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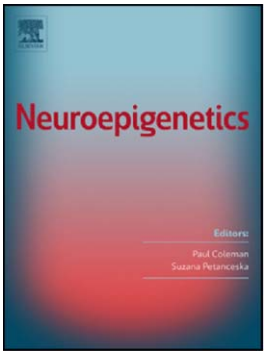

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Elucidating novel dysfunctional pathways in Alzheimer's disease by integrating loci identified in genetic and epigenetic studies

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ABSTRACT

Alzheimer's disease is a complex neurodegenerative disorder that affected 5.2 million people in America in 2014[1]. A large number of genome-wide association studies (GWAS) have been performed, which have been supplemented more recently by the first epigenome-wide association studies (EWAS), leading to the identification of a number of novel loci altered in disease. Twin studies have shown monozygotic twin discordance for Alzheimer's disease[2], leading to the conclusion that a combination of genetic and epigenetic mechanisms are likely to be involved in disease etiology[3]. This review focuses on identifying overlapping pathways between published GWAS and EWAS studies, highlighting dysfunctional synaptic, lipid metabolism, plasma membrane/cytoskeleton, mitochondrial and immune cell activation pathways. Identifying common pathways altered in genetic and epigenetic studies will aid our understanding of disease mechanisms and identify potential novel targets for pharmacological intervention.

KEYWORDS

Alzheimer's disease; AD; DNA methylation; GWAS; EWAS; exome sequencing

INTRODUCTION

Dementia encompasses a group of chronic neurodegenerative diseases that affected an estimated 44.4 million people worldwide in 2013. Due to an increasingly aging population, it is predicted that this figure will rise to an estimated 75.6 million by 2030, and 135.5 million by 2050[4]. The worldwide cost of dementia in predicted to be in excess of $1,000 billion by 2030[5]. Alzheimer's disease (AD) is the most common form of dementia accounting for ~60-80% cases worldwide[6]. AD is characterized by the accumulation of extracellular amyloid-β (Aβ) plaques, intracellular neurofibrillary tangles of hyperphosporylated tau, and widespread gliosis in the brain[7]. Despite the progress that has been made in understanding the cellular pathology of AD, available treatments only temporarily alleviate some symptoms and do not modify the underlying disease process. By the time an individual becomes symptomatic there is already considerable neuronal cell loss, plaque deposition and tangle burden within the brain, which can appear up to ten years before a clinical diagnosis is made[8]. Reflecting the growing public health and socioeconomic burden of AD there has been a year on year increase in the number of publications investigating the etiology of the disease (**Figure 1**) as researchers seek novel disease-modifying treatments.

Although the neuropathology associated with AD has been well-described, little is known about the mechanisms underlying disease onset and progression. Quantitative genetic analyses demonstrated high heritability estimates (58%-79%) for AD[9], and thus initial approaches to understanding etiology focused on uncovering a genetic contribution to disease susceptibility. In recent years, the recruitment of large cohorts and the relatively inexpensive cost of assessing genetic variation through genome-wide association studies (GWAS) have allowed the identification of multiple variants associated with an elevated risk of developing AD. Many of these genes have also been robustly associated with AD via subsequent meta-analyses[10-13] and, most recently, polygenic risk scores for AD have been developed[14]. Collectively, common SNPs are believed to only account for 33% of attributable risk[15] and the mechanism behind their action remains largely unknown. Exome-sequencing projects have also identified other variants e.g. *TREM2*[16], which have a larger effect size, yet these are relatively rare. In recent years researchers have used epigenome-wide association studies (EWAS) to identify epigenetic changes in disease with the aim to elucidate additional mechanisms of pathology, which may provide a link to environmental factors.

Epigenetic processes mediate the reversible regulation of gene expression, occurring independently of DNA sequence variation, acting principally through chemical modifications to DNA and nucleosomal histone proteins. Dynamic changes to the epigenome orchestrate a diverse range of important neurobiological and cognitive processes in the brain[3]. DNA methylation is the best characterized and most stable epigenetic modification which modulates the transcription of mammalian genomes. This is due to its ability to be interrogated using archived genomic DNA resources, which are the focus of most human epidemiological epigenetic research to date[3]. The methylation of a cytosine in a CpG dinucleotide by DNA methyltransferase (DNMT) enzymes, forms 5-

methylcytosine (5-mC), which can disrupt the cell's transcriptional machinery by blocking the binding of transcription factors and attracting methyl-binding proteins that initiate chromatin compaction and bring about gene silencing[17]. The predominant focus to date is methylation within CpG Islands (CGIs) located within the 5' promoters of many constitutively expressed housekeeping control genes. However recent data suggests that the relationship between DNA methylation and transcription may be more complex, with gene body methylation and non-CpG methylation often being associated with active gene expression[18-21] and alternative splicing[22, 23]. The mechanisms involved in cytosine demethylation have also been studied; its demethylation by Ten-eleven translocation (TET) enzymes leads to a stepwise change in the cytosine side chain state, from methylated cytosine to hydroxymethylated cytosine (5-hmC), to formyl cytosine (5-fC), to carboxyl cytosine (5-caC) and finally back to unmodified cytosine by a yet unclassified enzyme/mechanism[24]. Each of these intermediates may have their own effect on gene transcription, splicing and subsequent protein function, and recent studies have shown 5-hmC to be at high levels in the brain[25, 26], with variation across different anatomical regions[27]. Recent advances in genomic technology have allowed the first genome-scale studies assessing methylomic variation (EWAS) in AD. These studies have identified AD-associated DNA methylomic variation at numerous loci in the cortex, with consistent findings across multiple independent study cohorts, in addition to brain-region specific changes and blood DNA methylation signatures[28, 29]. In addition, a recent paper by Yu *et al.* combined genetic and epigenetic findings by examining DNA methylation patterns across genes that have previously been nominated by GWAS, identifying several overlapping loci[30].

Although GWAS and EWAS analyses have identified multiple genes associated with AD, the extent to which common pathways are shared in the findings across studies has not yet been explored. This review aims to integrate the most robust findings from GWAS, exome sequencing studies and EWAS performed to date in AD to highlight common molecular pathways, which could ultimately aid in the identification of novel pharmacological targets for the disease.

METHODS

Using the publically available online search – GWAS catalogue https://www.ebi.ac.uk/gwas/search?query=Alzheimer%27s%20disease#association and a P value cut off of $P< 5x10^{-8}$, we identified 45 unique GWAS in AD totalling 144 SNPs. We then removed studies based on poor sample size (<1000 total samples) as well as removing those studies that included samples that were non-European in origin. Following the study selection, SNPs in intronic regions were removed from the analysis. After filtering for associated disease outcome measures, including the terms: "Dementia and core Alzheimer's disease neuropathological changes", "Alzheimer's disease late onset", "Alzheimer's disease", "Psychosis and Alzheimer's disease", "Alzheimer's disease age of onset", "Alzheimer's disease biomarkers" and "Neurofibrillary tangles" we were left with 29 studies with 49 SNPs in 32 unique genes **(Table 1A)[10-13, 31-48]**. Four genes were identified from exome

sequencing studies[16, 49-51] by performing a literature search in PubMed using the phrases "Alzheimer's disease" and "Exome sequencing" alone and in combination **(Table 1B)**. Genes from EWAS were compiled from the 2014 publications by Lunnon *et al.* and De Jager *et al.* including probes with $P<1x10^{-7}$[28, 29]. The 2012 publication by Bakulsk *et al.* was excluded from the analysis based on sample size[52]. Gene names were checked against quoted genomic location using the UCSC genome browser, only genes containing a probe of interest were included. The resulting gene list contained 48 unique genes that met the criteria for inclusion for our study **(Table 2)**. Gene annotation for all genes of interest were taken from the Gene Ontology (GO) Consortium database, where available, and supplemented with information from the Entrez gene database. Two genes overlapped between GWAS and exome sequencing studies (*TREM2*, *SORL1*) and one gene overlapped between GWAS and EWAS (*BIN1*), bringing the total number of genes across all analyses to 81.

PATHWAYS

The 81 genes identified were compared in terms of their molecular/cellular function and grouped by pathways in which the identified genes operate. By taking significant loci across multiple study designs, we identified five common pathways altered at the genetic and/or epigenetic level in AD; plasma membrane and cytoskeletal processes, lipid homeostasis, synaptic signalling, immune cell processes and mitochondrial processes (**Figure 2**). The largest number of genes fell into the functional group plasma membrane and cytoskeletal processes (n=14), however this could be due to the fact that this is a proportionally larger pathway and is therefore more likely to contain an associated gene by chance. Of the pathways we have identified many of them have considerable overlap, for example lipid processes are intrinsically linked to the plasma membrane which is composed of phospholipids and a large percentage of cholesterol. To better understand the overlap between GWAS and EWAS nominated genes we looked at the cellular localization of genes from each type of study (**Figure 3**). The two largest localization groups (cellular membrane and nucleus) were consistent between methodologies. This would, to some degree, be expected as the majority of total proteins are involved in these locations and, in addition, current protein research has focused on these areas of the cell.

To provide a more structured approach to pathway analysis, all 81 genes were entered into the PANTHER pathway analysis using the enrichment analysis from gene ontology consortium[53]. Fourteen biological process and four cellular component pathways were identified after passing Bonferroni correction. Most pathways reflected an interaction with Aβ or other AD pathology **(Figure 4)**. As the data for these genes was most likely collected from AD publications the resulting pathways are not unexpected, but are most likely to be limited.

<u>Plasma Membrane / Cytoskeleton</u>

This is the pathway which contained the largest number of associated genes from our analysis (n=14). The plasma membrane insulates the intracellular components from the extracellular environment, as well as catalyzing the transport of specific compounds, including nutrients and ions. Phospholipids that make up the membrane provide suitable fluidity and permeability. Alterations in the receptor function, membrane integrity, and membrane-dependent processes seen in AD have been reviewed by A. Farooqui *et al*[54]. The cytoskeleton provides contractility and couples biochemical responses with mechanical stresses in cells. It is vital in the movement of cellular machinery around the cell and to the membrane, as well as orchestrating the procedures needed for cellular movement and re-shaping, a function specifically important to the microglial cells of the brain in the response to inflammation[55]. For an overview of cell mechanics and the cytoskeleton see the review by Fletcher and Mullins 2010[56]**.** The inability of neurons to regulate calcium homeostasis through cell surface ion channels is an aspect of AD pathogenesis that appears to be intimately involved in the dysfunction and death of neurons[57]. Familial AD mutations in *APP* and *PSEN1* support a role for perturbed calcium regulation in AD[57]. In addition, all of the enzymatic machinery responsible for the generation of the pathogenic Aβ plaque formation are plasma membrane based[58]; suggesting that damage to the plasma membrane may be a key factor in the Aβ pathology typical of AD.

BIN1 has been nominated by both GWAS and EWAS, and in addition to its role in synaptic signalling, it also has a role in plasma membrane/cytoskeletal processes as it acts as an amphiphysin, which are known to promote caspase-independent apoptosis as well as play an important role in neuronal membrane organization[59]. Major learning defects and seizures have been linked to decreased expression of amphiphysins in murine brain[60]. In addition, altered expression of *BIN1* has been shown in aging mouse models of AD[61], providing further evidence for its role in AD pathology. Despite having no previous link to AD, *ANK1* is now the one of the strongest reported candidate genes in AD EWAS, with strong links to cell structure. *ANK1* was found to be hypermethylated in AD brain in two separate studies, including one with two independent validation cohorts[28, 29]. The differentially methylated region (DMR) in this gene spans at least six CpG sites, and was significantly associated with neuropathology in cortical regions, but not cerebellum or pre-mortem blood[28], indicating tissue-specificity of the DMR to regions of neuropathology. *ANK1* is found in multiple different isoforms, with some transcript variants specific to the brain[62], and some evidence for differential splicing in AD[28]. As with *BIN1,* one of the main functions of *ANK1* is compartmentalization and maintenance of the plasma membrane, and it is possible that the altered expression of this gene could lead to neuronal membrane dysfunction in AD[28].

The *PVRL2* gene identified by GWAS encodes a single-pass type I membrane glycoprotein, which is one of the plasma membrane components of adherens junctions. Cell to cell connections brought about by adherens junctions are vital for effective neuronal signalling[63]. Interestingly, Marambaud *et al.* using various immunological based methods to investigate the *PSEN1*/γ-secretase system, where mutations are associated with familial AD, and showed it disrupted adherens junctions in AD[63]. Expression of *PVRL2* has been detected in many organs including the brain, and it was later

suggested it was associated with human longevity along with the AD GWAS nominated loci *TOMM40* and *APOE*[64]. In addition, Elias-Sonnenschein *et al.*, showed a significant correlation between the GWAS nominated locus *MS4A4A* and Aβ but not with tau pathology in AD[65]. Despite this, there is little-to-no research on the specific function of *MS4A4A*, although the gene product is associated with GO pathways that indicate it is an integral component of the plasma membrane. Two other genes within the *MS4A* gene cluster have also been nominated via GWAS; *MS4A4E* and *MS4A6A*[11, 12]. One recent study demonstrated that *MS4A6A* genotype and AD are associated with differential expression of isoform variants in blood and some brain regions[66].

Lipid Homeostasis

Recent epidemiological, molecular and biochemical evidence has strengthened the hypothesis that cholesterol is a risk factor for AD, and although cholesterol homeostasis in the brain is largely unexplored, new findings strongly support the involvement of cholesterol in both the generation and deposition of Aβ[67]. Specifically, the quantity of cholesterol in the neuronal plasma membrane has been shown to make neurons more susceptible to the damage caused by Aβ in AD[68]. Other studies suggest that cholesterol acts directly on the amyloid cascade by promoting amyloidogenic processing of *APP*[57]. Interestingly, statins, which are a class of cholesterol-lowering drugs, decrease Aβ levels as well as plaque deposition in *APP* transgenic mouse models[69]. In addition high cholesterol levels and changes to cholesterol metabolism can increase the production of Aβ in cell culture and murine models[67]. Three of the most significant genes from AD GWAS are associated with lipid metabolism (*APOE, APOC1, CLU*). *APOE* was first identified as a risk factor for AD in 1993[70], using immuno-staining and genotyping analysis of 30 AD cases and 91 controls. Since 2006 and the wide application of GWAS to AD research[71], the *APOE* polymorphism has been successfully replicated in several other studies[31, 41, 72-75], making *APOE* the most robust gene linked to late-onset AD (LOAD) risk to date. The proportion of genetic variance for LOAD risk attributed to *APOE* genotype is estimated to be 10–20%[76]. *APOE* is a 299 amino acid glycoprotein and the major protein component of very low-density lipoproteins (VLDL), the major apolipoprotein in the brain[67], as well as having a functional role in cholesterol and triglyceride metabolism[77]. There are three *APOE* alleles that affect one's risk of AD (ε2, ε3 and ε4), in addition to age of onset[78]. Of the three alleles; *APOE* ε2 demonstrates a protective effect, with an OR of 0.3 for possessing one ε2 allele, whilst *APOE* ε4 is associated with a higher LOAD risk, with an OR of 4.4 and 19.3 respectively for having one or two alleles[79], as well as a younger median age of dementia onset[79, 80]. It has been suggested that the mutated *APOE* hinders clearance of soluble Aβ protein from the brain, leading to Aβ aggregation into fibrils. Furthermore, *APOE* has been shown to promote neurodegeneration by directing the toxic Aβ oligomers to synapses[75]. However, a recent PET study to measure Aβ in 602 individuals found that the ε4 allele is neither necessary, nor sufficient, for the development of AD pathology[75].

SORL1 has been identified in several studies of AD, using GWAS and exome sequencing methods, in addition Yu *et al.* found epigenetic changes in this gene[30]. It has many functional domains with

different functions, including cargo transport, chaperone-like activity, signalling, and intracellular sorting[81]. When acting as a sorting receptor, the *SORL1* gene product protects *APP* from being directed to the endosome where it would be cleaved by β-secretase, producing Aβ[82]. Further, SORL1 can bind APOE, making *SORL1* an important component in the pathophysiology of AD[65].

Synaptic Signalling

Synaptic dysfunction is possibly the best-established of all the proposed pathological mechanisms for AD to date as it shows clear progression throughout the entire disease, including pre-symptomatic changes[83]. Early stages of AD are characterized by a 25-35% decrease in numerical density of synapse per cortical region[84]. There has also been evidence that the loss of synapses correlates with the soluble pool of cortical Aβ[85]. Stereological and biochemical analyses have shown that the reduction in synaptic density within AD brain correlates with cognitive defects better than the traditional hallmarks of Aβ plaques and neurofibrillary tangles[83].

We have identified four genes from GWAS and EWAS analyses of AD that have been linked to synaptic function. Two of these, *BIN1* and *PICALM,* have functions in vesicular trafficking. Specifically, studies have shown that the *BIN1* gene has roles in a number of specific pathways, including clathrin-mediated endocytosis (CME) which is an essential step in the intracellular trafficking of proteins and lipids such as nutrients, growth factors and neurotransmitters in synapses[86-88]. Originally identified as a tumour suppressor[89], the *BIN1* gene product is expressed most abundantly in brain and muscle[90], with several alternatively spliced brain specific isoforms. *BIN1* is one of the few genes that has been reproducibly identified by GWAS that does not fall near or within the *APOE* locus, in addition it is the only gene in our analysis to be significantly associated with AD in both GWAS and EWAS.

Like *BIN1*, *PICALM* is also involved in CME[87]. PICALM directs the trafficking of the VAMP2 protein. VAMP2 is a SNARE protein that plays a key role in the fusion of vesicles to the presynaptic membrane allowing neurotransmitter release into the synapse, a process essential to neuronal function[91]. *PICALM* has been robustly identified as a risk factor for AD via GWAS[10, 37], however, AD linked SNPs identified in *PICALM* may still be affected by *APOE* genotype, due to the large amount of attenuation seen when adjusted for *APOE* status[45]. Jun *et al.* have also reported this interaction observing that genotypes of *PICALM* conferred risk predominantly in *APOE* ε4–positive participants, providing strong evidence for a synergistic effect[92]. PICALM is also thought to affect amyloid precursor protein (APP) processing via endocytic pathways[10].

As a previously known risk factor gene for AD[12], *PTK2B* was shown via network analyses to be linked to *RHBDF2*, *ANK1* and *RPL13*, which were recently nominated from EWAS and providing further evidence for a role in AD pathology[29]. *PTK2B* has a number of roles including the induction of long term potentiation (LTP) of nerve cells, a central process of memory formation; cell migration and synaptic function[12].

Immune cell dysfunction (Astrocytes, Oligodendrocytes & Microglia)

There is a widely accepted link between inflammation, the immune system and AD pathology[93-97], more specifically the inflammation seen in AD has been proposed to exacerbate symptoms[94]. Microglia, which are the brain's resident macrophages, have been shown to increase their viability by 22.0~29.4% in response to fibular Aβ deposits of 0.2 to 5.0μM, which are commonly seen in AD. Oligomeric Aβ at a dose of 5.0μM results in cytotoxic microglia[98] and ultimately leads to synaptic degeneration and neuronal death[99]. However, relatively few genes that have shown robust associations with AD have been directly linked with inflammation or immune functions. Most noteworthy a rare variant in *TREM2*, was recently recognised by a number of AD exome sequencing studies and GWAS[16, 48, 100, 101]. *TREM2* encodes an innate immune system receptor on the surface of microglial cells within the brain. With the signalling counterpart DAP12 (also called TYROBP), TREM2 forms a molecular complex that promotes phagocytosis of bacteria[102]. Work by Takahashi *et al*. has shown that TREM2 also has a role in the clearance of apoptotic neurones, due to its ability to increase migration and phagocytosis of microglia[103]. Recently one study demonstrated correlation in *TREM2* and *CD33* gene expression in AD[104]. As *CD33* has also been nominated in various AD GWAS[11, 13, 35] this provides further evidence for an overlap of AD gene pathways in disease. As described above, recent protein-protein interaction data also demonstrated that several EWAS nominated loci (*ANK1, RHBDF2, PICLAM*) have a functional link to *PTK2B*[29]. *PTK2B* is an AD risk factor gene that plays a key role in the signalling cascade involved in the modulation of microglial and infiltrating macrophage cell activation[29].

A further gene related to immune function is *RHBDF2*, identified by EWAS. Differentially methylated CpG sites close to the *RHBDF2* gene were identified in two independent EWAS[28, 29], with recent studies showing this increases *RHBDF2* expression in AD brain[29]. *RHBDF2* transports TNFα converting enzyme (TACE, also called ADAM17), which is necessary for the release of TNFα from the cell surface[105]. *RHBDF2* absence in mice affects the release of TNFα from the cell surface[106] and therefore impairs systemic immune responses to pathogens[107], although the brain phenotype has yet to be researched.

Mitochondrial Processes

Mitochondrial dysfunction is one of the most prominent characteristics of AD, in both the brain and the periphery[108-110], with *TOMM40*, one of the most robust genes identified from GWAS, associated with mitochondrial function. This gene is located approximately 2kb downstream from *APOE* and due to the locality of these two genes there is strong linkage disequilibrium (LD) for *TOMM40* with the *APOE* locus[111], hence many studies have failed to find an association of *TOMM40* in AD after adjusting for *APOE* genotype[75, 112, 113]. However, one study reports *TOMM40* as a possible risk factor of AD independent of *APOE*[114]. Specifically this study found a poly-T track mutation in *TOMM40* that acts independently of *APOE* genotype, which has also seen been reported in another independent

study[115]. In addition to increasing risk of developing AD, *TOMM40* has also been linked to an earlier age of onset for the disease[116]. Other studies also suggest that *TOMM40* provides an additional risk for AD, in addition to *APOE*[117, 118]. However, until the extent of the LD between *TOMM40* and *APOE* is fully characterized, it will be difficult to pinpoint the exact effect the *TOMM40* mutation has on LOAD pathogenesis.

CLU has various nuclear and mitochondrial isoforms and is thought to regulate the rate of cell proliferation. *CLU* has been consistently replicated across many GWAS and holds a strong association with AD[10, 36, 92, 112]. The nuclear isoforms result in the promotion of apoptosis, whereas mitochondrial isoforms of *CLU* suppress BAX-dependent release of cytochrome c into the cytoplasm and inhibit apoptosis[119]. As an increased level of apoptosis in the brain is seen in AD, it could suggest a role of *CLU* mutations in pathogenesis[120]. *SPG7* was identified by EWAS and encodes a mitochondrial metalloprotease protein. Mitochondrial proteases degrade misfolded and non-assembled polypeptides. They also regulate the activity of specific substrates by mediating essential processing steps. These proteases have been hypothesized to play a role in neurodegenerative diseases by affecting neuronal maintenance and axonal function[121].

DISCUSSION

The use of GWAS to identify common disease variants in AD has been at the forefront of research to understanding disease etiology for 10 years. More recently, the falling cost of exome and whole genome sequencing has identified rarer variants with a larger effect size. However, only three EWAS have been reported in AD to date[28, 29, 52], which have solely focussed on DNA methylation, although further studies are highly anticipated. Of all the genes identified from GWAS and EWAS in AD, only one locus was found to be overlapping between these two methodologies (*BIN1*).

As with any pathway identification analysis there are caveats to our method. Some pathways are significantly larger than others containing more genes, therefore using this method we are more likely to find associated genes in these pathways over others. Secondly, cellular pathways that contain a gene which is either genetically or epigenetically altered may still be able to function normally, as similar proteins could “step-in” to fulfil the lost functionality. Thirdly, in our analysis we did not filter our results based on loss of function SNPs or reduced expression, therefore despite the alterations in AD the genes we have identified may well have no change in their functionality. Fourthly, AD is characterised by neuronal cell loss and gliosis, and thus the findings from EWAS may simply represent an alteration in cellular abundance and although EWAS studies can apply cell specific corrections to methylation data[122], this was not included in our analysis. The ability to look at single cell epigenetic profiles in disease would allow researchers to conclusively quantify changes that occur at both cellular and disease state levels, however single cell isolation in post mortem tissue, via laser capture microdissection (LCM) or florescent-assisted cell sorting (FACS), currently represents a considerable challenge to the field. Finally, epigenetic research in AD is still in its early stages with

only two EWAS included in our analysis, this coupled with the fact that current methylation data is the sum of two different cytosine modifications (5-mC and 5-hmC) means we may have an underrepresentation of significant EWAS genes in AD. A further caveat of epigenetic studies compared to genetic studies is that causality is more difficult to establish and thus further studies examining the functional role of nominated EWAS loci are warranted.

CONCLUSION

Looking at the most-significant genetic and epigenetic findings in AD to date we have identified several pathways that require further exploration and could ultimately aid in our understanding of AD etiology. Well characterized clinical cohorts will also allow the identification of further rare variants of AD, whilst advances in methodologies are also allowing the identification of other epigenetic marks, such as histone modifications and other DNA modifications at single nucleotide resolution[3, 123]. A number of recent studies have demonstrated altered global levels of 5-hmC in AD brain[124, 125], however studies to investigate loci-specific 5-hmC changes in AD are yet to be published. There is also the potential for further disease mechanisms to be identified from current studies as research moves to integrate GWAS and EWAS data in the same datasets to identify *cis* methylation quantitative trait loci (mQTLs). Ultimately integrating genomic and epigenomic data with other "omic" modalities will allow the identification of novel dysfunctional pathways in disease[3].

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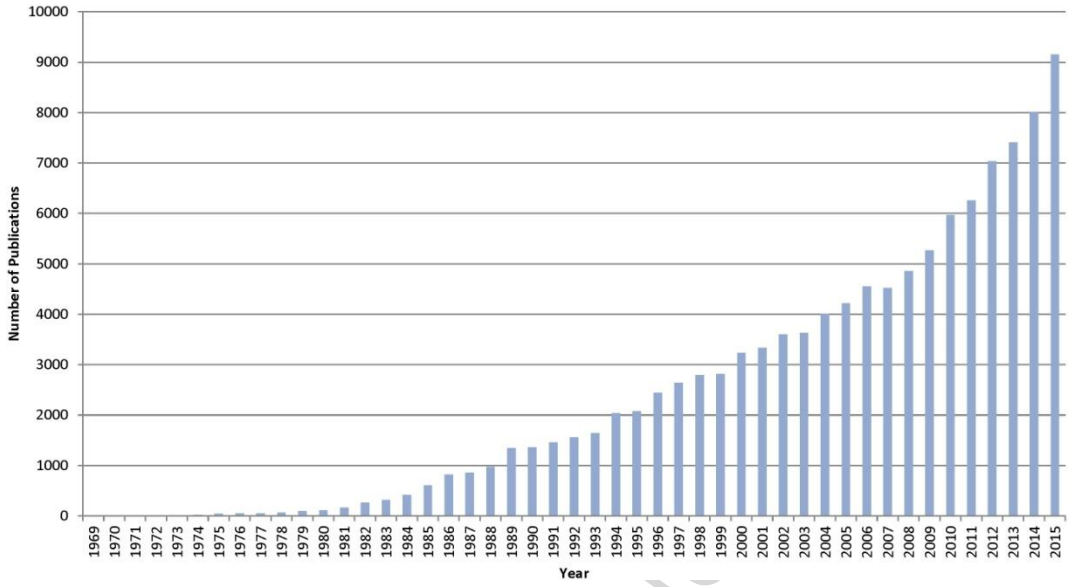

Fig. 1

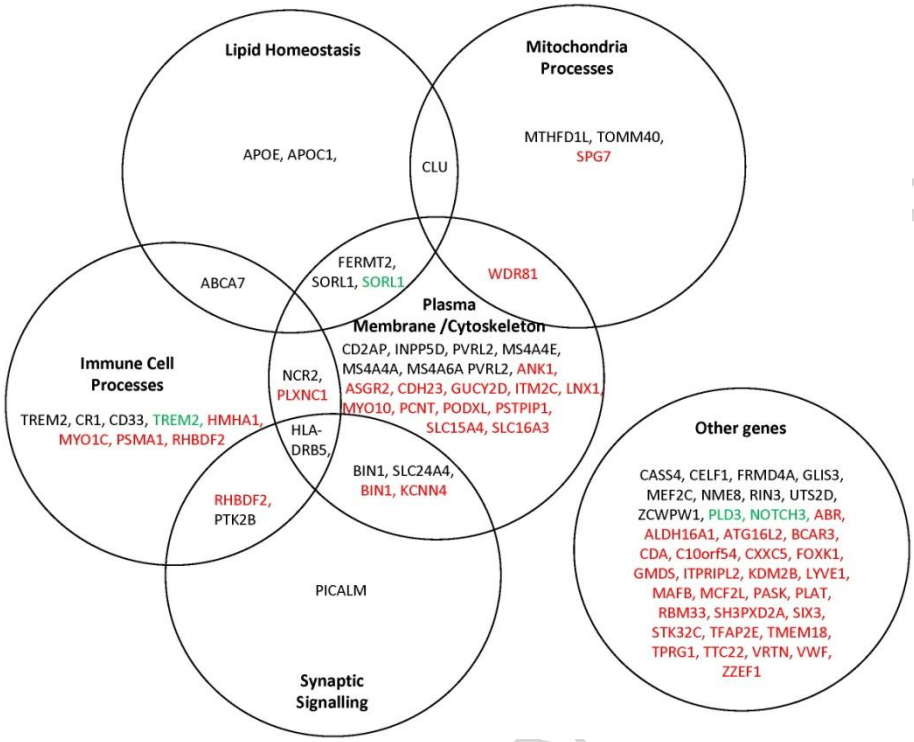

Fig. 2

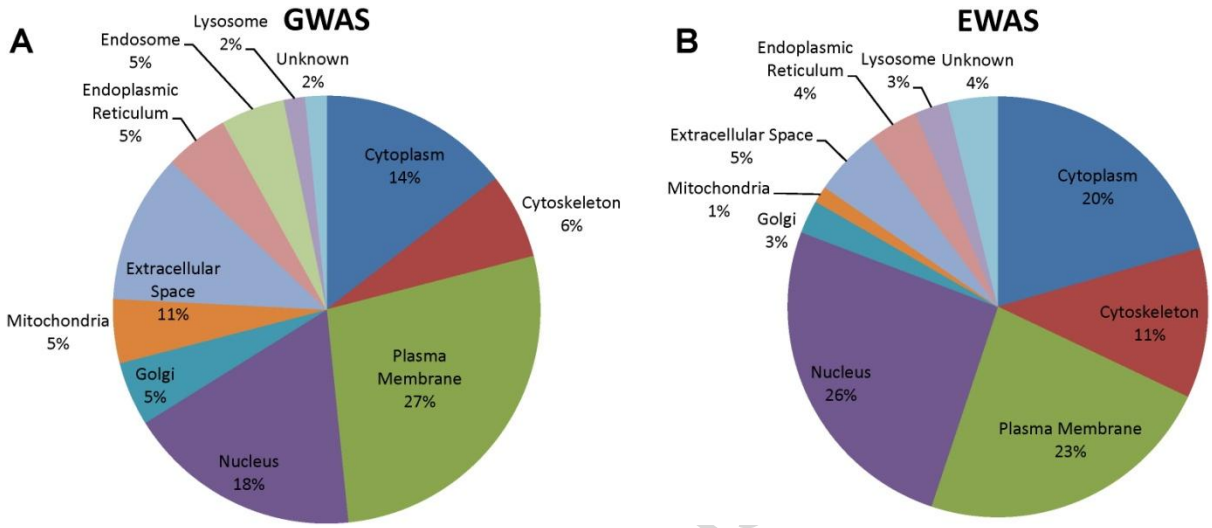

Fig. 3

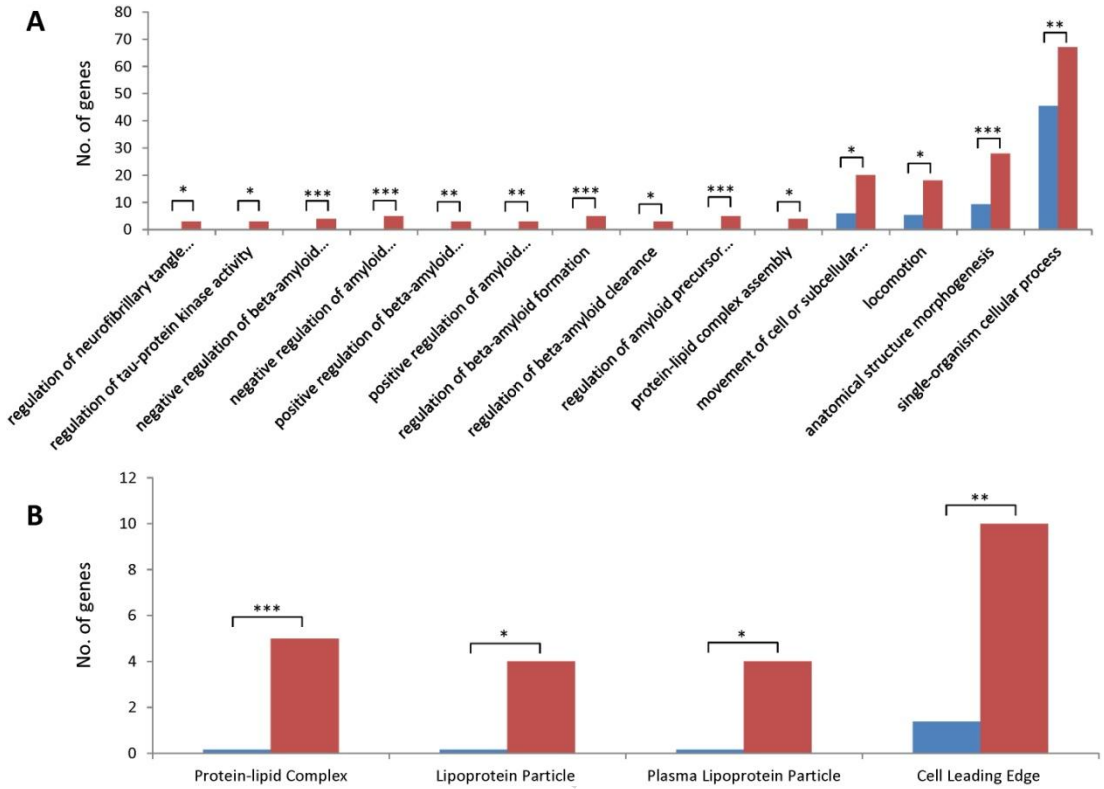

Fig. 4

Table 1A: Genes nominated from GWAS studies. Shown are the genes identified from GWAS studies and their respective SNPs, associated P-value, GO annotation, chromosome and genomic position and the relevant study. SNPs of P<5x10-8 were only included.

Gene	SNPs Identified	P-value	GO annotation	Chromosome	Position	Title of Paper	First Author and Year	Reference
ABCA7	rs3764650	5.00E-17	GO:0005215 transporter activity GO:0005524 ATP binding GO:0005548 phospholipid transporter activity GO:0016887 contributes_to ATPase activity	19	1046521	Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease.	Hollingworth, 2011	[11]
	rs4147929	1.00E-15		19	1063444	Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease.	Lambert, 2013a	[12]
APOE, APOC1,TOMM40	rs4420638	1.00E-39	APOE GO:0001540 beta-amyloid binding IDA 11305869 GO:0005319 lipid transporter activity IDA 17305370 GO:0005515 protein binding IPI 12950167 GO:0005543 phospholipid binding	19	44919689	A high-density whole-genome association study reveals that APOE is the major susceptibility gene for sporadic late-onset Alzheimer's disease.	Coon, 2007	[31]
		1.00E-39				Sorl1 as an Alzheimer's disease predispositio	Webster, 2008	[32]

					n gene?		
	2.00 E-44				Candidate single-nucleotide polymorphisms from a genomewide association study of Alzheimer disease.	Li, 2008	[33]
	1.00 E-12				Genome-wide association analysis of age-at-onset in Alzheimer's disease.	Kamboh, 2012a	[34]
	8.00 E-149				Genome-wide association study of Alzheimer's disease.	Kamboh, 2012b	[35]
rs2075650	2.00 E-157	APOC1 GO:0004859 phospholipase inhibitor activity GO:0005504 fatty acid binding GO:0031210 phosphatidylcholine binding GO:0055102 lipase inhibitor activity GO:0060228 phosphatidylcholine-sterol O-acyltransferase activator activity	19	44892362	Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease.	Harold, 2009	[10]
	2.00 E-16				Genome-wide association study identifies variants at CLU and CR1	Lambert, 2009	[36]

		associated with Alzheimer's disease.		
1.00 E-295		Genome-wide analysis of genetic loci associated with Alzheimer disease.	Seshadri, 2010	[37]
5.00 E-36		Dementia revealed: novel chromosome 6 locus for late-onset Alzheimer disease provides genetic evidence for folate-pathway abnormalities.	Naj, 2010	[38]
9.00 E-116		Overrepresentation of glutamate signalling in Alzheimer's disease: network-based pathway enrichment using meta-analysis of genome-wide association studies.	Perez-Palma, 2014	[39]
4.00 E-13	TOMM40 GO:0008320 protein transmembrane transporter activity GO:0015288 porin activity	ABCC9 gene polymorphism is associated with hippocampal sclerosis of aging	Nelson, 2014	[40]

						pathology.		
	rs6859	6.00 E-14		19	44878777	A genome-wide association study for late-onset Alzheimer's disease using DNA pooling.	Abraham, 2008	[41]
	rs157580	8.00 E-89		19	44892009	The membrane-spanning 4-domains, subfamily A (MS4A) gene cluster contains a common variant associated with Alzheimer's disease.	Antunez, 2011	[42]
	rs157582	9.00 E-52		19	44892962	Genome-wide association study of Alzheimer's disease with psychotic symptoms.	Hollingworth, 2012	[43]
	rs769449	2.00 E-18		19	44906745	GWAS of cerebrospinal fluid tau levels identifies risk variants for Alzheimer's disease.	Cruchaga, 2013	[44]
BIN1	rs7561528	4.00 E-14	GO:0005515 protein binding GO:0032403 protein complex binding GO:0042802 identical protein binding GO:0046982 protein	2	127132061	Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset	Naj, 2011	[13]

		heterodimerization activity GO:0048156 tau protein binding			Alzheimer's disease.		
	6.00E-11				Genome-wide association study of Alzheimer's disease.	Kamboh, 2012b	[35]
rs744373	3.00E-14		2	127137039	Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease.	Hollingworth, 2011	[11]
	1.00E-10				Meta-analysis for genome-wide association study identifies multiple variants at the BIN1 locus associated with late-onset Alzheimer's disease.	Hu, 2011	[45]
	2.00E-09				The membrane-spanning 4-domains, subfamily A (MS4A) gene cluster contains a common variant associated with Alzheimer's	Antunez, 2011	[42]

						disease.		
	rs6733839	7.00E-44		2	127135234	Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease.	Lambert, 2013a	[12]
	rs12989701	3.00E-10		2	127130409	Meta-analysis for genome-wide association study identifies multiple variants at the BIN1 locus associated with late-onset Alzheimer's disease.	Hu, 2011	[45]
CASS4	rs7274581	3.00E-08	GO:0000155 phosphorelay sensor kinase activity GO:0005515 protein binding	20	56443204	Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease.	Lambert, 2013a	[12]
CD2AP	rs10948363	5.00E-11	GO:0005200 structural constituent of cytoskeleton GO:0005515 protein binding GO:0017124 SH3 domain binding	6	47520026	Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease.	Lambert, 2013a	[12]
	rs9349407	9.00E-09		6	47485642	Common variants at MS4A4/MS4A6E,	Naj, 2011	[13]

						CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease.		
CD33	rs3865444	2.00E-09	GO:0004872 receptor activity GO:0005515 protein binding GO:0030246 carbohydrate binding	19	51224706	Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease.	Naj, 2011	[13]
CELF1	rs10838725	1.00E-08	GO:0000166 nucleotide binding GO:0000900 translation repressor activity, nucleic acid binding GO:0003723 RNA binding IDA 16946708 GO:0003729 mRNA binding	11	47536319	Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease.	Lambert, 2013a	[12]
CLU	rs9331896	3.00E-25	GO:0005515 protein binding GO:0016887 NOT ATPase activity GO:0031625 ubiquitin protein ligase binding GO:0051087 chaperone binding GO:0051787 misfolded protein binding	8	27610169	Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease.	Lambert, 2013a	[12]
	rs2279590	6.00E-10		8	27598736	Genome-wide association	Lambert, 2009	[36]

						study identifies variants at CLU and CR1 associated with Alzheimer's disease.		
	rs11136000	9.00E-10		8	27607002	Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease.	Harold, 2009	[10]
	rs569214	4.00E-08		8	27630273	The membrane-spanning 4-domains, subfamily A (MS4A) gene cluster contains a common variant associated with Alzheimer's disease.	Antunez, 2011	[42]
CR1	rs6656401	6.00E-24	GO:0001851 complement component C3b binding GO:0001855 complement component C4b binding GO:0001861 complement component C4b receptor activity GO:0004877 complement component C3b	1	207518704	Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease.	Lambert, 2013a	[12]

			receptor activity					
	rs3818361	4.00E-14		1	207611623	Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease.	Hollingworth, 2011	[11]
	rs6656401	3.00E-10		1	207518704	Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease.	Lambert, 2009	[36]
	rs6701713	5.00E-10		1	207612944	Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease.	Naj, 2011	[13]
FERMT2	rs17125944	8.00E-09	GO:0005515 protein binding GO:0005547 phosphatidylinositol-3,4,5-trisphosphate binding	14	52933911	Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease.	Lambert, 2013a	[12]
FRMD4A	rs7081208	1.00E-	GO:0030674 protein binding,	10	13949865	Genome-wide	Lambert, 2013b	[46]

		10	bridging			haplotype association study identifies the FRMD4A gene as a risk locus for Alzheimer's disease.		
GLIS3	rs514716	3.00E-09	GO:0000978 RNA polymerase II core promoter proximal region sequence-specific DNA binding sequence-specific DNA binding transcription factor activity involved in positive regulation of transcription GO:0001078 RNA polymerase II core promoter proximal region sequence-specific DNA binding transcription factor activity involved in negative regulation of transcription	9	3929424	GWAS of cerebrospinal fluid tau levels identifies risk variants for Alzheimer's disease.	Cruchaga, 2013	[44]
HLA-DRB5	rs9271192	3.00E-12	GO:0042605 peptide antigen binding	6	32610753	Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease.	Lambert, 2013a	[12]

INPP5D	rs35349669	3.00E-08	GO:0004445 inositol-polyphosphate 5-phosphatase activity GO:0005515 protein binding GO:0017124 SH3 domain binding GO:0034594 phosphatidylinositol trisphosphate phosphatase activity GO:0051425 PTB domain binding	2	233159830	Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease.	Lambert, 2013a	[12]
MEF2C	rs190982	3.00E-08	GO:0000977 RNA polymerase II regulatory region sequence-specific DNA binding GO:0000978 RNA polymerase II core promoter proximal region sequence-specific DNA binding GO:0000980 RNA polymerase II distal enhancer sequence-specific DNA binding GO:0000981 sequence-specific DNA binding RNA polymerase II transcription factor activity GO:0000983 RNA polymerase II core promoter sequence-specific DNA binding	5	88927603	Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease.	Lambert, 2013a	[12]

			transcription factor activity					
MS4A4A	rs4938933	8.00E-12	GO:0016021 integral component of membrane	11	60266956	Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease.	Naj, 2011	[13]
MS4A4E, MS4A6A	rs610932	2.00E-14	MS4A4E GO:0016021 integral component of membrane	11	60171834	Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease.	Holling worth, 2011	[11]
	rs983392	6.00E-16	MS4A6A GO:0016021 integral component of membrane	11	60156035	Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease.	Lambert, 2013a	[12]
MTHFD1L	rs11754661	2.00E-10	GO:0004329 formate-tetrahydrofolate ligase activity GO:0004477 NOT methenyltetrahydrofolate cyclohydrolase activity	6	150885942	Dementia revealed: novel chromosome 6 locus for late-onset Alzheimer disease provides genetic	Naj, 2010	[38]

			GO:0004488 NOT methylenetetrahydrofolate dehydrogenase (NADP+) activity GO:0005524 ATP binding			evidence for folate-pathway abnormalities.		
NME8	rs2718058	5.00E-09	GO:0004550 nucleoside diphosphate kinase activity IBA GO:0005524 ATP binding	7	37801932	Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease.	Lambert, 2013a	[12]
PICALM	rs10792832	9.00E-26	GO:0005515 protein binding GO:0005545 1-phosphatidylinositol binding GO:0030276 clathrin binding GO:0032050 clathrin heavy chain binding	11	86156833	Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease.	Lambert, 2013a	[12]
	rs561655	7.00E-11		11	86089237	Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease.	Naj, 2011	[13]
	rs3851179	1.00E-09		11	86157598	Genome-wide association study identifies variants at CLU and PICALM associated	Harold, 2009	[10]

						with Alzheimer's disease.		
	rs536841	3.00E-09		11	86076782	The membrane-spanning 4-domains, subfamily A (MS4A) gene cluster contains a common variant associated with Alzheimer's disease.	Antunez, 2011	[42]
	rs17817600	2.00E-08		11	85966428	Genome-wide association study of Alzheimer's disease.	Kamboh, 2012b	[35]
PTK2B	rs28834970	7.00E-14	GO:0004683 calmodulin-dependent protein kinase activity GO:0004713 protein tyrosine kinase activity GO:0004715 non-membrane spanning protein tyrosine kinase activity GO:0004871 signal transducer activity	8	27337604	Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease.	Lambert, 2013a	[12]
PVRL2	rs6857	2.00E-62	GO:0001618 virus receptor activity GO:0005515 protein binding GO:0015026 coreceptor	19	44888997	Genome-Wide Association Meta-analysis of Neuropathologic Features	Beecham, 2014	[47]

			activity GO:0042802 identical protein binding GO:0042803 protein homodimerization activity			of Alzheimer's Disease and Related Dementias.		
SLC24A4, RIN3	rs10498633	6.00E-09	SLC24A4 GO:0008273 calcium, potassium:sodium antiporter activity GO:0015293 symporter activity RIN3 GO:0005096 GTPase activator activity GO:0005515 protein binding GO:0017112 Rab guanyl-nucleotide exchange factor activity GO:0017137 Rab GTPase binding	14	92460608	Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease.	Lambert, 2013a	[12]
SORL1	rs11218343	1.00E-14	GO:0001540 beta-amyloid binding GO:0004888 transmembrane signaling receptor activity GO:0005515 protein binding GO:0030169 low-density lipoprotein particle binding GO:0030306 ADP-ribosylation factor binding	11	121564878	Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease.	Lambert, 2013a	[12]

NCR2	rs6922617	4.00E-08	GO:0004888 transmembrane signaling receptor activity	6	41368363	GWAS of cerebrospinal fluid tau levels identifies risk variants for Alzheimer's disease.	Cruchaga, 2013	[44]
TREM2	rs75932628	2.00E-12	GO:0001530 lipopolysaccharide binding GO:0004872 receptor activity GO:0005515 protein binding GO:0042834 peptidoglycan binding GO:0070891 lipoteichoic acid binding	6	41161514	Variant of TREM2 associated with the risk of Alzheimer's disease.	Jonsson, 2012	[48]
UTS2D	rs9877502	5.00E-09	GO:0001664 G-protein coupled receptor binding GO:0005179 hormone activity	3	190951729	GWAS of cerebrospinal fluid tau levels identifies risk variants for Alzheimer's disease.	Cruchaga, 2013	[44]
ZCWPW1	rs1476679	6.00E-10	GO:0008270 zinc ion binding	7	100406823	Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease.	Lambert, 2013a	[12]

Table 1B: Genes nominated from exome sequencing studies. Shown are the genes identified from exome sequencing studies and their respective SNPs, GO annotation, chromosome and genomic position and the relevant study.

Gene	SNPs identified	GO annotation	Chromosome	Position	Title of Paper	First Author and Year	Reference
TREM2	rs2234255	GO:0001530 lipopolysaccharide binding GO:0004872 receptor activity GO:0005515 protein binding GO:0042834 peptidoglycan binding GO:0070891 lipoteichoic acid binding	6	41127543	TREM2 Variants in Alzheimer's Disease	Guerreiro, 2013	[16]
	rs147564421		6	41129100			
	rs2234253		6	41129105			
	rs142232675		6	41129133			
	rs201258663		6	41129195			
	rs75932628		6	41129252			
	#N/A		6	41129279			
SORL1	#N/A	GO:0001540 beta-amyloid binding GO:0004888 transmembrane signaling receptor activity GO:0005515 protein binding GO:0030169 low-density lipoprotein particle binding GO:0030306 ADP-ribosylation factor binding	11	1.21E+08	High frequency of potentially pathogenic SORL1 mutations in autosomal dominant early-onset Alzheimer disease	Pottier, 2012	[51]
	#N/A		11	1.21E+08			
	#N/A		11	1.21E+08			

NOTCH3	rs10408676	GO:0005509 calcium ion binding GO:0005515 protein binding GO:0019899 enzyme binding	19	15290007	Exome sequencing reveals an unexpected genetic cause of disease: NOTCH3 mutation in a Turkish family with Alzheimer's disease	Guerreiro, 2012	[49]
PLD3	rs145999145	GO:0004630 phospholipase D activity GO:0005515 protein binding GO:0070290 N-acylphosphatidylethanolamine-specific phospholipase D activity	19	40877595	Rare coding variants in the phospholipase D3 gene confer risk for Alzheimer’s disease	Cruchaga, 2014	[50]

Table 2: Genes nominated from EWAS studies. Shown are the genes identified from EWAS studies and their respective probes, associated P-value, GO annotation, chromosome and genomic position and the relevant study. Probes of P<1x10-7 were only included.

Gene	Probes Identified	P-value	Functional summary, GO annotation	Chromosome	Position	Title of Paper	First Author and Year	Reference
ABR	cg25018458	1.89 E-10	GO:0005089 Rho guanyl-nucleotide exchange factor activity GO:0005096 GTPase activator activity	17	980014	Methylomic profiling implicates cortical deregulation of ANK1 in Alzheimer's disease	Lunnon, 2014	[28]
ALDH16A1	cg20618448	1.16 E-08	GO:0004029 aldehyde dehydrogenase (NAD) activity	19	49962324	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
ANK1	cg05066959	7.13 E-14	GO:0005198 structural molecule activity GO:0005200 structural constituent of cytoskeleton GO:0005515 protein binding GO:0008093 cytoskeletal adaptor activity GO:0019899 enzyme binding GO:0030507 spectrin binding GO:0051117 ATPase binding	8	41519308	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]

	1.24 E-09	8	41519308	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29
cg11823178	7.83 E-14	8	41519399	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
	3.42 E-11	8	41519399	Methylomic profiling implicates cortical deregulation of ANK1 in Alzheimer's disease	Lunnon, 2014	[28]
cg16140558	1.85 E-08	8	41514039	Methylomic profiling implicates cortical deregulation of ANK1 in Alzheimer's disease	Lunnon, 2014	[28]

ASGR2	cg18659586	1.06E-09	GO:0004873 asialoglycoprotein receptor activity GO:0005515 protein binding GO:0030246 carbohydrate binding	17	7017474	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
ATG16L2	cg21806242	3.71E-10	Regulation of autophagy	11	72532891	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
BCAR3	cg02342148	1.60E-08	GO:0005085 guanyl-nucleotide exchange factor activity GO:0005515 protein binding	1	94145223	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
BIN1	cg22883290	3.73E-08	GO:0005515 protein binding GO:0032403 protein complex binding GO:0042802 identical protein binding	2	127800646	Alzheimer's disease: early alterations in brain DNA methylati	De Jager, 2014	[29]

			GO:0046982 protein heterodimerization activity GO:0048156 tau protein binding			on at ANK1, BIN1, RHBDF2 and other loci		
CDA	cg26407544	2.10 E-10	GO:0001882 nucleoside binding GO:0004126 cytidine deaminase activity GO:0005515 protein binding GO:0008270 zinc ion binding	1	20945355	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
CDH23, C10orf54	cg23968456	1.09 E-08	GO:0005509 calcium ion binding GO:0005515 protein binding	10	73521631	Methylomic profiling implicates cortical deregulation of ANK1 in Alzheimer's disease	Lunnon, 2014	[28]
		3.97 E-10		10	73521631	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
CXXC5	cg07354506	9.26 E-08	GO:0004871 signal transducer activity GO:0005515 protein binding GO:0008134	5	139048148	Methylomic profiling implicates cortical deregulati	Lunnon, 2014	[28]

			transcription factor binding GO:0008270 zinc ion binding			on of ANK1 in Alzheimer's disease		
FOXK1	cg07180538	4.95E-08	GO:0000977 RNA polymerase II regulatory region sequence-specific DNA binding GO:0000981 sequence-specific DNA binding RNA polymerase II transcription factor activity GO:0005515 protein binding	7	4786899	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
	cg25594100	2.54E-11		7	4786943	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
GMDS	cg07714812	3.81E-08	GO:0005515 protein binding GO:0008446 GDP-mannose 4,6-dehydratase activity GO:0070401 NADP+ binding	6	1635611	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
GUCY2D	cg04157161	7.80E-08	GO:0004383 guanylate cyclase activity GO:0004672	17	7906847	Alzheimer's disease: early	De Jager, 2014	[29]

			protein kinase activity GO:0004872 receptor activity GO:0005524 ATP binding			alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci		
HMHA1	cg02308560	3.06E-08	GO:0005096 GTPase activator activity GO:0005515 protein binding GO:0046872 metal ion binding	19	1071176	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
ITM2C	cg18346707	3.30E-08	GO:0001540 beta-amyloid binding GO:0005515 protein binding GO:0005524 ATP binding	2	231732249	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
ITPRIPL2	cg16733298	5.24E-08	membrane-associated protein, intracellular calcium signalling	16	19127132	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2	De Jager, 2014	[29]

						and other loci		
KCNN4	cg22904711	1.08E-08	GO:0005515 protein binding GO:0005516 calmodulin binding GO:0015269 calcium-activated potassium channel activity GO:0016286 NOT small conductance calcium-activated potassium channel activity	19	44278628	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
KDM2B	cg11724984	4.76E-09	GO:0003677 DNA binding GO:0005515 protein binding GO:0008270 zinc ion binding GO:0019843 rRNA binding GO:0032452 histone demethylase activity	12	121890864	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
LNX1	cg12114584	5.81E-13	GO:0004842 ubiquitin-protein transferase activity GO:0005515 protein binding GO:0008270 zinc ion binding GO:0016874 ligase activity GO:0030165 PDZ domain binding	4	54518744	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
LYVE1	cg18343862	4.96E-08	GO:0004872 receptor activity GO:0004888 transmembrane signaling	11	105900003	Alzheimer's disease: early alteration	De Jager, 2014	[29]

			receptor activity GO:0005515 protein binding GO:0005540 hyaluronic acid binding			s in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci		
MAFB	cg13579486	3.25E-08	GO:0003700 sequence-specific DNA binding transcription factor activity GO:0005515 protein binding GO:0008134 transcription factor binding GO:0043565 sequence-specific DNA binding	20	39314091	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
MCF2L	cg07883124	6.31E-12	GO:0005089 Rho guanyl-nucleotide exchange factor activity GO:0005545 1-phosphatidylinositol binding	13	113634042	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
	cg09448088	6.43E-10		13	113635690	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other	De Jager, 2014	[29]

						loci		
MYO10	cg06742628	1.58E-10	GO:0005515 protein binding GO:0005516 calmodulin binding GO:0005524 ATP binding GO:0005547 phosphatidylinositol-3,4,5-trisphosphate binding	5	16886424	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
MYO1C	cg05417607	2.25E-08	GO:0003774 motor activity GO:0003779 actin binding GO:0005102 receptor binding GO:0005515 protein binding GO:0005516 calmodulin binding	17	1373605	Methylomic profiling implicates cortical deregulation of ANK1 in Alzheimer's disease	Lunnon, 2014	[28]
		2.52E-08		17	1373605	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
PASK	cg25488284	3.50E-08	GO:0004674 protein serine/threonine kinase activity GO:0004871 signal transducer activity	2	242048127	Methylomic profiling implicates cortical deregulation of	Lunnon, 2014	[28]

			GO:0005515 protein binding			ANK1 in Alzheimer's disease		
PCNT	cg00621289	6.48E-08	GO:0005515 protein binding GO:0005516 calmodulin binding	21	47855916	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
	cg04147621	1.39E-08		21	47856020	Methylomic profiling implicates cortical deregulation of ANK1 in Alzheimer's disease	Lunnon, 2014	[28]
	cg23449541	9.39E-08		21	47855893	Methylomic profiling implicates cortical deregulation of ANK1 in Alzheimer's disease	Lunnon, 2014	[28]
PLAT	cg17693222	2.14E-08	GO:0004252 serine-type endopeptidase activity GO:0005515 protein binding	8	42033472	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2	De Jager, 2014	[29]

						and other loci		
PLXNC1	cg12877335	2.36E-11	GO:0004872 receptor activity GO:0005102 receptor binding GO:0005515 protein binding	12	94539319	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
PODXL	cg08737189	3.37E-08	GO:0005515 protein binding	7	131223417	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
	cg19140834	1.22E-09		7	131217668	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
PSMA1	cg27443779	1.18E-08	GO:0003723 RNA binding GO:0004298 threonine-type	11	146647963	Alzheimer's disease: early	De Jager, 2014	[29]

			endopeptidase activity GO:0005515 protein binding			alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci		
PSTPIP1	cg11652496	2.57E-09	GO:0003779 actin binding GO:0005515 protein binding GO:0019903 protein phosphatase binding	15	77324526	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
RBM33	cg13639901	1.54E-13	GO:0000166 nucleotide binding GO:0044822 poly(A) RNA binding	7	155556590	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
	cg14430943	2.71E-10		7	155556652	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2	De Jager, 2014	[29]

						and other loci		
RHBDF2	cg05810363	9.42E-10	GO:0004252 serine-type endopeptidase activity GO:0019838 growth factor binding	17	74475270	Methylomic profiling implicates cortical deregulation of ANK1 in Alzheimer's disease	Lunnon, 2014	[28]
		3.68E-10		17	74475270	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
	cg12163800	2.66E-08		17	74475355	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
	cg13076843	3.81E-08		17	74475294	Methylomic profiling implicates cortical deregulation of ANK1 in	Lunnon, 2014	[28]

						Alzheimer's disease		
		1.68 E-09		17	74475294	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
SH3PXD2A	cg19007269	2.96 E-09	GO:0005515 protein binding GO:0035091 phosphatidylinositol binding	10	105420501	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
SIX3	cg22385702	4.47 E-10	GO:0000980 RNA polymerase II distal enhancer sequence-specific DNA binding GO:0001205 RNA polymerase II distal enhancer sequence-specific DNA binding transcription factor activity involved in positive regulation of transcription GO:0001222 transcription	2	45175881	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]

			corepressor binding GO:0003700 sequence-specific DNA binding transcription factor activity					
SLC15A4	cg06653632	9.77 E-08	GO:0005290 L-histidine transmembrane transporter activity GO:0015293 symporter activity	12	129281444	Methylomic profiling implicates cortical deregulation of ANK1 in Alzheimer's disease	Lunnon K	[28]
SLC16A3	cg07012687	6.19 E-09	GO:0005515 protein binding GO:0008028 monocarboxylic acid transmembrane transporter activity GO:0015129 lactate transmembrane transporter activity GO:0015293 symporter activity GO:0015355 secondary active monocarboxylate transmembrane transporter activity	17	80195180	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
SPG7	cg03169557	7.95 E-09	GO:0004222 metalloendopeptidase activity GO:0005515 protein binding GO:0005524 ATP binding GO:0008233 peptidase activity GO:0008270 zinc ion binding	16	89598950	Methylomic profiling implicates cortical deregulation of ANK1 in Alzheimer's disease	Lunnon K	[28]

		3.99 E-10		16	89598950	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
STK32C	cg25917732	9.98 E-08	GO:0004672 protein kinase activity GO:0004674 protein serine/threonine kinase activity GO:0004713 protein tyrosine kinase activity null GO:0005524 ATP binding GO:0016772 transferase activity, transferring phosphorus-containing groups	10	134038395	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
TFAP2E	cg17474422	1.56 E-08	GO:0000977 RNA polymerase II regulatory region sequence-specific DNA binding GO:0000981 sequence-specific DNA binding RNA polymerase II transcription factor activity GO:0042803 protein homodimerization activity	1	36039866	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]

TMEM18	cg21644387	7.25 E-08	GO:0003677 DNA binding	2	663024	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
TPRG1	cg04252044	2.42 E-10	GO:0042802 identical protein binding	3	188664747	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
	cg12307200	3.06 E-17		3	188664632	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
TTC22	cg15645660	4.05 E-08	Chaperone activity	1	55247356	Alzheimer's disease: early alterations in brain DNA methylati	De Jager, 2014	[29]

						on at ANK1, BIN1, RHBDF2 and other loci		
VRTN	cg21207436	2.24E-09	GO:0004803 transposase activity GO:0043565 sequence-specific DNA binding	14	74815316	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
VWF	cg27041424	4.47E-10	GO:0001948 glycoprotein binding GO:0002020 protease binding GO:0005178 integrin binding GO:0005515 protein binding GO:0005518 collagen binding	12	6232979	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]
WDR81	cg19803550	1.04E-08	GO:0016772 transferase activity, transferring phosphorus-containing groups	17	1637391	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]

ZZEF1	cg06753513	3.87 E-12	GO:0005509 calcium ion binding GO:0008270 zinc ion binding	17	3977385	Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci	De Jager, 2014	[29]