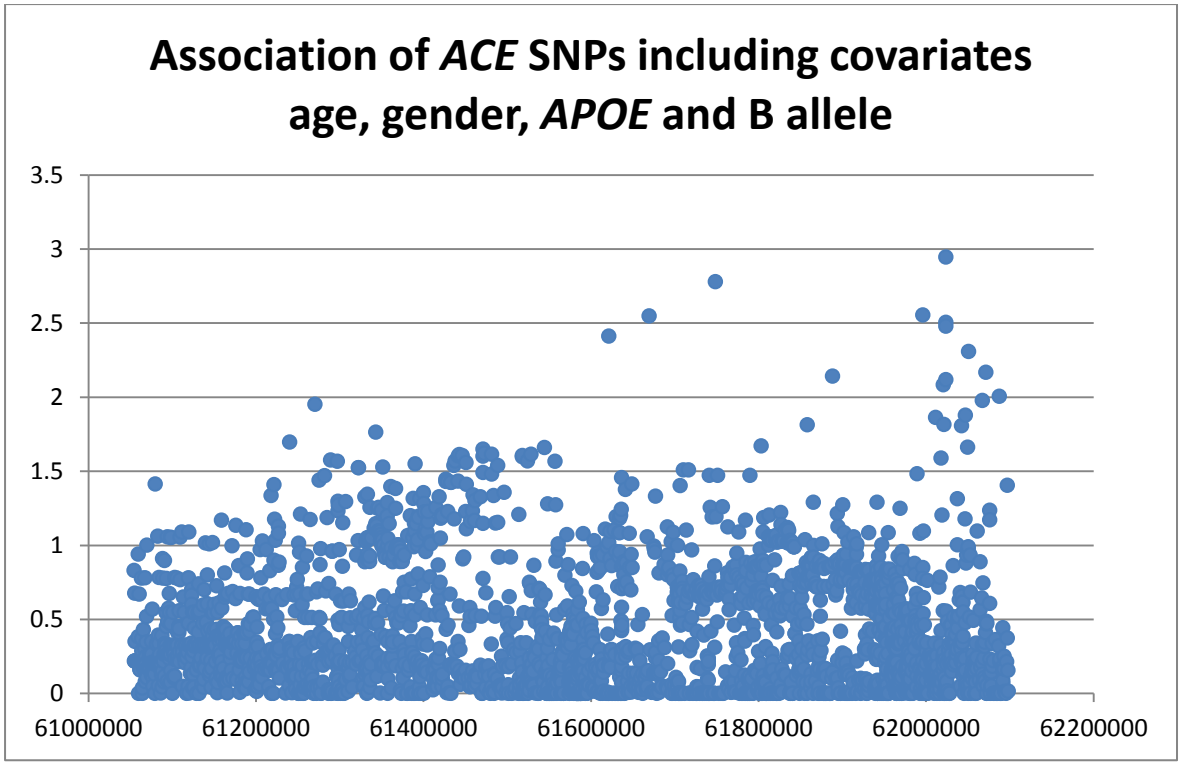
Suppl. Fig 1 – *ACE* (Chr17:61554422-61599205) association using logistic regression and no covariates. –log10(p-value) is on the x-axis and chromosomal position for Chromome 17 on the y-axis.



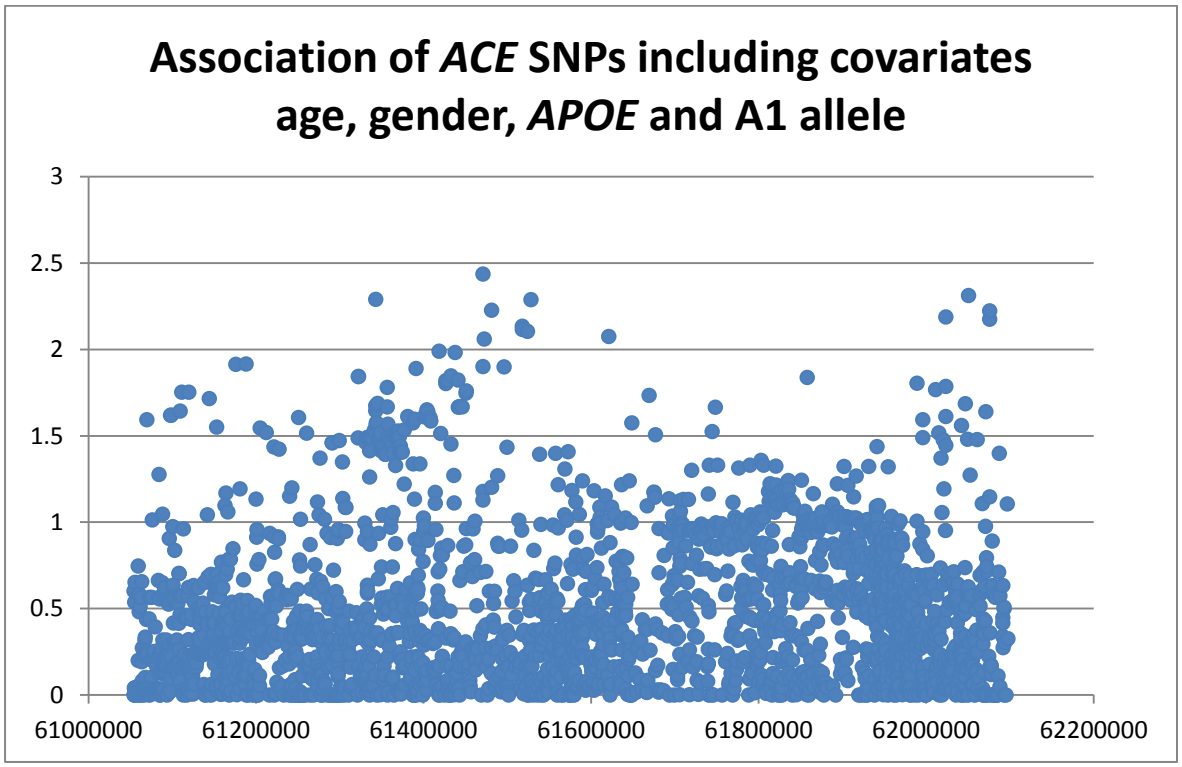
Suppl. Fig 2 - *ACE* SNP association using all covariates apart from ABO genotyping. –log10(p-value) is on the x-axis and chromosomal position for Chromome 17 on the y-axis.



Suppl. Fig 3. *ACE* SNP association using logistic regression including all covariates, interaction term ABO blood type and the additive effect of each genetic variant. –log10(p-value) is on the x-axis and chromosomal position for Chromome 17 on the y-axis.



Suppl. Fig 4. *ACE* SNP association using logistic regression including all covariates and the presence of a B allele (associated with increased ACE activity) as an interaction term. –log10(p-value) is on the x-axis and chromosomal position for Chromome 17 on the y-axis.



Suppl. Fig 5. *ACE* SNP association using logistic regression including all covariates and the presence of an A1 allele (associated with decreased ACE activity) as an interaction term. –log10(p-value) is on the x-axis and chromosomal position for Chromome 17 on the y-axis.