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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