
Article:
De Marco, M. and Venneri, A. (2015) 'O' blood type is associated with larger grey-matter volumes in the cerebellum. Brain Research Bulletin, 116. 1 - 6. ISSN 0361-9230

https://doi.org/10.1016/j.brainresbull.2015.05.005

Title: 'O' Blood Type is Associated with Larger Grey-Matter Volumes in the Cerebellum

Authors: Matteo De Marco[a], Annalena Venneri[a,b]

[a] Department of Neuroscience, University of Sheffield, Sheffield, United Kingdom

[b] Department of Functional Neuroimaging, IRCCS San Camillo Foundation Hospital, Venice, Italy

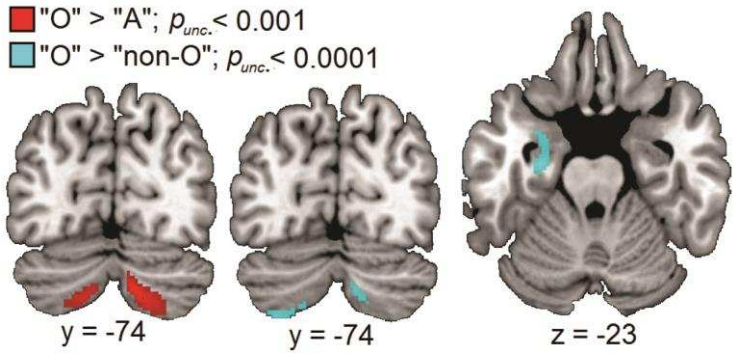
Matteo De Marco name specification: First Name: "Matteo"; Family Name: "De Marco"

Annalena Venneri name specification: First Name: "Annalena"; Family Name: "Venneri"

Corresponding author: Prof. Annalena Venneri, Department of Neuroscience - Medical School, University of Sheffield, Beech Hill Road, Royal Hallamshire Hospital, N floor, room N130, Sheffield - S10 2RX - United Kingdom, a.venneri@sheffield.ac.uk, Tel: +44 114 2713430, Fax: +44 114 2222290

Disclosures: Matteo De Marco reports no disclosures relevant to the manuscript. Annalena Venneri is partially funded by grant No 42/RF-2010-2321718, by the Italian Ministry of Health and by the European Union Seventh Framework Programme (FP7/2007 – 2013) under grant agreement no. 601055, VPH-DARE@IT

Graphical abstract



Highlights

- ‘O’ blood type adults have increased volumes in the posterior cerebellum.
- ‘O’ blood type might be protective against dementia.
- Biological explanations include possible fostering of endothelial dysfunction.

Abstract

Recent evidence indicated higher incidence of cognitive deficits in ABO blood-type system 'AB' individuals. Since this statistical difference might originate from the lack of protective effects exerted by 'O' alleles on the brain via vascular or non-vascular routes, this study investigated volumetric differences in grey matter between 'O' and non 'O' adults to explore the possibility of a structural endophenotype visible in 'O' adults without cognitive impairment or neurodegeneration.

A large sample of cognitively healthy adults who had previously undergone structural MRI for research purposes were contacted telephonically and enquired about their ABO blood type. Out of the 189 individuals who were able to retrieve and communicate this information, 'O' (n = 76) and 'A' adults (n = 65) were included in Model 1. In Model 2, all 'non-O' (n = 113) were instead collapsed in a single group. Voxel-Based Morphometry analyses were carried out on three-dimensional T1-weighted scans, and between-sample t tests were run to compare the maps of grey-matter volumes of the subgroups of interest, controlling for major nuisance variables.

In Model 1, 'O' adults had larger grey-matter volumes in two symmetrical clusters within the posterior ventral portion of the cerebellum. This was confirmed in Model 2. Additionally, 'non-O' adults showed lower volume values in temporal and limbic regions, including the left hippocampus.

The cerebellar clusters were located in regions previously found to be part of a network responsible for sensorimotor integration. It is speculated that the structural reductions seen in 'non-O' adults might result in a susceptibility to down-regulation of this network. This occurrence is likely to intensify along the ageing process and may contribute to foster cognitive decline. Although Model 2 seems to suggest that having a 'O' blood-type might play a role in protection against those conditions in which temporal and mediotemporal volumetric loss is observed (Alzheimer's disease), additional supporting evidence is needed.

A number of potential biological processes might sustain these between-group differences, including sensorimotor ontogenesis, hormonal function, and a regional impact of cerebral amyloid angiopathy. These findings identify the cerebellar tissue as a candidate for further studying ABO function, and support a general association between ABO blood type and variance in the development of the nervous system.

Keywords: blood group; volumetric MRI; brain structure; voxel-based morphometry; brain development;

1 - Introduction[1]

The main blood-type classification (the ABO system) is controlled by a single genetic locus, which defines four basic ABO phenotypes: blood types 'A', 'B', 'AB', and 'O' (Dean, 2005). The variability intrinsic to the ABO blood type seems to be significantly associated with the functionality of the cardiovascular system. In fact, an ABO-based modulation of the plasmatic amount of a coagulation glycoprotein, the von Willebrand factor, has been reported, with non-'O' adults having higher levels (Jenkins and O'Donnel, 2006). Consistently with higher plasma levels of a clotting-inducing factor, non-'O' adults carry a larger number of cardiovascular risk factors (Carpeggiani et al., 2010). Based on this evidence, many studies have investigated the association between blood type and the occurrence of vascular events affecting the brain (Wu et al., 2008). Remarkably, an 'O' vs non-'O' difference seems to be visible even at a young age, as ≤ 50 years old sufferers from ischemic stroke carrying both alleles ('AB' blood-type individuals) appear to have a poorer prognosis (Yang et al., 2014). In addition, a recent cohort-based investigation which tested the possibility that 'non-O' adults might show poorer cognitive trajectories, found that 'AB' blood type is associated with higher incidence of cognitive decline (Alexander et al., 2014). Although the cardiovascular pathway could be a major interpretational avenue for this evidence, the resulting association was not significantly modified by the inclusion in the models of the quantity of antigens for a molecule normally bound to the von Willebrand factor. This indicates that other mechanisms aside from a cardiovascular route might underlie the impact of ABO blood type on brain and cognition. We thus hypothesised that neurostructural differences may exist between 'O' and non-'O' adults, with potentially regions of smaller grey matter volumes in the non-'O' adults, and that these differences might be associated with a potential contribution to a scenario of structural susceptibility for cognitive decline. To explore a potential mechanism of translation to

[1] Abbreviations included in the manuscript: GM: Grey matter; RGM: Global ratio of grey matter.

the clinical setting, this hypothesis was tested in a cohort of healthy adults. Inferential models were created in accordance with the distribution of blood types within the final sample.

2 - Material and Methods

This study was designed and carried out following the principles of the Declaration of Helsinki (World Medical Association, 2013), and was approved by the Institutional Review Board of the IRCCS Fondazione Ospedale San Camillo (Venice, Italy). A retrospective dataset of 265 structural Turbo Field Echo Sequence T1-weighted images acquired at the IRCCS San Camillo Foundation Hospital (Venice, Italy) from November 2010 to November 2014 was put together. These three-dimensional images had been exclusively obtained for research purposes on clinically-established cognitively-healthy adults, who, at the time of scan, had given informed consent for participation in research projects. With the exception of four members of IRCCS San Camillo staff population, all adults older than 55 had been administered an extensive battery of cognitive tests to ascertain absence of cognitive deficits, as part of the research projects they had been originally recruited for (Table 1). This battery had been put together in order to be sensitive to cognitive impairment due to the most common forms of neurodegenerative and neurovascular conditions, and included tests of short-term and long-term memory, learning, attention, speed of processing, executive functions, lexical-semantic abilities, visuospatial processing and visuoconstructive skills (see Table 1 for a detailed list of tests). The acquisition parameters were for all as follows. Scanner: 1.5 T Philips Achieva; voxel dimension: 1.1 x 1.1 x 0.6 mm; field of view: 250 mm; matrix size 256 x 256 x 124; TR: 7.4 ms, TE: 3.4 ms; flip angle: 8°. All recruited adults were free from any subjective cognitive complaint at the time of scan and all neuro-radiologic responses (based on T1-weighted, T2-weighted and FLAIR images) revealed no sign of diagnostic entities which could alter brain

structure or brain function. Since the ABO phenotype is a variable acknowledged by a large proportion of the world population, telephonic interviews were carried out to collect blood types from all individuals. In addition to an overt degree of methodological convenience, this method of collection was preferred over lab testing because self-reported ABO blood types are valid measures to be used in associative studies (Bider-Canfield and Cotterchio, 2014; Ito et al., 2001). Upon informed consent, 189 adults were able to retrieve and report their ABO blood type. Twenty-three adults did not know and were unable to retrieve this information. While no adult refused to give consent, contact was not possible and was thus not made in the case of 53 individuals. Blood types distributed as follows: 'O': 76; 'A': 65; 'B': 34; 'AB': 14. These proportions did not differ (χ^2_3 = 4.019, p = 0.259) from those inferred from the large sample described in a retrospective study carried out in a hospital centre located about 77 miles (in a straight line) from our institution (Mengoli et al., 2014). Since 'O' adults (age range: 22-80) and 'A' adults (age range: 21-77) showed a similar distribution frequency, these two subgroups were selected for a first 'O ' vs non-'O' blood type comparison (Model 1). This choice was also made not to contaminate the group of non-'O' adults by conjoining qualitatively different phenotypes (and thus, possibly qualitatively-different underlying biological mechanisms). In a second model (Model 2), sample size was instead optimised, and 'O' adults were compared against all 'non-O' adults (age range: 21-77). T1-weighted scans were preprocessed and analysed with Statistical Parametric Mapping 8 software (Wellcome Trust Centre for Neuroimaging, London, UK) running in a Matlab 7 environment. Following standard Voxel-Based Morphometry procedures (Ashburner and Friston, 2000), a tissue-class probabilistic segmentation was initially run to separate grey matter (GM), white matter, and cerebrospinal fluid of each scan within its native space. This was carried out to calculate total intracranial volume and the corresponding global ratio of grey matter (RGM). GM was also separated in a modulated and normalised space, and was smoothed with an 8 mm^3 full-width at half maximum gaussian kernel. A between-group t test was run to compare smoothed GM maps of 'O' and 'A'/'non-O' blood-type individuals. Age at scan and gender were added in the model as

nuisance factors, together with RGM as a proxy of brain reserve (as suggested by the model of Stern (2009)), and years of education as a proxy of cognitive reserve (Liu et al., 2013; Stern, 2009). For Model 1, the threshold of significance was set at $p < 0.001$ (uncorrected). Since Model 2 was based on the potential combination of qualitatively-different biological mechanisms (whose intra-group variability might result in increased between-group separation), a more conservative threshold was adopted ($p < 0.0001$, uncorrected). Only clusters surviving a Family-Wise Error-corrected $p < 0.05$ were reported as significant. Peak Montreal National Institute coordinates were converted to Talairach space using a nonlinear transform (imaging.mrc-cbu.cam.ac.uk/downloads/MNI2tal/mni2tal-m), and the Talaraich Daemon client was used for the purpose of interpretation (Lancaster et al., 2000). According to the null hypothesis, no voxel-based difference in GM was expected. Considering the explorative nature of the study and, thus, the absence of a literature of reference for this between-group inferential test, a two-tailed directional alternative hypothesis was adopted.

Between-group differences in age, years of education, RGM, total intracranial volume and gender were investigated with t or χ^2 tests. These numeric variables were analysed with IBM SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA).

3 - Results

No between-group difference was found in any of the numeric variables (Table 2). In Model 1 'O' adults showed a bilateral pattern of larger GM volumes in the ventral posterior portion of the two cerebellar hemispheres (Table 3, Figure 1). This pattern of larger volume was more evident in the right cluster, which survived even a more conservative $p < 5e\text{-}05$ threshold ($p = 0.000010318$, MNI peak at 10, -70, -38). A similar pattern of larger volume showed by 'O' adults was maintained in

Model 2, extending this time slightly more within the left cerebellar hemisphere. In addition, 'non-O' adults also showed smaller volumes in a bilateral set of areas within the temporal lobe (especially in its orbital portion), in the left hippocampus, and in the right uncus (Table 4, Figure 2). Because of the possibility that these additional differences were driven by 'B' adults, we ran a post-hoc explorative model comparing 'O' and 'B' adults only (n = 34, age range: 23-73). These findings confirmed this thesis, with 'B' adults showing reduced GM volumes in cerebellar and temporal-mediotemporal/limbic areas (results not shown), but, given the inhomogeneous allocation ratio to the subgroups (0.447) and the too small size of the sample (73% of a sufficient size, as indicated by a power analysis), no further interpretational effort was made.

The inverse contrasts did not reveal any significant finding in any of the models.

4 - Discussion

Increasing evidence suggests that the ABO blood-type classification is involved in a number of human diseases (Franchini and Liumburno, 2013). On this note, the influence of ABO-system blood types on the nervous system has been a largely unexplored research area. Despite the association which has been frequently reported between 'O' and non-'O' blood types and cardiovascular risk factors (Carpeggiani et al., 2010; Wu et al., 2008; Yang et al., 2014), recent findings suggest that other, non-vasculogenic mechanisms might exist, such as, for instance, the involvement of glycosyltransferase enzymes encoded by the ABO genetic locus (Alexander et al., 2014). In a retrospective and explorative fashion, we carried out a voxel-based morphometric analysis of the differences of whole-brain GM volumes between a subgroup of healthy adults with 'O' blood type and comparable subgroups having other blood types. Particular attention was paid to the methodology of the main analyses. No between-group differences were found in age and

proportion of males/females, and, moreover, groups did not differ in terms of proxies of brain reserve (RGM) and cognitive reserve (levels of education). Despite the non-significance these four variables were added anyhow in the model as nuisance variables.

The portion of the cerebellum where a volumetric inter-group difference was found was located in the territory of the medial branch of the posterior-inferior cerebellar artery. This finding emerged from both Model 1 and Model 2, suggesting that this region might be the preferential regional locus of an 'O' endophenotype. The two outcome clusters overlapped with good accuracy with the cerebellar regions known to be part of a cerebro-cerebellar network responsible for sustaining sensorimotor integration (Buckner et al., 2011). Although this is not a functional circuit primarily involved in the most common forms of dementia of major aetiology (Seeley et al., 2011), a significantly smaller ABO-related GM volume in these areas might concur in fostering vulnerability for cognitive decline in old age.

It is fair to acknowledge that, in the original study by Alexander and colleagues (2014), no significant statistic was found between cognitive trajectory of 'O' and 'A' adults. Methodological factors might account for this discrepancy, as in that study inter-group differences were measured as presence/absence of cognitive impairment in a dichotomised form, whereas in this study differences were explored testing continuous variables within the domain of normality.

Even in the presence of a clear-cut pattern of results and the idea of network down-regulation as a neat interpretational avenue, an important theoretical aspect has to be taken in consideration. It is not clear, in fact, whether the observed morphometric difference represents a volumetric loss consistent with atrophy, or, rather, it reflects a simpler and more efficient structural level of complexity. On this note, the analysis of the ABO-dependent differences in the pattern of functional connectivity within the sensorimotor-integration network or, alternatively, in association with a cerebellar region of interest would provide unequivocal evidence to answer this question. Although such an evocative hypothesis can be put forward in the attempt of interpreting the

potential consequence of these volumetric differences in terms of brain and cognitive function, it will not suggest a biological candidate mechanism which could explain the smaller GM volumes seen in 'A' adults and, particularly, the regionality of this difference and why it is specific to a well-established cerebellar portion.

Findings from Model 2 indicate larger GM volumes in 'O' adults in temporal and limbic regions, bilaterally, with the largest Z score found in the hippocampus. This pattern suggests that 'O' might also confer an endophenotype of reduced volumetric susceptibility to those conditions in which volume reduction of these areas is visible (e.g. Alzheimer's disease). This conclusion, however, has to be drawn cautiously because these anatomical differences were not visible in Model 1, not even when findings were uncorrected, at a cluster level. For this reason, we chose to interpret the results of Model 2 as a trend. Since it was possible that these additional cluster of significance were actually driven by adults with a 'B' blood type, a third, post-hoc model was run to compare 'O' and 'B' adults. This additional inference confirmed this trend, although, with similar inhomogeneously-distributed subgroups, our sample was not sufficiently powered to sustain this evidence as a significant finding.

Over the past decades, the ABO blood type has not been studied systematically as a possible modulator of brain structure or brain function. There is, however, a set of studies which offers possible interpretative or speculative frameworks which might, at least partially, account for these volumetric differences. Four major candidates were identified: the "cardiovascular hypothesis", the antigenic moulding of neurosensory pathways, the "cortisol hypothesis", and the "angiotensin hypothesis". A link between the functionality of the cardiovascular system and regional brain volume is supported by a very recent study by Wang and colleagues (2014), who found that white-matter hyperintensity burden in healthy elderly adults is negatively associated with whole-brain and hippocampal GM volume, and positively associated with ventricular enlargement. If the variability for the ABO blood type regulated cerebellar GM volumes via a route of cardiovascular nature, this

would imply that one or more mechanisms exist by which the biology of ABO blood types affects the circulation within the territory of the posterior cerebellar artery with higher affinity than any other vascular region. At present, however, no published study supports this possibility. Another piece of evidence which might account for a regional, rather than global effect of ABO blood type has emerged from a study carried out on rodents. Experimental evidence collected on a transgenic mouse model revealed that ABO antigen 'A' (associated with 'A' blood type in man) and antigen precursor 'H' (associated with 'O' blood type in man) are expressed on the cellular membrane of the olfactory axonal structure, which influences the neural development of these sensory pathways (St John et al., 2006). This leaves the association between a possible expression of blood-type antigens on human sensory neurons open to speculation. Another, older study found an association between blood type and timecourse plasma levels of the "stress hormone" cortisol, which had been experimentally raised in study participants in response to an artificial stressor (Neumann et al., 1992). In this study 'A' adults were found to have a quicker recovery from the hormonal up-regulation, but, at the same time, had also higher absolute levels of cortisol than 'O' adults at all stages of the experimental manipulation. Although the brain receptorial population for glucocorticoids is mainly located in mediotemporal/limbic and prefrontal areas (Lupien et al., 2007), no GM difference was visible in these regions between the two subgroups in Model 1 (which tested exactly the two blood types as in the study by Neumann and colleagues, 1992), suggesting that blood type probably does not regulate cerebellar GM volumes via a chronic effect of stress-hormones. A fourth, endocrine route with a significant interaction with the cardiovascular system is that illustrated by the findings by Terao and colleagues (2013). These authors found that levels of angiotensin-I converting enzyme (which is involved in inducing vasoconstriction) differ according to the ABO alleles, with the 'B' allele being associated to higher plasmatic levels, the 'A_2' and 'O' alleles being associated with intermediate levels, and the 'A_1' allele being associated with lower levels. Although the pattern of enzymatic variability generated by blood types does not associate with the presence of a diagnosis of Alzheimer's disease (Braae et al., 2015), the possibility that this

mechanism might have a significant impact on the variability of regional cortical volumes remains open to speculation.

Alzheimer's disease is the most common cause of dementia, but is not associated with volumetric reductions in the posterior cerebellum (Colloby et al., 2014). Nonetheless, the presence of Alzheimer's pathology within the entire cerebellar complex is common amongst those showing an early disease onset (Cole et al., 1993). Although no concrete evidence has yet been found in support of an association between ABO blood type, neural development, and neural protection, a recent histological study found that in 'A' blood type the expression of the ABO antigens is modulated at the level of the brain vascular endothelium (Wang et al., 2013). Since endothelial dysfunction is molecularly associated with neurodegeneration of the Alzheimer's type (Grammas et al., 2011; Lyros et al., 2014), it is possible that ABO-dependent modulation of endothelial function might contribute as a risk factor for (or protection factor against) accumulation of Alzheimer's pathology. Although this is only a speculation, a major mechanism by which the 'H' antigen precursor might be implicated in a systemic protection against the vascular events which contribute to the initiation of the neurodegenerative cascade of Alzheimer's disease might be a positive influence exerted by an "H antigen endothelium" on the neighbouring tunica media and tunica adventitia, where cerebral beta-amyloid angiopathy is observed (Thal et al., 2008). A specific study is needed to test this speculative hypothesis.

In conclusion, 'O' adults were found to have larger GM volumes in the posterior portion of the cerebellum, bilaterally, and this symmetrical pattern cannot be accounted for by an effect of age, gender, or neurocognitive reserve. Furthermore, trends of larger GM volumes seem to be visible in this group also in temporal and mediotemporal-limbic regions. Although the biological bases of such difference are not clear, these findings suggest that the cerebellar tissue (particularly, within the territory of the posterior-inferior cerebellar artery) might be the most adequate regional candidate for the future study of ABO function within the human brain.

Acknowledgements

The authors thank Dr. Francesca Meneghello and Dr. Marco Pitteri for their crucial contribution to data collection. We would also like to thank the MRI Unit staff at the IRCCS San Camillo Foundation Hospital for coordinating brain scanning procedures, together with the neuroradiologists clinically involved in this project. The research leading to these results has received partial funding from the European Union Seventh Framework Programme (FP7/2007 – 2013) under grant agreement no. 601055, VPH-DARE@IT

References

Alexander, K.S., Zakai, N.A., Gillett, S., McClure, L.A., Wadley, V., Unverzagt, F. and Cushman, M. (2014). ABO blood type, factor VIII, and incident cognitive impairment in the REGARDS cohort. Neurology, 83, 1271-1276. DOI: 10.1212/WNL.0000000000000844

Ashburner, J. and Friston, K.J. (2000). Voxel-based morphometry--the methods. Neuroimage, 6, 805-821. DOI: 10.1006/nimg.2000.0582

Bider-Canfield, Z. and Cotterchio, M. (2014). Self-reported ABO blood type compared with DNA-derived blood group. Epidemiology, 25, 936-937. DOI: 10.1097/EDE.0000000000000156

Braae, A., Medway, C., Carrasquillo, M., Younkin, S., the Alzheimer's Research UK (ARUK) Consortium, Kehoe, P.G. and Morgan, K. (2015). Blood type gene locus has no influence on ACE association with Alzheimer's disease. Neurobiol. Aging, [Epub ahead of print] 22th January 2015. DOI: 10.1016/j.neurobiolaging.2015.01.013

Buckner, R.L., Krienen, F.M., Castellanos, A., Diaz, J.C. and Yeo, B.T. (2011). The organization of the human cerebellum estimated by intrinsic functional connectivity. J. Neurophysiol., 106, 2322-2345. DOI: 10.1152/jn.00339.2011

Carpeggiani, C., Coceani, M., Landi, P., Michelassi, C. and L'Abbate, A. (2010). ABO blood group alleles: a risk factor for coronary artery disease. An angiographic study. Atherosclerosis, 211, 461-466. DOI: 10.1016/j.atherosclerosis.2010.03.012

Cole, G., Neal, J.W., Shingrao, S.K., Jasani, B. and Newman G.R. (1993). The distribution of amyloid plaques in the cerebellum and brain stem in Down syndrome and Alzheimer's disease: A light microscopical analysis. Acta Neuropathol., 85, 542-552.

Colloby, S.J., O'Brien, J.T. and Taylor, J.P. (2014). Patterns of cerebellar volume loss in dementia with Lewy bodies and Alzheimer's disease: A VBM-DARTEL study. Psychiatry Res. Neuroimaging, 223, 187-191. DOI: 10.1016/j.pscychresns.2014.06.006

Dean, L. (2005). The ABO blood group In: Blood groups and red cell antigens. [online]. Available at: http://www.ncbi.nlm.nih.gov/books/NBK2267/. Accessed October 30, 2014.

Franchini, M. and Liumburno, G.M. (2013). ABO blood group: Old dogma, new perspectives. Clin. Chem. Lab. Med., 51, 1545-1553. DOI: 10.1515/cclm-2013-0168

Grammas, P. (2011). Neurovascular dysfunction, inflammation and endothelial activation: Implications for the pathogenesis of Alzheimer's disease. J. Neuroinflammation, 8, 26. DOI: 10.1186/1742-2094-8-26

Ito, H., Matsuo, K., Saito, T., Hirose, K., Inoue, M., Takezaki, T., Hamajima, N., Kuroishi, T. and Tajima, K. (2001). Valid Responses to ABO Blood Type Question in Self-reporting Questionnaire. Asian Pac. J. Cancer Prev., 2, 315-317.

Jenkins, P.V. and O'Donnel, J.S. (2006). ABO blood group determines plasma von Willebrand factor levels: a biologic function after all? Transfusion, 46, 1836-1844. DOI: 10.1111/j.1537-2995.2006.00975.x

Lancaster, J.L., Woldorff, M.G., Parsons, L.M., Liotti, M., Freitas, C.S., Rainey, L., Kochunov, P.V., Nickerson, D., Mikiten, S.A. and Fox, P.T. (2000). Automated Talairach Atlas labels for functional brain mapping. Hum. Brain Mapp., 10, 120-131. DOI: 10.1002/1097-0193(200007)10:3<120::AID-HBM30>3.0.CO;2-8

Liu, Y., Cai, Z.L., Xue, S., Zhou, X. and Wu, F. (2013). Proxies of cognitive reserve and their effects on neuropsychological performance in patients with mild cognitive impairment. J. Clin. Neurosci., 20, 548-553. DOI: 10.1016/j.jocn.2012.04.020

Lupien, S.J., Maheu, F., Tu, M., Fiocco, A. and Schramek, T.E. (2007). The effects of stress and stress hormones on human cognition: Implications for the field of brain and cognition. Brain Cogn., 65, 209-237. DOI: 10.1016/j.bandc.2007.02.007

Lyros, E., Bakogiannis, C., Liu, Y. and Fassbender, K. (2014). Molecular links between endothelial dysfunction and neurodegeneration in Alzheimer's disease. Curr. Alzheimer Res., 11, 18-26. DOI: 10.2174/1567205010666131119235254#sthash.RIXQRJMz.dpuf

Mengoli, C., Bonfanti, C., Rossi, C. and Franchini, M. (2014). Blood group distribution and life-expectancy: a single-centre experience. Blood Transfus., [Epub ahead of print] 29th October 2014. DOI: 10.2450/2014.0159-14

Neumann, J.K., Abrogast, B.A., Chi, D.S. and Abrogast, L.Y. (1992). Effects of stress and blood type on cortisol and VLDL toxicity preventing activity. Psychosom. Med., 54, 612-619.

Seeley, W.W., Crawford, R.K., Zhou, J., Miller, B.L. and Greicius, M.D. (2009). Neurodegenerative diseases target large-scale human brain networks. Neuron, 62, 42-52. DOI: 10.1016/j.neuron.2009.03.024

Stern, Y. (2009) Cognitive reserve. Neuropsychologia, 47, 2015-2028. DOI: 10.1016/j.neuropsychologia.2009.03.004

St John, J.A., Claxton, C., Robinson, M.W., Yamamoto, F., Domino, S.E. and Key, B. (2006). Genetic manipulation of blood group carbohydrates alters development and pathfinding of primary sensory axons of the olfactory systems. Dev. Biol., 298, 470-484. DOI: 10.1016/j.ydbio.2006.06.052

Terao, C., Bayoumi, N., McKenzie, C.A., Zelenika, D., Muro, S., Mishima, M., Connell, J.M., Vickers, M.A., Lathrop, G.M., Farrall, M., Matsuda, F., Keavney, B.D. and the Nagahama Cohort Research Group. (2013). Quantitative variation in plasma angiotensin-I converting enzyme activity shows allelic heterogeneity in the ABO blood group locus. Ann. Hum. Genet., 77, 465-471. DOI: 10.1111/ahg.12034

Thal, D.R., Griffin, W.S.T., de Vos, R.A.I. and Ghebremedhin, E. (2008). Cerebral amyloid angiopathy and its relationship to Alzheimer's disease. Acta Neuropathol. 115, 599–609. DOI: 10.1007/s00401-008-0366-2

Wang, M.M., Lee, S.J., Kim, J., Majersik, J.J., Blaivas, M. and Borjigin, J. (2013). ABO blood antigens define human cerebral endothelial diversity. Neuroreport, 24, 79-83. DOI: 10.1097/WNR.0b013e32835c93a2

Wang, R., Fratiglioni, L., Laveskog, A., Kalpouzos, G., Ehrenkrona, C.H., Zhang, Y., Bronge, L., Wahlund, L.O., Bäckman, L. and Qiu, C. (2014). Do cardiovascular risk factors explain the link

between white matter hyperintensities and brain volumes in old age? A population-based study. Eur. J. Neurol., 21, 1076-1082 DOI: 10.1111/ene.12319

World Medical Association. (2013). Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. JAMA, 310, 2191-2194.

Wu, O., Bayoumi, N., Vickers, M.A. and Clark, P. (2008). ABO(H) blood groups and vascular disease: a systematic review and meta-analysis. J. Thromb. Haemost., 6, 62-69. DOI: 10.1111/j.1538-7836.2007.02818.x

Yang, N., Zhang, B., Xie, L.C., Yin, J., He, Y., Yang, X. and Gao, C. (2014). The association baseline NIH Stroke Scale score with ABO blood subtypes in young patients with acute ischemic stroke. Atherosclerosis, 236, 144-149. DOI: 10.1016/j.atherosclerosis.2014.06.019

Figure captions

Figure 1: Cerebellar clusters where ‘O’ adults had larger GM volumes than ‘A’ adults.

Figure 2: Significant clusters of GM difference where ‘O’ adults had larger GM volumes than ‘non-O’ (i.e. A, B, AB) adults.

Table 1: Overview of the cognitive profile shown by the subgroup of adults older than 55 years included in the study. The median performance captures the central tendency of the sub-sample taking into account the skewed distribution normally seen for some of the scores. Mean and standard deviations are also indicated.

Test	Mean (sd)	Median Performance
Mini Mental State Examination	29.16 (1.23)	30
Raven Progressive Matrices	31.41 (3.42)	32
Phonemic Fluency	36.19 (12.16)	36.5
Semantic Fluency	42.87 (9.02)	42
Digit Cancellation	54.56 (4.19)	55.5
Similarities Test	21.37 (4.09)	22
Token Test	34.6 (2.10)	36
Rey-Osterrieth Complex Figure - Copy	32.90 (3.08)	34
Rey-Osterrieth Complex Figure - Recall	15.62 (5.14)	15.5
Stroop Test - Time	22.09 (8.40)	20
Stroop Test - Errors	0.76 (2.72)	0
Digit Span - Forward	6.19 (0.79)	6
Digit Span - Backwards	4.31 (0.97)	4
Visuospatial Span	4.98 (0.89)	5
Visuospatial Supraspan	21.65 (5.51)	23.335
Prose Memory - Immediate Recall	10.19 (3.23)	10
Prose Memory - Delayed Recall	13.31 (4.53)	12.5
Paired Associates Test	13.49 (4.57)	13
Confrontational Naming	19.52 (0.92)	20

Table 2: Demographic characteristics of the sample included in the analyses

Demographic Variable	'O' Adults n = 76	'A' Adults n = 65	'non-O' Adults n = 113	p Model 1	p Model 2
Age at Scan (years)	41.18 (17.11)	44.86 (18.52)	44.56 (17.58)	0.223	0.193
Education Level (years)	15.32 (4.24)	14.28 (4.70)	14.64 (5.03)	0.170	0.335
Gender (M/F)	25/51	14/51	34/79	0.133	0.683
Total Intracranial Volume (cm^3)	1686.27 (190.00)	1636.82 (165.12)	1641.32 (161.02)	0.104	0.082
RGM	0.39 (0.04)	0.38 (0.04)	0.39 (0.04)	0.262	0.272

Between-group differences in gender were inferred with a chi-square test. The remaining variables were analysed with independent-sample t tests. RGM: Ratio of Grey Matter.

Table 3: Cerebellar GM regions showing larger volumes in the group of 'O' adults compared to "A" adults (Model 1)

Cluster Number	Cluster Extent (voxels)	Cluster-Level pFWE	Cerebellar Area	Side	Peak-Level p	Z Score at Local Maximum	Talairach Coordinates		
							x	y	z
1	812	< 0.001	Posterior Lobe - Pyramis	R	<0.001	4.48	10	-69	-28
			Posterior Lobe - Cerebellar Tonsil	R	<0.001	3.83	6	-58	-34
			Posterior Lobe - Cerebellar Tonsil	R	<0.001	3.41	18	-53	-44
2	243	0.033	Posterior Lobe - Uvula	L	<0.001	3.99	-12	-70	-32
			Posterior Lobe - Pyramis	L	<0.001	3.62	-14	-79	-31
			Posterior Lobe - Cerebellar Tonsil	L	<0.001	3.17	-24	-60	-37

Clusters were significant with a set-level uncorrected $p < 0.001$. L: Left; R: Right; FWE: Family-Wise Error.

Table 4: Brain GM regions showing larger volumes in the group of 'O' adults compared to "non-O" adults (Model 2)

Cluster Number	Cluster Extent (voxels)	Cluster-Level pFWE	Brain Area	Side	Peak-Level p	Z Score at Local Maximum	Talairach Coordinates		
							x	y	z
1	361	< 0.001	Hippocampus	L	< 0.001	4.58	-28	-15	-19
			Superior Temporal Gyrus (BA 38)	L	< 0.001	4.57	-36	14	-33
			Inferior Temporal Gyrus (BA 20)	L	< 0.001	4.40	-32	-8	-37
2	76	0.046	Uncus (BA 20)	R	< 0.001	4.34	32	-17	-28
			Uncus (BA 28)	R	< 0.001	4.14	28	-9	-30
3	74	0.049	Middle Temporal Gyrus (BA 21)	R	< 0.001	4.32	46	4	-36
			Superior Temporal Gyrus (BA 38)	R	< 0.001	4.30	32	12	-34
			Superior Temporal Gyrus (BA 38)	R	< 0.001	3.80	26	6	-32
4	97	0.025	Cerebellum -Uvula	R	< 0.001	4.22	8	-69	-27
5	147	0.007	Cerebellum - Inferior Semi-Lunar Lobule	L	< 0.001	4.12	-30	-74	-42
			Cerebellum - Inferior Semi-Lunar Lobule	L	< 0.001	4.04	-18	-79	-35
			Cerebellum - Pyramis	L	< 0.001	3.97	-12	-81	-30

Clusters were significant with a set-level uncorrected $p < 0.0001$. L: Left; R: Right; FWE: Family-Wise Error; BA: Brodmann Area

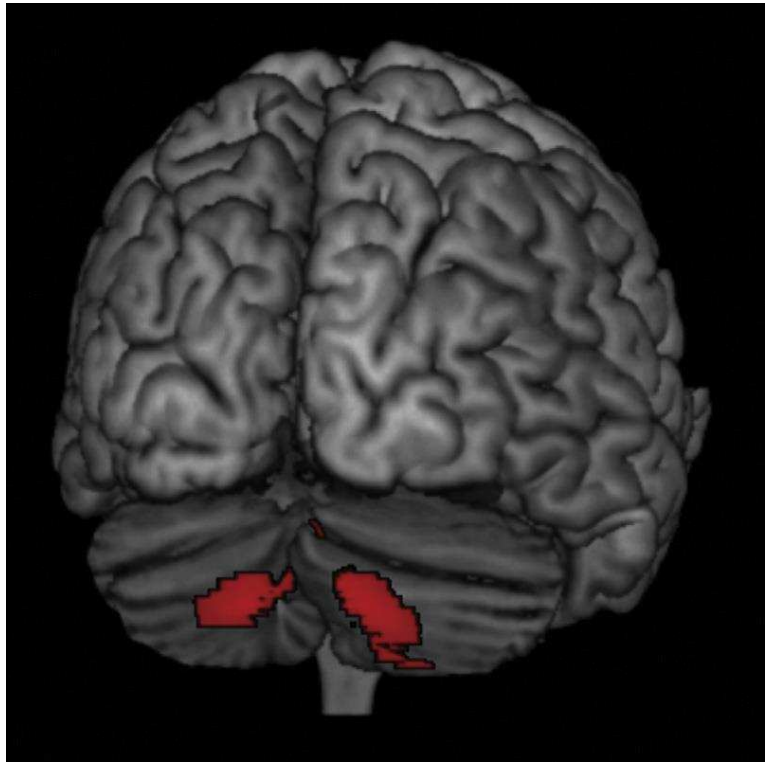


Figure 1

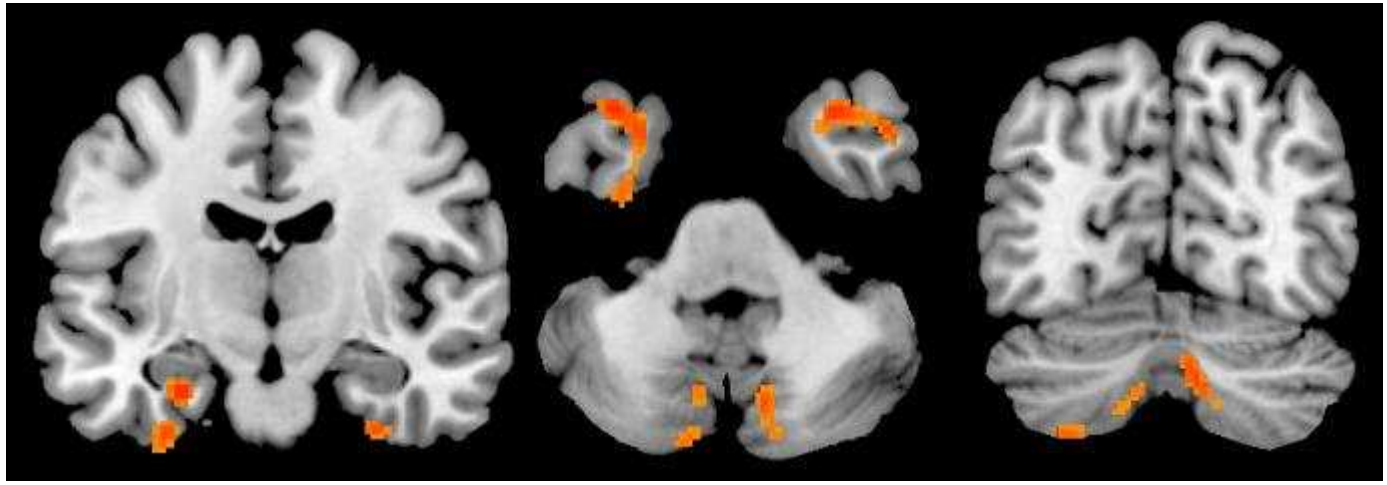


Figure 2