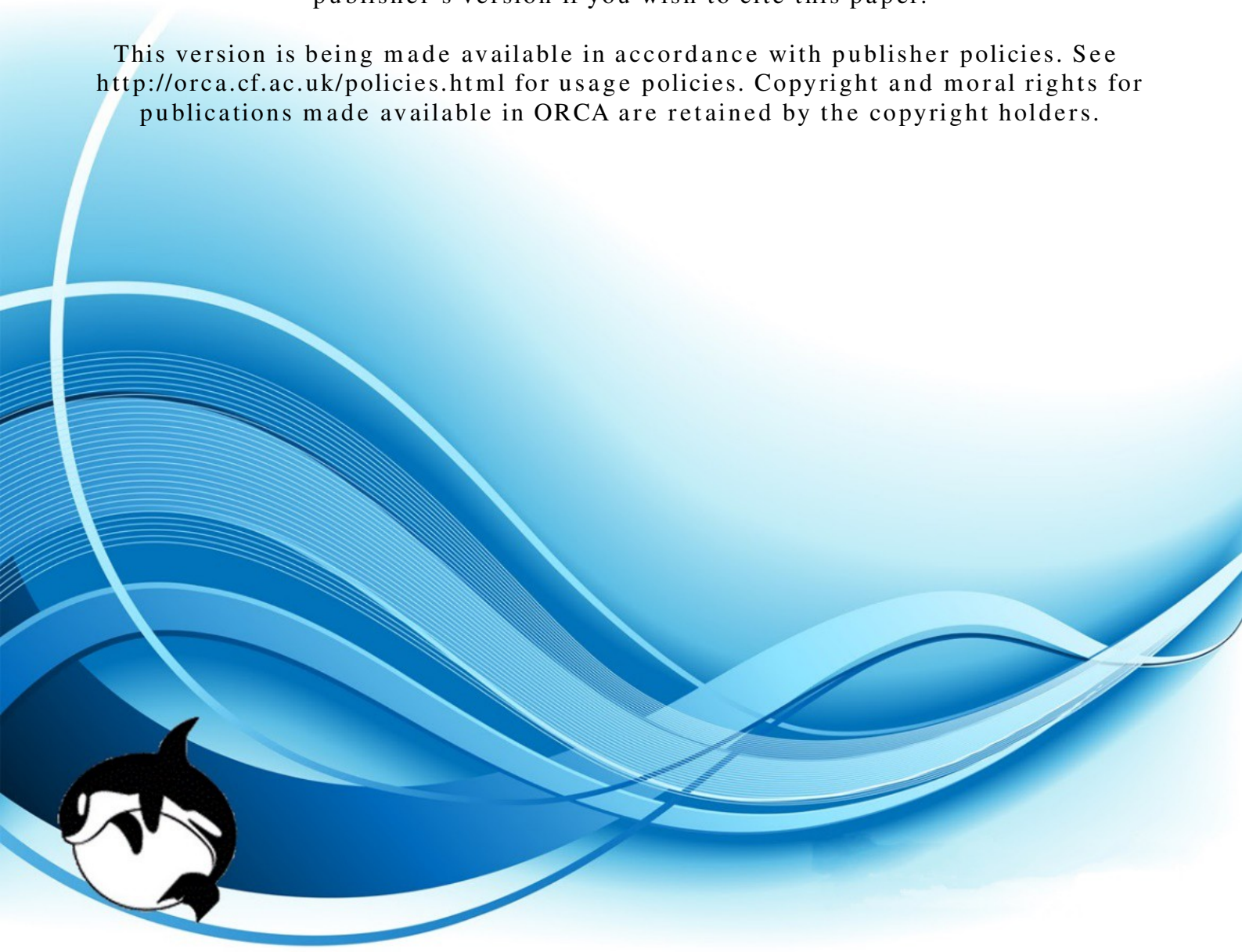

Genes, pathways and risk prediction in Alzheimer's disease

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Alzheimer's disease is the most common neurodegenerative disorder and afflicts close to 1 million people in the UK (out of a population of 66 million) and 3 million in the US (out of a population of 325 million) (https://institute.progress.im/en/node/2688). At present there is no disease modifying therapy and only moderately efficacious symptomatic treatment. Genetic analysis of the autosomal dominant forms of the disease clearly implicated APP processing and Aβ deposition as the initiating factor in the disease process (1) but therapies based on preventing amyloid deposition have so far failed in clinical trials. While the reason for these failures may be varied (2), amyloid deposition begins many years before symptom onset (3) leading to a prevalent view that for therapies aimed at preventing amyloid deposition to be successful they may need to be given much earlier in the disease process to persons who are clinically normal. A corollary of this is that we need to understand other elements of the pathogenic process to identify other targets for therapeutic intervention later in the disease process.

Genetic analysis offers a route both to understanding the pathways involved in disease pathogenesis and to identifying people in the general population who are at high risk of developing the disease. In this review, we discuss the pathways that have been identified as important in determining the risk of late onset disease and also the utility of genetic analysis for predicting those at high risk of developing disease.

Genetic evidence for pathways involved in the pathogenesis of Alzheimer's disease.

APP processing and Aβ production.

All the early onset autosomal dominant Alzheimer cases have mutations in either the amyloid precursor protein (APP) gene or in presenilin 1 or 2 (PSEN1/2). The presenilins are the major component of the γ-secretase complex. The work of Ihara and colleagues and Chavez Gutierrez/De Strooper and colleagues have revealed in great detail how APP and PSEN mutations perturb APP processing and Aβ production (5, 6). All of the pathogenic mutations increase the amounts

of longer forms of Aβ which form the central component of plaques: they do this by gene dosage effects (Downs syndrome and APP gene duplication cases), greater flux through the β-secretase pathway (Swedish mutation), or increasing the proportion of longer Aβ (Aβ42 or Aβ43) made during processing. Conversely, an APP mutation which reduces the flux through the β-secretase pathway reduces the risk of dementia (7). More recently, mutations that reduce the flux through the alternative, α-secretase pathway, have been reported to increase the risk of dementia presumably by a compensatory increase in flux through the β-secretase pathway (8, 9).

One of the few candidate genes which have been shown to have a clear association with Alzheimer's disease is SORL1 which is involved in the endosome/lysosome trafficking of APP. Common variability at this locus was shown to have an association with Alzheimer's disease (10) and later, loss of function mutations were shown to give rise to almost fully penetrant disease (11).

All these data in autosomal dominant disease are therefore consistent with the central importance of Aβ deposition in the initiation of the disease process and this has been the major intellectual foundation of the amyloid hypothesis for the disease. This simple hypothesis, as originally conceived was, however, postulated in an almost exclusively neuronal way…. APP metabolism in a neuron… giving rise to Aβ outside the neuron…. which, somehow, caused a toxic reaction inside a neuron…. and then tangle formation and cell death (12). Although this has been the dominant disease pathogenesis model for more than 20 years, in fact, demonstration of relevant toxicity of Aβ has not been forthcoming and this, together with an appreciation of the long time frame between Aβ deposition and clinical dementia has led to the suggestion that a more complex formulation of the hypothesis was called for which was not, necessarily, neuronal cell autonomous (13). Genetic analysis of sporadic late onset disease was carried out, in part, to shed new light on whatever other pathways may be involved in the disease pathogenesis.

The innate immune system and lipid metabolism

With, first genome wide association studies and later, exome sequencing, genetic determinants of late onset "sporadic" disease, beyond the apoe locus started to be identified (14, 15). With the first GWAS it became clear that the major pathways being identified were in the innate immune system (16). With the identification of TREM2 through exome sequencing (17, 18) the importance of microglia became ever more apparent.

A conceptual breakthrough came with the realization that, not only were there many microglial loci, but also that they formed part of a network of genes regulated by the PU.1 myeloid transcription factor and that the gene for this transcription factor, SPI1 was also a locus for disease (19). Thus, many of the loci were part of a co-regulated network of microglial genes. In agreement with this finding was the observation, that in APP transgenic mice, many of the Alzheimer loci were co-upregulated with TREM2 in response to amyloid deposition (20). This latter analysis showed that genetic variability in microglial response to Aβ was one factor in determining ones risk of developing disease.

These genetic data are consistent with *in vivo* data using non-radioactive labelling to track APP metabolism in both autosomal dominant disease and in late onset sporadic disease. In the former, overproduction of longer Aβ species was observed: in the latter, less Aβ clearance was seen (21, 22).

Integrating the Genetic Findings Concerning Amyloid Production and Amyloid Response

The genetic data summarized above is therefore consistent with the view that amyloid deposition is one initiating event in Alzheimer pathogenesis. However, it is notable that many of the microglial genes involved in pathogenesis are lipid metabolism genes, apoe, a cholesterol and phospholipid carrier (23), ABCA7 a phospholipid transporter (24) and TREM2 a phospholipid sensing receptor (25) as examples. With this in mind, and considering the initiating event in APP processing is intramembranous cleavage, we suggest that the hypothesis that the link between the production and aggregation of Aβ and the microglial reaction is

the disruption of the neuronal membrane which is sensed and removed, at least in part by the activated microglia (26). We would suggest that it is the slow failure of this removal and repair process that underlies the long prodrome of Alzheimer's disease.

The Time Course of Alzheimer's Disease

The evidence that Alzheimer's disease had a long prodromal phase, essentially symptom free has been in the literature for a very long time, especially from the work on Down syndrome from Mann and from Wisniewski and her colleagues (27, 28). However, it was with the development of imaging and CSF biomarkers that the importance of this long prodrome was more generally recognized (3). This realization has partially resolved the longstanding argument about the large number of people who die apparently with intact cognition, but with extensive amyloid pathology (29). This long timeframe is difficult to reconcile with a simple metabolic amyloid cascade and is more consistent with a complex tissue response being important in the determination of events after amyloid deposition. This timeframe also may explain the failure of at least some of the anti-amyloid trials since most of these drugs did not remove amyloid, but rather prevented further deposition (2).

Genetic Prediction and Alzheimer's Disease

While the reasons for the failure of the anti-amyloid clinical trials is likely to have been varied (2, 4), it is likely that one of the important confounds has been the poor diagnostic accuracy in those trials carried out without amyloid imaging or CSF assessment (34, 35). Of course, this diagnostic accuracy is worse early in the disease and in those individuals who were apoe4 negative (35). Although apoe is the strongest predictor of late onset AD, the genetic heritability explained by this locus is not high (h^2=4.8% [95% CI: 3.2-6.3%) (30) as compared to genome-wide estimates (h^2=24-53%)(31-33). The late onset (60+) AD risk prediction accuracy by apoe alone varies with age with a better prediction in younger group, and is about AUC=68% [95% CI: 66%-69%] with e4 + e2 alleles

as predictors (36). However this accuracy is not sufficient for clinical applications or trials (34).

Genome-wide association studies allow researchers to summarise genome-wide genotype data into a variables that measures genetic liability to a disorder or a trait. Two very closely related approaches have been taken called polygenic risk score (PRS) analysis and polygenic hazard score analysis (PHS). We have recently formally compared these statistical approaches using the recent GWAS data and show that they give essentially identical results under most circumstances (37). PRS is calculated from genome-wide association study summary statistics, summing the number of risk alleles carried by an individual, weighted by the effect size from the discovery GWAS. Since GWAS captures a much higher proportion of genetic variability than apoe alone, the prediction accuracy by PRS is higher with an area under the curve (AUC) of between 75% and 84% in clinical and pathology confirmed samples, respectively (36, 30). These AUC estimates are very close the maximal prediction accuracy (introduced by Wray and colleagues (40)) which can be achieved based upon SNP-based heritability captured by the whole genome (39). When apoe is included to the PRS, the majority of the people at high extreme of the PRS distribution contain e4 allele(s), however the predictive accuracy of PRS in pathologically confirmed E3 homozygotes is also to high and equivalent to the predictive accuracy of the whole dataset (38). Figure 1 illustrates the clear differences in the distributions of PRS for the cases split by apoe genotypes, and controls.

While the polygenic scores predict general liability of an individual to develop AD within lifetime, this approach can be taken further to predict particular aspects of the disease such as the age at onset (37, 40). Targeting risk associated with disease specific pathways (16, 42) may additionally provide insight for predicting the endophenotypes of AD. As two examples Ahmad and colleagues (43) have shown that the *endocytosis* pathway is relevant in the prodromal phase of AD in subjects with MCI and that *immune response* and *clathrin/AP2 adaptor complex* pathways may be relevant for brain-related early endophenotypes of AD and we have shown that the risk of amyloid deposition is almost entirely determined by apoe genotype while the risk of Alzheimer's disease is has an

additive dependence on the other risk genes implying that different genetic loci have risk conveying effects at different stages of disease aetiology (44). Thus, in the design of clinical trials and interventions, one can imagine a using polygenic analysis as a rapid and cheap screening tool to select persons for biomarker analysis as part of the clinical trial section process. One could then use this analysis as a tool to select individuals for iPSC generation either based on their overall score or by their pathway specific score.

What is left to be done?

The work summarised above illustrates the considerable progress that has been made in dissecting the genetic architecture of Alzheimer's disease in terms of both the pathways involved in the disease and in the prediction of the disease. In these broad areas, there is progress still to be made, but this is likely to be incremental. There are, however, large gaps in our knowledge. The published data is almost entirely based on simple case control analysis of Caucasian samples. There has been little attempt to see whether there are epistatic interactions between loci or whether different loci contributed to different aspects of the pathology. Nor has there been much attempt to understand the genetics of the rate of decline of disease. Finally, other syndromes, such as Dementia with Lewy Bodies (DLB) and Progressive Supranuclear Palsy (PSP) have related pathologies to Alzheimer's disease but these have not had the resources or sample numbers to make as much progress as has occurred in AD.

Analysis of non-Caucasian Populations

While it is likely that in other populations the same general pathways to pathogenesis will operate, the precise alleles and loci involved in disease will be different. This is certainly true in Parkinson's disease where the haplotype structure differences mean, for example, that the MAPT locus, prominent in Caucasians, is not seen in Asian populations (45 *q.v.* 46). So far there is limited information on Asian populations (47). These early Asian studies resemble the early studies in Caucasians before large consortia were formed to allow large sample numbers to be achieved. Analysis of African American samples has been initiated and has already yielded interesting results with, for example, the

identification of a reasonably common reduced function variant in ABCA7 that contributes to disease risk (48). This latter finding illustrates how analysis of non-Caucasian populations can help in the identification of informative alleles for disease modeling. However, far more analysis of non-Caucasian samples will be required before we can apply effective polygenic risk analysis to the prediction of disease in these populations.

Epistasis

A discussion point in the genetics of complex diseases is whether non-additive genetic interaction effects contribute to risk. The standard approach to GWAS has assumed an additive model, which, in statistical terms, is equivalent to looking for the main effects of variants contributing to disease risk. The assumption of additivity has been effective, but it is also pragmatic, since looking at the effects of many 100,000s of SNPs would be computationally expensive if all combinations of interactions were considered (49). Although computing is getting more powerful, extremely large sample sizes will be required to achieve sufficient power to detect small genetic interaction effect sizes, as would be expected in most complex genetic traits, given the multiple testing constraints. The extent to which genetic interactions contribute to risk in AD is unknown. The usual practice is to analyse for interaction only the most associated SNPs, however some analyses suggested that testing for interactions between SNPs with known main effects (e.g. genome-wide significant SNPs) is unlikely to be the best strategy, as the majority of interactions involved SNPs that did not have a significant main effect on gene expression (50).

We would anticipate that with the current widely accepted statistical methodology (testing for SNPxSNP pairwise interaction with regression modelling), interaction analyses are unlikely to provide informative results and generate reliable biological hypotheses. Instead these studies could be hypothesis driven rather than hypothesis free. The AD field recently produced a number of interesting biologically relevant hypotheses concerning interactions (e.g. 19) which could be further explored and tested for interactions with regression models, accounting for interactions and/or hierarchical models allowing multiple levels of random effects. These studies all require access to the

primary genotype data rather than summary statistics and assembling data sets of cases and controls in such a format as to allow these analyses is a major impediment for these analyses.

Genetic Analyses of Alzheimer-Related markers, Rates of Progression and Atypical Phenotypes

Nearly all of the analyses so far have been case-control design and these, as reviewed above, have been productive. There have been relatively few attempts to assess genetic risk for more complex phenotypes. Such phenotypes could include PET amyloid positivity, rate of disease of progression amongst others. None of these studies, so far has been large enough to completely dissociate these endophenotypes from disease risk, but there are already indications, if not proof, that they are likely to yield different loci from simple case control analyses. The rate of progression in AD has been found to vary widely between individuals, with numerous factors thought to drive this heterogeneity. So far GWAS for cognitive decline has only been reported for relatively small samples (51-53). These studies rule out a large effect of APOE and this suggests different mechanisms underlying disease initiation and progression. Understanding the genetic factors underlying rates of decline is clearly of interest since our primary therapeutic goal is to slow the decline in those with disease and thus the pathways identified by this approach will be of interest in drug development.

Most cases of AD have a hippocampal presentation with initial problems in creating new memories. There are two exceptions: some individuals, especially with presenilin mutations, have initial symptoms including spastic paraparesis (54) and a proportion of AD cases have initial symptoms of visual disturbances and initiating pathology in the occipital, visual cortex (55). Genome wide association studies of this variant of AD, suggests that there may be subtle differences in the genetic risk loci for this syndrome, but larger numbers are required for this to be established (56).

Genetic Analysis of Progressive Supranuclear Palsy (Tangle Disease) and Dementia with Lewy Bodies (Plaques and Lewy bodies).

In AD, the predominant (but not the only) pathologies are amyloid plaques and tau tangles. The relationship between these pathologies has been endlessly debated. The genetic analysis of these related diseases may provide insight into genetic risk factors influencing the formation of tangles (56) and the relationship between plaques and Lewy bodies as a contrast to the relationship between plaques and tangles (57). Genetic analyses of both these diseases has been hampered by the fact that clinical diagnosis is rather poor and relatively few samples have been available through brain banks.

Concluding Remarks

The findings from genetic analysis have been the driving force for our understanding of the pathogenesis of the dementias so far. With the huge increase in the number of loci that genetic analysis has discovered has come a deeper understanding of that pathogenesis. However, so far, this increase in knowledge has not led to better treatments. We have to believe that with a deeper understanding of the pathogenic processes will come effective treatments and more accurate and earlier diagnoses. Over the last 5 years, the emphasis has started to switch from locus identification, to the integration of disparate genetic and phenotypic data. We can expect that this process will continue and our views on how to prevent the disease will move from stopping a linear models of disease to ones in which we see our goal as rebalancing a dysfunctional network.

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References

1. Selkoe,D.J. and Hardy,J. 2016) The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol. Med.* , **8** ,595-608.
2. Karran,E. and Hardy,J. (2014) A critique of the drug discovery and phase 3 clinical programs targeting the amyloid hypothesis for Alzheimer disease. *Ann. Neurol.* , **76** , 185-205.
3. Jack,C.R.jr. and Holtzman,D.M. (2013) Biomarker modeling of Alzheimer's disease. *Neuron.* , **80** , 1347-1358.
4. Hardy,J. and De Strooper,B. (2017) Alzheimer's disease, where next for anti-amyloid therapies? *Brain* , **140** , 853-855.
5. Qi-Takahara,Y., Morishima-Kawashima,M., Tanimura,Y., Dolios,G., Hirotani,N., Horikoshi,Y., Kametani,F., Maeda,M., Saido,T.C., Wang,R. and Ihara,Y. (2005) Longer forms of amyloid beta protein, implications for the mechanism of intramembrane cleavage by gamma-secretase. *J. Neurosci.* , **25** , 436-445.
6. Chávez-Gutiérrez,L., Bammens,L., Benilova,I., Vandersteen,A., Benurwar,M., Borgers,M., Lismont,S., Zhou,L., Van Cleynenbreugel,S., Esselmann,H., Wiltfang,J., Serneels,L., Karran E., Gijsen,H., Schymkowitz,J., Rousseau,F., Broersen K. and De Strooper,B. (2012) The mechanism of γ-Secretase dysfunction in familial Alzheimer disease. *EMBO J.* , **31** , 2261-2274.
7. Jonsson,T., Atwal,J.K., Steinberg,S., Snaedal,J., Jonsson,P.V., Bjornsson,S., Stefansson,H., Sulem,P., Gudbjartsson,D. and Maloney,J., et al. (2012) A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. *Nature* , **488** , 96-99.
8. Kim,M., Suh,J., Romano,D., Truong,M.H., Mullin,K., Hooli,B., Norton,D., Tesco,G., Elliott,K. and Wagner,S.L., et. al. (2009) Potential late-onset Alzheimer's disease-associated mutations in the ADAM10 gene attenuate alpha-secretase activity. *Hum. Mol. Genet.* , **18** , 3987-3996.
9. Hartl,D., May,P., Gu,W., Mayhaus,M., Pichler,S., Spaniol,C., Glaab,E., Bobbili,D.R., Antony,P. and Koegelsberger,S., et al. (2018) A rare loss-of-function variant of ADAM17 is associated with late-onset familial Alzheimer disease. *Mol. Psychiatr.* , 10.1038/s41380-018-0091-8
10. Rogaeva,E., Meng,Y., Lee,J.H., Gu,Y., Kawarai,T., Zou,F., Katayama,T., Baldwin,C.T., Cheng,R., Hasegawa,H. and Chen,F., et al. (2007) The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease. *Nat. Genet.* , **39** , 168-177.
11. Holstege,H., van der Lee,S.J., Hulsman,M., Wong,T.H., van Rooij,J.G., Weiss,M., Louwersheimer,E., Wolters,F.J., Amin,N., Uitterlinden,A.G., Hofman,A., Ikram,M.A., van Swieten,J.C., Meijers-Heijboer,H., van der Flier,W.M., Reinders, M.J., van Duijn C.M. and Scheltens,P., et al. (2017) Characterization of pathogenic SORL1 genetic variants for association with Alzheimer's disease: a clinical interpretation strategy. *Eur. J. Hum. Genet.* , **25** , 973-981.

12. Hardy,J., Duff,K., Hardy,K.G., Perez-Tur,J. and Hutton,M. (1998) Genetic dissection of Alzheimer's disease and related dementias: amyloid and its relationship to tau. *Nat. Neurosci.* , **1** , 355-358.

13. De Strooper,B. and Karran,E. (2016) The Cellular Phase of Alzheimer's Disease. *Cell* , **164** , 603-615.

14. Harold,D., Abraham,R., Hollingworth,P., Sims,R., Gerrish,A., Hamshere, M.L., Pahwa,J.S., Moskvina,V., Dowzell,K. and Williams,A., et al. (2009) Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nat. Genet.* , **41** , 1088-1093.

15. Lambert,J.C., Heath,S., Even,G., Campion,D., Sleegers,K., Hiltunen,M., Combarros,O., Zelenika,D., Bullido,M.J. and Tavernier,B. et al. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. *Nat. Genet.* , **41** , 1094-1099.

16. Jones,L., Holmans,P.A., Hamshere,M.L., Harold,D., Moskvina,V., Ivanov,D., Pocklington,A., Abraham,R., Hollingworth,P. and Sims,R., et al. (2010) Genetic evidence implicates the immune system and cholesterol metabolism in the aetiology of Alzheimer's disease. *PLoS One*, **5** , e13950.

17. Jonsson,T., Stefansson,H., Steinberg,S., Jonsdottir,I., Jonsson,P.V., Snaedal,J., Bjornsson,S., Huttenlocher,J., Levey,A.I. and Lah,J.J., et al. (2013) Variant of TREM2 associated with the risk of Alzheimer's disease. *N. Engl. J. Med.* , **10** , 107-116.

18. Guerreiro,R., Wojtas,A., Bras,J., Carrasquillo,M., Rogaeva,E., Majounie,E., Cruchaga,C., Sassi,C., Kauwe,J.S. and Younkin,S., et al. (2013) TREM2 variants in Alzheimer's disease. *N. Engl. J. Med.* , **368** , 117-127.

19. Huang,K.L., Marcora,E., Pimenova,A.A., Di Narzo,A.F., Kapoor,M., Jin,S.C., Harari,O., Bertelsen,S., Fairfax,B.P. and Czajkowski,J., et al. (2017) A common haplotype lowers PU.1 expression in myeloid cells and delays onset of Alzheimer's disease. *Nat. Neurosci.* , **20** , 1052-1061.

20. Matarin,M., Salih,D.A., Yasvoina,M., Cummings,D.M., Guelfi,S., Liu,W., Nahaboo Solim,M.A., Moens,T.G., Paublete,R.M. and Ali,S.S., et al. (2015) A genome-wide gene-expression analysis and database in transgenic mice during development of amyloid or tau pathology. Cell Rep. , **10** , 633-644.

21. Potter,R., Patterson,B.W., Elbert,D.L., Ovod,V., Kasten,T., Sigurdson,W., Mawuenyega,K., Blazey,T., Goate,A. and Chott, R., et al. (2013) Increased in vivo amyloid-β42 production, exchange, and loss in presenilin mutation carriers. *Sci. Transl. Med.* , **5** , 189ra7.

22. Patterson,B.W., Elbert,D.L., Mawuenyega,K.G., Kasten,T., Ovod,V., Ma,S., Xiong,C., Chott,R., Yarasheski,K. and Sigurdson,W., et al. (2015) Age and amyloid effects on human central nervous system amyloid-beta kinetics. *Ann. Neurol.* , **78** , 439-453.

23. Weisgraber,K.H. and Mahley,R.W. (1996) Human apolipoprotein E: the Alzheimer's disease connection. *FASEB J.* , **10** , 1485-1494.

24. Quazi,F. and Molday,R.S. (2013) Differential phospholipid substrates and directional transport by ATP-binding cassette proteins ABCA1, ABCA7,

and ABCA4 and disease-causing mutants. *J. Biol. Chem.* , **288** , 34414-34426.

25. Wang,Y., Cella,M., Mallinson,K., Ulrich,J.D., Young,K.L., Robinette,M.L., Gilfillan,S., Krishnan,G.M., Sudhakar,S., Zinselmeyer,B.H., Holtzman,D.M., Cirrito,J.R. and Colonna,M. (2015) TREM2 lipid sensing sustains the microglial response in an Alzheimer's disease model. *Cell* , **160** , 1061-1071.
26. Salih,D.A., Bayram,S., Guelfi,M.S.,Reynolds,R.H., Shoai,M., Ryten,M., Brenton, J., Zhang,D., Matarin,M. and Botia,J., et al. (2018) Genetic variability in response to Aβ deposition influences Alzheimer's risk. *BioRxiv* , 437657.
27. Wisniewski,K.E., Wisniewski,H.M. and Wen,G.Y. (1985) Occurrence of neuropathological changes and dementia of Alzheimer's disease in Down's syndrome. *Ann. Neurol.* , **17** , 278–282.
28. Mann,D.M.A., Younis,N., Jones,D. and Stoddart,R.W. (1992) The time course of pathological events concerned with plaque formation in Down's syndrome with particular reference to the involvement of microglial cells. *Neurodegen.* , **1** , 201–215.
29. Dickson,D.W., Crystal,H.A., Mattiace,L.A., Masur,D.M., Blau,A.D., Davies,P., Yen,S.H. and Aronson,M.K. (1992) Identification of normal and pathological aging in prospectively studied nondemented elderly humans. *Neurobiol. Aging.* , **13** , 179-189.
30. Escott-Price,V., Myers,A.J., Huentelman,M. and Hardy,J. (2017) Polygenic risk score analysis of pathologically confirmed Alzheimer's disease. *Ann. Neurol.* , **82** , 311-314.
31. Ridge,P.G., Hoyt,K.B., Boehme,K., Mukherjee,S., Crane,P.K., Haines,J.L., Mayeux,R., Farrer,L.A., Pericak-Vance,M.A., Schellenberg,G.D., Kauwe,J.S. and Alzheimer's Dis. Genet. C. (2016) Assessment of the genetic variance of late-onset Alzheimer's disease. *Neurobiol. Aging.* , **41** , 200 e213-220.
32. Ridge,P.G., Mukherjee,S., Crane,P.K., Kauwe,J.S. and Alzheimer's Disease Genetics, C. (2013) Alzheimer's disease - analyzing the missing heritability. PLoS One , **8** , e79771. DOI,10.1371/journal.pone.0079771.
33. Lee,S.H., Harold,D., Nyholt,D.R., ANZGene Consortium, International Endogene Consortium, Genetic and Environmental Risk for Alzheimer's disease Consortium, Goddard,M.E., Zondervan,K.T., Williams,J. and Montgomery,G.W., et al. (2013) Estimation and partitioning of polygenic variation captured by common SNPs for Alzheimer's disease, multiple sclerosis and endometriosis. *Hum. Mol. Genet.* , **22** , 832-841.
34. Beach,T.G., Monsell,S.E., Phillips,L.E. and Kukull,W. (2012) Accuracy of the Clinical Diagnosis of Alzheimer disease at National Institute on Aging Alzheimer Disease Centers, 2005-2010. *J. Neuropathol. Exp. Neurol.* , **71** , 266-273.

35. Escott-Price,V., Baker,E., Shoai,M., Leonenko,G., Myers,A., Huentelman,M., and Hardy,J. Genetic analysis suggests high misassignment rates in clinical Alzheimer's cases and controls. *Neurobiol. Aging* , (in press).

36. Escott-Price,V., Sims,R., Bannister,C., Harold,D., Vronskaya,M., Majounie,E,. Badarinarayan,N., GERAD/PERADES, IGAP consortia, and Morgan,K., et al. (2015) Common polygenic variation enhances risk prediction for Alzheimer's disease. *Brain* , **138** , 3673-3684.

37. Leonenko G, Sims R, Shoai M, Frizzati A, Bossù P, Spalletta G, Fox NC, Williams J; GERAD consortium, Hardy J, Escott-Price V. Polygenic risk and hazard scores for Alzheimer's disease prediction. Ann Clin Transl Neurol. 2019 Feb 18;6(3):456-465.

38. Escott-Price,V., Myers,A., Huentelman,M., Shoai,M. and Hardy,J. (2018) Polygenic Risk Score Analysis of Alzheimer's Disease in Cases without APOE4 or APOE2 Alleles. *J. Prev. Alzheimers Dis.* , dx.doi.org/10.14283/jpad.2018.46.

39. Wray,N.R., Yang,J., Goddard,M.E. and Visscher,P.M. (2018) The genetic interpretation of area under the ROC curve in genomic profiling. PLoS Genet 2018 **6** , e1000864.

40. Desikan,R.S., Fan,C.C., Wang,Y., Schork,A.J., Cabral,H.J., Cupples,L.A., Thompson,W.K., Besser,L., Kukull,W.A. and Holland,D., et al. (2017) Genetic assessment of age-associated Alzheimer disease risk - Development and validation of a polygenic hazard score. *PLOS Med.* , doi.org/10.1371/journal.pmed.1002258

41. Sims,R., van der Lee,S.J., Naj,A.C., Bellenguez,C., Badarinarayan,N., Jakobsdottir,J., Kunkle,B.W., Boland,A., Raybould,R. and Bis,J.C., et al. (2017) Rare coding variants in PLCG2, ABI3, and TREM2 implicate microglial-mediated innate immunity in Alzheimer's disease. *Nat. Genet.* , **49** , 1373-1384.

42. Ahmad,S., Bannister,C., van der Lee,S.J., Vojinovic,D., Adams,H.H.H., Ramirez,A., Escott-Price,V., Sims,R., Baker,E. and Williams,J., et al. (2018) Disentangling the biological pathways involved in early features of Alzheimer's disease in the Rotterdam Study. *Alzheimer's. Dement.* ,**14** , 848-857.

43. Satake,W., Nakabayashi,Y., Mizuta,I., Hirota,Y., Ito,C., Kubo,M., Kawaguchi,T., Tsunoda,T., Watanabe,M. and Takeda,A., et al. (2009) Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. *Nat. Genet.* , **41** , 1303-1307.

44. Leonenko G, Shoai M, Bellou E, Sims R, Williams J, Hardy J, Escott-Price V; Alzheimer's Disease Neuroimaging Initiative. Genetic risk for Alzheimer's disease is distinct from genetic risk for amyloid deposition. Ann Neurol. 2019 Jun 14. doi: 10.1002/ana.25530

45. Simón-Sánchez,J., Schulte,C., Bras,J.M., Sharma,M., Gibbs,J.R., Berg,D., Paisan-Ruiz,C., Lichtner,P., Scholz,S.W. and Hernandez,D.G., et al. (2009) Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nat. Genet.* , **41** , 1308-1312.

46. Zhou,X., Chen,Y., Mok,K.Y., Zhao,Q., Chen,K., Chen,Y., Hardy,J., Li,Y., Fu,A.K.Y., Guo, Q., Ip,N.Y. and Alzheimer's Disease Neuroimaging Initiative. (2018) Identification of genetic risk factors in the Chinese population implicates a role of immune system in Alzheimer's disease pathogenesis. *Proc. Natl. Acad. Sci. USA.* , **115** , 1697-1706.

47. Reitz,C., Jun,G., Naj,A., Rajbhandary,R., Vardarajan,B.N., Wang,L.S., Valladares,O., Lin,C.F., Larson,E.B. and Graff-Radford,N.R., et al. (2013) Variants in the ATP-binding cassette transporter (ABCA7), apolipoprotein E ϵ4,and the risk of late-onset Alzheimer disease in African Americans. *JAMA.* , **309** , 1483-1492.

48. Cordell,H.J. (2009) Detecting gene-gene interactions that underlie human diseases. Nat. Rev. Genet. , **10** , 392-404.

49. Hemani,G., Shakhbazov,K., Westra,H.J., Esko,T., Henders,A.K., McRae,A.F., Yang,J., Gibson,G., Martin,N.G. and Metspalu,A., et al. (2014) Detection and replication of epistasis influencing transcription in humans. *Nature* , **508** , 249-253.

50. Sherva,R., Tripodis,Y., Bennett,D.A., Chibnik,L.B., Crane,P.K., de Jager,P.L., Farrer,L.A., Saykin,A.J., Shulman,J.M., and Naj,A., et al. (2014) Genome-wide association study of the rate of cognitive decline in Alzheimer's disease. *Alzheimer's Dement.* , **10** , 45-52.

51. Wang,X., Lopez,O.L., Sweet,R.A., Becker,J.T., DeKosky,S.T., Barmada,M.M., Demirci,F.Y. and Kamboh,M.I. (2015) Genetic determinants of disease progression in Alzheimer's disease. *J. Alzheimers Dis.* , **43** , 649-655.

52. Lim,Y.Y., Villemagne,V.L., Laws,S.M., Pietrzak,R.H., Snyder,P.J., Ames,D., Ellis,K.A., Harrington,K., Rembach,A., and Martins,R.N., et al. (2015) APOE and BDNF polymorphisms moderate amyloid β-related cognitive decline in preclinical Alzheimer's disease. *Mol. Psychiatry.* , **20** , 1322-1328.

53. Crook,R., Verkkoniemi,A., Perez-Tur,J., Mehta,N., Baker,M., Houlden,H., Farrer,M., Hutton,M., Lincoln,S. and Hardy, J., et al. (1998) A variant of Alzheimer's disease with spastic paraparesis and unusual plaques due to deletion of exon 9 of presenilin 1. *Nat. Med.* , **4** , 452-455.

54. Schott,J.M. and Crutch,S.J. (2019) Posterior Cortical Atrophy. *Continuum (Minneap. Minn).* , **25** , 52-75.

55. Schott,J.M., Crutch,S.J., Carrasquillo,M.M., Uphill,J., Shakespeare,T.J., Ryan,N.S., Yong,K.X., Lehmann,M., Ertekin-Taner,N. and Graff-Radford,N.R., et al. (2016) Genetic risk factors for the posterior cortical atrophy variant of Alzheimer's disease. *Alzheimer's Dement.* , **12** , 862-871.

56. Höglinger,G.U., Melhem,N.M., Dickson,D.W., Sleiman,P.M., Wang,L.S., Klei,L., Rademakers,R., de Silva,R., Litvan,I. and Riley,D.E., et al. (2011) Identification of common variants influencing risk of the tauopathy progressive supranuclear palsy. *Nat. Genet.* , **43** , 699-705.

57. Guerreiro,R., Ross,O.A., Kun-Rodrigues,C., Hernandez,D.G., Orme,T., Eicher,J.D., Shepherd,C.E., Parkkinen,L., Darwent,L., and Heckman,M.G., et

al. (2018) Investigating the genetic architecture of dementia with Lewy bodies - a two-stage genome-wide association study. *Lancet Neurol.* , **17** , 64-74.

Figure 1. The distribution of standardized polygenic risk scores (PRS) in the pathologically confirmed sample of controls and cases, the latter split by apoe genotypes.