Commentary

The three new pathways leading to Alzheimer's disease

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Genome-wide association studies (GWAS) promise a significant impact on the understanding of late-onset Alzheimer's disease (LOAD) as the genetic components have been estimated to account for 60–80% of the disease. The recent publication of results from large GWAS suggests that LOAD is now one of the best-understood complex disorders. Four recent large LOAD GWAS have resulted in the identification of nine novel loci. These genes are *CLU* – clusterin, *PICALM* – phosphatidylinositol-binding clathrin assembly protein, *CR1* – complement receptor 1, *BIN1* – bridging integrator 1, *ABCA7* – ATP-binding cassette transporter, *MS4A* cluster – membrane-spanning 4-domains subfamily A, *CD2AP* – CD2-associated protein, *CD33* – sialic acid-binding immunoglobulin-like lectin and *EPHA1* – ephrin receptor A1. Collectively, these genes now explain around 50% of LOAD genetics and map on to three new pathways linked to immune system function, cholesterol metabolism and synaptic cell membrane processes. These three new pathways are not strongly linked to the amyloid hypothesis that has driven so much recent thinking and open up avenues for intensive research with regard to the potential for therapeutic intervention.

Keywords: Alzheimer, amyloid, dementia, genetics, GWAS

The search for genetic contributions to late-onset Alzheimer's disease

The advent of genome-wide association studies (GWAS) has undoubtedly increased our understanding of the role played by common variation in complex diseases. This can be no better illustrated than by noting the impact such studies have had on our understanding of late-onset Alzheimer's disease (LOAD). In contrast to early-onset familial Alzheimer's disease (which accounts for approximately 2% of AD cases) finding the genetic components of LOAD (which have been estimated to account for 60–80% of the disease) has proven to be a much more challenging task [1].

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The amyloid hypothesis has been a central driver in AD research for 20 years and proposes that Aβ plays a pivotal role in the neurodegenerative process as a result of an imbalance between neurotoxic Aβ generation and clearance [2]. One of the classical pathological hallmarks of AD, the amyloid plaque, is composed of aggregated Aβ. Mutations in the three genes which cause early-onset familial Alzheimer's disease – amyloid precursor protein (*APP*) and the presenilins (*PSEN1* and *PSEN2*) – overwhelmingly support the role of amyloid metabolism as the common pathway in this Mendelian form of the disorder [3]. *APP* harbours within its sequence the Aβ peptide, which is liberated following cleavage by β- and γ-secretases, and the *PSENs* are components of the γ-secretase complex responsible for proteolytic cleavage of *APP*. The vast majority, if not all, of the familial Alzheimer's disease causing mutations result in altered processing of *APP*. Consequently, these observations

informed the search for genetic factors in LOAD with the belief that the late-onset form of the disease must also have the processing of *APP* at its core.

The discovery of the readily detectable risk for LOAD associated with the ε4 allele of the *APOE* gene [4] (one allele gives an increased risk of AD of 2.5-fold, two alleles approximately 16-fold) together with the observation that *APOE* could be involved with Aβ transport added further support for the amyloid hypothesis. However, since this observation over 15 years ago little progress was made in uncovering further replicable genetic associations despite intensive worldwide effort. With the recent publication of results from large GWAS, the situation has changed dramatically; indeed, to such an extent that it could be argued that LOAD is now one of the best-understood complex disorders.

The new genetic associations with late-onset Alzheimer's disease

Apart from the well-known association with *APOE*, what are the new genetic associations? Four large LOAD GWAS have been published in the last 18 months and have resulted in the identification of nine novel loci associated with the disorder [5–9]. These genes are *CLU* – clusterin, *PICALM* – phosphatidylinositol-binding clathrin assembly protein, *CR1* – complement receptor 1, *BIN1* – bridging integrator 1, *ABCA7* – ATP-binding cassette transporter, *MS4A* cluster – membrane-spanning 4-domains subfamily A, *CD2AP* – CD2-associated protein, *CD33* – sialic acid-binding immunoglobulin-like lectin and *EPHA1* – ephrin receptor A1. Collectively, if the relative risk/population-attributable risk for these new genes is totalled, we find that they explain around 50% of LOAD genetics [10]. On the plus side, this means that we now understand a considerable component of LOAD genetics and have made remarkable progress; on the negative side, it means that 50% is still remaining to be found – the so-called 'missing heritability' that GWAS has failed to identify. Larger GWAS and/or extended meta-analysis may well reveal additional genes but the current belief is that some of the hidden effects are due to rare variation (which GWAS are not designed to detect) or possibly due to epistatic (gene–gene) interactions which again could well remain undetected using conventional GWAS approaches. These are by no means the only explanations; other considerations such as copy number variation, epigenetic variation or non-coding RNA processing may play additional roles.

Pathways leading to Alzheimer's disease that do not link to amyloid

Having found new genes associated with LOAD, what impact are these likely to have? Perhaps, one of the most exciting things to come out of these findings so far is the belief that these genes have identified new pathways involved in the disease process which do not directly link to amyloid [3,8,11]. This raises the possibility that the impact amyloid plays in LOAD might be less than we had previously thought. These pathways highlight additional mechanisms associated with the disease process and are legitimate potential therapeutic targets. If an in-depth look is taken at the genes identified it is possible to see something quite remarkable – all nine new genes map onto three pathways (with considerable overlap shown by some genes). The new pathways implicated in LOAD (Figure 1) together with the genes that track to these pathways are as follows:

- **immune system function** (both innate and adaptive) – *CLU*, *CR1*, *ABCA7*, *MS4A* cluster, *CD33* and *EPHA1*;
- **cholesterol metabolism** – *APOE*, *CLU* and *ABCA7*; and
- **synaptic dysfunction and cell membrane processes** – *PICALM*, *BIN1*, *CD33*, *CD2AP* and *EPHA1*.

Each of these pathways makes sense from a biological perspective with some previous supporting scientific and anecdotal observations. While *APOE*, *CLU* and *ABCA7* do play a role in Aβ metabolism, a mechanism that clearly plays a central role in familial Alzheimer's disease, perhaps as a result of finding these new susceptibility factors it is possible to consider that Aβ may have a lesser role in LOAD pathogenesis. It can now be argued that the evidence for the involvement of amyloid in the late-onset form of the disease (LOAD) is diminishing. However, it is still feasible that toxic Aβ may well have a modulatory effect on these new pathways (Figure 1).

Why have genome-wide association studies given the answers?

So, why did GWAS deliver when the plethora of candidate gene studies attempted previously did not? One of the problems of candidate gene studies is that the gene of study is selected using a priori biochemical and/or genetic data implicating that gene. Studying genes on a one-by-one basis is an expensive and labour-intensive process. Genetic variation throughout the entire human genome, that is, all genes, can be assessed using single-nucleotide
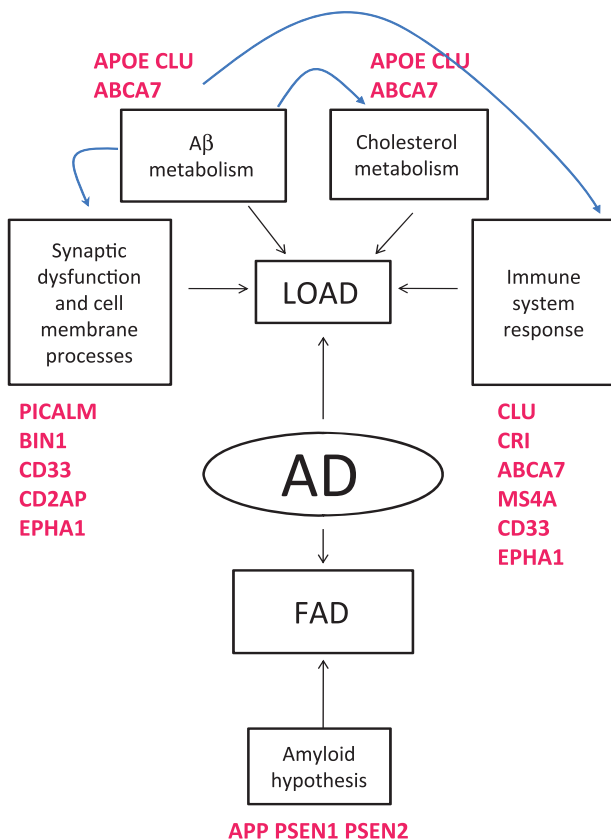
Figure 1. New genes and disease pathways in Alzheimer's disease implicated from recent genome-wide association studies. The genes involved in each pathway are shown in red; it is evident that there is considerable overlap seen with several of the newly identified risk factors being involved in more than a single pathway. Aβ may have a modulatory effect on these new pathways as indicated by the blue arrows. AD, Alzheimer's disease; FAD, familial Alzheimer's disease; LOAD, late-onset Alzheimer's disease.

polymorphisms (SNPs). By exploiting microarray technology, GWAS has permitted hundreds of thousands of SNPs to be interrogated in a single experiment. Determining if there are differences in SNP frequencies between disease and control cohorts permits identification of loci associated with the disease. Multiple testing issues in GWAS are a valid concern because so many SNPs are being assayed at once. For this reason, more stringent criteria are applied and *P*-values of between 5×10^{-7} and 5×10^{-8} are generally accepted as being genome-wide significant [1]. It has been possible to conduct GWAS because the HapMap project provided the genetic community with a catalogue of common genetic variation (SNPs with minor allele frequency ≥5%) distributed throughout the genome. This information then enabled

the generation of commercial 'SNP chips' that could be assayed in a high throughput manner. Finally, the availability of large collections of samples (in the thousands if not tens of thousands) permitted adequately powered studies to be undertaken. Herein lies a fundamental premise of GWAS – these studies are very good at detecting association of common variants with disease but the power to detect an effect comes from the ability to analyse large-sample collections.

Late-onset Alzheimer's disease genetic research has at long last started to 'bear fruit'. These new pathways open up avenues for intensive research with regard to the potential for therapeutic intervention, perhaps to multiple targets in the more elderly population. While AD diagnostics and the development of disease-modifying drugs still lag behind the recent advances made in genetics, the sincere hope of all is that these new findings will prompt renewed vigour for their search and that these endeavours will also make significant advances in the not-too-distant future.

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