REVIEW ARTICLE

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Alzheimer's disease genetics: current knowledge and future challenges

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Alzheimer's disease (AD) is highly heritable, but genetically complex. Recently, three large-scale genome-wide association studies have made substantial breakthroughs in disentangling the genetic architecture of the disease. These studies combined include data from over 43 000 independent individuals and provide compelling evidence that variants in four novel susceptibility genes (*CLU*, *PICALM*, *CR1*, *BIN1*) are associated with disease risk. These findings are tremendously exciting, not only in providing new avenues for exploration, but also highlighting the potential for further gene discovery when larger samples are analysed. Here we discuss progress to date in identifying risk genes for dementia, ways forward and how current findings are refining previous ideas and defining new putative primary disease mechanisms. Copyright © 2010 John Wiley & Sons, Ltd.

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Introduction

It is estimated that there are 35.6 million people worldwide suffering with dementia, a figure that, baring a cure, is set to double every 20 years, reaching 115.4 million by 2050 (Prince and Jackson, 2009). Alzheimer's disease (AD) is the most common form of dementia with heritability estimates as high as 79% (Gatz *et al*., 2006). Much of the initial work on the molecular genetics of AD has come from studies of rare families where early-onset AD is transmitted in an autosomal-dominant fashion, resulting from fully penetrant mutations in three genes: *APP*, *PSEN1* and *PSEN2*. These mutations alter production of the Aβ peptide, the principal component of senile plaques (Tanzi and Bertram, 2005). Early-onset familial AD accounts for less than 1% of all AD cases (Campion *et al*., 1999). Genetic variation in the *APP*, *PSEN1* and *PSEN2* genes has little or no effect on susceptibility to late-onset AD (Bertram *et al*., 2007; Harold *et al*., 2009), suggesting a degree of aetiological heterogeneity.

Genetics of late-onset AD

Until recently the Apolipoprotein gene (*APOE*) was the only unequivocally established susceptibility gene for LOAD (Corder *et al*., 1993; Saunders *et al*., 1993; Schmechel *et al*., 1993; Strittmatter *et al*., 1993). Compared to those with no ε4 alleles, the increased risk for AD is two-to-four fold in those with one ε4 allele and about 12-fold in ε4 homozygotes (Farrer *et al*., 1997; Bertram *et al*., 2007). The neuropathological pathway by which APOE increases the disease risk is not well understood. However, prevailing evidence suggests that the differential effects of ApoE isoforms on Aβ aggregation and clearance play a major role in AD pathogenesis (Kim *et al*., 2009). Others have implicated homeostasis of cholesterol and phospholipids, synaptic plasticity, neuroinflammation, amyloid metabolism, accumulation of neurofibrillary tangles and neuronal survival (Poirier, 2008; Kim *et al*., 2009).

Aside from APOE, over 1200 papers have been published claiming or refuting association between AD and over 500 putative risk genes (Bertram *et al*., 2007). Results are often contradictory. Non-replication of association findings is common for complex diseases and largely reflects the fact that most studies are insufficiently powered to detect the small genetic effects (Colhoun *et al*., 2003). Linkage studies of late-onset AD also suffered a similar fate, where numerous studies have been reported. The strongest support for linkage has been to regions on chromosomes 9, 10 and

12 (Pericak-Vance *et al*., 1997; Hamshere *et al*., 2007; Butler *et al*., 2009); however, the elucidation of specific risk loci in these regions has not been forthcoming. Linkage studies have shown that late-onset AD is unlikely to be explained by rarer variants of large effect ($OR > 5$). The failure to categorically identify further disease loci has perpetuated the commonly held belief that late-onset AD is governed by an array of low penetrance common risk alleles across a number of different loci (Avramopoulos, 2009). The best method currently available to detect common susceptibility variants is genome-wide association (GWA).

Genome wide association studies

GWA studies represent a recent advance from candidate gene studies. Rapid improvements in genotyping technology now permit genotyping and analysis of millions of genetic variants, providing >80% coverage of common variation in the human genome (Barrett and Cardon, 2006; Pe'er *et al*., 2006). Given the number of hypotheses being tested simultaneously a *P*-value below 5.0×10^{-8} is usually required to declare a locus genome-wide significant (Dudbridge and Gusnanto, 2008; Moskvina and Schmidt, 2008).

To date more than 400 GWA studies have been published (Hindorff *et al*., 2010), identifying over 250 common genetic variants that show replicated association with polygenic traits (Altshuler *et al*., 2008; Lettre and Rioux, 2008; Mohlke *et al*., 2008; Hirschhorn and Lettre, 2009; Hindorff *et al*., 2010). This represents one of the most prolific periods of discovery in human genetics (Hirschhorn, 2009). The majority of newly identified risk marker alleles have small relative risks, ranging from 1.1 to 1.5 (Manolio *et al*., 2008) and even in combination explain only a small proportion of heritability to complex traits (Manolio *et al*., 2009). Despite this, findings emerging from GWA studies are providing valuable insights into primary cause of disease and reshaping how we think about complex disease. For example, the analysis of >20 loci for Type 2 diabetes identified through GWAS has highlighted the importance of insulin secretion, rather than insulin resistance, as a primary cause of disease (Zeggini *et al*., 2008).

Genome wide association studies of Alzheimer's disease

Until last year results from 14 GWA studies of AD had been published (see Table 1). The majority have employed samples sizes <2000 subjects. These studies have been reviewed elsewhere (Avramopoulos, 2009; Bertram and Tanzi, 2009). Most notably they have

Table 1 Summary for GWA studies of Alzheimer's disease

Study	Sample size		Sample source	Tested phenotype	Genotyping array
	Cases	Controls			
Harold *et al*., 2009 [a,b]	3941	7848	UK, Germany, US	AD [e,f]	Ilumina 300k, 550k, 610k
Seshadri *et al*., 2010 [b,c]	3006	14 648	US, Iceland	AD [e,f]	Illumina 330k, 370k, 550k Affymetrix 500k
Lambert *et al*., 2009	2032	5328	French	AD [e]	Illumina 610k
Abraham *et al*., 2008 [a]	1082	1239	UK	AD [e]	Illumina 300k, 340k (pooled)
Bertram *et al*., 2008	941	404	US	AD [e,f], Onset Age	Affymetrix 500K
Carrasquillo *et al*., 2009	844	1255	US	AD [e,f]	Illumina 300k
Li *et al*., 2008	753	736	Canada	AD [e]	Affymetrix 500K
Coon *et al*., 2007 [c]	664	422	US, Netherlands	AD [f]	Affymetrix 500K
Beecham *et al*., 2009	492	496	US	AD [e]	Illumina 550K
Reiman *et al*., 2007	446	290	US, Netherlands	AD, APOE ε4 + Carriers	Affymetrix 500K
Grupe *et al*., 2007 [a]	380	396	UK, US	AD [e]	Celera cSNPs
Heinzen *et al*., 2010 [d]	331	368	US	AD [e,f]	Illumina 550k
Potkin *et al*., 2009	172	209	US	AD [e], Hippocampal Atrophy	Illumina 610k
Poduslo *et al*., 2009	9	10	US	AD [e]	Affymetrix 500K

Studies are listed in descending order of case sample size.
[a]These studies use overlapping samples from the MRC Genetic Resource for AD.
[b]Overlaps with Carrasquillo *et al*. (Carrasquillo *et al*., 2009).
[c]Overlaps with Reiman *et al*. (Reiman *et al*., 2007).
[d]Overlaps with Beecham *et al*. (Beecham *et al*., 2009).
[e]Clinically diagnosed Alzheimer's disease.
[f]Neuropathological defined Alzheimer's disease.

identified SNPs within *TNK1* (Grupe *et al*., 2007), *GAB2* (Reiman *et al*., 2007), *GOLM1* (Li *et al*., 2008), *CD33* (Bertram *et al*., 2008), *ATXN1* (Bertram *et al*., 2008), *FAM113B* (Beecham *et al*., 2009), *DISC1* (Beecham *et al*., 2009), *ZNF224* (Beecham *et al*., 2009), *PCDH11X* (Carrasquillo *et al*., 2009) and *TRPC4AP* (Poduslo *et al*., 2009). Although it is still early days, with the exception of the *APOE* locus, replication in independent samples for many of these loci have been inconsistent (for up to date meta-analysis see www.alzgene.org).

It is notable that GWA studies, in contrast to traditional linkage-based approaches, have invariably identified the APOE locus as having a significant association with late-onset AD. The failure to identify any other locus of similar effect has perpetuated the view that LOAD is caused by a large number of low penetrance common alleles, across a range of loci (Bertram and Tanzi, 2009). The effect sizes of these loci are likely to be of the magnitude of 1.05–1.5. Given the stringent criteria for genome-wide significance, samples sizes in the order of thousands are required to detect loci of this effect size (Wang *et al*., 2005). In September 2009, two such studies were reported. More recently, Seshadri and colleagues (2010) have performed a meta-analysis of new and previous GWA studies, incorporating data from four population-based studies.

Large-scale GWA studies of AD

First, Harold *et al*. (Harold *et al*., 2009) undertook a large two-stage genome-wide association study. In stage 1, 3941 AD cases were compared to 7848 controls. The most significant SNPs were genotyped in an independent replication sample of 2023 cases and 2340 controls. Second, Lambert *et al*. performed a GWA study using 2032 AD cases and 5328 controls ascertained in France, replicating in an independent European sample of 3978 AD cases and 3297 controls. Combined these studies include eight times the number of individuals in the largest previous GWA study. Outside of the *APOE* locus, Harold *et al*. reported genome-wide significant evidence for association in Stage 1, with support in an independent extension sample, for two novel susceptibility loci. These were rs11136000 in the *CLU* or *APOJ* gene ($p = 8.5 \times 10^{-10}$, OR = 0.86) and two SNPs 5′ to the *PICALM* gene (rs3851179: $p = 1.3 \times 10^{-9}$, OR = 0.86; rs541458: 8.3×10^{-10}, OR = 0.86). Remarkably, Lambert *et al*. identified association, with an identical effect size, to the same allele of rs11136000 as the top 'non-*APOE*' SNP ($p = 7.5 \times 10^{-9}$, OR = 0.86). They also found support for the *PICALM* locus ($p = 0.03$ and $p = 3 \times 10^{-3}$ for rs3851179 and rs541458, respectively) and genome-wide significant association with rs6656401 in *CR1* in their combined sample ($p = 3.7 \times 10^{-9}$, OR = 1.21). Harold *et al*. detected association with rs3818361 also in the *CR1* gene (Harold *et al*. $p = 9.2 \times 10^{-6}$, OR = 1.17; Lambert *et al*. $p = 8.9 \times 10^{-8}$, OR = 1.19). Taken together these studies provide compelling evidence that *CLU*, *PICALM* and *CR1* are genuine risk genes for AD. It is also interesting that in addition to SNPs meeting stringent criteria for genome wide significance, a significant excess of loci showing 'sub-threshold' association ($p < 1 \times 10^{-5}$) with AD were observed, including variants 5′ to the bridging integrator 1 (*BIN1*) gene. This locus has received further support from a recent genome-wide association study by Seshadri and colleagues (2010), who performed a three-stage analysis of new and previously published GWA study data (Seshadri *et al*., 2010). In stage one, new data from four population-based studies were included in a meta-analysis with publicly available GWA datasets. The most significant SNPs were then meta-analysed with data from Lambert *et al*. (Stage 2) and Harold *et al*. (Stage 3). In stage 1, rs744373 located 5′ to the *BIN1* gene, showed evidence of association with AD ($p = 4.93 \times 10^{-4}$, OR = 1.13); when combined with the Harold *et al*. and Lambert *et al*. data, this SNP surpasses the threshold for genome-wide significance ($p = 1.59 \times 10^{-11}$, OR = 1.15). Notably, Seshadri *et al*. replicated association with the *CLU* and *PICALM* SNPs (stage 1 $p = 4.98 \times 10^{-4}$ and $p = 1.22 \times 10^{-5}$, respectively). They failed to replicate the *CR1* association in their stage 1 data; however, when combined with stage 2 and 3, this SNP still showed very strong evidence for association with AD ($p = 1.04 \times 10^{-11}$). The functional variation contributing to AD susceptibility at each of the newly identified loci is unknown. Further work is required to fine-map each locus, to identify the true risk variants and to characterise their functional nature. A summary of the association results for each of the confirmed AD susceptibility loci can be found in Table 2.

What do GWA studies of Alzheimer's disease tell us about the disease?

AD is interesting in that it is governed by rare autosomal dominant mutations (*APP*, *PSEN1*, *PSEN2*), a common variant with moderate to large effect (*APOE*) and common variants of smaller effect

Table 2 Summary of confirmed susceptibility loci for Alzheimer's disease

Gene	SNP	Minor allele frequency in controls[a]	Harold *et al.* *p*-value	Lambert *et al.* *p*-value	Seshadri *et al.* Stage 1 *p*-value	Seshadri *et al.* replication *p*-value	Meta-analysis *p*-value	Meta-analysis OR (95% CI)	Population attributable risk (OR) [a,b]	Population attributable risk (RR) [a,b]
APOE locus	rs2075650	0.15	1.8×10^{-157}	9.0×10^{-112}	3.2×10^{-68}	N/A	1.0×10^{-295}	2.53 (2.41–2.66)	18.5%	13.6%
CLU	rs11136000	0.40	8.5×10^{-10}	7.5×10^{-9}	5.0×10^{-4}	0.030	8.9×10^{-19}	0.87 (0.84–0.89)	8.2%	3.2%
PICALM	rs3851179	0.37	1.3×10^{-9}	0.037	1.2×10^{-5}	0.007	4.1×10^{-15}	0.87 (0.84–0.90)	8.6%	3.1%
	rs541458	0.32	8.3×10^{-10}	2.8×10^{-3}	N/A	N/A	9.3×10^{-12}	0.87 (0.83–0.90)	9.3%	2.9%
CR1	rs3818361	0.18	9.2×10^{-6}	8.9×10^{-8}	>0.05	N/A	1.0×10^{-11}	1.18 (1.13–1.23)	3.2%	2.5%
	rs6656401	0.19	N/A	3.5×10^{-9}	N/A	N/A	N/A	N/A	3.8%	3.0%
BIN1	rs744373	0.28	3.2×10^{-6}	5.7×10^{-4}	4.9×10^{-4}	0.020	1.4×10^{-12}	1.15 (1.11–1.20)	2.0%	2.8%

[a,b]Allele frequencies used are based on those presented by Harold *et al.* (Harold *et al.*, 2009), except for rs6656401 which was only genotyped in the study by Lambert *et al.* (Lambert *et al.*, 2009); OR (95% CI) = 1.21 (1.14–1.29).

Here we present Population Attributable Risk (PAR; the expected reduction in disease load following removal of a risk factor) calculated using the odds ratio (OR) and also the relative risk (RR). PAR based on RR is more conservative. PAR was calculated according to the following formula: $PAR = Fcon(risk - 1)/[Fcon(risk - 1) + 1]$. Where *risk* is the RR or OR accordingly. RR is the relative risk associated with the risk allele and was calculated as $Fcase/Fcon$, where $Fcon$ is the observed frequency of the risk allele in controls and $Fcase = \text{Meta-analysisOR} \times Fcon/(1 - Fcon + \text{Meta-analysisOR} \times Fcon)$. By estimating $Fcase$ we take account of stratification issues that were controlled for in the estimation of the OR in each of the individual studies.

(*CLU*, *PICALM*, *CR1*, *BIN1*). It is clear that additional loci containing common variation with an effect size similar to *APOE* do not exist. The genes known to be responsible for Mendelian early-onset AD appear to have little or no effect on susceptibility to common late-onset AD. This is surprising, and contradicts what has been observed in other complex traits, where genes with rare variants of large effect often contain common variation of small effect. For example, nearly one fifth of the approximately 90 loci, which show positive association with type 2 diabetes, lipid levels, obesity or height include a gene that is mutated in a corresponding single-gene disorder (Lettre and Rioux, 2008; Mohlke *et al.*, 2008; Hirschhorn and Lettre, 2009).

What is clear about the identified susceptibility genes is that they are not random, as argued by some (Goldstein, 2009), but show patterns of putative functional relationships. For example, APOE and CLU are both brain apolipoproteins, whilst both *PICALM* and *BIN1* are involved in vesicle formation. As most of these new susceptibility genes have been identified in the last year it is too early to be confident of the disease mechanisms they highlight. However, evidence already exists which allows some speculation about potential disease related function effects, including amyloid clearance, lipid transport, endocytosis and intracellular trafficking and inflammatory response/innate immunity.

Possible disease mechanisms

CLU is a versatile protein which has nuclear, cytoplasmic and secreted isoforms (Nuutinen *et al.*, 2009). The functional relevance of the nuclear and cytoplasmic forms has not been clarified (Leskov *et al.*, 2003), but the secreted form has been shown to have chaperone properties (Nuutinen *et al.*, 2009).

CLU is expressed in nearly all mammalian tissues, with high levels in the brain (Jones and Jomary, 2002) and like *APOE*, is one of the major apolipoproteins in the brain. In fact, with respect to involvement in AD, CLU appears to mirror APOE in many ways. In individuals with AD, CLU expression is increased in affected cortical areas of the brain and like APOE, is present in amyloid plaques and in the cerebrospinal fluid (McGeer *et al.*, 1992; Giannakopoulos *et al.*, 1998; Calero *et al.*, 2000; Liang *et al.*, 2008). Furthermore, Aβ is one of the ligands that both CLU and APOE chaperone (Nuutinen *et al.*, 2009). The two apolipoproteins are involved in the clearance of Aβ from the brain by either enhancing endocytosis (Bartl *et al.*, 2001) or through transport across the

blood–brain barrier (Bell *et al*., 2007). Lipids are abundant in the brain and most are generated in the glial cells and transported to neurons (Bjorkhem and Meaney, 2004). As lipids are insoluble they need to be solubilised before they can be transported between cells and this is achieved by coating the lipids with proteins so that the lipids are transported in soluble lipoprotein particles, which bind to cells and internalise their cargo using receptor mediated endocytosis (RME), through a series of lipoprotein receptors. The main brain cholesterol transport lipoprotein is ApoE (Beffert *et al*., 1998). Differences between ApoE isoforms have been established. ApoE ε4 does not operate as efficiently in delivery of cholesterol to neurons as ApoE ε3 (Gong *et al*., 2002) and the different isoforms bind to different populations of lipoproteins (Weisgraber, 1990). ApoE ε4 is a more unstable protein than ApoE ε3 or ApoE ε2 (Morrow *et al*., 2002). ApoE ε3 is better at stimulating neurite outgrowth than apo E ε4 (Holtzman *et al*., 2000). Aβ can be cleared across the blood brain barrier through interactions with lipoprotein receptors and the different ApoE isoforms interact preferentially with different receptors (Deane *et al*., 2008) such that apo ε4 bound lipoprotein particles export Aβ less efficiently than ε2- or ε3-bound particles (Bates *et al*., 2009). Clusterin has also been shown to have a role in the Aβ clearance (DeMattos *et al*., 2004) and its role in lipid trafficking that is parallel to that of ApoE could be pertinent to Aβ clearance (Jenne *et al*., 1991; Calero *et al*., 2000).

PICALM, located on chromosome 11q14.2, encodes phosphatidylinositol-binding clathrin assembly protein, which is ubiquitously expressed in all tissue types with prominent expression in neurons, where it is non-selectively distributed at the pre- and post-synaptic structures (Tebar *et al*., 1999). *BIN1* (AMPH2, amphiphysin), is located on chromosome 2q14.3. *BIN1* expression is not brain specific, but there are several isoforms with enriched expression in brain (Pant *et al*., 2009). Both PICALM and BIN1 function in clathrin mediated endocytosis (CME) (Pant *et al*., 2009). CME internalises ligands bound to the cell surface and releases secretory cargoes from the transGolgi. CME plays an essential role in the intracellular trafficking of large molecules including proteins and lipids (Dreyling *et al*., 1996; Tebar *et al*., 1999; Yao *et al*., 2005). Cell culture experiments have shown that full-length APP is retrieved from the cell surface by CME (Nordstedt *et al*., 1993) and that inhibition of endocytosis reduces APP internalisation and reduces Aβ production and release (Koo and Squazzo, 1994; Carey *et al*., 2005). Mice that have a functional knockout of the PICALM gene show dysfunctional haematopoiesis and abnormal iron metabolism, though they have no overt neurological phenotype (Klebig *et al*., 2003).

PICALM also plays a role in directing trafficking of VAMP2, a protein receptor (SNARE) protein that has a prominent role in the fusion of synaptic vesicles to the presynaptic membrane in neurotransmitter release. This process is crucial to neuronal function and memory formation (Harel *et al*., 2008). Brains affected with AD show a reduced number of synapses, and stereological and biochemical analysis has shown that this reduction in synaptic density correlates better with cognitive decline than with the accumulation of plaques (Masliah *et al*., 2001). There is also evidence that synapses within the brains of those with AD may be dysfunctional even before they visibly degenerate (Fitzjohn *et al*., 2001). It is therefore possible that genetically directed changes in *PICALM* function result in perturbations at the synapse, possibly through synaptic vesicle cycling, thereby contributing to neurodegeneration.

Finally, both CLU and CR1 play significant roles in inflammation and in innate and adaptive immunity. The idea that inflammation is associated with AD is not new (Zotova *et al*., 2010). What is new is the possibility, implied by the genetic data, that inflammatory processes play a primary role in disease development. Markers of inflammation have been associated with amyloid plaques in AD (McGeer and McGeer, 2001) and inflammatory processes proposed as pathogenic contributors (Bates *et al*., 2009). There is also evidence that those at genetic risk show greater expression of an innate pro-inflammatory cytokine profile in middle age (van Exel *et al*., 2009). Epidemiological studies have shown that long-term use of anti-inflammatory drugs reduces the risk for AD and Parkinson's disease (McGeer *et al*., 1996; Chen *et al*., 2005; Vlad *et al*., 2008). CLU is an important inhibitor of complement activation, modulating the membrane attack complex (Kirszbaum *et al*., 1992), and it has been suggested that it acts to prevent the inflammatory response associated with complement activation downstream of protein aggregation (e.g., Aβ accumulation). One major difference between apoE and CLU is that the latter is highly expressed in response to stress (Michel *et al*., 1997). Elevated plasma CLU levels have been observed in other forms of neurodegeneration (Dalrymple *et al*., 2007) and in AD (Thambisetty *et al*., 2009). Interestingly, *Bin1* knockout mosaic mice show reduced inflammation with ageing (Chang *et al*., 2007). CR1 is predominantly involved in adaptive immunity and is abundantly expressed on red blood cells, especially on intravascular

erythrocytes and has been detected on neurons, both observed in AD brains (Zanjani *et al.*, 2005). CR1 is integral to the plasma membrane. The protein mediates cellular binding to particles and immune complexes that have activated complement. CR1 can act as a negative regulator of the complement cascade, mediate immune adherence and phagocytosis and inhibit both the classical and alternative complement pathways (Morgan and Harris, 1999).

One intriguing possibility is that changes to the complement system could trigger synaptic pruning. It is known that components of the complement cascade, including C1q and C3, tag unwanted synapses for elimination during neurodevelopment (Stevens *et al.*, 2007). It is possible that changes to the complement system caused by AD risk variants could re-ignite programmed synaptic loss, which we know is an early disease change that correlates well with cognitive dysfunction (Masliah *et al.*, 2001). Conversely, we know that cholesterol promotes synapse formation (Barres and Smith, 2001), so interference with cholesterol processing through AD risk gene activity could also impinge on synaptic health. These findings therefore suggest the novel hypothesis that AD is principally a disease of synaptic disintegration.

Clinical implications

At present the identified genetic risk factors for AD have little clinical utility in predicting AD risk. Common variants identified by GWA studies almost universally have modest predictive power (Aulchenko *et al.*, 2009; De Jager *et al.*, 2009). Even with APOE, which has a relatively large effect, the predictive utility is questionable as most carriers will not become affected and around a half of AD patients do not carry the allele. Simulation studies suggest that 100 loci with allele frequencies similar to those of *CLU* would be required to reach discriminative accuracy of ~70% (van der Net *et al.*, 2009), whereas methods which employ polygenic methods (e.g. selecting all SNPs below a reduced p threshold) lack accuracy (Evans *et al.*, 2009). It therefore seems that accurate disease prediction will not be possible without the elucidation of all genetic risk loci, along with a comprehensive knowledge of gene–gene and gene–environment interactions.

Next generation strategies, including exome and whole genome sequencing, will be required to fully disentangle the complex genetic architecture of AD. Using these approaches it should be possible to identify a large proportion of the genetic variance of AD. The current genetic data are providing new avenues for exploration, but also highlight the potential for gene discovery when larger samples are analysed. We must not overlook the important fact that genes and their associated pathways are primary events in disease development and our ability to prevent disease for the next generation will depend on knowing what the true causes are. It seems likely that the majority of AD sufferers have an accumulation of risk that crosses a threshold triggering disease. Most people possess some of the risk factors, be they genetic or not, for common traits and diseases, but it is only when the accumulated effects of these cross such a threshold that disease occurs. Consequently, we may only need to remove the effects of some risk factors to significantly reduce the amount of disease in the population. The identification of further risk loci will deliver an array of new drug targets that could lead to better treatment or prevention. It is also important to understand that although AD is likely caused by multiple genetic and environmental factors, it is unlikely that all these risk factors need to be controlled or eliminated to have a significant impact on disease prevalence or treatment. However, we should bear in mind that new biological insights do not guarantee a rapid translation into clinical practice; the latter will require great effort by basic, translational, and clinical researchers.

Ways forward

Phenotypic refinement

Improved phenotyping by expanding to subtler, more precise phenotypes offers another avenue for exploration. The first generation of GWA studies have indicated that traditional psychiatric diagnostic phenotypes might not provide the most powerful means of mapping disease loci (Sabb *et al.*, 2009). It is becoming increasingly apparent that within diagnostic categories, such as bipolar disorder and schizophrenia, extensive aetiological and genetic heterogeneity operates (O'Donovan *et al.*, 2009). By incorporating clinically and neuropathologically derived phenotypic information in GWA studies we may detect association with variants contributing different effects to sub groups of individuals, which would otherwise be overlooked by considering all cases as a homogenous group. The utility of this approach has been demonstrated in studies of other psychiatric phenotypes (Hamshere *et al.*, 2009; Papolos *et al.*, 2009; Van Deerlin *et al.*). So far GWA studies of AD have largely focused on disease risk. The most notable exception being the Alzheimer's

Disease Neuroimaging Initiative (ADNI) GWA study (Potkin *et al*., 2009), which supplemented a traditional case–control GWA study with analysis of hippocampal grey matter density as a quantitative trait.

Perhaps the most obvious candidate for sub-phenotypic investigation in AD is age at disease onset. The ability to predict, modify and manage variation in age at onset would have a huge impact on society and health policy. For example, therapies that delay the onset of AD symptoms, even if only briefly would have a major impact on public health. Delaying the onset of AD by just 2 years would result in 2 million fewer cases in the US over the next 50 years, whilst a delay of 5 years would reduce prevalence by half (Brookmeyer *et al*., 1998). Segregation analyses provide evidence that a number of other loci, in addition to APOE, influence age at onset (Daw *et al*., 1999). It is therefore important to investigate age at onset as a quantitative trait.

Psychotic symptoms in AD have also been proposed as a marker for a discrete form of the disease suitable for gene mapping efforts (Demichele-Sweet and Sweet, 2009). Psychotic symptoms are more common in AD than in the general population (Savva *et al*., 2009), affecting around 40% of patients (Ropacki and Jeste, 2005). They are of serious clinical concern and are associated with decreased quality of life for caregivers and patients (Shin *et al*., 2005), more rapid decline (Lopez *et al*., 1999; Wilkosz *et al*., 2009) and premature institutionalisation (Shin *et al*., 2005). Family studies indicate that AD with psychotic symptoms (AD + P) is heritable (Sweet *et al*., 2002; Bacanu *et al*., 2005; Hollingworth *et al*., 2007) and candidate genes for other psychiatric symptoms have shown evidence for association with their presence (Holmes *et al*., 1998; Sweet *et al*., 1998; Craig *et al*., 2004; Go *et al*., 2005; Sweet *et al*., 2005). Risk variants may either act as disease modifiers, influencing susceptibility to psychotic symptoms in the presence of AD resulting from other environmental or genetic factors. Such variants may also alter the course of other neurodegenerative illnesses to yield psychosis. Second, one or more susceptibility genes may exist for a biologically distinct phenotype, characterised by the presence of psychotic symptoms. Ours and other groups are beginning to investigate these questions by combining clinical data with available GWA data.

GWAS: bigger is better

GWA studies have surpassed early expectations, however in most complex traits the identified loci only explain a small proportion of heritability (Manolio *et al*., 2009). For example, over 40 loci have been identified which influence human height, however, in total they only explain 5% of the phenotypic variance despite robust heritability estimates of around 80%. In AD we estimate that *APOE*, *CLU*, *BIN1*, *PICALM* and *CR1* combined only account for 19% of disease risk, suggesting that further disease loci remain to be identified. Indeed, in our recent GWA study paper we observed significantly more sub-threshold hits ($p < 1 \times 10^{-5}$) than would be expected by chance (Harold *et al*., 2009). It is therefore essential that we continue to identify these loci and to seek functionally relevant patterns of association. Even our recent study had limited power to detect loci with effect sizes similar to those of CLU, PICALM, CR1 and BIN1 (Harold *et al*., 2009). Studies in several phenotypes have clearly demonstrated that the number of detected variants increases with increasing samples sizes (Barrett *et al*., 2008; Zeggini *et al*., 2008; Ahmed *et al*., 2009; Kathiresan *et al*., 2009; Kraft and Hunter 2009). Meta-analysis of data from over 100 000 subjects, utilising genotyped samples from across the world, is a feasible within the next year and must now be a priority.

Conclusions

Eight genes are now known to contribute to the development of AD, three through the activity of highly penetrant rare variants (*APP, PSEN1, PSEN2*) and five through the activity of common risk variants of moderate to small effect (*APOE*, *CLU*, *PICALM*, *CR1* and *BIN1*). Further research using more powerful GWA and whole genome sequencing approaches is likely to define more of the genetic architecture of AD.

Key Points

- Alzheimer's disease (AD) is highly heritable. For many years *APP*, *PSEN1*, *PSEN2* and *APOE* have been the only unequivocally established susceptibility genes for AD. Recent large-scale genome-wide association studies have identified a further four risk loci (*CLU*, *PICALM*, *CR1* and *BIN1*). These findings have refined previous ideas and defined new putative disease mechanisms, providing new impetus for focused studies aimed at understanding AD pathogenesis. Further research using more powerful samples and methods will undoubtedly define more of the genetic architecture of AD.

Current findings have already refined previous ideas and defined new putative disease mechanisms including, amyloid clearance from the brain, lipid processing, endocytosis/trafficking and innate/adaptive immunity.

Conflicts of interest

None declared.

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