King's Research Portal

DOI:

[10.3233/JAD-170027](https://doi.org/10.3233/JAD-170027)

Document Version

Peer reviewed version

[Link to publication record in King's Research Portal](#)

Citation for published version (APA):

Lupton, M. K., Benyamin, B., Proitsi, P., Nyholt, D. R., Ferreira, M. A., Montgomery, G. W., Heath, A. C., Madden, P. A., Medland, S. E., Gordon, S. D., Lovestone, S., Tsolaki, M., Kloszewska, I., Soininen, H., Mecocci, P., Vellas, B., Powell, J. F., Bush, A. I., Wright, M. J., ... Whitfield, J. B. (2017). No Genetic Overlap between Circulating Iron Levels and Alzheimer's Disease. *JOURNAL OF ALZHEIMERS DISEASE*, 59(1), 85-99. <https://doi.org/10.3233/JAD-170027>

Citing this paper

Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher's website for any subsequent corrections.

General rights

Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Research Portal

Take down policy

If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

No genetic overlap between circulating iron levels and Alzheimer's disease

Michelle K Lupton^{a*}, Beben Benyamin^b, Petroula Proitsi^c, Dale R Nyholt^{a,d}, Manuel A Ferreira^a, Grant W Montgomery^a, Andrew C Heath^e, Pamela A Madden^e, Sarah E Medland^a, Scott D Gordon^a, GERAD1 Consortium⁺, the Alzheimer's Disease Neuroimaging Initiative⁺⁺, Simon Lovestone^f, Magda Tsolaki^g, Iwona Kloszewska^h, Hilka Soininenⁱ, Patrizia Mecocci^j, Bruno Velas^k, John F Powell^c, Ashley I Bush^l, Margaret J Wright^{a,b,m}, Nicholas G Martin^a, John B Whitfield^a.

- a. *QIMR Berghofer Medical Research Institute, Brisbane, Australia.*
- b. *Queensland Brain Institute, University of Queensland, Brisbane, Australia.*
- c. *Institute of Psychiatry Psychology and Neuroscience, Kings College London, UK*
- d. *Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia.*
- e. *Washington University School of Medicine, St Louis, MO, USA.*
- f. *University of Oxford, Department of Psychiatry, Warneford Hospital, Oxford, United Kingdom.*
- g. *Memory and Dementia Centre, 3rd Department of Neurology, Aristotle University of Thessaloniki, Thessaloniki, Greece.*
- h. *Department of Old Age Psychiatry & Psychotic Disorders, Medical University of Lodz, Lodz, Poland.*
- i. *Department of Neurology, University of Eastern Finland and Kuopio University Hospital, Kuopio, Finland.*
- j. *Section of Gerontology and Geriatrics, Department of Medicine, University of Perugia, Perugia, Italy.*
- k. *Gerontopole, CHU, UMR INSERM 1027, University of Toulouse, France .*
- l. *Florey Institute of Neuroscience & Mental Health, University of Melbourne, Parkville, Australia.*
- m. *Centre for Advanced Imaging, University of Queensland, Brisbane, Australia.*

* Correspondence to Michelle K Lupton PhD. QIMR Berghofer Medical Research Institute. 300 Herston Road, Herston QLD 4030, Australia. Tel.: 61-7-3845-3947; Fax: 61-7-3362-0101.E-mail address: Michelle.Lupton@QIMRBerghofer.edu.au

⁺Data used in the preparation of this article were obtained from the Genetic and Environmental Risk for Alzheimer's disease (GERAD1) Consortium. As such, the investigators within the GERAD1 consortia contributed to the design and implementation of GERAD1 and/or provided data but did not participate in analysis or writing of this report (except those who are named authors). Membership of the GERAD1 Consortium is provided in the Acknowledgments section.

⁺⁺Data used in preparing this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators may be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

Running title: Effect of circulating iron levels on AD risk

Abstract

Iron deposition in the brain is a prominent feature of Alzheimer's disease (AD). Recently, peripheral iron measures have also been shown to be associated with AD status. However, it is not known whether these associations are causal: do elevated or depleted iron levels throughout life have an effect on AD risk?

We evaluate the effects of peripheral iron on AD risk using a genetic profile score (GPS) approach by testing whether variants affecting iron, transferrin or ferritin levels selected from GWAS meta analysis of approximately 24,000 individuals are also associated with AD risk in an independent case-control cohort (n~10,000). Conversely, we test whether AD risk variants from a GWAS meta analysis of approximately 54,000 account for any variance in iron measures (n~9000).

We do not identify a genetic relationship, suggesting that peripheral iron is not causal in the initiation of AD pathology.

Keywords

Alzheimer's disease; Dementia; Iron; Transferrin; Ferritin; Genome Wide Association Study; Population Genetics; Apolipoproteins E; Genetic Profile Scores.

Introduction

Iron is the most abundant metal in the brain, where it is vital for neurotransmitter synthesis, myelination of neurons and energy generation by mitochondria [1]. However excess iron contributes to the generation of reactive oxygen species, and consequent tissue damage [2]. Dysfunctional brain iron homeostasis is believed to play an important role in Alzheimer's disease (AD) [3]. Iron accumulation is seen in the AD post-mortem brain [4] and iron content correlates with disease duration and mini-mental state examination (MMSE) score [5, 6]. Individuals with mild cognitive impairment (MCI) with high risk of AD, showed higher cortical iron in vivo using MRI (measured using quantitative susceptibility mapping (QSM) techniques), which spatially co-localised with A β plaques and correlated with higher plaque load [7]. In addition, transferrin (an iron transport protein) and ferritin (an intracellular iron storage protein) are both elevated in AD brain tissue in neurodegenerative regions [8]. Ferritin levels in cerebrospinal fluid (CSF) negatively correlated with cognitive performance and predicted conversion from mild cognitive impairment (MCI) to AD [9]. Ferritin levels were also associated with CSF apolipoprotein E levels and were elevated by the Alzheimer's risk allele, APOE- ϵ 4, suggesting that ferritin may reflect the mechanism by which APOE- ϵ 4 is a risk factor for AD.

Iron trafficking across the blood brain barrier is tightly regulated and early studies suggested that the brain is protected from systemic fluctuations in iron, with a lack of correlation between liver and brain iron concentrations post-mortem [10, 11]. Animal studies went on to challenge this view, showing that excess dietary iron increased brain iron levels in specific brain regions [12]. Quantitative MRI studies measuring the proton transverse relaxation rate (R_2) now allow iron concentrations to be assessed in the brain in vivo. One such study in cognitively normal elderly men found that iron levels in basal ganglia structures were correlated with serum iron measures [13]. In an investigation in the large Australian Imaging

Biomarker and Lifestyle (AIBL) cohort of healthy controls, MCI and AD patients had disturbed brain iron metabolism reflected in the periphery by a decrease in plasma iron and haemoglobin [14], which was due to a deficiency of iron-loading onto Transferrin [15]. Several mechanisms have been suggested to cause dysregulation of iron transport across the blood brain barrier in AD including the involvement of amyloid precursor protein (APP) fragments and chronic inflammation [11]. A deficit in brain iron trafficking, which is essential for heme formation, neurotransmitter synthesis, and myelination of axons, could contribute to the pathophysiology of AD. But results are inconsistent, with two meta analyses having differing conclusions on whether differences in circulating iron levels can be detected between AD cases and controls, and reporting heterogeneity between studies [16, 17].

It is clear that iron dysregulation has a role in AD, and that to a limited extent plasma iron might reflect changes in brain iron levels, but there has been little investigation of whether peripheral iron levels over the long term affect risk of AD. Apart from the lack of suitable and adequately powered prospective studies, a limitation of observational studies is the inability to distinguish between causal associations and those due to confounding and reverse causation. A systematic review found that, in a limited number of trials, testing whether depletion or supplementation of iron changed a person's risk of AD provided no conclusive evidence, and that additional studies are necessary [18].

Drug development and randomised clinical trials are expensive and take many years to reach fruition, especially for a slowly progressive disease where treatment needs to start early in the disease course. An alternative approach, which overcomes the problem of reverse causation, is Mendelian Randomization (MR). Here the genetic variants affecting the putative causal variable are used as instrumental variables to test for an effect on disease risk. A

demonstration that genetic polymorphisms known to modify the phenotype level also modify disease risk provides indirect evidence of a causal association between phenotype and disease. MR analysis has the following assumptions: firstly the genetic variant used is only associated with the risk factor of interest; secondly it is independent of all confounding variables; and finally there is no causal pathway leading from the genetic variant to the disease except through the risk factor of interest. For highly polygenic traits a large number of genetic polymorphisms can be combined to explain a larger proportion of the variance of the trait. The large numbers of variants included means that some are likely to violate the assumptions for a MR analysis. But a lack of association between appropriate SNPs and the outcome, given a dataset large enough to give reasonable power suggests that there is no causal relationship. A shared genetic basis indicates either, pleiotropy where a variant affects multiple traits independently, or a causal relationship between the two correlated traits; with the requirement that any potential confounders must be taken into account. If a shared genetic basis is found then a quantitative MR approach would then be required to compare direct and mediated paths between variants affecting the postulated causal variables and the outcome. This method has been widely used, both confirming and refuting suggested causal relationships based on epidemiological findings [19]. For example, this approach has had significant success in clarifying relationships between lipid levels and ischemic heart disease [20]. In addition a recent study compared 42 traits or diseases with available large GWAS where among other findings the authors found evidence that increased BMI causally increases triglyceride levels [21].

MR was recently used to test for an effect of serum iron on Parkinson's Disease (PD) risk, using three genetic variants influencing iron levels (*HFE* rs1800562, *HFE* rs1799945, and *TMPRSS6* rs855791)[22]. The combined MR estimate showed a statistically significant

protective effect of increased serum iron in PD, suggesting that over the course of a life time alteration in tissue iron homeostasis reflected by a decrease in serum iron levels is on the causal pathway in the pathogenesis of PD. Twelve iron associated SNPs identified through GWAS were also used to investigate the role of iron in atherosclerosis, and identified a potential causal role in women [23].

Single genetic variants that influence serum iron levels have not been shown to have a large effect on AD risk. The transferrin genetic variant C2 has been investigated and shown to have a small but significant association (OR=1.11, 95% CI 1.05 to 1.17, in a meta analysis of 19 studies [24]). Several studies previously reported an increased frequency of the *HFE* H63D (rs1799945) mutation in AD patients [25], but these findings have not been replicated in the largest AD GWAS meta analysis [26]. There is evidence of interaction effects, which would not be apparent in GWAS meta analyses, involving H63D and *APOE* ϵ 4 alleles where the combination appears to affect age of onset and, to a lesser extent, risk [27-29].

Since several genes are well characterized for their impact on peripheral iron variation, we sought to determine their combined causal effect on AD risk. We test the effect of a large number of genetic variants affecting the iron-related measures of serum iron concentration, transferrin (the major iron transporter), ferritin (which reflects iron storage in bone marrow) and transferrin saturation (ratio between serum iron and total iron binding capacity) on AD risk, in combination using a genetic profile score (GPS) approach. Variants are selected from an iron GWAS meta analysis discovery cohort [30] (n=23,986) and tested in large independent target AD case-control datasets (n=9,251). In addition we test for the converse scenario, whether those at a high genetic risk for AD have higher peripheral iron levels throughout life, using SNPs identified by the AD GWAS meta analysis discovery cohort [26]

(from the International Genomics of Alzheimer's Project, IGAP n=54,162) in an independent population based target sample with available iron measures (n=8893). Previously an AD polygenic score analysis has shown that disease prediction accuracy is greatest including SNPs with P value <0.5. Including the full polygenic score significantly improved prediction over use of *APOE* alone where including both *APOE* and PRS gave AUC=78.2% [31]. Examples of the AD PRS based on the IGAP discovery analysis demonstrating genetic overlap with other traits include neuroimaging measures of subcortical brain volumes, plasma C-reactive protein and lipids [32, 33].

Finally to confirm our findings using an alternative method we used SNP Effect Concordance Analysis (SECA) with only the discovery datasets, to examine whether SNPs found to be associated with the serum iron measures are enriched within associated SNPs with AD risk, and vice versa.

Material and Methods

Subjects

The AD case-control cohort comprises the datasets shown in table 1. All individuals were of European descent and all AD case-control cohort individuals were age ≥ 60 years. Controls were screened for dementia using either MMSE, ADAS-cog, determined to be free from characteristic AD plaques at neuropathological examination or had a Braak score ≤ 2.5 . Individuals with AD met criteria for either probable (NINCDS-ADRDA, DSM-IV) or definite (CERAD) AD. Individuals classed as MCI were excluded. The WTCCC2 1958 BC samples are population samples aged 54 years at collection and are included as unscreened controls in this analysis.

The population based sample set comprises (a) adult twins, their spouses and first degree relatives who volunteered for studies on risk factors or biomarkers for physical or psychiatric conditions (N=8380); (b) people with self-reported endometriosis and unaffected relatives (N=830) [34, 35]. The mean age is 47 years (ranged 15-92 years) with 62% female.

Biochemical markers of iron status were measured using standard clinical methods on Roche/Hitachi 917 or Modular P analysers [30]. Serum iron was measured by colorimetry with Ferrozine reagent, serum transferrin by immunoturbidimetry, and ferritin by latex particle immunoturbidimetry. Transferrin saturation was calculated from the iron and transferrin results. The values for ferritin were log transformed to produce a normal distribution.

Genetic Profile Scores (GPS)

GPS for serum iron, transferrin, transferrin saturation and ferritin (log) were calculated in target AD case-control cohorts, using stage 1 summary data from the discovery sample of a GWAS meta analysis combining 11 population based studies of biochemical markers of iron status, with a sample size of 23,986 [30] using the method previously described ([36] and supplementary methods). In brief, linkage disequilibrium based clumping was used to select SNPs in the discovery data, providing the most significantly associated SNP available in the target data set. The total score is calculated by the number of risk alleles weighted by the standardised per-allele effects for P value thresholds of 1×10^{-6} , 1×10^{-4} , 1×10^{-3} , 0.01, 0.05, 0.1, 0.5 and 1 (all SNPs) (Supplementary table 1).

The AD GPS was generated in the target population based cohort using summary data from the AD GWAS meta analysis from the IGAP discovery sample consisting of 17,008 Alzheimer's disease cases and 37,154 controls [26]. GPS were calculated as described above,

with the number of risk alleles weighted by the effect on AD risk (log odds ratio). All *APOE* associated signal was removed and *APOE* genotype assessed separately.

***APOE* Genotype**

In the AD cohorts a subset of samples have available *APOE* genotypes (table 1) inferred from rs429358 and rs7412 SNPs genotyped using TaqMan SNP genotyping assays. In the Australian dataset *APOE* genotype was estimated from imputed rs429358 and rs7412 SNP genotypes (Supplementary methods).

GPS Association analysis

In the AD cohort data sets we tested for an association between iron, transferrin, transferrin saturation and ferritin GPS at each P value threshold with AD case-control status using logistic regression (performed in STATA v11) controlling for age, sex, and four ancestry principal components. Results for each dataset were combined in a Meta analysis allowing a test for between study heterogeneity (STATA METAN specifying a random effects model). Finally all datasets were combined in a mega analysis also controlling for study. In addition we separately assessed the effect of the three iron level influencing variants that have previously been shown to associate with PD risk [22]. We tested for an association with the following SNPs; HFE rs1800562, HFE rs1799945, and TMPRSS6 rs855791 using logistic regression under an additive model and then combined the three variants in a GPS. To investigate any potential interaction effect of *APOE* ϵ 4 genotype we also repeated these analyses controlling for *APOE* ϵ 4 carrier status and also in *APOE* ϵ 4 positive and *APOE* ϵ 4 negative groups.

In the population based dataset we tested for an association of AD GPS and number of *APOE* ϵ 4 alleles with peripheral iron measures (iron, transferrin, transferrin saturation and ferritin) using Genome-wide Efficient Mixed Model Association algorithm (GEMMA) software [37]. The sample contains related individuals including monozygotic and dizygotic twin pairs, and other first degree relatives. We used linear mixed model regression using the likelihood ratio test, including sex, age and four ancestry principal components as covariates and controlling for family structure using a genetic relatedness matrix estimated from genome wide genotypes.

SNP effect concordance analysis (SECA)

We carried out SECA analysis of large scale GWAS meta analysis summary statistics to examine the genetic overlap between AD and each iron measure using the default approach [38]. SECA allows a larger sample size to be examined without the need for individual level genotype data. The GWAS meta-analysis results for AD (meta analysis N=74,046) [26] and iron measures (iron, transferrin, transferrin saturation and ferritin, meta analysis N=23,986) [30] were used to test for an excess of SNPs associated in the AD and iron phenotype data sets, and whether the SNP effect directions are concordant. SNP effects across the two GWAS summary results were aligned (AD and iron) to the same effect allele and independent SNPs were extracted via LD clumping identifying a subset of independent SNPs with the most significant P-values in the AD dataset. Restricting to SNPs associated with $P_1 \leq 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0$ in the AD dataset, exact binomial statistical tests determine whether there is an excess of SNPs associated in both datasets for the subset of SNPs associated with $P_2 \leq 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0$ in the iron dataset. Fisher's exact test is then used to determine whether there is an excess of SNPs where the effect directions are concordant across the datasets for each P value subset.

Due to the larger sample size the AD GWAS summary statistics were initially used as dataset 1, and each of the iron measures as dataset 2, providing the greatest possible power. Because the analysis is restricted to those SNPs which are most highly associated in dataset 1, we also repeated the analysis with the iron GWAS summary statistics as dataset 1 (in case of a scenario where SNPs strongly affecting iron phenotypes had an effect on AD risk, but SNPs strongly affecting AD risk did not affect iron phenotypes).

Results

Genetic profile score (GPS) analysis

The discovery GWAS meta analysis datasets used in the study contain large sample sizes (in total 54,162 for AD and 23,986 for serum iron status) and show both AD and serum iron measures to have a strong polygenic components^{27, 31}. For serum iron measures using replication cohorts, the lead SNPs at the 11 significant loci explained 3.4, 7.2, 6.7 and 0.9% of the phenotypic variance for iron, transferrin, saturation and (log-transformed) ferritin, respectively[30]. There is large deviation from the expected distribution of association test statistics compared to observed values, with association signals observed far below the level of genome-wide significance (figure 1). Therefore using SNPs below genome-wide significance will increase power to detect an association.

Association analysis conducted in each AD disease case-control data set identified no effect of any serum iron status GPS (serum iron, transferrin, ferritin and transferrin saturation) on AD risk, and the Meta analysis identified no significant between study heterogeneity (Supplementary figure 1). When combined in a mega analysis no effect of any serum iron status GPS (serum iron, transferrin, ferritin and transferrin saturation) on AD risk was identified with a sample size of 6,381 controls and 2,870 AD cases (Table 3). Controlling for *APOE* genotype did not significantly affect the results, and no significant association was

identified in separate *APOE* $\epsilon 4$ carrier and non-carrier groups (data not shown). Previously three iron level influencing genetic variants (HFE rs1800562, HFE rs1799945, and TMPRSS6 rs855791) have been shown to be associated with PD risk[22]. There was no association of these SNPs with AD status in our dataset and no interaction identified with *APOE* $\epsilon 4$ status (Supplementary Table 2). In addition, the GPS constructed from these three SNPs did not have an effect on AD risk (Supplementary Table 2). There was no association of AD GPS or *APOE* $\epsilon 4$ with any peripheral iron measure (Table 4).

SNP Effect Concordance Analysis (SECA)

In agreement with the GPS analysis we did not identify any significant pleiotropy between datasets or concordant effects using SECA. We tested for an excess of SNPs associated with AD also associating with iron phenotypes. Using a binomial test we compared the AD dataset with each of the iron phenotype datasets in turn examining 144 SNP subsets (testing twelve P value threshold combinations). No SNP sets were found to have nominally significant pleiotropy (figure 2). Using Fisher's test we also tested for an excess of SNPs where the effect directions (BETA) are concordant between SNP subsets in each dataset. Again we identified no significant concordance (supplementary figure 2). Additionally no significant pleiotropy or concordant effects were seen when switching the primary dataset, i.e. testing for an excess of SNPs associated with each iron phenotype also associating with AD.

Discussion

It is becoming increasingly clear from investigations of iron homeostasis and recent advances in iron imaging methods that iron dysregulation is an important feature of AD, and therefore

lowering of iron content in the brain is a potential therapeutic target [39]. But there is limited understanding of the importance of peripheral iron levels in AD risk, and whether prolonged increased or decreased iron levels may be a risk factor for AD. We investigated whether there is a shared genetic basis between AD and peripheral iron levels using a PRS approach. We identified no effect of genetic variants affecting peripheral iron biomarkers (including iron, transferrin, transferrin saturation and ferritin) on AD risk. Nor did we find increased serum iron levels in those who are at increased genetic risk of developing AD, including both *APOE* $\epsilon 4$ carriers and those with a higher load of other common risk variants. In addition, in an investigation of the genetic overlap between AD and each iron measure, we do not find any significant overlap of genetic loci from the results of large-scale GWAS meta analysis studies.

Taken together, our results suggest that the causes of variation in brain iron that might contribute to AD are distinct from those causing variation in circulating iron (serum iron) or in iron stores in bone marrow or other organs (serum ferritin). Iron retention is complex in different organs, and our current data on peripheral iron measurement cannot exclude causation by other genes that affect iron levels in the brain that are not reflected by serum values. In addition the peripheral iron measurements used are standard clinical pathology measures. Non-standard and possibly more direct measures (such as transferrin saturation using size exclusion chromatography-inductively coupled plasma-mass spectrometry) have been shown to be more sensitive to differences in the blood between AD patients and controls [15].

It is also possible that, even if iron is not a primary cause of increase in AD risk, it accumulates after the initiation of cell damage by other mechanisms, and exacerbates it. Evidence for this comes from recent work showing that once A β forms aggregates they

induce iron accumulation [40]. Iron-related therapies could still be relevant for patients who are in the early stages of AD.

Iron accumulation in tissues is a feature of many diseases, and may prove to be causal for some. Our current results for AD are in contrast to previous evidence of a causal association of increased peripheral iron measures with a decreased risk of Parkinson's disease (PD) [22]. The authors hypothesised that low peripheral iron may decrease neuronal iron storage through a reduction in ferritin, resulting in free iron accumulation in the brain. To investigate whether a similar effect exists for AD we tested a larger number of iron-affecting variants against the most recent GWAS meta analysis on AD risk. These explain a larger proportion of the variance and therefore we would expect them to have more power to detect any effect.

However, our analysis has limitations that need to be considered. Firstly, the multi-SNP GPS includes a large number of genetic variants of unknown effect or multiple effects, therefore we cannot rule out that as well as affecting iron levels, some also affect AD risk through other pathways and could potentially do so in opposite directions. To attempt to address this issue we also tested for an effect of three genetic variants (in *HFE* and *TMPRSS6*) known to have a direct role in peripheral iron levels and previously shown to have an effect on PD risk [22], where we also did not find an effect. In addition we cannot rule out the possibility that other genomic variations such as epigenetic dysregulation affect iron levels which are then causal for AD.

Secondly, as in other complex diseases and phenotypes, discovered genetic variants only represent a small proportion of the variance in both iron levels and AD risk. This study utilizes summary data from the two largest GWAS meta analysis discovery cohorts for both

AD and biochemical markers of iron status (total sample sizes of 54,162 and 23,986 respectively [26, 30]) to compute comprehensive GPS. In addition the GPS were applied to large samples with individual level genotype and phenotype data (For AD cases-control: 2813 AD cases, and 6438 controls (of which 4926 are unscreened for AD, aged 54), and ≥ 8751 for iron measures). Even so, we cannot rule out a small effect that is not detectable with this sample size.

Thirdly, effects on iron in relevant brain areas may differ from effects on circulating iron or iron in other organs. Previous studies identified an association between iron accumulation in the basal ganglia of elderly men and peripheral iron measures [13]. However, only 9% of the variance of cerebrospinal fluid ferritin can be explained by plasma ferritin [9], highlighting the separation between these compartments. It is also possible that there are genetic loci more relevant to iron-homeostasis in elderly people, as the sample used to construct the iron phenotypes GPS have a mean age of 47.

Our results suggest that there is not a causal connection between lifetime peripheral iron measures and increased risk of AD. We did not replicate the previous finding of an effect of *HFE* SNPs on risk of AD and an epistatic interaction for risk with *APOE* $\epsilon 4$ genotype, but we cannot yet rule out an association of *HFE* SNPs with AD age of onset or phenotypic interactions [25, 27, 28].

It has been suggested that public recommendations for AD risk reduction should caution the use of iron supplementation for those whom it is not required [18, 41, 42]. Dietary patterns such as a Mediterranean diet and reduced red meat consumption that associate with lower AD risk do tend to have a low iron intake, but also have other unrelated health benefits for example high intake of vegetables and low saturated fat. Consistent with our genetic findings,

there is no clear evidence that dietary intervention affecting iron intake alters the risk of AD [18]. More work is needed to assess the effect of iron on the progression (as opposed to the initiation) and age of onset of AD.

In conclusion, although iron deposition is an important feature of AD brain tissues, these results suggest that there is not a significant causal relationship between lifetime peripheral iron levels and AD.

Acknowledgements

Genetic and Environmental Risk for Alzheimer's Disease Consortium (GERAD1) Collaborators

Denise Harold¹, Rebecca Sims¹, Amy Gerrish¹, Jade Chapman¹, Valentina Escott-Price¹, Nandini Badarinarayan¹, Richard Abraham¹, Paul Hollingworth¹, Marian Hamshere¹, Jaspreet Singh Pahwa¹, Kimberley Dowzell¹, Amy Williams¹, Nicola Jones¹, Charlene Thomas¹, Alexandra Stretton¹, Angharad Morgan¹, Kate Williams¹, Sarah Taylor¹, Simon Lovestone², John Powell³, Petroula Proitsi³, Michelle K Lupton³, Carol Brayne⁴, David C. Rubinsztein⁵, Michael Gill⁶, Brian Lawlor⁶, Aoibhinn Lynch⁶, Kevin Morgan⁷, Kristelle Brown⁷, Peter Passmore⁸, David Craig⁸, Bernadette McGuinness⁸, Janet A Johnston⁸, Stephen Todd⁸, Clive Holmes⁹, David Mann¹⁰, A. David Smith¹¹, Seth Love¹², Patrick G. Kehoe¹², John Hardy¹³, Rita Guerreiro^{14,15}, Andrew Singleton¹⁴, Simon Mead¹⁶, Nick Fox¹⁷, Martin Rossor¹⁷, John Collinge¹⁶, Wolfgang Maier¹⁸, Frank Jessen¹⁸, Reiner Heun¹⁸, Britta Schürmann^{18,19}, Alfredo Ramirez¹⁸, Tim Becker²⁰, Christine Herold²⁰, André Lacour²⁰, Dmitriy Drichel²⁰, Hendrik van den Bussche²¹, Isabella Heuser²², Johannes Kornhuber²³, Jens Wiltfang²⁴, Martin Dichgans^{25,26}, Lutz Frölich²⁷, Harald Hampel²⁸, Michael Hüll²⁹, Dan Rujescu³⁰, Alison Goate³¹, John S.K. Kauwe³², Carlos Cruchaga³³, Petra Nowotny³³, John C. Morris³³, Kevin Mayo³³, Gill Livingston³⁴, Nicholas J. Bass³⁴, Hugh Gurling³⁴, Andrew McQuillin³⁴, Rhian Gwilliam³⁵, Panagiotis Deloukas³⁵, Markus M. Nöthen²⁰, Peter Holmans¹, Michael O'Donovan¹, Michael J. Owen¹, Julie Williams¹.

1 Medical Research Council (MRC) Centre for Neuropsychiatric Genetics and Genomics, Neurosciences and Mental Health Research Institute, Department of Psychological Medicine and Neurology, School of Medicine, Cardiff University, Cardiff, UK,

2 Department of Psychiatry, Medical Sciences Division, University of Oxford, Oxford, UK,

3 Kings College London, Institute of Psychiatry, Department of Neuroscience, De Crespigny Park, Denmark Hill, London, UK,

4 Institute of Public Health, University of Cambridge, Cambridge, UK,

5 Cambridge Institute for Medical Research, University of Cambridge, Cambridge, UK,

6 Mercers Institute for Research on Aging, St. James Hospital and Trinity College, Dublin, Ireland,

7 Institute of Genetics, Queens Medical Centre, University of Nottingham, UK,

8 Ageing Group, Centre for Public Health, School of Medicine, Dentistry and Biomedical Sciences, Queens University Belfast, UK,

9 Division of Clinical Neurosciences, School of Medicine, University of Southampton, Southampton, UK,

10 Clinical Neuroscience Research Group, Greater Manchester Neurosciences Centre, University of Manchester, Salford, UK,

11 Oxford Project to Investigate Memory and Ageing (OPTIMA), University of Oxford, Department of Pharmacology, Mansfield Road, Oxford, UK,

12 University of Bristol Institute of Clinical Neurosciences, School of Clinical Sciences, Frenchay Hospital, Bristol, UK,

13 Department of Molecular Neuroscience and Reta Lilla Weston Laboratories, Institute of Neurology, UCL, London, UK,

14 Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Bethesda, Maryland, United States of America,

15 Department of Molecular Neuroscience, Institute of Neurology, University College London, Queen Square, London WC1N 3BG, UK,

16 MRC Prion Unit, Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK,

17 Dementia Research Centre, Department of Neurodegenerative Diseases, University College London, Institute of Neurology, London, UK,

18 Department of Psychiatry, University of Bonn, Sigmund-Freud-Straße 25, 53105 Bonn, Germany,

19 Institute for Molecular Psychiatry, University of Bonn, Bonn, Germany,

20 Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany,

21 Institute of Primary Medical Care, University Medical Center Hamburg-Eppendorf, Germany,

22 Department of Psychiatry, Charité Berlin, Germany,

23 Department of Psychiatry, Friedrich-Alexander-University Erlangen-Nürnberg, Germany,

24 Department of Psychiatry and Psychotherapy, University Medical Center (UMG), Georg-August-University, Göttingen, Germany,

- 25** Institute for Stroke and Dementia Research, Klinikum der Universität München, Marchioninstr. 15, 81377, Munich, Germany,
- 26** Department of Neurology, Klinikum der Universität München, Marchioninstr. 15, 81377, Munich, Germany,
- 27** Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Germany,
- 28** Institute for Memory and Alzheimer's Disease & INSERM, Sorbonne Universities, Pierre and Marie Curie University, Paris, France; Institute for Brain and Spinal Cord Disorders (ICM), Department of Neurology, Hospital of Pitié-Salpêtrière, Paris, France,
- 29** Centre for Geriatric Medicine and Section of Gerontopsychiatry and Neuropsychology, Medical School, University of Freiburg, Germany,
- 30** Department of Psychiatry, University of Halle, Halle, Germany,
- 31** Neuroscience Department, Icahn School of Medicine at Mount Sinai, New York, US,
- 32** Department of Biology, Brigham Young University, Provo, UT, 84602, USA,
- 33** Departments of Psychiatry, Neurology and Genetics, Washington University School of Medicine, St Louis, MO 63110, US,
- 34** Department of Mental Health Sciences, University College London, UK,
- 35** The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK.

For the GERAD1 Consortium; Cardiff University was supported by the Wellcome Trust, Medical Research Council (MRC), Alzheimer's Research UK (ARUK), and the Welsh Assembly Government. ARUK supported sample collections at the Kings College London; the South West Dementia Bank; and Universities of Cambridge, Nottingham, Manchester, and Belfast. The Belfast group acknowledges support from the Alzheimer's Society, Ulster Garden Villages, N.Ireland R&D Office, and the Royal College of Physicians/Dunhill Medical Trust. The MRC and Mercer's Institute for Research on Ageing supported the Trinity College group. The South West Dementia Brain Bank acknowledges support from Bristol Research into Alzheimer's and Care of the Elderly. The Charles Wolfson Charitable Trust supported the OPTIMA group. Washington University was funded by NIH grants, Barnes Jewish Foundation, and the Charles and Joanne Knight Alzheimer's Research Initiative. Patient recruitment for the MRC Prion Unit/UCL Department of Neurodegenerative Disease collection was supported by the UCLH/UCL Biomedical Centre. LASER-AD was funded by Lundbeck SA. The Bonn group was supported by the German Federal Ministry of Education and Research (BMBF), Competence Network Dementia and Competence Network Degenerative Dementia, and by the Alfried Krupp von Bohlen und Halbach-Stiftung. The GERAD1 Consortium also used samples ascertained by the NIMH AD Genetics Initiative.

AddNeuroMed is part of InnoMed (Innovative Medicines in Europe), an integrated project funded by the European Union of the Sixth Framework program priority ((FP6-2004-LIFESCIHEALTH-5); the Alzheimer's Research Trust UK; the John and Lucille van Geest Foundation; and the NIHR Biomedical Research Centre for Mental Health and Biomedical Research Unit for Dementia at the South London, Maudsley NHS Foundation Trust and Kings College London, and a joint infrastructure grant from Guy's and St Thomas' Charity and the Maudsley Charity; Academy of Finland, Kuopio University Hospital (HS) and funding from UEF-BRAIN (HS).

The Kings Health Partners Dementia Case Register is funded by the National Institute for Health Research (NIHR) Mental Health Biomedical Research Centre and Dementia Unit at South London and Maudsley NHS Foundation Trust and (Institute of Psychiatry, Psychology and Neuroscience) King's College London.

Data collection and sharing for ADNI was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defence award number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions

from the following: Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Araclon Biotech; BioClinica, Inc.; Biogen Idec Inc.; Bristol-Myers Squibb Company; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; EuroImmun; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; Fujirebio; GE Healthcare; ; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Medpace, Inc.; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Synarc Inc.; and Takeda Pharmaceutical Company. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Disease Cooperative Study at the University of California, San Diego. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California

This study makes use of data generated by the Wellcome Trust Case-Control Consortium. A full list of the investigators who contributed to the generation of the data is available from www.wtccc.org.uk. Funding for the project was provided by the Wellcome Trust under award 076113 and 085475" and cite the relevant primary WTCCC publication (details of which can be found on the WTCCC website).

For the Australian study, we acknowledge funding from the Australian National Health and Medical Research Council (NHMRC grants 241944, 389875, 389891, 389892, 389938, 442915, 442981, 496739 and 552485), US National Institutes of Health (NIH grants AA07535, AA10248, AA014041, AA011998, AA013320, AA013321, AA017688, DA012854) and the Australian Research Council (ARC grant DP0770096). MKL is supported by a Perpetual Foundation Wilson Fellowship for early career researchers.

BB was supported by the Australian National Health and Medical Research Council (APP1084417 and APP1079583).

We thank the International Genomics of Alzheimer's Project (IGAP) for providing AD meta-analysis summary results data for these analyses. The investigators within IGAP contributed to the design and implementation of IGAP and/or provided data but did not participate in analysis or writing of this report. IGAP was made possible by the generous participation of the control subjects, the patients, and their families. The i-Select chips were funded by the French National Foundation on Alzheimer's disease and related disorders. EADI was supported by the LABEX (laboratory of excellence program investment for the future) DISTALZ grant, Inserm, Institut Pasteur de Lille, Université de Lille 2 and the Lille University Hospital. GERAD was supported by the Medical Research Council (Grant n° 503480), Alzheimer's Research UK (Grant n° 503176), the Wellcome Trust (Grant n° 082604/2/07/Z) and German Federal Ministry of Education and Research (BMBF): Competence Network Dementia (CND) grant n° 01GI0102, 01GI0711, 01GI0420. CHARGE was partly supported by the NIH/NIA grant R01 AG033193 and the NIA AG081220 and AGES contract N01-AG-12100, the NHLBI grant R01 HL105756, the Icelandic Heart Association, and the Erasmus Medical Centre and Erasmus University. ADGC was supported by the NIH/NIA grants: U01 AG032984, U24 AG021886, U01 AG016976, and the Alzheimer's Association grant ADGC-10-196728.

We acknowledge the Genetics of Iron Status Consortium as the source of the iron meta-analysis SNP association data. The following individuals are part of the Iron Status Consortium and therefore

contributed to the design and implementation of the iron meta analysis study but did not participate in analysis or writing of this paper (unless named as authors).

Genetics of Iron Status Consortium

Beben Benyamin^{1,2}, Tonu Esko^{3,4}, Janina S. Ried⁵, Aparna Radhakrishnan⁶, Sita H. Vermeulen^{7,8}, Michela Traglia^{9,10}, Martin Goëgele¹¹, Denise Anderson¹², Linda Broer^{13,14}, Clara Podmore¹⁵, Jian'an Luan¹⁵, Zoltan Kutalik^{16,17}, Serena Sanna¹⁸, Peter van der Meer¹⁹, Toshiko Tanaka²⁰, Fudi Wang²¹, Harm-Jan Westra²², Lude Franke²², Evelin Mihailov^{3,23}, Lili Milani³, Jonas Haïldin³, Juliane Winkelmann^{24,25,26,27}, Thomas Meitinger^{26,28}, Joachim Thiery^{29,30}, Annette Peters^{31,32}, Melanie Waldenberger^{31,32}, Augusto Rendon^{6,33,34}, Jennifer Jolley^{6,33}, Jennifer Sambrook^{6,33}, Lambertus A. Kiemeny^{7,35}, Fred C. Sweep³⁶, Cinzia F. Sala³⁷, Christine Schwenbacher¹¹, Irene Pichler¹¹, Jennie Hui^{38,39}, Ayse Demirkan^{13,40}, Aaron Isaacs^{13,41}, Najaf Amin¹³, Maristella Steri¹⁸, Ge'ard Waeber⁴², Niek Verweij¹⁹, Joseph E. Powell^{1,43}, Dale R. Nyholt², Andrew C. Heath⁴⁴, Pamela A.F. Madden⁴⁴, Peter M. Visscher^{1,43}, Margaret J. Wright², Grant W. Montgomery², Nicholas G. Martin², Dena Hernandez⁴⁵, Stefania Bandinelli⁴⁶, Pim van der Harst^{19,47,48}, Manuela Uda¹⁸, Peter Vollenweider⁴², Robert A. Scott¹⁵, Claudia Langenberg¹⁵, Nicholas J. Wareham¹⁵, InterAct Consortium, Cornelia van Duijn^{13,41,49}, John Beilby^{38,39}, Peter P. Pramstaller^{11,50}, Andrew A. Hicks¹¹, Willem H. Ouwehand^{6,33}, Konrad Oexle²⁸, Christian Gieger^{5,31,32}, Andres Metspalu³, Clara Camaschella^{9,51}, Daniela Toniolo⁹, Dorine W. Swinkels³⁶ & John B. Whitfield²

1 The University of Queensland, Queensland Brain Institute, Brisbane, Queensland 4072, Australia.

2 QIMR Berghofer Medical Research Institute, Brisbane, Queensland 4029, Australia.

3 Estonian Genome Center, University of Tartu, Riia 23b, 51010 Tartu, Estonia.

4 The Broad Institute of Massachusetts Institute of Technology and Harvard University, 320 Charles St, Cambridge, Massachusetts 02141, USA.

5 Institute of Genetic Epidemiology, Helmholtz Zentrum München, Ingolstädter Landstrasse 1, 85764 Neuherberg, Germany.

6 Department of Haematology, University of Cambridge, Cambridge Biomedical Campus Box 139, Hills Road, Cambridge CB2 0XY, UK.

7 Department for Health Evidence, Radboud University Medical Centre, Geert Grooteplein-Zuid 10, 6525GA Nijmegen, The Netherlands.

8 Department of Genetics, Radboud University Medical Centre, Geert Grooteplein-Zuid 10, 6525 GA, Nijmegen, The Netherlands.

9 Division of Genetics and Cell Biology, San Raffaele Scientific Institute, 20132 Milano, Italy.

10 Institute for Maternal and Child Health-IRCCS "Burlo Garofolo"- Trieste, 34137 Trieste, Italy. **11** Center for Biomedicine, European Academy of Bolzano/Bozen (EURAC), Via Galvani 31, 39100 Bolzano, Italy.

12 Telethon Institute for Child Health Research, Centre for Child Health Research, The University of Western Australia, PO Box 855, West Perth, Western Australia 6872, Australia.

13 Department of Epidemiology, Subdivision Genetic Epidemiology, Erasmus Medical Center, Postbus 2040, 3000 CA Rotterdam, The Netherlands.

14 Department of Internal Medicine, Erasmus Medical Center, Postbus 2040, 3000 CA Rotterdam, The Netherlands.

15 MRC Epidemiology Unit, University of Cambridge School of Clinical Medicine, Box 285 Institute of Metabolic Science, Cambridge Biomedical Campus, Cambridge CB2 0QQ, UK.

16 Institute of Social and Preventive Medicine (IUMSP), Centre Hospitalier Universitaire Vaudois (CHUV), Rte de la Corniche 10, 1010 Lausanne, Switzerland.

17 Swiss Institute of Bioinformatics, 1015 Lausanne, Switzerland.

18 Istituto di Ricerca Genetica e Biomedica (IRGB), Consiglio Nazionale delle Ricerche (CNR), Monserrato, 09042 Cagliari, Italy.

19 University of Groningen, University Medical Center Groningen, Department of Cardiology, 9700 RB Groningen, The Netherlands.

20 Translational Gerontology Branch, National Institute on Aging, 251 Bayview Boulevard, Suite 100, Baltimore, Maryland 21224-6825, USA.

21 School of Public Health, Zhejiang University, Hangzhou 310058, P. R. China.

22 Department of Genetics, University Medical Center Groningen, P.O. Box 30001, 9700 RB Groningen, The Netherlands.

- 23** Institute of Molecular and Cell Biology, University of Tartu, Tartu 51010, Estonia.
- 24** Department of Neurology and Neurosciences and Center for Sleep Sciences and Medicine, Stanford University, Palo Alto, California 94304, USA.
- 25** Neurologische Klinik und Poliklinik, Klinikum rechts der Isar, Technische Universität München, 81675 Munich, Germany.
- 26** Institut für Humangenetik, Helmholtz Zentrum München, 85764 Neuherberg, Germany.
- 27** Munich Cluster for Systems Neurology (SyNergy), 81675 Munich, Germany.
- 28** Institute of Human Genetics, Klinikum Rechts der Isar, Technische Universität München, Ismaninger Straße 22, 81675 München, Germany.
- 29** Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, Universitätsklinikum Leipzig, Liebigstraße 27, 04103 Leipzig, Germany.
- 30** LIFE Research Center for Civilization Diseases, Medical Faculty, University Leipzig, Philipp-Rosenthal-Straße 27, 04103 Leipzig, Germany.
- 31** Institute of Epidemiology II, Helmholtz Zentrum München - German Research Center for Environmental Health, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany.
- 32** Research Unit of Molecular Epidemiology, Helmholtz Zentrum München – German Research Center for Environmental Health, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany.
- 33** NHS Blood and Transplant, Cambridge Biomedical Campus, Long Road, Cambridge CB2 0PT, UK.
- 34** Medical Research Council Biostatistics Unit, Cambridge Biomedical Campus, Cambridge CB2 0SR, UK.
- 35** Department of Urology, Radboud University Medical Centre, Geert Grooteplein-Zuid 10, 6525GA Nijmegen, The Netherlands.
- 36** Laboratory of Genetic, Endocrine and Metabolic Diseases (LGEM), Department of Laboratory Medicine, Radboud University Medical Centre, Geert Grooteplein-Zuid 10, 6525 GA Nijmegen, The Netherlands.
- 37** Division of Genetics and Cell Biology, San Raffaele Scientific Institute, 20132 Milano, Italy.
- 38** PathWest Laboratory Medicine of WA, Nedlands, Western Australia 6009, Australia.
- 39** Schools of Pathology and Laboratory Medicine, and Population Health, The University of Western Australia, Nedlands, Western Australia 6009, Australia.
- 40** Department of Human Genetics, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands.
- 41** Center of Medical Systems Biology, Einthovenweg 20, 2333 ZC Leiden, The Netherlands.
- 42** Department of Medicine, Internal Medicine, CHUV, University of Lausanne, Rue du Bugnon 46, 1011 Lausanne, Switzerland.
- 43** University of Queensland Diamantina Institute, University of Queensland, Princess Alexandra Hospital, Brisbane, Queensland 4072, Australia.
- 44** Department of Psychiatry, Washington University, 660S Euclid Avenue, St. Louis, Missouri 63110, USA.
- 45** Laboratory of Neurogenetics, National Institute on Aging, Bethesda, Maryland 20892, USA.
- 46** Geriatric Unit, Azienda Sanitaria Firenze (ASF), Via delle Casine, 16, 50122 Firenze, Italy.
- 47** Durrer Center for Cardiogenetic Research, ICIN-Netherlands Heart Institute, 3511 GC Utrecht, The Netherlands.
- 48** University of Groningen, University Medical Center Groningen, Department of Genetics, 9700 RB Groningen, The Netherlands.
- 49** Member of Netherlands Consortium for Healthy Aging sponsored by Netherlands Genomics Initiative, Postbus 9600, 2300 RC Leiden, The Netherlands.
- 50** Department of Neurology, General Central Hospital, Lorenz-Bohler-Straße, 5, 39100 Bolzano, Italy.
- 51** Vita-Salute University, 20132 Milan, Italy. WA

Conflict of Interest/Disclosure Statement

The authors have no conflict of interest to declare.

References

- [1] Gerlach M, Ben-Shachar D, Riederer P, Youdim MB (1994) Altered brain metabolism of iron as a cause of neurodegenerative diseases? *J Neurochem* **63**, 793-807.
- [2] Padurariu M, Ciobica A, Lefter R, Serban IL, Stefanescu C, Chirita R (2013) The oxidative stress hypothesis in Alzheimer's disease. *Psychiatr Danub* **25**, 401-409.
- [3] Hare D, Ayton S, Bush A, Lei P (2013) A delicate balance: Iron metabolism and diseases of the brain. *Front Aging Neurosci* **5**, 34.
- [4] Antharam V, Collingwood JF, Bullivant JP, Davidson MR, Chandra S, Mikhaylova A, Finnegan ME, Batich C, Forder JR, Dobson J (2012) High field magnetic resonance microscopy of the human hippocampus in Alzheimer's disease: quantitative imaging and correlation with iron. *Neuroimage* **59**, 1249-1260.
- [5] Zhu WZ, Zhong WD, Wang W, Zhan CJ, Wang CY, Qi JP, Wang JZ, Lei T (2009) Quantitative MR phase-corrected imaging to investigate increased brain iron deposition of patients with Alzheimer disease. *Radiology* **253**, 497-504.
- [6] Ding B, Chen KM, Ling HW, Sun F, Li X, Wan T, Chai WM, Zhang H, Zhan Y, Guan YJ (2009) Correlation of iron in the hippocampus with MMSE in patients with Alzheimer's disease. *J Magn Reson Imaging* **29**, 793-798.
- [7] van Bergen JM, Li X, Hua J, Schreiner SJ, Steininger SC, Quevenco FC, Wyss M, Gietl AF, Treyer V, Leh SE, Buck F, Nitsch RM, Pruessmann KP, van Zijl PC, Hock C, Unschuld PG (2016) Colocalization of cerebral iron with Amyloid beta in Mild Cognitive Impairment. *Sci Rep* **6**, 35514.
- [8] Crichton RR, Dexter DT, Ward RJ (2011) Brain iron metabolism and its perturbation in neurological diseases. *J Neural Transm (Vienna)* **118**, 301-314.
- [9] Ayton S, Faux NG, Bush A, Alzheimer's Dis Neuroimaging I (2015) Ferritin levels in the cerebrospinal fluid predict Alzheimer's disease outcomes and are regulated by APOE. *Nat Commun* **6**, 6760.

- [10] Koeppen AH (2003) A brief history of brain iron research. *J Neurol Sci* **207**, 95-97.
- [11] McCarthy RC, Kosman DJ (2015) Mechanisms and regulation of iron trafficking across the capillary endothelial cells of the blood-brain barrier. *Front Mol Neurosci* **8**, 31.
- [12] Pinero DJ, Li NQ, Connor JR, Beard JL (2000) Variations in dietary iron alter brain iron metabolism in developing rats. *J Nutr* **130**, 254-263.
- [13] House MJ, St Pierre TG, Milward EA, Bruce DG, Olynyk JK (2010) Relationship between brain R(2) and liver and serum iron concentrations in elderly men. *Magn Reson Med* **63**, 275-281.
- [14] Faux NG, Rembach A, Wiley J, Ellis KA, Ames D, Fowler CJ, Martins RN, Pertile KK, Rumble RL, Trounson B, Masters CL, Bush AI (2014) An anemia of Alzheimer's disease. *Mol Psychiatry* **19**, 1227-1234.
- [15] Hare DJ, Doecke JD, Faux NG, Rembach A, Volitakis I, Fowler CJ, Grimm R, Doble PA, Cherny RA, Masters CL, Bush AI, Roberts BR (2015) Decreased plasma iron in Alzheimer's disease is due to transferrin desaturation. *ACS Chem Neurosci* **6**, 398-402.
- [16] Tao Y, Wang Y, Rogers JT, Wang F (2014) Perturbed iron distribution in Alzheimer's disease serum, cerebrospinal fluid, and selected brain regions: a systematic review and meta-analysis. *J Alzheimers Dis* **42**, 679-690.
- [17] Wang ZX, Tan L, Wang HF, Ma J, Liu J, Tan MS, Sun JH, Zhu XC, Jiang T, Yu JT (2015) Serum Iron, Zinc, and Copper Levels in Patients with Alzheimer's Disease: A Replication Study and Meta-Analyses. *J Alzheimers Dis* **47**, 565-581.
- [18] Loef M, Walach H (2012) Copper and iron in Alzheimer's disease: a systematic review and its dietary implications. *Br J Nutr* **107**, 7-19.
- [19] Dudbridge F (2016) Polygenic Epidemiology. *Genet Epidemiol*.
- [20] Burgess S, Freitag DF, Khan H, Gorman DN, Thompson SG (2014) Using multivariable Mendelian randomization to disentangle the causal effects of lipid fractions. *PLoS One* **9**, e108891.

- [21] Pickrell JK, Berisa T, Liu JZ, Segurel L, Tung JY, Hinds DA (2016) Detection and interpretation of shared genetic influences on 42 human traits. *Nat Genet* **48**, 709-717.
- [22] Pichler I, Del Greco MF, Gogele M, Lill CM, Bertram L, Do CB, Eriksson N, Foroud T, Myers RH, Nalls M, Keller MF, Benjamin B, Whitfield JB, Pramstaller PP, Hicks AA, Thompson JR, Minelli C (2013) Serum iron levels and the risk of Parkinson disease: a mendelian randomization study. *PLoS Med* **10**, e1001462.
- [23] Galesloot TE, Janss LL, Burgess S, Kiemeny LA, den Heijer M, de Graaf J, Holewijn S, Benjamin B, Whitfield JB, Swinkels DW, Vermeulen SH (2015) Iron and hepcidin as risk factors in atherosclerosis: what do the genes say? *BMC Genet* **16**, 79.
- [24] Wang Y, Xu S, Liu Z, Lai C, Xie Z, Zhao C, Wei Y, Bi JZ (2013) Meta-analysis on the association between the TF gene rs1049296 and AD. *Can J Neurol Sci* **40**, 691-697.
- [25] Ali-Rahmani F, Schengrund CL, Connor JR (2014) HFE gene variants, iron, and lipids: a novel connection in Alzheimer's disease. *Front Pharmacol* **5**, 165.
- [26] Lambert JC, Ibrahim-Verbaas CA, Harold D, Naj AC, Sims R, Bellenguez C, Jun G, Destefano AL, Bis JC, Beecham GW, Grenier-Boley B, Russo G, Thornton-Wells TA, Jones N, Smith AV, Chouraki V, Thomas C, Ikram MA, Zelenika D, Vardarajan BN, Kamatani Y, Lin CF, Gerrish A, Schmidt H, Kunkle B, Dunstan ML, Ruiz A, Bihoreau MT, Choi SH, Reitz C, Pasquier F, Hollingworth P, Ramirez A, Hanon O, Fitzpatrick AL, Buxbaum JD, Campion D, Crane PK, Baldwin C, Becker T, Gudnason V, Cruchaga C, Craig D, Amin N, Berr C, Lopez OL, De Jager PL, Deramecourt V, Johnston JA, Evans D, Lovestone S, Letenneur L, Moron FJ, Rubinsztein DC, Eiriksdottir G, Sleegers K, Goate AM, Fievet N, Huentelman MJ, Gill M, Brown K, Kamboh MI, Keller L, Barberger-Gateau P, McGuinness B, Larson EB, Green R, Myers AJ, Dufouil C, Todd S, Wallon D, Love S, Rogaeva E, Gallacher J, St George-Hyslop P, Clarimon J, Lleó A, Bayer A, Tsuang DW, Yu L, Tsolaki M, Bossu P, Spalletta G, Proitsi P, Collinge J, Sorbi S, Sanchez-Garcia F, Fox NC, Hardy J, Naranjo MC, Bosco P, Clarke R, Brayne C, Galimberti D, Mancuso M, Matthews F, European Alzheimer's Disease I, Genetic, Environmental Risk in Alzheimer's D,

- Alzheimer's Disease Genetic C, Cohorts for H, Aging Research in Genomic E, Moebus S, Mecocci P, Del Zompo M, Maier W, Hampel H, Pilotto A, Bullido M, Panza F, Caffarra P, Nacmias B, Gilbert JR, Mayhaus M, Lannfelt L, Hakonarson H, Pichler S, Carrasquillo MM, Ingelsson M, Beekly D, Alvarez V, Zou F, Valladares O, Younkin SG, Coto E, Hamilton-Nelson KL, Gu W, Razquin C, Pastor P, Mateo I, Owen MJ, Faber KM, Jonsson PV, Combarros O, O'Donovan MC, Cantwell LB, Soininen H, Blacker D, Mead S, Mosley TH, Jr., Bennett DA, Harris TB, Fratiglioni L, Holmes C, de Bruijn RF, Passmore P, Montine TJ, Bettens K, Rotter JJ, Brice A, Morgan K, Foroud TM, Kukull WA, Hannequin D, Powell JF, Nalls MA, Ritchie K, Lunetta KL, Kauwe JS, Boerwinkle E, Riemenschneider M, Boada M, Hiltunen M, Martin ER, Schmidt R, Rujescu D, Wang LS, Dartigues JF, Mayeux R, Tzourio C, Hofman A, Nothen MM, Graff C, Psaty BM, Jones L, Haines JL, Holmans PA, Lathrop M, Pericak-Vance MA, Launer LJ, Farrer LA, van Duijn CM, Van Broeckhoven C, Moskvina V, Seshadri S, Williams J, Schellenberg GD, Amouyel P (2013) Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet* **45**, 1452-1458.
- [27] Percy M, Moalem S, Garcia A, Somerville MJ, Hicks M, Andrews D, Azad A, Schwarz P, Beheshti Zavareh R, Birkan R, Choo C, Chow V, Dhaliwal S, Duda V, Kupferschmidt AL, Lam K, Lightman D, Machalek K, Mar W, Nguyen F, Rytwinski PJ, Svava E, Tran M, Wheeler K, Yeung L, Zanibbi K, Zener R, Ziraldo M, Freedman M (2008) Involvement of ApoE E4 and H63D in sporadic Alzheimer's disease in a folate-supplemented Ontario population. *J Alzheimers Dis* **14**, 69-84.
- [28] Percy M, Somerville MJ, Hicks M, Garcia A, Colelli T, Wright E, Kitaygorodsky J, Jiang A, Ho V, Parpia A, Wong MK (2014) Risk factors for development of dementia in a unique six-year cohort study. I. An exploratory, pilot study of involvement of the E4 allele of apolipoprotein E, mutations of the hemochromatosis-HFE gene, type 2 diabetes, and stroke. *J Alzheimers Dis* **38**, 907-922.

- [29] Alizadeh BZ, Njajou OT, Millan MR, Hofman A, Breteler MM, van Duijn CM (2009) HFE variants, APOE and Alzheimer's disease: findings from the population-based Rotterdam study. *Neurobiol Aging* **30**, 330-332.
- [30] Benyamin B, Esko T, Ried JS, Radhakrishnan A, Vermeulen SH, Traglia M, Gogele M, Anderson D, Broer L, Podmore C, Luan J, Kutalik Z, Sanna S, van der Meer P, Tanaka T, Wang F, Westra HJ, Franke L, Mihailov E, Milani L, Haldin J, Winkelmann J, Meitinger T, Thiery J, Peters A, Waldenberger M, Rendon A, Jolley J, Sambrook J, Kiemeny LA, Sweep FC, Sala CF, Schwienbacher C, Pichler I, Hui J, Demirkan A, Isaacs A, Amin N, Steri M, Waeber G, Verweij N, Powell JE, Nyholt DR, Heath AC, Madden PA, Visscher PM, Wright MJ, Montgomery GW, Martin NG, Hernandez D, Bandinelli S, van der Harst P, Uda M, Vollenweider P, Scott RA, Langenberg C, Wareham NJ, InterAct C, van Duijn C, Beilby J, Pramstaller PP, Hicks AA, Ouwehand WH, Oexle K, Gieger C, Metspalu A, Camaschella C, Toniolo D, Swinkels DW, Whitfield JB (2014) Novel loci affecting iron homeostasis and their effects in individuals at risk for hemochromatosis. *Nat Commun* **5**, 4926.
- [31] Escott-Price V, Sims R, Bannister C, Harold D, Vronskaya M, Majounie E, Badarinarayan N, Morgan K, Passmore P, Holmes C, Powell J, Brayne C, Gill M, Mead S, Goate A, Cruchaga C, Lambert JC, van Duijn C, Maier W, Ramirez A, Holmans P, Jones L, Hardy J, Seshadri S, Schellenberg GD, Amouyel P, Williams J (2015) Common polygenic variation enhances risk prediction for Alzheimer's disease. *Brain* **138**, 3673-3684.
- [32] Lupton MK, Strike L, Hansell NK, Wen W, Mather KA, Armstrong NJ, Thalamuthu A, McMahon KL, de Zubicaray GI, Assareh AA, Simmons A, Proitsi P, Powell JF, Montgomery GW, Hibar DP, Westman E, Tsolaki M, Kloszewska I, Soininen H, Mecocci P, Velas B, Lovestone S, Brodaty H, Ames D, Trollor JN, Martin NG, Thompson PM, Sachdev PS, Wright MJ (2016) The effect of increased genetic risk for Alzheimer's disease on hippocampal and amygdala volume. *Neurobiol Aging* **40**, 68-77.

- [33] Desikan RS, Schork AJ, Wang Y, Thompson WK, Dehghan A, Ridker PM, Chasman DI, McEvoy LK, Holland D, Chen CH, Karow DS, Brewer JB, Hess CP, Williams J, Sims R, O'Donovan MC, Choi SH, Bis JC, Ikram MA, Gudnason V, DeStefano AL, van der Lee SJ, Psaty BM, van Duijn CM, Launer L, Seshadri S, Pericak-Vance MA, Mayeux R, Haines JL, Farrer LA, Hardy J, Ulstein ID, Aarsland D, Fladby T, White LR, Sando SB, Rongve A, Witoelar A, Djurovic S, Hyman BT, Snaedal J, Steinberg S, Stefansson H, Stefansson K, Schellenberg GD, Andreassen OA, Dale AM (2015) Polygenic Overlap Between C-Reactive Protein, Plasma Lipids, and Alzheimer Disease. *Circulation* **131**, 2061-2069.
- [34] Benjamin B, Ferreira MA, Willemsen G, Gordon S, Middelberg RP, McEvoy BP, Hottenga JJ, Henders AK, Campbell MJ, Wallace L, Frazer IH, Heath AC, de Geus EJ, Nyholt DR, Visscher PM, Penninx BW, Boomsma DI, Martin NG, Montgomery GW, Whitfield JB (2009) Common variants in TMPRSS6 are associated with iron status and erythrocyte volume. *Nat Genet* **41**, 1173-1175.
- [35] Painter JN, Anderson CA, Nyholt DR, Macgregor S, Lin J, Lee SH, Lambert A, Zhao ZZ, Roseman F, Guo Q, Gordon SD, Wallace L, Henders AK, Visscher PM, Kraft P, Martin NG, Morris AP, Treloar SA, Kennedy SH, Missmer SA, Montgomery GW, Zondervan KT (2011) Genome-wide association study identifies a locus at 7p15.2 associated with endometriosis. *Nat Genet* **43**, 51-54.
- [36] Purcell SM, Wray NR, Stone JL, Visscher PM, O'Donovan MC, Sullivan PF, Sklar P (2009) Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature* **460**, 748-752.
- [37] Zhou X, Stephens M (2012) Genome-wide efficient mixed-model analysis for association studies. *Nat Genet* **44**, 821-824.
- [38] Nyholt DR (2014) SECA: SNP effect concordance analysis using genome-wide association summary results. *Bioinformatics* **30**, 2086-2088.

- [39] Belaidi AA, Bush AI (2016) Iron neurochemistry in Alzheimer's disease and Parkinson's disease: targets for therapeutics. *J Neurochem* **139 Suppl 1**, 179-197.
- [40] Everett J, Cespedes E, Shelford LR, Exley C, Collingwood JF, Dobson J, van der Laan G, Jenkins CA, Arenholz E, Telling ND (2014) Ferrous iron formation following the co-aggregation of ferric iron and the Alzheimer's disease peptide beta-amyloid (1-42). *J R Soc Interface* **11**, 20140165.
- [41] Barnard ND, Bush AI, Ceccarelli A, Cooper J, de Jager CA, Erickson KI, Fraser G, Kesler S, Levin SM, Lucey B, Morris MC, Squitti R (2014) Dietary and lifestyle guidelines for the prevention of Alzheimer's disease. *Neurobiol Aging* **35 Suppl 2**, S74-78.
- [42] Hare DJ, Arora M, Jenkins NL, Finkelstein DI, Doble PA, Bush AI (2015) Is early-life iron exposure critical in neurodegeneration? *Nat Rev Neurol* **11**, 536-544.
- [43] Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere ML, Pahwa JS, Moskvina V, Dowzell K, Williams A, Jones N, Thomas C, Stretton A, Morgan AR, Lovestone S, Powell J, Proitsi P, Lupton MK, Brayne C, Rubinsztein DC, Gill M, Lawlor B, Lynch A, Morgan K, Brown KS, Passmore PA, Craig D, McGuinness B, Todd S, Holmes C, Mann D, Smith AD, Love S, Kehoe PG, Hardy J, Mead S, Fox N, Rossor M, Collinge J, Maier W, Jessen F, Schurmann B, van den Bussche H, Heuser I, Kornhuber J, Wiltfang J, Dichgans M, Frolich L, Hampel H, Hull M, Rujescu D, Goate AM, Kauwe JS, Cruchaga C, Nowotny P, Morris JC, Mayo K, Sleegers K, Bettens K, Engelborghs S, De Deyn PP, Van Broeckhoven C, Livingston G, Bass NJ, Gurling H, McQuillin A, Gwilliam R, Deloukas P, Al-Chalabi A, Shaw CE, Tsolaki M, Singleton AB, Guerreiro R, Muhleisen TW, Nothen MM, Moebus S, Jockel KH, Klopp N, Wichmann HE, Carrasquillo MM, Pankratz VS, Younkin SG, Holmans PA, O'Donovan M, Owen MJ, Williams J (2009) Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nat Genet* **41**, 1088-1093.
- [44] Lovestone S, Francis P, Kloszewska I, Mecocci P, Simmons A, Soininen H, Spenger C, Tsolaki M, Vellas B, Wahlund LO, Ward M, AddNeuroMed C (2009) AddNeuroMed--the European

collaboration for the discovery of novel biomarkers for Alzheimer's disease. *Ann N Y Acad Sci* **1180**, 36-46.

- [45] Hye A, Riddoch-Contreras J, Baird AL, Ashton NJ, Bazenet C, Leung R, Westman E, Simmons A, Dobson R, Sattlecker M, Lupton M, Lunnon K, Keohane A, Ward M, Pike I, Zucht HD, Pepin D, Zheng W, Tunnicliffe A, Richardson J, Gauthier S, Soininen H, Kłoszewska I, Mecocci P, Tsolaki M, Vellas B, Lovestone S (2014) Plasma proteins predict conversion to dementia from prodromal disease. *Alzheimer's & Dementia* **10**, 799-807.
- [46] Mueller SG, Weiner MW, Thal LJ, Petersen RC, Jack CR, Jagust W, Trojanowski JQ, Toga AW, Beckett L (2005) Ways toward an early diagnosis in Alzheimer's disease: the Alzheimer's Disease Neuroimaging Initiative (ADNI). *Alzheimers Dement* **1**, 55-66.
- [47] (2007) Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* **447**, 661-678.

Cohorts	N AD cases	N Control s	Mean Age (range, SD)	% Female	<i>APOE</i> ϵ 4 Frequency
Genetic and Environmental Risk for Alzheimer's disease (GERAD1) [43]	2361	942	79.0 (60-108, 7.7)	64.6	AD=0.33 (N=2183) CN=0.13 (N=906)
Innovative Medicines in Europe (AddNeuroMed) [44]	223	280	77.5 (60-98, 6.9)	59.8	AD=0.33 (N=217) CN=0.15 (N=143)
Kings Health Partners- Dementia Case Register (KPH-DCR) [45]	64	85	79.5 (61-93, 6.8)	59.7	AD=0.38 (N=52) CN=0.14 (N=65)
Alzheimer's Disease Neuroimaging Initiative (ADNI) [46]	165	205	76.3 (60-91, 6.0)	44.9	AD=0.42 (N=165) CN=0.14 (N=204)
Wellcome Trust Case Control Consortium 1958 British Birth Cohort (WTCCC2) [47]	0	4926	54 (all 54)	49.7	CN=0.16 (N=4862)

Table 1. Alzheimer's disease case-control cohort data sets.

The Alzheimer's disease cohorts which contributed data to the assessment of the effect of iron genetic profile scores to risk of AD. The *APOE* ϵ 4 frequency is shown for the individuals where *APOE* genotype data was available, with the sample size in brackets.

Abbreviations: AD, Alzheimer's disease; CN, controls.

Serum measure	N	Mean	Range	SD
Iron ($\mu\text{mol/L}$)	8751	19.54	0.10-50.50	6.74
Transferrin Saturation (%)	8800	28.71	0.12-95.3	10.80
Transferrin (g/L)	8891	2.82	1.40-5.19	0.44
Ferritin (log10) ($\mu\text{g/L}$)	8892	2.00	0.00-3.26	0.50

Table 2. Serum iron measures in the Australian data set

GPS		Association with AD risk (n=9251)		
		β	SE	P
Iron	p $\leq$ 1	0.04	0.03	0.278
	p $\leq$ 0.5	0.03	0.03	0.365
	p $\leq$ 0.1	0.01	0.03	0.868
	p $\leq$ 0.05	0.02	0.03	0.638
	p $\leq$ 0.01	-0.01	0.03	0.695
	p $\leq$ 0.001	-0.01	0.03	0.839
	p $\leq$ 0.0001	0.02	0.03	0.624
	p $\leq$ 0.000001	0.02	0.33	0.632
Transferrin Saturation	p $\leq$ 1	0.03	0.03	0.291
	p $\leq$ 0.5	0.03	0.03	0.330
	p $\leq$ 0.1	0.03	0.03	0.381
	p $\leq$ 0.05	0.02	0.03	0.584
	p $\leq$ 0.01	0.02	0.03	0.510
	p $\leq$ 0.001	0.02	0.03	0.590
	p $\leq$ 0.0001	0.02	0.03	0.628
	p $\leq$ 0.000001	0.03	0.03	0.408
Transferrin	p $\leq$ 1	0.00	0.03	0.933
	p $\leq$ 0.5	0.00	0.03	0.950
	p $\leq$ 0.1	0.02	0.03	0.589
	p $\leq$ 0.05	0.01	0.03	0.797
	p $\leq$ 0.01	-0.02	0.03	0.517
	p $\leq$ 0.001	-0.03	0.03	0.299
	p $\leq$ 0.0001	-0.03	0.03	0.404
	p $\leq$ 0.000001	-0.02	0.03	0.467
Ferritin	p $\leq$ 1	0.02	0.03	0.577
	p $\leq$ 0.5	0.03	0.04	0.465
	p $\leq$ 0.1	0.03	0.04	0.465
	p $\leq$ 0.05	0.05	0.04	0.196
	p $\leq$ 0.01	0.03	0.03	0.347
	p $\leq$ 0.001	0.03	0.03	0.355
	p $\leq$ 0.0001	0.03	0.03	0.377
	p $\leq$ 0.000001	0.04	0.03	0.170

Table 3. The association of serum iron measure genetic profile score (GPS) at different P value thresholds with AD risk.

The association analysis was carried out using logistic regression controlling for sex, age, four ancestry principal components and study

Abbreviations: β , standardised Beta

Serum Iron Measure	AD GPS	N	β	SE	P
Iron	p $\leq$ 1	8751	0.02	0.01	0.153
	p $\leq$ 0.5	8751	0.02	0.01	0.148
	p $\leq$ 0.1	8751	0.01	0.01	0.349
	p $\leq$ 0.05	8751	0.01	0.01	0.594
	p $\leq$ 0.01	8751	0.00	0.01	0.747
	p $\leq$ 0.001	8751	0.01	0.01	0.405
	p $\leq$ 0.0001	8751	0.01	0.01	0.615
	p $\leq$ 0.000001	8751	0.02	0.01	0.119
	APOE ϵ 4	8494	0.00	0.01	0.843
Transferrin Saturation	p $\leq$ 1	8800	371.45	224.20	0.097
	p $\leq$ 0.5	8800	201.12	136.43	0.140
	p $\leq$ 0.1	8800	46.40	54.11	0.391
	p $\leq$ 0.05	8800	13.37	38.99	0.732
	p $\leq$ 0.01	8800	2.82	18.46	0.878
	p $\leq$ 0.001	8800	0.76	6.58	0.908
	p $\leq$ 0.0001	8800	0.25	2.15	0.908
	p $\leq$ 0.000001	8800	3.19	1.27	0.012
	APOE ϵ 4	8531	0.02	0.02	0.477
Transferrin	p $\leq$ 1	8891	-218.75	225.19	0.331
	p $\leq$ 0.5	8891	-78.29	137.03	0.568
	p $\leq$ 0.1	8891	9.86	54.36	0.856
	p $\leq$ 0.05	8891	23.12	39.16	0.555
	p $\leq$ 0.01	8891	5.87	18.52	0.751
	p $\leq$ 0.001	8891	16.29	6.58	0.013
	p $\leq$ 0.0001	8891	4.97	2.15	0.021
	p $\leq$ 0.000001	8891	-1.77	1.28	0.166
	APOE ϵ 4	8619	-0.02	0.02	0.466
Ferritin	p $\leq$ 1	8892	156.22	192.51	0.417
	p $\leq$ 0.5	8892	81.98	117.14	0.484
	p $\leq$ 0.1	8892	35.61	46.42	0.442
	p $\leq$ 0.05	8892	7.49	33.47	0.822
	p $\leq$ 0.01	8892	11.05	15.85	0.485
	p $\leq$ 0.001	8892	2.53	5.64	0.654
	p $\leq$ 0.0001	8892	-0.64	1.84	0.728
	p $\leq$ 0.000001	8892	0.85	1.09	0.435
	APOE ϵ 4	8621	0.01	0.02	0.486

Table 4. The association of AD GPS at different P value thresholds (excluding APOE) and number of APOE ϵ 4 alleles with iron phenotypes. The association analysis was carried out using linear mixed models implemented in GEMMA (genome-wide efficient mixed-model association) [37] using the likelihood ratio test. Family relationships were controlled for using a genetic relatedness matrix estimated from genotypes. Sex, age and four ancestry principal components were also included as covariates.

Abbreviations: β , standardised Beta

Figure 1. Q-Q plots of the association P-values from the discovery GWAS meta analyses.

Including the GWAS meta analysis of biochemical markers of iron status[30] and the International Genomics of Alzheimer's Project (IGAP)[26]. SNPs in the *APOE* region (within 500kb either side of *APOE* locus) are excluded from the AD plot. The red line is the line of equivalence, observed=expected.

Figure 2. Genetic overlap between dataset 1 (AD) and dataset 2 (Serum iron)

In the SECA analysis exact binomial statistical tests are performed to determine whether there is an excess of SNPs associated in both datasets for 144 SNP subsets from 12x12 P-value threshold combinations. A binomial test 'heatmap' plot is generated to graphically summarize the proportion of SNP subsets with an excess [observed(obs) $\geq$ expected (exp)] or deficit (obs<exp) number of associated SNPs, and empirical P-values (adjusted for testing all 144 subsets) are calculated via permutation.

Supplementary Methods

1. GWAS data and imputation methods.

All AD cohorts were genotyped on the Illumina 610-Quad or Illumina 666W-Quad chip. All GWAS data were imputed to the 1000G phase 1 integrated reference panel (April 2012 National Center for Biotechnology Information [NCBI] build 37). As genotype data was used from multiple sources stringent quality control filters were applied. GWAS data quality control, merging and imputation steps have been described in detail previously [1].

The population based sample set was genotyped on several different genome wide platforms (Illumina Human317K, HumanCNV370v1, HumanCNV370-Quadv3, Human610-Quadv1). Sample QC included omitting ethnic outliers, duplicate samples, and samples with unresolved sex, identity or pedigree issues (if not correctable after investigation). Mendelian error genotypes per marker were removed across families. Exclusion criteria for markers were $MAF < 1\%$, call rate < 0.99 , $P_{HWE} < 10^{-6}$, mean GenCall score < 0.7 . Approximately 281,000 markers are observed in all genotyping projects. Imputation of approximately 12,000,000 SNPs was carried out using the 1000 Genomes reference panel (August 4, 2010 release with European haplotypes) using minimac. After imputation 7,262,077 markers passed QC ($R^2 \geq 0.3$).

In the Australian dataset *APOE* genotype was estimated from imputed rs429358 and rs7412 SNP genotypes, which are not perfectly imputed (R^2 values are 0.68 and 0.63 respectively). We found the concordance between the imputed and genotyped *APOE* $\epsilon 4$ was 93%. This was calculated by comparing genotyped and imputed *APOE* (from the Queensland Twin Imaging (QTIM) cohort, which had available directly genotyped *APOE* and was included in the same imputation dataset) in a sample size of 3879 [2].

2. Genetic Profile Scores

SNPs with $MAF \leq 0.02$, genotyping rate ≤ 0.99 and $HWP < 1 \times 10^{-6}$ in the target sample were excluded. Linkage Disequilibrium (LD) based clumping was carried out on all SNPs in the discovery data, providing the most significantly associated SNP available in the target data set, in each region of LD (using PLINK clumping command with a pairwise r^2 threshold of 0.2 and a physical distance threshold of 300kb). SNPs were checked for flip strands between the discovery and target sample. The total score is calculated by the number of risk alleles weighted by the standardised per-allele effects, beta using PLINK score function. The risk score was calculated for P value thresholds of 1×10^{-6} , 1×10^{-4} , 1×10^{-3} , 0.01, 0.05, 0.1, 0.5 and 1 (all SNPs). The iron GPS were calculated separately in three imputed AD case-control datasets (as described in detailed imputation methods [1]; set 1 consists of GERAD1 and WTCCC2, set 2 of ADNI and part of AddNeuroMed, and set 3 the remaining Addneuromed and KPH-DCR). SNPs within 500kb either side of the *APOE* locus were excluded from the GPS to ensure all *APOE* associated signal was removed. The *APOE* effect is not well represented within a GRS owing to the $\epsilon 4$ allele being a diplotype acting under a co-dominant genetic model, and with a much larger effect size than the other common AD risk variants[3].

- [1] Proitsi P, Lupton MK, Velayudhan L, Newhouse S, Fogh I, Tsolaki M, Daniilidou M, Pritchard M, Kloszewska I, Soininen H, Mecocci P, Vellas B, Williams J, Stewart R, Sham P, Lovestone S, Powell JF (2014) Genetic predisposition to increased blood cholesterol and triglyceride lipid levels and risk of Alzheimer disease: a mendelian randomization analysis. *PLoS Med* **11**, e1001713.
- [2] Lupton MK, Strike L, Hansell NK, Wen W, Mather KA, Armstrong NJ, Thalamuthu A, McMahon KL, de Zubicaray GI, Assareh AA, Simmons A, Proitsi P, Powell JF, Montgomery GW, Hibar DP, Westman E, Tsolaki M, Kloszewska I, Soininen H, Mecocci P, Velas B, Lovestone S, Brodaty H, Ames D, Trollor JN, Martin NG, Thompson PM, Sachdev PS, Wright

MJ (2016) The effect of increased genetic risk for Alzheimer's disease on hippocampal and amygdala volume. *Neurobiol Aging* **40**, 68-77.

- [3] Genin E, Hannequin D, Wallon D, Sleegers K, Hiltunen M, Combarros O, Bullido MJ, Engelborghs S, De Deyn P, Berr C, Pasquier F, Dubois B, Tognoni G, Fievet N, Brouwers N, Bettens K, Arosio B, Coto E, Del Zompo M, Mateo I, Epelbaum J, Frank-Garcia A, Helisalmi S, Porcellini E, Pilotto A, Forti P, Ferri R, Scarpini E, Siciliano G, Solfrizzi V, Sorbi S, Spalletta G, Valdivieso F, Vepsäläinen S, Alvarez V, Bosco P, Mancuso M, Panza F, Nacmias B, Bossu P, Hanon O, Piccardi P, Annoni G, Seripa D, Galimberti D, Licastro F, Soininen H, Dartigues JF, Kamboh MI, Van Broeckhoven C, Lambert JC, Amouyel P, Campion D (2011) APOE and Alzheimer disease: a major gene with semi-dominant inheritance. *Mol Psychiatry* **16**, 903-907.

Supplementary Tables and Figures

AD				
P Value Threshold	Australian			
p≤1	833350			
p≤0.5	483466			
p≤0.1	127186			
p≤0.05	69634			
p≤0.01	17566			
p≤0.001	2108			
p≤0.0001	514			
p≤0.000001	42			
Fe				
P Value Threshold	GERAD1--WTCCC	AddNeuroMed_1	AddNeuroMed2-DCR	ADNI
p≤1	252456	244606	253672	240836
p≤0.5	179022	172554	178822	170344
p≤0.1	53802	51316	53124	50772
p≤0.05	30488	28972	29954	28656
p≤0.01	7990	7576	7854	7504
p≤0.001	1222	1172	1218	1158
p≤0.0001	276	264	284	256
p≤0.000001	92	88	98	82
Sat				
P Value Threshold	GERAD1- WTCCC	AddNeuroMed_1	AddNeuroMed2-DCR	ADNI
p≤1	253590	240732	240918	236990
p≤0.5	179912	170458	170380	168114
p≤0.1	54280	51132	51100	50692
p≤0.05	30986	28980	28996	28742
p≤0.01	8190	7670	7664	7594
p≤0.001	1302	1214	1202	1214
p≤0.0001	352	338	324	330
p≤0.000001	164	158	148	148
Trans				
P Value Threshold	GERAD1- WTCCC	AddNeuroMed_1	AddNeuroMed2-DCR	ADNI
p≤1	254286	242506	242854	238678
p≤0.5	182046	173622	174096	171426
p≤0.1	57478	54324	54370	53534
p≤0.05	33606	31760	31766	31414
p≤0.01	9236	8732	8754	8654
p≤0.001	1620	1534	1536	1530
p≤0.0001	420	400	398	388
p≤0.000001	162	148	158	150
Ferri				
P Value Threshold	GERAD1- WTCCC	AddNeuroMed_1	AddNeuroMed2-DCR	ADNI
p≤1	242692	232648	232986	228938
p≤0.5	173016	165518	165664	163236
p≤0.1	53188	50292	50072	49560
p≤0.05	30394	28840	28736	28380
p≤0.01	8140	7702	7690	7622
p≤0.001	1204	1138	1138	1126
p≤0.0001	212	198	202	198
p≤0.000001	38	40	36	38

Supplementary Table 1. Number of SNPs included in each Genetic profile score for each imputation dataset.

Abbreviations: AD, Alzheimer's disease; Fe, Iron; sat, Transferrin Saturation; Trans, Transferrin; Ferri, Ferritin.

Variant	All (n=9251)			APOE ε4 +ve (n=3676)			APOE ε4 -ve (n=5575)		
	β	SE	P	β	SE	P	β	SE	P
HFE rs1799945	-0.009	0.062	0.885	-	0.095	0.320	0.023	0.090	0.803
HFE	0.098	0.090	0.279	0.105	0.138	0.444	0.081	0.133	0.540
TMPRSS6 rs855791	-0.048	0.046	0.295	-	0.069	0.962	-	0.067	0.164
Three SNP GPS	-0.002	0.032	0.960	-	0.049	0.900	-	0.048	0.691

Supplementary Table 2. The association of iron influencing mutations with AD risk.

Analysis was carried out using logistic regression controlling for sex, age, four ancestry principal components and study. Genotypes were tested under an additive model with the risk allele being that associated with increased iron levels. The genetic profile score (GRS) is generated from the three genotypes. Standardised Betas (β) are shown.

Supplementary figure 1. Meta analysis for the effect of Serum iron measures genetic profile scores at P≤0.5 threshold.

The meta-analysis used effect size estimates and standard errors with a random effects model. ES represents the effect size which is the combined β value. I² is a measure of between study heterogeneity. Results shown for P≤0.5 threshold only, but no significant association or heterogeneity between datasets was observed at any P value threshold. Group 1 is GERAD1 together with WTCCC21958 British Birth Cohort, Group 2 is AddNeuroMed (second batch) with DCR, Group 3 is AddNeuroMed (first batch) and Group 4 is ADNI.

Transferrin Saturation GPS

Iron GPS

Supplementary Figure 2. SNP effect direction between dataset 1 (AD) and dataset 2 (Serum iron)

In the SECA analysis Fisher's exact statistical tests are performed to determine whether there is an excess of SNPs where the effect directions (BETA) are concordant across dataset1 and dataset2 for 144 SNP subsets from 12x12 P-value threshold combinations. A Fisher's test 'heatmap' plot is generated to graphically summarize the proportion of SNP subsets with concordant (Fisher's test odds ratio, $OR_{FT} \geq 1$) and discordant ($OR_{FT} < 1$) SNP effects, and an empirical P-value ($P_{FTsig-permuted}$) is calculated via permutation for the observed number of subsets (n_{FTsig}) with nominally significant concordance ($OR_{FT} \geq 1$ and $P_{FT} \leq 0.05$).