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Review

The broken Alzheimer's disease genome

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SUMMARY

The complex pathobiology of late-onset Alzheimer's disease (AD) poses significant challenges to therapeutic and preventative interventions. Despite these difficulties, genomics and related disciplines are allowing fundamental mechanistic insights to emerge with clarity, particularly with the introduction of high-resolution sequencing technologies. After all, the disrupted processes at the interface between DNA and gene expression, which we call the broken AD genome, offer detailed quantitative evidence unrestrained by preconceived notions about the disease. In addition to highlighting biological pathways beyond the classical pathology hallmarks, these advances have revitalized drug discovery efforts and are driving improvements in clinical tools. We review genetic, epigenomic, and gene expression findings related to AD pathogenesis and explore how their integration enables a better understanding of the multicellular imbalances contributing to this heterogeneous condition. The frontiers opening on the back of these research milestones promise a future of AD care that is both more personalized and predictive.

INTRODUCTION

Alzheimer's disease (AD) has traditionally been considered first and foremost a neurodegenerative condition. This neuron-centric view of AD is not wholly unjustified, as synapse and neuronal loss are cornerstone features of the worsening cognitive outcomes associated with disease progression.[1,2] In addition, two primary histopathological hallmarks, extracellular β-amyloid deposition and intraneuronal neurofibrillary tangles of hyperphosphorylated tau protein, have informed much of the research on AD pathogenesis and are still fundamental scoring criteria of present molecular attempts to stage disease trajectory.[3] However, we now know that the disease is more multifaceted than this, comprising different cell types, inflammatory overloads, the vasculature, and uniquely vulnerable brain regions, among others.[4] Therefore, the limited success of AD therapies, which have focused largely on mitigating β-amyloid pathology,[5] may stem from our inability to tackle the complexity of the disease and the heterogenicity of those suffering from it.

The genome holds the key to many of these individual differences. Genetics account for up to 58%–79% of AD risk,[6] and about 75 susceptibility loci have been discovered to date.[7–13] For comparison, the genetic component of Parkinson's disease is about 15%.[14] In fact, the heritability of AD is so great that parental disease history has been employed to identify AD-by-proxy cases in attempts to increase the power of genetic association studies.[7,9] Still, it has not been trivial to translate these genetic links into mechanistic breakthroughs and therapeutic targets, as the resulting functional outcomes and causal genes linked to each polymorphism remain mostly unresolved. Research efforts have also been dedicated to dynamic gene expression processes, namely the epigenome, the transcriptome, and the proteome. Such analyses provide a more nuanced assessment of AD-associated imbalances across the different stages of the disease than that afforded by genetic association studies. At any rate, both approaches are complementary, and their integration is crucial to elucidating the mechanisms that underlie AD.

Here, we explore how genomic research has advanced the understanding of late-onset AD. This is, for us, the first meaning of the "broken" AD genome, akin to unraveling a code. But various processes centered on our DNA become dysfunctional in AD, imparting an equally significant connotation to the term; i.e., "broken" in this context alludes to the genome as a driver of disease. We primarily highlight findings originating from human datasets, as existing disease models often fail to recapitulate the full pathological spectrum of AD. We recognize the importance of these tools and, when appropriate, reference insights obtained using them. We also identify challenges for the field and discuss strategies for amassing the wealth of genomic information now available for developing therapeutics and clinical tools.

The genetic etiology of AD

Genetic studies have transformed our understanding of AD etiology and promise to revolutionize treatment by allowing for personalized care and focused pharmaceutical development programs. Genetic associations also provide corroboration of the importance of β-amyloid in the pathogenesis of familial AD,

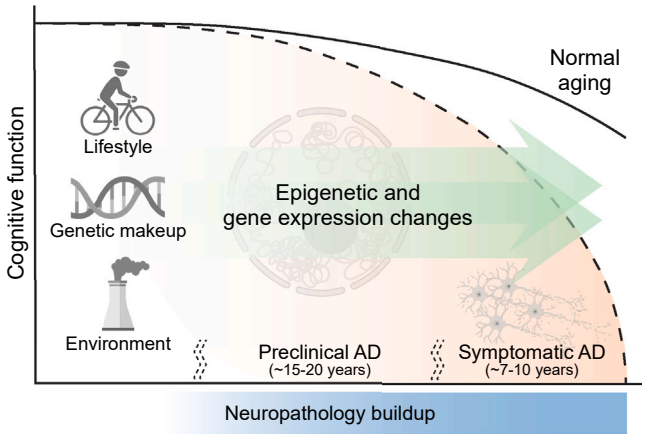

Figure 1. Intersecting paths: The genome and AD

Despite the etiology of AD being dominated by a strong genetic component, nonheritable elements also play a role in shaping disease risk. These encompass behavioral factors, such as nutrition, physical activity, and sleep, in addition to environmental determinants, including pollution and socio-economical status. Together, they affect gene expression across the lifespan. The genome further interacts with the gradual accrual of neuropathological alterations in the brain, comprising both protective and disease-driving programs in a multicellular web of feedback and feedforward responses.

as pro-amyloidogenic mutations in genes involved in its metabolism (*APP*, *PSEN1*, and *PSEN2*) are generally causative of this rare form of the condition.[15] How our genes contribute to sporadic AD, which accounts for >99% of all cases, is less clear, as their interaction with non-heritable factors, such as lifestyle, pre-existing conditions, and environmental exposures, will influence clinical outcomes to a greater degree (Figure 1). We can therefore distinguish two types of DNA variation: polymorphisms associated with "deterministic" genes (*APP*, *PSEN1*, and *PSEN2*) at one end of the spectrum, which predict with high certainty that carriers will develop AD, and those that increase its likelihood but do not directly cause AD, known as "risk" genes.[16] The latter underlie the genetic component of sporadic AD and are relatively frequent in the population, but their contribution to total individual risk is generally small.[16] The *APOE* ε4 (*APOE4*) allele is an important exception in that it increases lifetime AD risk by more than 50% in homozygotic carriers and about 20%–30% in those who inherit *APOE4* from one parent and the more common *APOE3* allele from the other.[17] Given its prevalence, our discussion will be focused on sporadic (also known as late-onset) AD.

Despite *APOE4* being the major genetic risk factor for AD, not all *APOE*-related genetic signals are harmful. Indeed, the ε2 allele is associated with a significantly reduced disease risk, and homozygous *APOE2* carriers have a particularly low likelihood of developing the condition.[18] Globally, there are three main *APOE* alleles encoding protein isoforms that diverge at only two amino acid positions. Each isoform influences disease differently (lifetime risk ε4 > ε3 > ε2) and does so in a dose-dependent manner.[19,20] In relation to the ε3/ε3 genotype, each additional copy of the ε4 allele amounts to a higher risk of AD and a younger age of onset.[21] *APOE4*, which is found in 40%–50% of AD individuals,[22] is also associated with other neurological disorders[23,24] and increases the risk for age-related cognitive impairment in non-demented individuals,[25] underscoring the key role played by apoE in brain health. Recent evidence indicates that its contribution to AD risk stems partly from the differential impact that each apoE isoform has on myelination.[26]

Overall, the *APOE4* allele is associated with an estimated 3–4 times increased risk, but it does not fully account for the heritability of AD. Genome-wide association studies (GWASs) have identified around 75 loci that collectively contribute to disease risk, highlighting the polygenic nature of sporadic AD.[11] On a pathway level, GWAS hits have implicated endosomal trafficking, the immune response, lipid metabolism, and vascular factors as pivotal in pathogenesis, among others (Figure 2A).[11,13,16,27] It is important to note, however, that most GWAS signals occur in non-coding regions of the genome, and, therefore, it has not always been easy to establish how these polymorphisms affect disease susceptibility (Figure 2B).[10,28] For example, non-coding DNA variants can disturb gene expression by disrupting transcription factor recognition sequences.[29] While they are typically assigned to the gene that maps closest to the lead single-nucleotide polymorphism (SNP),[30] regulatory domains can be located far outside the core transcription unit of a gene (i.e., coding sequences and proximal gene promoters/enhancers).[31] Hence, once located, the functional mapping of most GWAS signals requires further mechanistic exploration. As a result, despite some successes, such as the identification of altered splicing as the likely mechanism behind the effects of the *PICALM*, *CLU*, and *PTK2B* risk alleles,[32] it remains unresolved how most genetic variants affect disease susceptibility. Until then, gene annotations should be interpreted cautiously.

The untangling of AD's genetic architecture has further been hampered by a poor understanding of the spatiotemporal context by which risk loci modulate pathogenesis. Indeed, the effects of genetic variation in non-coding regions of our genome often have a strong cell-type-specific component.[36] Interestingly, enhancers exhibiting tissue-specific activity are enriched within intronic regions of the genome,[37] exactly where most AD GWAS signals accumulate (Figure 2B). Consecutively larger GWAS studies reporting an ever-greater number of AD risk loci are not the solution; instead, it is necessary to precisely link non-coding genetic variants to their functional consequences, which requires studying when and where they interact with the disease pathological cascade. The recent availability of cell-type-specific resources makes this increasingly feasible in AD.[26,27,38–40] As an example, only recently did the brain vasculature emerge as a site of AD risk gene expression.[27] A tailored single-nucleus profiling protocol was at the core of this insight,[27] illustrating how GWAS discoveries require proper contextualization (Figure 3). While this finding was incidental, others have taken a more directed approach toward dissecting the impact of non-coding genomic variation in AD.[41] Using massively parallel reporter assays to systematically test the regulatory consequences of thousands of common SNPs associated with AD, a plurality of variants that affect gene expression were found to converge within microglial enhancers.[41]

Most risk genes highlighted in Figures 2 and 3 are linked with what are called "common" variants. These are defined by their

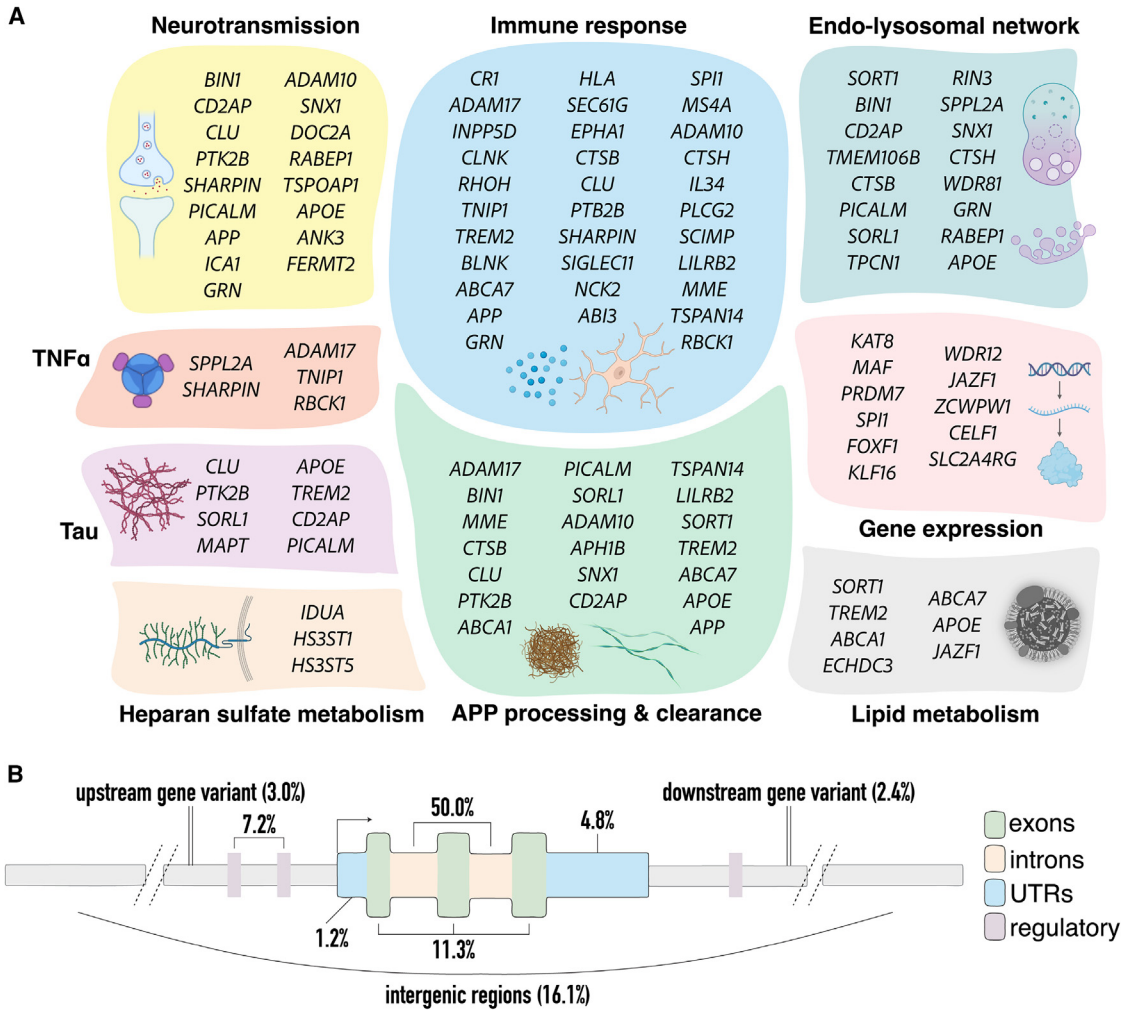

Figure 2. Functional mapping of late-onset AD genetic etiology

(A) Pathway enrichment of genetic loci linked to AD based on statistically significant GWAS signals identified by Bellenguez et al.,[11] a two-stage case-control study totaling 111,326 clinically diagnosed/"proxy" AD cases and 677,663 control individuals, published in 2022. Their analysis, the largest to date, led to the identification of 75 risk loci, including 42 novel genetic associations. GWAS hits are genetic variants that occur more frequently in individuals with a particular trait or disease. These polymorphisms are most common in non-coding genomic regions and, as a rule of thumb, require additional validations before annotations can be unambiguously assigned to a specific gene. We used Metascape, SynGO, expression profiles, and manual curation to group genes according to their function;[33,34] a small fraction of hits could not be interpreted confidently and were omitted. Unsurprisingly, various genes overlap across distinct functional processes, particularly with regards to APP metabolism, immunity, and the endo-lysosomal pathway, such are the interdependencies between these processes. We note, in addition, that *ADAM10* and *ADAM17* are α-secretases; their identification suggests that altered non-amyloidogenic APP processing can influence AD pathogenesis.

(B) Genomic distribution of lead SNPs associated with increased AD risk. GWAS data were mined from panel compiled by Andrews et al.[35] Functional annotations were performed using gnomAD and GWAS Catalog.

occurrence in more than 5% of the population. There are, however, other informative genetic signals below that frequency threshold, collectively known as "rare" variants.[42] Indeed, not all genetic variance associated with the disease is captured by commonly occurring SNPs.[42,43] Despite not being found in many individuals, these rare variants typically impact disease risk more strongly than common polymorphisms, and, hence, their identification can lead to mechanistic breakthroughs. The discovery of rare *TREM2* variants that as much as triple an individual's disease susceptibility is paradigmatic of this potential.[44]

Notably, many of the loci with known rare variants are also associated with common polymorphisms in GWAS studies, including *TREM2*, *SORL1*, *ADAM10*, and *ABCA7*,[45] highlighting how both avenues of exploration ultimately serve a shared purpose.[42] While we have focused our present survey on SNPs linked to disease risk, the heritability of AD is also thought to be affected by large genomic variations (>50 bp) known globally as structural variants.[43] They include copy number variations, insertions, inversions, and translocations, among other alterations. Even if quite rare, the finding of duplication events in the *APP* locus of

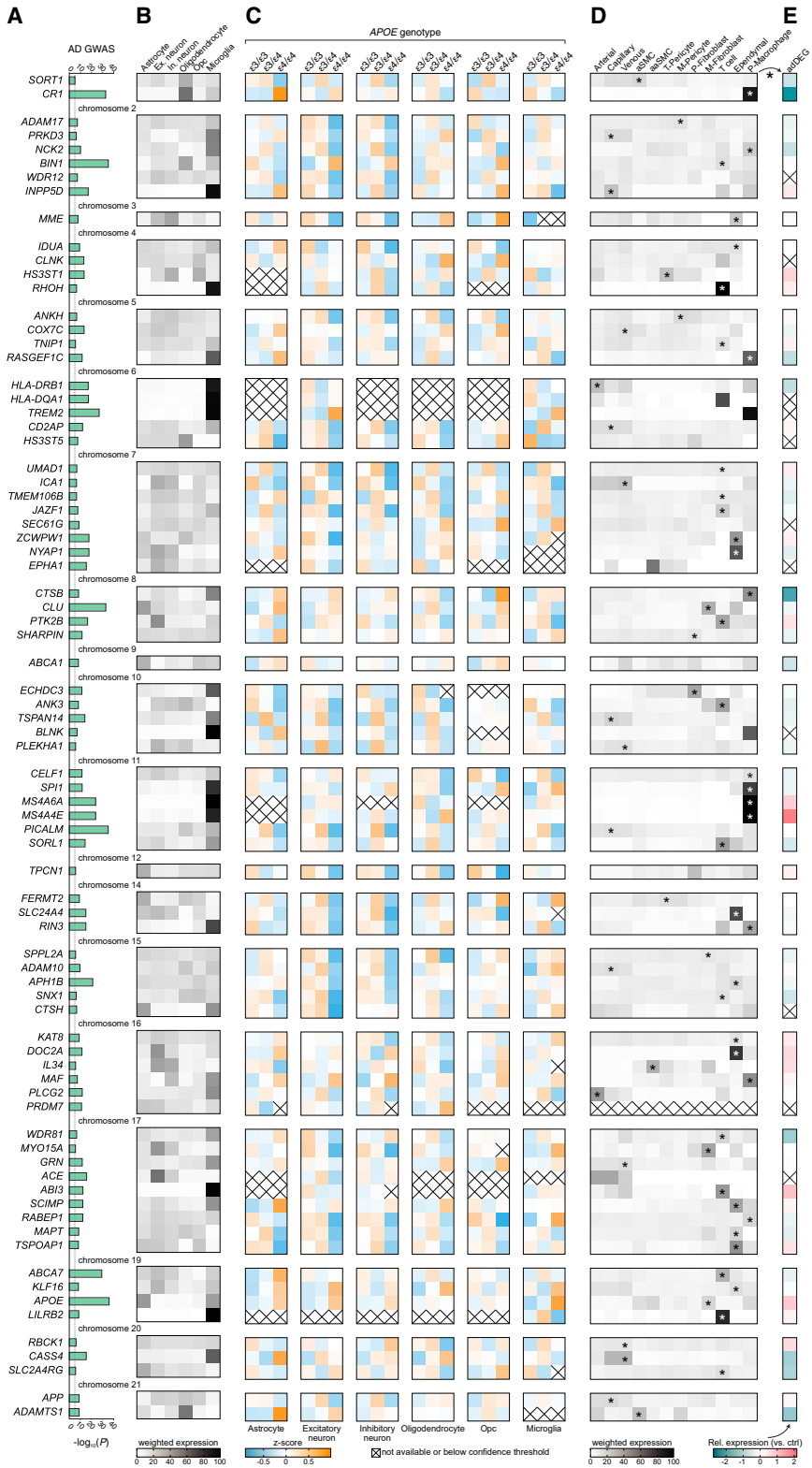

Figure 3. Cellular map of AD genetic risk

Shown is an expression profile overview of prioritized genetic loci harboring genome-wide significant signals linked to AD risk.

(A) Simplified Manhattan plot derived from the dataset collected by Bellenguez et al.[11] The *p* values were calculated using a fixed-effect meta-analysis. Brackets indicate different prioritized genes in the same locus. Note also that GWAS signals from independent studies linked to *EPHA1* stem from two adjacent loci.

(B) Proportional expression of GWAS hits across parenchymal dorsolateral prefrontal cortex cells. Ex. neuron, excitatory neuron; In. neuron, inhibitory neuron; OPC, oligodendrocyte precursor cell. Expression values are weighted on a 0–100 scale; darker colors indicate progressively higher expression values.

(C) Differential gene expression analysis centered on the interaction between *APOE* genotype and other AD GWAS hits. Data points in (B) and (C) were mined from snRNA-seq analyses performed by Blanchard et al.[26]

(D) Proportional expression of GWAS hits across major vascular cell types (hippocampus and superior frontal cortex). Asterisks mark the cell type carrying the strongest overall expression for each gene. aSMC, vascular smooth muscle cell; aaSMC, arteriolar smooth muscle cell; T-Pericyte, solute-transport pericyte; M-Pericyte, ECM, extracellular matrix-regulating pericyte; P-Fibroblast, perivascular fibroblast; M-Fibroblast, meningeal fibroblast; P-Macrophage, perivascular macrophage. Vessel isolation and nuclei extraction for sequencing (VINE-seq) data were extracted from Yang et al.[27]

(E) Gene expression comparison between AD and control cases. This analysis was circumscribed to the cell type most highly enriched in each gene (i.e., those marked with an asterisk in D). All transcriptional trends shown should be regarded as indicative due to limited sample sizes and, at times, high interindividual variation.

families with early-onset AD remains a clear example of the deleterious consequences these genomic events can have in affected individuals.[46] Overall, research on this topic has been impacted by the limitations of array-based and short-read technologies typically employed in the field, but that may soon change with the increasing implementation of long-read sequencing platforms.

Despite these challenges, AD research has advanced to the point that polygenic risk scores can be calculated with accuracy.[47] These metrics attempt to quantify how much disease risk is affected by one's genetic landscape. As mentioned above, most SNPs negligibly contribute to AD risk and have little predictive value on their own (note that *APOE* signals are treated outside of polygenic risk score frameworks).[47] But when multiple variants are assessed together, an individual's risk can be more confidently extrapolated. Polygenic risk scores are particularly useful when combined with other factors that affect disease risk. For example, models incorporating *APOE* status, polygenic risk scores, sex, and age reliably predict AD with 75%–85% accuracy.[48,49] Overall, compared to age or *APOE* status, the effect size of polygenic risk scores is relatively small. Still, their inclusion in risk models overall improves AD risk prediction by 1.6- to 1.9-fold, a modest but certainly not negligible increment.[11] While genetic profiling is not yet ready for widespread clinical use, there is hope that these metrics can gradually start to be implemented as a tool for assessing disease risk as well as defining treatment regimens and patient stratification in clinical trials. *APOE4* carriers have a higher risk of side effects caused by the recently approved aducanumab anti-amyloid therapy,[50] highlighting how genomics can inform therapeutic decisions. However, to deliver on this promise, ancestry diversity among study participants has to be improved to ensure that genetic research equally benefits all racial and ethnic groups.[51] These and other related questions have been the focus of a recent review on the genetic causes of AD.[12] Similarly of note, the Alzheimer's Disease Variant Portal (https://advp.niagads.org) is a curated, up-to-date collection of genetic association findings across >80 cohorts and 8 populations.[28]

Somatic genomic damage in AD neurons

The genetic variants linked to AD risk discussed in the previous section are inherited through the germline from one's parents and are present in all somatic cells of the body. However, as we age, DNA damage, including strand breaks and base modifications, occurs due to errors in DNA repair.[52] *De novo* mutations can also be triggered by by-products of cellular metabolism (e.g., genotoxic reactive oxygen species),[53] among other mechanisms. While DNA replication and cell division have traditionally been seen as major mutagenic forces,[54,55] recent evidence suggests that neurons accumulate mutations throughout life at similar rates as mitotically active cells.[56] Interestingly, neurons in AD accrue hundreds more somatic single-nucleotide variants (sSNVs) relative to the levels seen in normal aging (874 more mutation events, on average, per neuron, a 49% increase over control cells).[57] This observation suggests that there are additional DNA damage-driving processes at play in AD. For example, cytosine-to-adenine substitutions, an enriched sSNV in AD neurons,[57] are associated with oxidative damage,[58] which has been documented previously in AD.[59] The distribution of somatic variants in AD is also telling, in that sSNVs are found broadly dispersed across the genome.[57] Mechanistically, it is worth remarking that base excision repair, a pathway for removing damaged bases, is impaired in AD.[60] The genomic damage seen in AD may thus be a consequence of a higher mutational potential (i.e., oxidative burden), deficient DNA repair processes,[60] or a combination of both (Figure 4).

Other works shed light on the functional outcomes driven by somatic DNA changes. Links between loci with high accumulation of DNA damage and gene expression abnormalities have been documented,[62] whereas neuronal genome structural variations associated with DNA double-strand breaks aligned with transcriptional abnormalities have also been described.[61] While our discussion up until there focused on neuronal cells, a preprint using single-cell full-transcript RNA sequencing technology similarly points to an increased somatic mutational burden being present in glial cell types from AD individuals.[63] Tellingly, as in neurons, these somatic changes correlate with gene expression alterations.[63] Collectively, these studies suggest that dysregulated gene expression is influenced by genomic damage. This could happen via disruptions in gene regulatory elements, changes in 3D genome organization, or, as documented by Miller and colleagues, coding region alterations that essentially create genetic knockouts.[57] In light of the recently identified link between neuronal activity and DNA repair mechanisms in neurons,[64] it is also conceivable that synaptic dysfunction, a hallmark feature of AD decline,[2] has a compounding effect on genome integrity.

Epigenetic hallmarks

Until now, we have discussed the interplay between DNA polymorphisms and AD pathophysiology. However, genetics does not explain all phenotypic variance. Our environment and lifestyle interact with the genome through epigenetic mechanisms and impact key AD-related genes and pathways.[65] Various forms of epigenetic regulation exist, with DNA methylation and histone post-translational modifications being prominent paradigms discussed below.

DNA methylation

The first large-scale studies of epigenetic changes associated with AD centered on CpG methylation.[66,67] CpG sites are defined by two consecutive (5′ → 3′) cytosine and guanine nucleotides on the same strand of DNA, and many CpG sites clustered together at high frequency on a genomic stretch form "CpG islands," a typical feature of mammalian gene promoters. CpG sites are enriched in 5-methylcytosine (5mC), with up to 60%–90% of all CpG cytosines being methylated in mammalian genomes.[68] Interest by AD researchers in the 5mC modification can be understood from landmark reports likening certain DNA methylation signatures to an "epigenetic clock," in that they enable accurate age estimates across the entire lifespan of both mice and humans.[69] Links to learning and memory mechanisms are also well characterized.[70] A genome-wide analysis of differentially methylated DNA regions across hundreds of AD brain samples revealed a robust association between *ANK1 (*ankyrin 1) hypermethylation and AD-related neuropathology.[67] This observation was substantiated across multiple regions of the

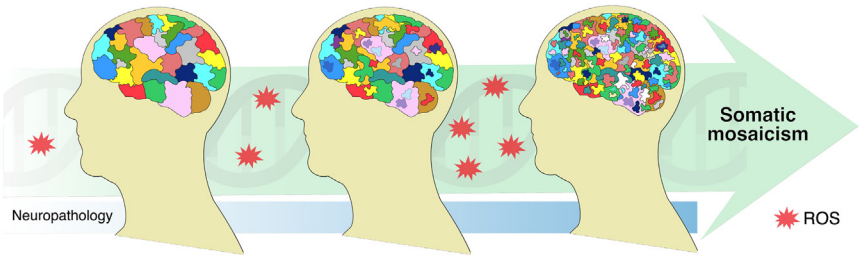

Figure 4. Somatic mosaicism in the AD brain

As we age, DNA damage accumulates due to oxidative stress and other factors. Building on top of previous studies, a recent high-resolution survey of genomic integrity in the brain revealed that, compared to control cells, mutational rates are higher in excitatory neurons from AD donors than in normal aging. The mutational signatures in AD neurons are also different, hinting that the underlying processes sparking these changes are unique to AD.[57] In addition to SNVs, which are discussed in detail in the main text, DNA double-strand breaks leading to gene fusions in AD excitatory neurons have also been reported recently.[61] It is speculated that increased oxidative damage related to AD pathology acts as a primary genotoxic trigger. ROS, reactive oxygen species.

cortex in independent sample cohorts, including the entorhinal cortex,[67] an early focal point of AD lesions.[71] Another report found 71 differentially methylated CpG sites significantly correlated with AD pathology.[66] In addition to *ANK1*, altered CpG methylation signals in two loci known to harbor AD susceptibility polymorphisms, *ABCA7* and *BIN1*, were detected in AD cohorts.[66] The authors also identified direct and indirect (i.e., association through common interactors) connections to various other AD genes,[66] consistent with the hypothesis that AD risk is determined by a combination of different sources of genomic variation (genetic and epigenetic). A subsequent study focusing on AD GWAS genes found that 3 other loci—*SORL1*, *HLA-DRB5*, and *SLC24A4*—have altered DNA methylation profiles in AD.[72] More recently, a meta-analysis of six independent DNA methylation datasets identified 220 differentially methylated CpGs linked to AD, annotatable to 121 genes.[73] Comparably, CpG methylation changes in the AD parahippocampal gyrus clustered around 270 genomic regions.[74] Most relevant perhaps was the authors' integration of these signals with various gene expression metrics generated from the same individuals.[74] Their model predicts that differential methylation explains, on average, 39% of the variance associated with protein expression in AD.[74] Overall, it is noteworthy that these studies unanimously report that AD-related DNA methylation patterns emerge in discrete chromosomal regions. It is therefore unlikely that they constitute a generalized (i.e., unspecific), genome-wide process. In a clear illustration of this phenomenon, genomic regions with AD-associated methylation patterns were less likely to be linked to genes actively transcribed in the healthy aging brain, suggesting that these changes are driven by AD-specific triggers.[66]

Histone modifications and chromatin accessibility

Differently to the relatively stable DNA methylation marks, histone modifications are more dynamically regulated, and, as a rule of thumb, thousands of differentially enriched domains are picked out in profiling studies centered on these signals. Of the various modifications known, histone acetylation has attracted most attention in AD research, not least given its links to cognition and the therapeutic promise of histone deacetylase inhibitors in preclinical models of AD.[75] Among these, the lysine H4K16 acetylation (H4K16ac) mark offers a good illustration of the importance of epigenetics for understanding aging biology, from old yeast cells to human disease.[76,77] In *S. cerevisiae*, Dang et at. identified an age-associated increase in H4K16ac at specific subtelomeric regions linked with dysfunctional transcriptional silencing at these loci.[77] Extending on these findings, comparative H4K16ac genome-wide analyses of AD individuals performed against both younger and age-matched cognitively normal controls by the same laboratory uncovered that, while normal aging predominantly leads to increases in H4K16ac, AD is associated with H4K16ac loss in the lateral temporal lobe, including in the proximity of AD susceptibility loci.[76] Broad dysregulation of lysine H3K27 acetylation (H3K27ac) in the AD entorhinal cortex has also been reported.[78] Of particular note, tau neuropathology, unlike β-amyloid, correlates with lysine H3K9 acetylation (H3K9ac) dysregulation in the AD dorsolateral prefrontal cortex, being linked to significant variation (both gains and losses) in up to 23% of all H3K9ac domains measured.[79] In contrast, tau-related H3K9ac alterations tend to cluster in large genomic segments covering several megabase pairs, indicating that tau pathology drives widespread chromatin remodeling in AD.[79] This is consistent with earlier evidence in *Drosophila* linking neuronal overexpression of tau to global heterochromatin relaxation.[80] The disease relevance of these analyses is further supported by an unbiased proteomics screen that singled out H3K27ac and H3K9ac enrichments as the main histone modifications specific to AD.[81]

Some studies are remarkable also for their push to turn the correlative epigenomic analyses at the core of their work into testable hypotheses. For instance, by manipulating histone modification levels, Nativio et al. showed that increased H3K27ac and H3K9ac levels worsened β-amyloid toxicity in flies,[81] whereas Klein and colleagues, in addition to confirming key aspects of their model in mouse models of tau pathology and induced pluripotent stem cell (iPSC)-derived human neurons, used their datasets to identify a small-molecule inhibitor that attenuates tau-related alterations of chromatin structure.[79] Despite important limitations, especially their reliance on bulk brain tissue preparations, these studies collectively underscore the complexity of the AD epigenome and its links to disease-associated transcriptional programs. Looking forward, single cell-resolved analyses, which are likely to become a mainstay of the field in the future,[82] will be key to finely dissect the role of histone modifications and other epigenetic events in AD.

Work by Roussos and colleagues is a major step forward in this direction and demonstrates the power of measuring cell-specific disease signatures.[83] Using fluorescence-activated sorting to initially separate neuronal ($NeuN^+$) and non-neuronal ($NeuN^-$) nuclei, the authors then resorted to assay for

transposase-accessible chromatin with sequencing (ATAC-seq) to produce genome-wide chromatin accessibility maps from AD and control brains. Overall, thousands of regulatory sequences were found to display disease-associated chromatin changes, including instances of cell-type- and/or brain-region-specific responses.[83] Building on the availability of RNA sequencing (RNA-seq) data from the same individuals, their analyses revealed that, globally, more than 70% of transcriptional variance can be explained by chromatin accessibility.[83] This inference implies that epigenetic mechanisms affecting chromatin structure are likely major determinants of AD-associated gene expression.

Technological developments have also allowed multi-omics analyses that combine single-nucleus RNA-seq (snRNA-seq) and single-nucleus ATAC-seq to probe cell-type-specific regulatory regions involved in AD-associated gene expression.[40,84] Here, different from previous efforts, where the two readouts were integrated from separate pipelines,[85] transcriptional changes and chromatin accessibility were captured from the same nuclei, increasing the likelihood of linking the two phenomena in a more relevant manner.[40,84] Particularly of note, using clinical and pathological measurements to stratify AD sufferers into early- and late-stage groups, Xiong et al. found that disease progression is associated with epigenomic erosion, a type of dysregulation marked by global shifts in chromatin accessibility and overall loss of cell identity.[40] They further observed that microglial enhancers are hotspots for AD GWAS hits, consistent with similar observations by other groups.[41,86]

The transcriptome at single-cell resolution

The first snRNA-seq survey of the embryonic human prefrontal cortex was followed soon after by the publication of two single-cell atlases of the AD brain.[38,39,87] The study by Grubman and colleagues is an important landmark because it profiled the entorhinal cortex,[39] one of the earliest regions affected by AD.[71] However, the substantially higher statistical power of Mathys et al. (80,660 nuclei across 48 individuals versus 13,214 nuclei from six control and six disease cases in Grubman et al.) contributed decisively to this resource becoming the most widely used. Like many previous bulk transcriptomic studies, the prefrontal cortex was the tissue of choice for their analyses.[38] The study design adopted by Mathys and coworkers is also notable for capturing more fully the pathological progression of AD. Specifically, β-amyloid levels were used for segregating individuals into early- and late-stage disease subgroups, revealing that transcriptional changes (both gene up- and downregulation) were strongest and highly cell-type-specific early on.[38] By contrast, as the disease advances, a generalized activation of proteostasis-related pathways and stress response genes was detectable in all cell types.[38] In addition, gene expression programs linked to AD pathology were substantially different between males and females, particularly in neurons and oligodendrocytes[38] (Figure 5A). Myelination-related processes were another key aspect of AD biology that emerged from their investigation.[38] They appeared recurrently as a top functional category not just in oligodendrocytes and oligodendrocyte precursor cells but in other cell types as well.[38] These concerted changes, also documented by Grubman et al., possibly indicate a compensatory response to myelin loss and/or a widespread "last-ditch" regulatory program aimed at maintaining myelin integrity.[38,39] Further work from Tsai's group has since uncovered a link between cholesterol dysregulation and impaired myelination in *APOE4* carriers (Figure 5B).[26] Substantial transcriptional differences between *APOE3* and *APOE4* genotypes correlated with cognitive impairment were also found enriched in cerebrovascular cells,[88] helping to explain, at the molecular level, how *APOE4*-dependent AD decline leads to brain vasculature dysregulation.[89]

Recent developments have addressed some of the limitations of earlier snRNA-seq resources, whose reduced sample sizes were insufficient to capture in full the clinicopathological and cellular heterogeneity of AD. Using a new computational framework, CelMod, an expanded cellular map of the AD neocortex was constructed from a snRNA-seq cohort of 24 individuals by exploiting an existing large-scale bulk RNA-seq database.[92] This analysis provided a robust and unprecedented glimpse into the diverse cell populations and coordinated cellular responses associated with AD, among which the detection of an overall decrease in somatostatin inhibitory neurons and the identification of two oligodendrocyte transcriptional states strongly linked with tau pathology and cognitive decline stand out as significant findings.[92] Of particular note, Mathys and colleagues recently produced the most comprehensive AD snRNA-seq atlas to date.[93] They sequenced 2.3 million prefrontal cortex nuclei from 427 Religious Orders Study and Rush Memory and Aging Project (ROSMAP) participants covering individuals with varying degrees of pathology and cognitive profiles.[93] Echoing the findings by Consens et al. and those obtained using CelMod,[92,94] this new dataset highlighted the particular vulnerability of somatostatin inhibitory neurons to AD pathology.[93] Differences in cell type composition were also evident in association with cognitive impairment: remarkably, two distinct subtypes of inhibitory neurons (Reelin [*RELN*]-positive LAMP5 neurons and the entire subclass of somatostatin-expressing GABAergic neurons) were overrepresented in individuals with the highest levels of global cognitive function.[93] At the molecular level, a coordinated increase in the expression of DNA damage response genes and cohesin, a ring-link protein complex that regulates chromatid cohesion, DNA repair, and other functions,[95] was observed across excitatory neurons subtypes and oligodendrocytes in individuals with high levels of AD pathology.[93]

Our understanding of AD microglia has perhaps benefitted the most from this push for more comprehensive single cell-resolved datasets. Despite important successes, including the isolation of live microglia from fresh surgical samples,[96] their low abundance in the brain and diverse cellular states has been challenging to circumvent. In an analysis of 443 subjects, including 217 AD patients with varying degrees of disease progression and 194,000 nuclei, Sun et al. provided the most complete profile yet of these cells in the human brain.[97] Interestingly, the authors identified a discrepancy between the rich diversity of microglia transcriptional states (12 in total) and the comparatively limited heterogeneity of these cells at the chromatin accessibility level (3 epigenomic states). The study also questions the significance of disease-associated microglia (DAMs) in AD. DAM states were initially identified in AD transgenic mice,[98] but these signatures were generally poorly enriched in the human brain.[97]

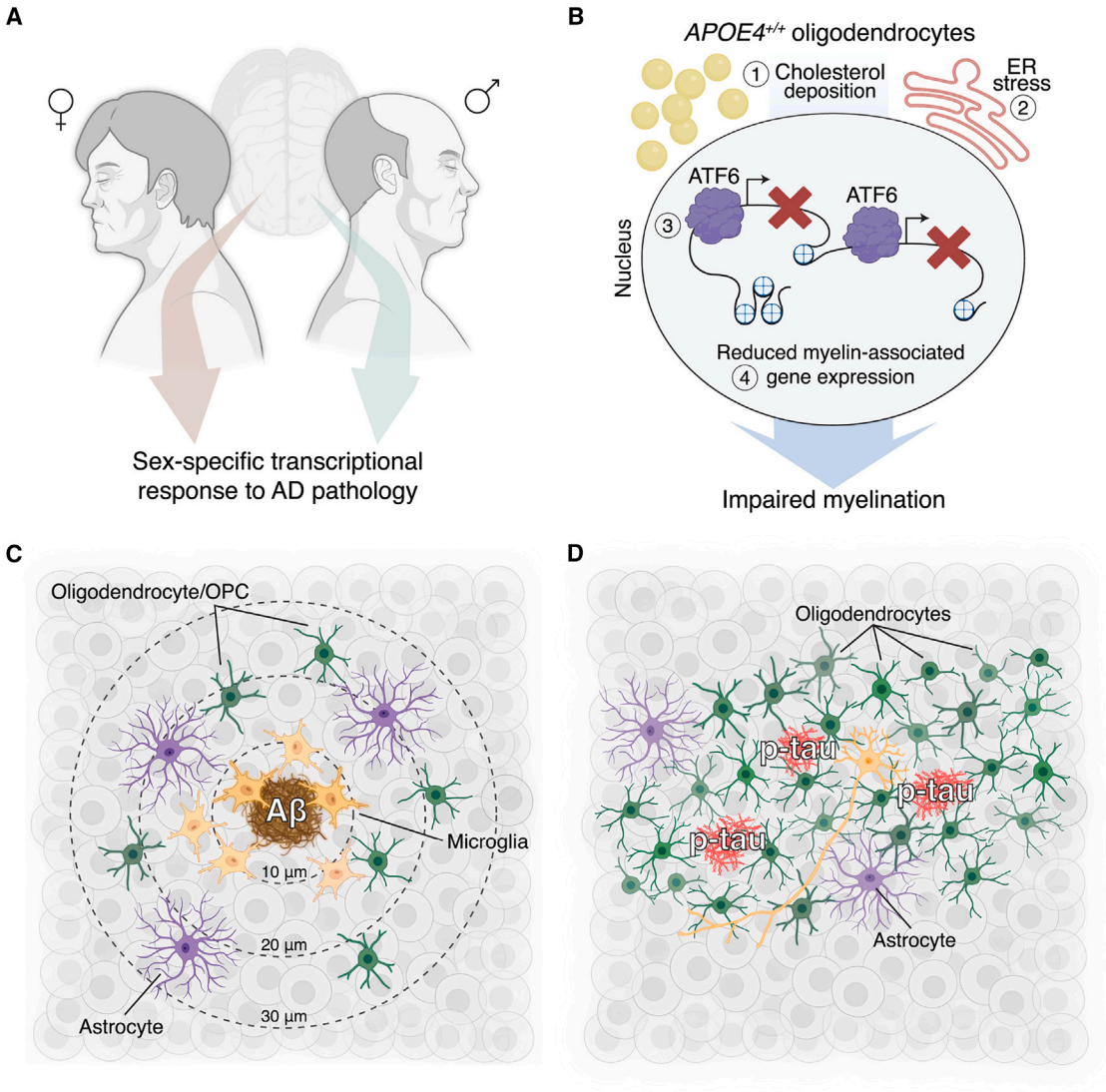

Figure 5. The AD transcriptome at scale

(A) It has long been known that women are disproportionately affected by AD. Investigations into gene expression patterns associated with the disease have revealed that this sexual dimorphism is evident also at the transcriptional level, with female and male individuals responding differentially to pathology. snRNA-seq analyses have made it possible to pinpoint these differences to specific cell types, with neurons and oligodendrocytes emerging as hotspots of sex-biased responses.[38]

(B) Single-nucleus transcriptional profiling has also been instrumental in furthering our understanding of the effects mediated by APOE4 on the human brain. This led researchers to zero in on cholesterol dyshomeostasis in oligodendrocytes as a root cause of myelination-related defects in *APOE4* carriers. Specifically, aberrant cholesterol deposition in *APOE4* oligodendrocytes leads to endoplasmic reticulum stress pathway activation and ATF6 translocation to the nucleus, a transcription factor known to mediate lipotoxic responses. While the underlying molecular mechanisms remain incompletely defined, the upregulation of cholesterol metabolism genes in *APOE4* oligodendrocytes coincides with decreased expression of myelin-associated genes and reduced overall myelination levels.[26]

(C and D) High-resolution spatial transcriptomics applied to the TauPS2APP animal model has revealed that Aβ plaques are surrounded in their immediate vicinity by a core shell structure of DAMs. Disease-associated astrocyte-like cells, oligodendrocytes, OPCs, and endothelial cells are found more distally, showing small but significant enrichments within 10–30 μm from plaques.[90] In contrast, hyperphosphorylated tau (p-tau), which is found primarily in hippocampal CA1 excitatory neurons, is associated with a localized enrichment of oligodendrocyte subtypes.[90] Interestingly, while both pathologies trigger oligodendrocyte reactivity, these analyses suggest that different subpopulations are recruited in response to amyloid and tau.[90] The finding that microglia accumulate around Aβ plaques is not new,[91] but methods like STARmap PLUS are opening doors to an unprecedented view of the molecular and cellular features related to AD neuropathology.

We feel compelled to highlight two additional reports. First, the transcriptomic profiling of AD neuronal somata bearing neurofibrillary tangles revealed with detail the molecular changes specifically associated with these lesions.[99] Among other findings, a common set of dysregulated genes related to synaptic transmission pathways were identified across various subtypes of neurons with tau pathology.[99] Activation of cellular stress-related genes was another shared feature among tangle-positive neurons.[99] Notably, *ATF4*, a central transcriptional effector of the integrated stress response,[100] was highly upregulated in these cells,[99] in line with recent observations linking ATF4-dependent transcription to tau pathology in neurons via its dimerization with another transcription factor, CREB3L2.[101] Second, given that synaptic dysfunction and loss are tightly correlated with cognitive impairment in AD,[2] the development of a droplet-based platform for the transcriptional profiling of individual synapses is particularly meaningful.[102] At the time of writing, the new methodology has only been applied to the study of an amyloidopathy mouse model; it was, however, validated to work with frozen human brain samples.[102] While the specificity of this new approach is currently under debate,[103] we are hopeful a catalog of AD-associated synaptic gene expression changes will be available to the community in the future.

Spatial transcriptomics: The new frontier

The emergence of high-throughput spatially resolved methods is revolutionizing our ability to query gene expression relationships between neighboring cells within tissues and their interaction with localized neuropathology.[104] "Spatial transcriptomics" was first used by AD researchers aiming to elucidate how gene expression is impacted in the immediate vicinity of β-amyloid plaques.[105] Initially investigated in a mouse model ($APP^{NL\text{-}G\text{-}F}$) and subsequently validated in human AD brains, two distinct gene co-expression networks reactive to β-amyloid deposition were discovered,[105] namely, an early response module activated under mild amyloid stress enriched in myelination-related genes and expressed primarily by oligodendrocytes, followed by a multicellular program involving complement and endo-lysosomal genes that develops gradually with advancing pathology.[105] Parenthetically, the finding that plaques trigger strong transcriptional effects in their vicinity rebukes suggestions that β-amyloid pathology plays a bystander role in the neurodegenerative cascade driving AD decline.[106] Another group expanded on these observations by also cataloging gene expression alterations proximal to tau aggregates in the human middle temporal gyrus.[107] Among other findings, overlapping but also dissimilar transcriptional responses were documented adjacent to β-amyloid and tau lesions.[107]

As remarkable as these findings are, the field of AD spatial transcriptomics is still in its infancy, not least because the small sample sizes in these studies preclude inferences about key AD covariates, such as sex, genotype, and subject age. The technologies backing the analyses described above also do not reach single-cell resolution and cannot acquire mRNA and protein signals from the same tissue section; rather, transcriptomic snapshots originate from thousands of circular tissue domains 55–100 μm in diameter, and neuropathology markers are stained in contiguous brain slices.[105,107] The recent development of STARmap PLUS partially addresses these limitations in that it affords subcellular spatial resolution with simultaneous detection of RNA and protein in the same tissue section (Figures 5C and 5D).[90] The trade-off is its current reduced genome coverage, particularly in the original implementation of the method.[108] Notably, when applied to the study of TauPS2APP mice, which display both amyloid and tau pathology, STARmap PLUS revealed that hippocampal tau lesions are strongly associated with the accumulation of three oligodendrocyte subtypes independent of local β-amyloid status.[90] The authors also found that a subpopulation of microglia transcriptionally reminiscent of DAMs establishes intimate contacts with β-amyloid plaques in early disease stages (Figure 5C).[90] The existence of DAMs, a protective subtype of microglia, had been first detected by snRNA-seq in transgenic AD mice,[98] underlining the fact that both methodologies should be seen as complementary. snRNA-seq is useful to characterize the global cellular heterogeneity of tissues; in turn, spatial transcriptomics brings to view gene expression relationships between cells and their surroundings. Recent work by Sadick al. provides a roadmap in this direction; by combining snRNA-seq with existing spatial transcriptomic resources, they pinpointed the location of AD-associated astrocyte subtypes to specific cortical layers.[109]

Diamond in the rough: AD brain proteomics

Historically, protein sequencing predates that of DNA and RNA.[110] That early start has since been overshadowed by the growth of genomics, powered by the development of PCR. The absence of amplification strategies for proteins and the complexity of these molecules help explain why the field of proteomics has lagged.[110,111] Regardless, proteins often act as the *de facto* biological effectors of the information stored in our genome and are thus of central importance to understanding cell phenotypes. While mRNA measurements are used as a proxy for protein levels, mRNA and protein profiles exhibit only partial consistency.[112] Observations in AD confirm this trend, with 40%–50% of protein co-expression modules with disease-associated changes not being seen in RNA networks derived from the same individuals.[113–115] This realization implies that not all gene expression changes involved in AD pathophysiology occur through mechanisms manifested at the mRNA level despite our substantially better account of the AD transcriptome. Ultimately, AD is a proteinopathy and, as such, has protein dysmetabolism at its core.[116]

A robust finding from proteomics surveys of the AD brain pertains to the high preservation of protein networks across different cortical regions.[117,118] This suggests an overlap in the biological processes and cell types contributing to the expression patterns observed in AD. Consistent with this idea, many of the protein network alterations associated with the disease appear to be linked to specific cell types and can sometimes show strong associations with neuropathology and other relevant clinical features.[117,119] For instance, in a recent large-scale proteomics study, a module related to mitogen-activated protein kinase (MAPK) signaling and metabolism was the most highly correlated with cognitive function.[115] By contrast, another set of co-expressed proteins, encompassing a collection of extracellular matrix-related genes, was shown to be influenced by

APOE4.[115] This effect was, however, independent of the cognitive trajectory.[115] Revealingly, neither of these modules was preserved at the mRNA level, underscoring the value of considering the proteome for understanding AD pathophysiology.[115] The implication of altered MAPK signaling in AD pathogenesis is supported by Bai et al. using a different cohort of patients.[119] Their work is also noteworthy for its stratification of cases across the AD clinicopathological continuum, in addition to whole proteome and phosphoproteome profiling.[119] In this vein, evidence from a proteomics study focusing specifically on resilience to AD implicates actin filament-based processes and injury responses as core molecular features of resilience.[120] In relation to potential cell-type-specific programs, an earlier study of >2,000 brains identified a module linked to glial sugar metabolism suggested to function as part of an anti-inflammatory response.[117] These analyses tentatively pinpoint astrocytes and microglia as the source of this co-expressed protein network.[117] As proteomic methodologies with single-cell resolution are still not widespread, weighted gene correlation network analysis (WGCNA) algorithms are now being used to cluster and simplify data from large-scale proteomics experiments. Simply put, WGCNA algorithms are used to contextualize proteomics readouts via computational inferences. In other words, without additional validations, presently available proteome-wide profiles cannot yet be ascribed to a particular cell type with absolute confidence. This outlook may soon change, as protein imaging approaches with multiplex potential have recently been described.[121,122] While proteome coverage is nowhere near that afforded by conventional mass spectrometry-based protocols, these technologies boast the kind of detail needed to visualize gene expression at the protein level *in situ* in the diseased brain.

Toward a unified view of AD

No matter how rich in detail, none of the modalities surveyed above paint a complete picture on their own. From genetics to proteomics, all biological layers contribute linked pieces of evidence. However, when considered separately, each approach falls short of fully capturing the processes that affect AD, resulting in potential missed insights and misleading signals. The demand for integrative analyses that merge different types of data into a common framework is demonstrated by the finding that RNA and protein networks are not aligned in AD.[115] We have also underscored how efforts to interpret genetic variants related to AD risk have gained momentum since the emergence of single-cell transcriptomic and epigenomic resources,[27] among other synergies. Except for AD genetics, we are, in essence, probing different facets of the same question. Ultimately, after many landmark contributions and significant investment, we must maximize what can be learned from existing omics catalogs. This goes beyond bringing together different molecular readouts, as the coherent incorporation of these with clinical, epidemiological, and brain imaging datasets is bound to further enrich our ability to capture sources of intra- and interindividual variability within the heterogeneous AD landscape.

Implementations of this vision are already underway. The ROSMAP project, which is part of the wider Accelerating Medicines Partnership-Alzheimer's Disease consortium, has emerged as a particularly amenable platform for multilevel integration strategies. The ROSMAP cohort combines two large, decades-long studies of aging and dementia launched in the 1990s, comprising annual psychological and clinical evaluations, including blood draws, plus harmonized neuropathological examination after death. The cross-modality characterization of participants has since allowed specific molecular and cellular responses to be connected to AD endophenotypes, such as cognitive decline and neuropathology features.[92,123] The power of this effort is epitomized by the recent back-to-back publication of four landmark papers using the ROSMAP cohort.[40,61,93,97] Through their integrative analyses that correlate detailed genomic profiles with clinicopathological features, the authors achieved what is a transformative multiscale resource poised to shape AD research for years to come.[40,61,93,97] The Seattle Alzheimer's Disease Cell Atlas (SEA-AD; available through SEA-AD.org) is also emerging as a landmark for the field.[124] Drawing on prior knowledge in the BRAIN Initiative Cell Census Network, SEA-AD combines single-cell profiling and spatial genomics with image-based quantitative neuropathology scoring schemes and deep clinical phenotyping. In a preprint,[124] the SEA-AD consortium reports, among other observations, that a subset of GABAergic interneurons are impacted in AD, corroborating independent findings from other patient cohorts.[92–94] They advance earlier evidence by pinpointing these affected cell subtypes predominantly to upper cortical layers.[124] Another recent approach has been to look for patterns across epigenomics, transcriptomics, proteomics, and metabolomics data to differentiate AD subtypes at the molecular level.[125] Using the postmortem brain as a starting point, three robust AD subtypes were identified, with the first characterized by metabolic alterations and the other two presenting unique RNA and epigenetic signatures.[125] Critically, this multidimensional classification index of AD was extrapolatable to blood samples,[125] opening doors to potential future utilization of minimally invasive molecular profiling as a clinical tool. In the same vein, Yang et al. jointly leveraged bulk RNA-seq, DNA methylation, histone acetylation, proteomics, and metabolomics datasets in the same model in their attempt to better stratify aging individuals at the molecular level.[126] Of these data modalities, histone acetylation, DNA methylation, and RNA abundance were found to be the most useful metrics in capturing cognitive trajectories.[126] Methods for cross-querying brain-imaging biomarkers (e.g., hippocampal volume) and molecular readouts are also in development. For example, the Alzheimer's Disease Neuroimaging Initiative has recently proposed a comprehensive strategy to integrate genetic and transcriptomic measurements with brain structural magnetic resonance imaging.[127] A different approach altogether has been the application of deep learning tools to infer disease-associated protein changes linked to AD clinical features from transcriptomic profiles.[113]

Genomic biomarkers

The clinical and pathological presentation of AD is highly heterogeneous, being influenced by interactions between genotype, environment, cognitive reserve, and a range of demographic factors, among other determinants. Besides β-amyloid and tau, which capture only a portion of the biological

mechanisms underlying AD, there is a growing appreciation for the co-occurrence of cerebrovascular disease and other concurrent pathologies,[128] complicating reductionist attempts to quantify heterogeneity among patients based on traditional metrics (e.g., β-amyloid 42 [$A\beta_{42}$]/$A\beta_{40}$ ratios or phospho-tau levels). Understandably, the search for novel biomarkers continues to be a major focus of investigation.

More than any other "omics" field, proteomics has been extensively deployed as a systems-based approach to biomarker discovery. While blood-based analyses have also been performed and offer undeniable advantages in clinical settings due to their less invasive nature,[129] cerebrospinal fluid (CSF) remains a promising option for biomarker studies in AD research based on its association with the brain's biochemical milieu.[130] Consistent with this idea, the degree of correspondence between CSF and brain proteomes in AD is indeed substantial.[114,117] For example, in the most comprehensive study to date, 15 of the 44 protein modules identified in the brain were conserved in the CSF.[114] Further analyses grouped these overlapping modules into five distinct biomarker panels reflecting various pathophysiological processes associated with AD, including synaptic, vascular, glial, and metabolic dysfunction.[114] Interestingly, glial-enriched myelination and immunity panels were increased in both brain and CSF proteomes, whereas those linked to synaptic and metabolic function displayed opposing trends, being decreased in the brain of individuals with AD but elevated in the CSF. By contrast, despite marginal increases in the AD brain, proteins in the vascular panel exhibited a sharp decline in CSF.[114]

Non-coding RNAs (ncRNAs) constitute upward of 90% of all the RNAs made from the human genome, yet their importance was largely unrecognized until recently. The intricacies of each family of ncRNAs are outside of the purview of this text, and we choose instead to highlight here recent investigations employing CSF ncRNAs signatures as biomarkers of AD pathology.[131] Interestingly, a combined set of three microRNAs (miRNAs) (miR-27a-3p, miR-30a-5p, and miR-34c, all increased in AD) and three PIWI-interacting RNAs (piRNAs; piR_019324 [decreased] and piR_019949 and piR_020364 [both increased]) adequately detects AD and is also suitable for predicting the conversion of mild cognitive impairment to AD dementia.[131] When considered together with phospho-tau and $A\beta_{42/40}$ ratio measures, this miRNA-piRNA signature performed even better, achieving striking area under the curve (AUC) values of 0.98, "diagnosing" AD.[131] In a first, Wingo et al. developed an integrative approach to define new molecular players underlying variation in cognitive trajectory (rate of change in cognitive performance over time) using global brain miRNA profiles as a starting point.[132] Among these, two miRNAs, miR-132-3p and miR-29a-3p, emerged as particularly significant factors in determining cognitive trajectories. The findings further indicate that both miRNAs influence cognitive trajectories, at least to some extent, independent of various common age-related pathologies, including Aβ and neurofibrillary tangles.[132]

Much remains to be done to fully connect complex biomarker profiles to specific disease trajectories. In any case, these efforts already represent a significant departure from our reliance on hallmark pathology markers as measures of AD states. Ultimately, they should allow for better stratification of patients, paving the way for improved therapeutic interventions and early disease monitoring.

Genomics-driven drug development

Besides helping shape much of the discourse on the molecular and cellular underpinnings of AD, genomics promises to unlock new treatment avenues. To begin with, drug targets backed by genetic evidence are twice as likely to lead to approved therapies.[133] In a field marred by failures despite enormous investment by governments and industry, these improved odds of success are non-negligible. With about 75 loci currently associated with AD risk,[11–13] GWASs have undoubtedly changed the playing field. Anti-amyloid therapies do continue to garner attention, but pharmaceutical development pipelines are diversifying.[134] These include apoE/lipid metabolism and the endo-lysosomal network, two pathways whose involvement in AD pathophysiology finds direct support in the genetic etiology of sporadic AD.[134] Treatment modalities are also expanding; for example, a tau-targeting antisense oligonucleotide reduced total tau CSF levels in a phase 1b trial in adults with mild AD.[135] A small interfering RNA therapeutic targeting *APP* mRNA is also in development (ClinicalTrials.gov: NCT05231785). In light of this wider landscape, it is worth stressing that the conceptual view that prevailed in the past three decades—the amyloid cascade hypothesis—was fueled by a series of genetic findings.[136] Back then, the assumption was that late-onset AD shared essentially the same pathogenic mechanism as familial forms of the condition, albeit on a slower time frame.[136] The pathobiology of sporadic AD proved to be more nuanced than the simplicity those genetic discoveries suggested, and we are today in a better position to implement drug development strategies informed by genetic and genomic data.

Most academic laboratories lack the resources and expertise to develop novel drugs from the ground up. Therefore, we see value in exploring an alternative strategy that democratizes the use of existing genomic datasets. It combines drug repurposing with an ever-growing initiative by the Broad Institute and the Library of Integrated Network-based Cellular Signatures (LINCS) project that has characterized, to date, more than 1.5 million gene expression profiles for a range of drugs and other perturbations.[137,138] A key feature of this platform, named the Connectivity Map (CMap; https://clue.io), is allowing users to compare disease-linked gene expression changes against drug-induced transcriptomic patterns to identify prospective therapeutic compounds based on high dissimilarity scores. It discovers molecules with the potential to counteract disease-associated transcriptional responses and normalize gene expression. At the other end of the spectrum, it can also pick out drugs that mimic specific protective transcriptional programs as candidates for bolstering resilience to disease. Unlike traditional approaches to drug development, which focus on single-target or single-pathway interventions, CMap is agnostic to the underlying pathobiology and operates under no mechanistic preconceptions. Rather, by focusing on an unbiased measure, gene expression, CMap circumvents our incomplete understanding of complex diseases such as AD and provides a way of achieving wide-reaching cellular outcomes.

Several successful implementations of CMap in preclinical AD studies have already been reported.[79,101,139] The study conducted by Williams et al. represents an important step forward in overcoming a major hurdle posed by CMap for repurposing efforts in AD.[139] Specifically, they addressed the platform's current reliance on perturbation-induced transcriptional profiles originating from cell lines with limited disease relevance. Their approach began with mining drug candidates predicted by CMap to oppose different aspects of the AD transcriptional landscape, including early- and late-stage disease responses. These compounds were then screened in iPSC-derived human cortical neurons using microarrays. Remarkably, of an initial set of 153 compounds identified by CMap, 51 produced desirable transcriptional outcomes in neuronal cultures. Enrichment analyses further revealed that the best-performing interventions frequently upregulated mitochondrial processes, consistent with the well-established role of mitochondrial dysfunction in AD pathogenesis.[139] In addition, an alternative strategy has been to focus on how individual transcriptional pathways are modulated in disease and explore candidate drugs based on those activities.[101] It is similarly noteworthy that AD-related epigenomic signatures have been shown to be a viable input for CMap.[79] As discussed above, tau pathology is associated with broad changes in H3K9ac domains and chromatin organization.[79] This knowledge informed the subsequent identification of a heat shock protein inhibitor that attenuates tau-induced chromatin rearrangements.[79]

Alternative approaches to drug repurposing targeting gene expression have also been explored.[140] DRIAD (Drug Repurposing in AD) is a machine learning framework that quantifies associations between pathological stages and any biological process that can be described by a list of genes.[140] It was trained on two types of transcriptomic data, AD brain profiles stratified by Braak scores and drug-elicited responses measured in neuron-glia cocultures. The initial cohort of chemical entities tested by DRIAD comprised 80 kinase inhibitors, many of which are US Food and Drug Administration-approved molecules. Analysis of top-ranked drugs revealed some interesting trends, including prioritization of innate immunity, autophagy, and microtubule dynamics modulators[140] as well as molecules also highlighted by CMap.[101]

Globally, the concept of drug repurposing for AD has received significant attention, with 28% of the 187 ongoing clinical trials involving repurposed molecules.[141] These agents exhibit higher success rates than new chemical entities and, overall, offer a cost-effective approach to expedite the development of treatments. Lamivudine, a common antiretroviral medication used to treat human immunodeficiency virus (HIV) and hepatitis B virus (HBV) infections, is a relevant case in point.[141] Currently in phase 2 (ClinicalTrials.gov: NCT04552795), this inhibitor has been shown to interfere with age-associated inflammation mediated by retrotransposon activation.[142] While much remains unknown regarding how DNA transposable elements contribute to AD neurodegeneration, lamivudine illustrates what there is to be gained by learning about our genome. That a drug listed by the World Health Organization as an essential medicine for priority conditions may one day be used to treat another urgent human health crisis would have been nothing less than a remarkable exercise in foresight.

Outlook

How do we effectively utilize the vast wealth of genomics data currently at our disposal? On the one hand, the complex and interconnected nature of the genome necessitates approaches that integrate data modalities into a unified framework. However, while the field has benefitted from recent technological advances in genomics, we must recognize that the availability of data does not equate to proportional increments in knowledge. Such large-scale ventures are futile without proper context and careful interpretation. The focus should be the pursuit of pathophysiological insights and their translation into consequential outcomes for patients. Improved study designs are generally warranted to capture the heterogeneity of AD. Indeed, despite the fact that two-thirds of AD cases are women (US estimates), genomic research has generally failed to address these differences. We have extensively discussed the findings by Mathys et al.,[38] but two additional studies deserve acknowledgment for their landmark contributions. The first report documented how menopause-related changes in follicle-stimulating hormone levels are tied to worse disease phenotypes in a mouse model of AD via upregulation of the transcription factor C/EBPβ.[143] In the same vein, Yan and colleagues found that a deubiquitinase located on chromosome X, USP11, escapes X inactivation and leads to compromised tau homeostasis in women.[144] Sex-specific genetic associations have also been identified, such as an intergenic variant linked to *TSPAN13* that protects against tau pathology in male carriers.[145] These works provide clarity on the sexual dimorphism of AD, which we hope will inspire further research. AD also disproportionately impacts certain racial and ethnic populations, such as Black and Hispanic adults,[146] but the specificities within their genomes remain understudied. Efforts such as the Health & Aging Brain among Latino Elders, the Washington Heights-Hamilton Heights-Inwood Community Aging Project, the National Alzheimer's Coordinating Center retention drive of American Indian and Alaska Native participants,[147] and the recent initiative targeting Asian Americans and Asian Canadians are examples of initiatives to address this gap.[148–150] Considering that the genetic interactions that influence disease onset and progression might differ, we further see potential in attempts to disambiguate between genes that act early on from those that shape the ensuing disease cascade. Doing so is key to prioritizing targets for drug discovery and expanding the predictive value of genetic results in the clinic. Ultimately, and paraphrasing John von Neumann, it might be that AD "is much too complicated to allow anything but approximations." Still, the genomic readouts we have today constitute our clearest shot at unraveling this multilayered disorder. The promise is real, and the technology is at our fingertips. It is now up to us as a community to make it happen.

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AUTHOR CONTRIBUTIONS

C.G.R. conceived the project, performed literature searches, and drafted the manuscript. H.P. and U.H. provided feedback, guidance, and research support. All authors reviewed and edited the manuscript before submission.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Long, J.M., and Holtzman, D.M. (2019). Alzheimer Disease: An Update on Pathobiology and Treatment Strategies. Cell *179*, 312–339. https://doi.org/10.1016/j.cell.2019.09.001.
2. Tzioras, M., McGeachan, R.I., Durrant, C.S., and Spires-Jones, T.L. (2023). Synaptic degeneration in Alzheimer disease. Nat. Rev. Neurol. *19*, 19–38. https://doi.org/10.1038/s41582-022-00749-z.
3. Nakamura, A., Kaneko, N., Villemagne, V.L., Kato, T., Doecke, J., Doré, V., Fowler, C., Li, Q.X., Martins, R., Rowe, C., et al. (2018). High performance plasma amyloid-beta biomarkers for Alzheimer's disease. Nature *554*, 249–254. https://doi.org/10.1038/nature25456.
4. De Strooper, B., and Karran, E. (2016). The Cellular Phase of Alzheimer's Disease. Cell *164*, 603–615. https://doi.org/10.1016/j.cell.2015.12.056.
5. Musiek, E.S., and Bennett, D.A. (2021). Aducanumab and the "post-amyloid" era of Alzheimer research? Neuron *109*, 3045–3047. https://doi.org/10.1016/j.neuron.2021.09.007.
6. Gatz, M., Reynolds, C.A., Fratiglioni, L., Johansson, B., Mortimer, J.A., Berg, S., Fiske, A., and Pedersen, N.L. (2006). Role of genes and environments for explaining Alzheimer disease. Arch. Gen. Psychiatry *63*, 168–174. https://doi.org/10.1001/archpsyc.63.2.168.
7. Jansen, I.E., Savage, J.E., Watanabe, K., Bryois, J., Williams, D.M., Steinberg, S., Sealock, J., Karlsson, I.K., Hägg, S., Athanasiu, L., et al. (2019). Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer's disease risk. Nat. Genet. *51*, 404–413. https://doi.org/10.1038/s41588-018-0311-9.
8. Marioni, R.E., Harris, S.E., Zhang, Q., McRae, A.F., Hagenaars, S.P., Hill, W.D., Davies, G., Ritchie, C.W., Gale, C.R., Starr, J.M., et al. (2018). GWAS on family history of Alzheimer's disease. Transl. Psychiatry *8*, 99. https://doi.org/10.1038/s41398-018-0150-6.
9. Kunkle, B.W., Grenier-Boley, B., Sims, R., Bis, J.C., Damotte, V., Naj, A.C., Boland, A., Vronskaya, M., van der Lee, S.J., Amlie-Wolf, A., et al. (2019). Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Abeta, tau, immunity and lipid processing. Nat. Genet. *51*, 414–430. https://doi.org/10.1038/s41588-019-0358-2.
10. Andrews, S.J., Fulton-Howard, B., and Goate, A. (2020). Interpretation of risk loci from genome-wide association studies of Alzheimer's disease. Lancet Neurol. *19*, 326–335. https://doi.org/10.1016/S1474-4422(19)30435-1.
11. Bellenguez, C., Küçükali, F., Jansen, I.E., Kleineidam, L., Moreno-Grau, S., Amin, N., Naj, A.C., Campos-Martin, R., Grenier-Boley, B., Andrade, V., et al. (2022). New insights into the genetic etiology of Alzheimer's disease and related dementias. Nat. Genet. *54*, 412–436. https://doi.org/10.1038/s41588-022-01024-z.
12. Reitz, C., Pericak-Vance, M.A., Foroud, T., and Mayeux, R. (2023). A global view of the genetic basis of Alzheimer disease. Nat. Rev. Neurol. *19*, 261–277. https://doi.org/10.1038/s41582-023-00789-z.
13. Lambert, J.C., Ibrahim-Verbaas, C.A., Harold, D., Naj, A.C., Sims, R., Bellenguez, C., DeStafano, A.L., Bis, J.C., Beecham, G.W., Grenier-Boley, B., et al. (2013). Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. Nat. Genet. *45*, 1452–1458. https://doi.org/10.1038/ng.2802.
14. Bandres-Ciga, S., Diez-Fairen, M., Kim, J.J., and Singleton, A.B. (2020). Genetics of Parkinson's disease: An introspection of its journey towards precision medicine. Neurobiol. Dis. *137*, 104782. https://doi.org/10.1016/j.nbd.2020.104782.
15. Hsu, S., Gordon, B.A., Hornbeck, R., Norton, J.B., Levitch, D., Louden, A., Ziegemeier, E., Laforce, R., Jr., Chhatwal, J., Day, G.S., et al. (2018). Discovery and validation of autosomal dominant Alzheimer's disease mutations. Alzheimer's Res. Ther. *10*, 67. https://doi.org/10.1186/s13195-018-0392-9.
16. Scheltens, P., De Strooper, B., Kivipelto, M., Holstege, H., Chételat, G., Teunissen, C.E., Cummings, J., and van der Flier, W.M. (2021). Alzheimer's disease. Lancet *397*, 1577–1590. https://doi.org/10.1016/S0140-6736(20)32205-4.
17. Genin, E., Hannequin, D., Wallon, D., Sleegers, K., Hiltunen, M., Combarros, O., Bullido, M.J., Engelborghs, S., De Deyn, P., Berr, C., et al. (2011). APOE and Alzheimer disease: a major gene with semi-dominant inheritance. Mol. Psychiatry *16*, 903–907. https://doi.org/10.1038/mp.2011.52.
18. Reiman, E.M., Arboleda-Velasquez, J.F., Quiroz, Y.T., Huentelman, M.J., Beach, T.G., Caselli, R.J., Chen, Y., Su, Y., Myers, A.J., Hardy, J., et al. (2020). Exceptionally low likelihood of Alzheimer's dementia in APOE2 homozygotes from a 5,000-person neuropathological study. Nat. Commun. *11*, 667. https://doi.org/10.1038/s41467-019-14279-8.
19. Castellano, J.M., Kim, J., Stewart, F.R., Jiang, H., DeMattos, R.B., Patterson, B.W., Fagan, A.M., Morris, J.C., Mawuenyega, K.G., Cruchaga, C., et al. (2011). Human apoE isoforms differentially regulate brain amyloid-beta peptide clearance. Sci. Transl. Med. *3*, 89ra57. https://doi.org/10.1126/scitranslmed.3002156.
20. Holtzman, D.M., Herz, J., and Bu, G. (2012). Apolipoprotein E and apolipoprotein E receptors: normal biology and roles in Alzheimer disease. Cold Spring Harb. Perspect. Med. *2*, a006312. https://doi.org/10.1101/cshperspect.a006312.
21. Corder, E.H., Saunders, A.M., Strittmatter, W.J., Schmechel, D.E., Gaskell, P.C., Small, G.W., Roses, A.D., Haines, J.L., and Pericak-Vance, M.A. (1993). Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science *261*, 921–923. https://doi.org/10.1126/science.8346443.
22. Crean, S., Ward, A., Mercaldi, C.J., Collins, J.M., Cook, M.N., Baker, N.L., and Arrighi, H.M. (2011). Apolipoprotein E epsilon4 prevalence in Alzheimer's disease patients varies across global populations: a systematic literature review and meta-analysis. Dement. Geriatr. Cogn. Disord *31*, 20–30. https://doi.org/10.1159/000321984.
23. Bras, J., Guerreiro, R., Darwent, L., Parkkinen, L., Ansorge, O., Escott-Price, V., Hernandez, D.G., Nalls, M.A., Clark, L.N., Honig, L.S., et al. (2014). Genetic analysis implicates APOE, SNCA and suggests lysosomal dysfunction in the etiology of dementia with Lewy bodies. Hum. Mol. Genet. *23*, 6139–6146. https://doi.org/10.1093/hmg/ddu334.
24. Tsuang, D., Leverenz, J.B., Lopez, O.L., Hamilton, R.L., Bennett, D.A., Schneider, J.A., Buchman, A.S., Larson, E.B., Crane, P.K., Kaye, J.A., et al. (2013). APOE epsilon4 increases risk for dementia in pure synucleinopathies. JAMA Neurol. *70*, 223–228. https://doi.org/10.1001/jamaneurol.2013.600.
25. Schiepers, O.J.G., Harris, S.E., Gow, A.J., Pattie, A., Brett, C.E., Starr, J.M., and Deary, I.J. (2012). APOE E4 status predicts age-related cognitive decline in the ninth decade: longitudinal follow-up of the Lothian Birth Cohort 1921. Mol. Psychiatry *17*, 315–324. https://doi.org/10.1038/mp.2010.137.
26. Blanchard, J.W., Akay, L.A., Davila-Velderrain, J., von Maydell, D., Mathys, H., Davidson, S.M., Effenberger, A., Chen, C.Y., Maner-Smith, K., Hajjar, I., et al. (2022). APOE4 impairs myelination via cholesterol dysregulation in oligodendrocytes. Nature *611*, 769–779. https://doi.org/10.1038/s41586-022-05439-w.

27. Yang, A.C., Vest, R.T., Kern, F., Lee, D.P., Agam, M., Maat, C.A., Losada, P.M., Chen, M.B., Schaum, N., Khoury, N., et al. (2022). A human brain vascular atlas reveals diverse mediators of Alzheimer's risk. Nature *603*, 885–892. https://doi.org/10.1038/s41586-021-04369-3.

28. Kuksa, P.P., Liu, C.L., Fu, W., Qu, L., Zhao, Y., Katanic, Z., Clark, K., Kuzma, A.B., Ho, P.C., Tzeng, K.T., et al. (2022). Alzheimer's Disease Variant Portal: A Catalog of Genetic Findings for Alzheimer's Disease. J. Alzheimers Dis. *86*, 461–477. https://doi.org/10.3233/JAD-215055.

29. Frydas, A., Wauters, E., van der Zee, J., and Van Broeckhoven, C. (2022). Uncovering the impact of noncoding variants in neurodegenerative brain diseases. Trends Genet. *38*, 258–272. https://doi.org/10.1016/j.tig.2021.08.010.

30. Brodie, A., Azaria, J.R., and Ofran, Y. (2016). How far from the SNP may the causative genes be? Nucleic Acids Res. *44*, 6046–6054. https://doi.org/10.1093/nar/gkw500.

31. Visel, A., Rubin, E.M., and Pennacchio, L.A. (2009). Genomic views of distant-acting enhancers. Nature *461*, 199–205. https://doi.org/10.1038/nature08451.

32. Raj, T., Li, Y.I., Wong, G., Humphrey, J., Wang, M., Ramdhani, S., Wang, Y.C., Ng, B., Gupta, I., Haroutunian, V., et al. (2018). Integrative transcriptome analyses of the aging brain implicate altered splicing in Alzheimer's disease susceptibility. Nat. Genet. *50*, 1584–1592. https://doi.org/10.1038/s41588-018-0238-1.

33. Zhou, Y., Zhou, B., Pache, L., Chang, M., Khodabakhshi, A.H., Tanaseichuk, O., Benner, C., and Chanda, S.K. (2019). Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nat. Commun. *10*, 1523. https://doi.org/10.1038/s41467-019-09234-6.

34. Koopmans, F., van Nierop, P., Andres-Alonso, M., Byrnes, A., Cijsouw, T., Coba, M.P., Cornelisse, L.N., Farrell, R.J., Goldschmidt, H.L., Howrigan, D.P., et al. (2019). SynGO: An Evidence-Based, Expert-Curated Knowledge Base for the Synapse. Neuron *103*, 217–234.e4. https://doi.org/10.1016/j.neuron.2019.05.002.

35. Andrews, S.J., Renton, A.E., Fulton-Howard, B., Podlesny-Drabiniok, A., Marcora, E., and Goate, A.M. (2023). The complex genetic architecture of Alzheimer's disease: novel insights and future directions. EBioMedicine *90*, 104511. https://doi.org/10.1016/j.ebiom.2023.104511.

36. Hekselman, I., and Yeger-Lotem, E. (2020). Mechanisms of tissue and cell-type specificity in heritable traits and diseases. Nat. Rev. Genet. *21*, 137–150. https://doi.org/10.1038/s41576-019-0200-9.

37. Borsari, B., Villegas-Mirón, P., Pérez-Lluch, S., Turpin, I., Laayouni, H., Segarra-Casas, A., Bertranpetit, J., Guigó, R., and Acosta, S. (2021). Enhancers with tissue-specific activity are enriched in intronic regions. Genome Res. *31*, 1325–1336. https://doi.org/10.1101/gr.270371.120.

38. Mathys, H., Davila-Velderrain, J., Peng, Z., Gao, F., Mohammadi, S., Young, J.Z., Menon, M., He, L., Abdurrob, F., Jiang, X., et al. (2019). Single-cell transcriptomic analysis of Alzheimer's disease. Nature *570*, 332–337. https://doi.org/10.1038/s41586-019-1195-2.

39. Grubman, A., Chew, G., Ouyang, J.F., Sun, G., Choo, X.Y., McLean, C., Simmons, R.K., Buckberry, S., Vargas-Landin, D.B., Poppe, D., et al. (2019). A single-cell atlas of entorhinal cortex from individuals with Alzheimer's disease reveals cell-type-specific gene expression regulation. Nat. Neurosci. *22*, 2087–2097. https://doi.org/10.1038/s41593-019-0539-4.

40. Xiong, X., James, B.T., Boix, C.A., Park, Y.P., Galani, K., Victor, M.B., Sun, N., Hou, L., Ho, L.L., Mantero, J., et al. (2023). Epigenomic dissection of Alzheimer's disease pinpoints causal variants and reveals epigenome erosion. Cell *186*, 4422–4437.e21. https://doi.org/10.1016/j.cell.2023.08.040.

41. Cooper, Y.A., Teyssier, N., Dräger, N.M., Guo, Q., Davis, J.E., Sattler, S.M., Yang, Z., Patel, A., Wu, S., Kosuri, S., et al. (2022). Functional regulatory variants implicate distinct transcriptional networks in dementia. Science *377*, eabi8654. https://doi.org/10.1126/science.abi8654.

42. Khani, M., Gibbons, E., Bras, J., and Guerreiro, R. (2022). Challenge accepted: uncovering the role of rare genetic variants in Alzheimer's disease. Mol. Neurodegener. *17*, 3. https://doi.org/10.1186/s13024-021-00505-9.

43. Wang, H., Wang, L.S., Schellenberg, G., and Lee, W.P. (2022). The role of structural variations in Alzheimer's disease and other neurodegenerative diseases. Front. Aging Neurosci. *14*, 1073905. https://doi.org/10.3389/fnagi.2022.1073905.

44. Carmona, S., Zahs, K., Wu, E., Dakin, K., Bras, J., and Guerreiro, R. (2018). The role of TREM2 in Alzheimer's disease and other neurodegenerative disorders. Lancet Neurol. *17*, 721–730. https://doi.org/10.1016/S1474-4422(18)30232-1.

45. Holstege, H., Hulsman, M., Charbonnier, C., Grenier-Boley, B., Quenez, O., Grozeva, D., van Rooij, J.G.J., Sims, R., Ahmad, S., Amin, N., et al. (2022). Exome sequencing identifies rare damaging variants in ATP8B4 and ABCA1 as risk factors for Alzheimer's disease. Nat. Genet. *54*, 1786–1794. https://doi.org/10.1038/s41588-022-01208-7.

46. Rovelet-Lecrux, A., Hannequin, D., Raux, G., Le Meur, N., Laquerrière, A., Vital, A., Dumanchin, C., Feuillette, S., Brice, A., Vercelletto, M., et al. (2006). APP locus duplication causes autosomal dominant early-onset Alzheimer disease with cerebral amyloid angiopathy. Nat. Genet. *38*, 24–26. https://doi.org/10.1038/ng1718.

47. Leonenko, G., Baker, E., Stevenson-Hoare, J., Sierksma, A., Fiers, M., Williams, J., de Strooper, B., and Escott-Price, V. (2021). Identifying individuals with high risk of Alzheimer's disease using polygenic risk scores. Nat. Commun. *12*, 4506. https://doi.org/10.1038/s41467-021-24082-z.

48. Escott-Price, V., Sims, R., Bannister, C., Harold, D., Vronskaya, M., Majounie, E., Badarinarayan, N., Morgan, K., et al.; Gerad/Perades, consortia, I., IGAP consortia (2015). Common polygenic variation enhances risk prediction for Alzheimer's disease. Brain *138*, 3673–3684. https://doi.org/10.1093/brain/awv268.

49. Escott-Price, V., Myers, A.J., Huentelman, M., and Hardy, J. (2017). Polygenic risk score analysis of pathologically confirmed Alzheimer disease. Ann. Neurol. *82*, 311–314. https://doi.org/10.1002/ana.24999.

50. Cummings, J., Rabinovici, G.D., Atri, A., Aisen, P., Apostolova, L.G., Hendrix, S., Sabbagh, M., Selkoe, D., Weiner, M., and Salloway, S. (2022). Aducanumab: Appropriate Use Recommendations Update. J. Prev. Alzheimers Dis. *9*, 221–230. https://doi.org/10.14283/jpad.2022.34.

51. Derks, E.M., Thorp, J.G., and Gerring, Z.F. (2022). Ten challenges for clinical translation in psychiatric genetics. Nat. Genet. *54*, 1457–1465. https://doi.org/10.1038/s41588-022-01174-0.

52. Vijg, J., and Dong, X. (2020). Pathogenic Mechanisms of Somatic Mutation and Genome Mosaicism in Aging. Cell *182*, 12–23. https://doi.org/10.1016/j.cell.2020.06.024.

53. Poetsch, A.R. (2020). The genomics of oxidative DNA damage, repair, and resulting mutagenesis. Comput. Struct. Biotechnol. J. *18*, 207–219. https://doi.org/10.1016/j.csbj.2019.12.013.

54. Tomasetti, C., and Vogelstein, B. (2015). Cancer etiology. Variation in cancer risk among tissues can be explained by the number of stem cell divisions. Science *347*, 78–81. https://doi.org/10.1126/science.1260825.

55. Gao, Z., Wyman, M.J., Sella, G., and Przeworski, M. (2016). Interpreting the Dependence of Mutation Rates on Age and Time. PLoS Biol. *14*, e1002355. https://doi.org/10.1371/journal.pbio.1002355.

56. Abascal, F., Harvey, L.M.R., Mitchell, E., Lawson, A.R.J., Lensing, S.V., Ellis, P., Russell, A.J.C., Alcantara, R.E., Baez-Ortega, A., Wang, Y., et al. (2021). Somatic mutation landscapes at single-molecule resolution. Nature *593*, 405–410. https://doi.org/10.1038/s41586-021-03477-4.

57. Miller, M.B., Huang, A.Y., Kim, J., Zhou, Z., Kirkham, S.L., Maury, E.A., Ziegenfuss, J.S., Reed, H.C., Neil, J.E., Rento, L., et al. (2022). Somatic genomic changes in single Alzheimer's disease neurons. Nature *604*, 714–722. https://doi.org/10.1038/s41586-022-04640-1.

58. Cheng, K.C., Cahill, D.S., Kasai, H., Nishimura, S., and Loeb, L.A. (1992). 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G-—T and A-—C substitutions. J. Biol. Chem. *267*, 166–172. https://doi.org/10.1016/S0021-9258(18)48474-8.

59. Nunomura, A., Perry, G., Aliev, G., Hirai, K., Takeda, A., Balraj, E.K., Jones, P.K., Ghanbari, H., Wataya, T., Shimohama, S., et al. (2001). Oxidative damage is the earliest event in Alzheimer disease. J. Neuropathol. Exp. Neurol. *60*, 759–767. https://doi.org/10.1093/jnen/60.8.759.

60. Weissman, L., Jo, D.G., Sørensen, M.M., de Souza-Pinto, N.C., Markesbery, W.R., Mattson, M.P., and Bohr, V.A. (2007). Defective DNA base excision repair in brain from individuals with Alzheimer's disease and amnestic mild cognitive impairment. Nucleic Acids Res. *35*, 5545–5555. https://doi.org/10.1093/nar/gkm605.

61. Dileep, V., Boix, C.A., Mathys, H., Marco, A., Welch, G.M., Meharena, H.S., Loon, A., Jeloka, R., Peng, Z., Bennett, D.A., et al. (2023). Neuronal DNA double-strand breaks lead to genome structural variations and 3D genome disruption in neurodegeneration. Cell *186*, 4404–4421.e20. https://doi.org/10.1016/j.cell.2023.08.038.

62. Zhu, Q., Niu, Y., Gundry, M., and Zong, C. (2021). Single-cell damage-nome profiling unveils vulnerable genes and functional pathways in human genome toward DNA damage. Sci. Adv. *7*, eabf3329. https://doi.org/10.1126/sciadv.abf3329.

63. Kousi, M., Boix, C., Park, Y.P., Mathys, H., Sledzieski, S., Peng, Z., Bennett, D.A., Tsai, L.-H., and Kellis, M. (2022). Single-cell mosaicism analysis reveals cell-type-specific somatic mutational burden in Alzheimer's Dementia. Preprint at bioRxiv, 489103. https://doi.org/10.1101/2022.04.21.489103.

64. Pollina, E.A., Gilliam, D.T., Landau, A.T., Lin, C., Pajarillo, N., Davis, C.P., Harmin, D.A., Yap, E.L., Vogel, I.R., Griffith, E.C., et al. (2023). A NPAS4-NuA4 complex couples synaptic activity to DNA repair. Nature *614*, 732–741. https://doi.org/10.1038/s41586-023-05711-7.

65. Migliore, L., and Coppedè, F. (2022). Gene-environment interactions in Alzheimer disease: the emerging role of epigenetics. Nat. Rev. Neurol. *18*, 643–660. https://doi.org/10.1038/s41582-022-00714-w.

66. De Jager, P.L., Srivastava, G., Lunnon, K., Burgess, J., Schalkwyk, L.C., Yu, L., Eaton, M.L., Keenan, B.T., Ernst, J., McCabe, C., et al. (2014). Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci. Nat. Neurosci. *17*, 1156–1163. https://doi.org/10.1038/nn.3786.

67. Lunnon, K., Smith, R., Hannon, E., De Jager, P.L., Srivastava, G., Volta, M., Troakes, C., Al-Sarraj, S., Burrage, J., Macdonald, R., et al. (2014). Methylomic profiling implicates cortical deregulation of ANK1 in Alzheimer's disease. Nat. Neurosci. *17*, 1164–1170. https://doi.org/10.1038/nn.3782.

68. Kinde, B., Gabel, H.W., Gilbert, C.S., Griffith, E.C., and Greenberg, M.E. (2015). Reading the unique DNA methylation landscape of the brain: Non-CpG methylation, hydroxymethylation, and MeCP2. Proc. Natl. Acad. Sci. USA *112*, 6800–6806. https://doi.org/10.1073/pnas.1411269112.

69. Horvath, S., and Raj, K. (2018). DNA methylation-based biomarkers and the epigenetic clock theory of ageing. Nat. Rev. Genet. *19*, 371–384. https://doi.org/10.1038/s41576-018-0004-3.

70. Kim, S., and Kaang, B.K. (2017). Epigenetic regulation and chromatin remodeling in learning and memory. Exp. Mol. Med. *49*, e281. https://doi.org/10.1038/emm.2016.140.

71. de Toledo-Morrell, L., Goncharova, I., Dickerson, B., Wilson, R.S., and Bennett, D.A. (2000). From healthy aging to early Alzheimer's disease: in vivo detection of entorhinal cortex atrophy. Ann. N. Y. Acad. Sci. *911*, 240–253. https://doi.org/10.1111/j.1749-6632.2000.tb06730.x.

72. Yu, L., Chibnik, L.B., Srivastava, G.P., Pochet, N., Yang, J., Xu, J., Kozubek, J., Obholzer, N., Leurgans, S.E., Schneider, J.A., et al. (2015). Association of Brain DNA methylation in SORL1, ABCA7, HLA-DRB5, SLC24A4, and BIN1 with pathological diagnosis of Alzheimer disease. JAMA Neurol. *72*, 15–24. https://doi.org/10.1001/jamaneurol.2014.3049.

73. Smith, R.G., Pishva, E., Shireby, G., Smith, A.R., Roubroeks, J.A.Y., Hannon, E., Wheildon, G., Mastroeni, D., Gasparoni, G., Riemenschneider, M., et al. (2021). A meta-analysis of epigenome-wide association studies in Alzheimer's disease highlights novel differentially methylated loci across cortex. Nat. Commun. *12*, 3517. https://doi.org/10.1038/s41467-021-23243-4.

74. Wang, E., Wang, M., Guo, L., Fullard, J.F., Micallef, C., Bendl, J., Song, W.M., Ming, C., Huang, Y., Li, Y., et al. (2023). Genome-wide methylomic regulation of multiscale gene networks in Alzheimer's disease. Alzheimers Dement. *19*, 3472–3495. https://doi.org/10.1002/alz.12969.

75. Cuadrado-Tejedor, M., Garcia-Barroso, C., Sánchez-Arias, J.A., Rabal, O., Pérez-González, M., Mederos, S., Ugarte, A., Franco, R., Segura, V., Perea, G., et al. (2017). A First-in-Class Small-Molecule that Acts as a Dual Inhibitor of HDAC and PDE5 and that Rescues Hippocampal Synaptic Impairment in Alzheimer's Disease Mice. Neuropsychopharmacology *42*, 524–539. https://doi.org/10.1038/npp.2016.163.

76. Nativio, R., Donahue, G., Berson, A., Lan, Y., Amlie-Wolf, A., Tuzer, F., Toledo, J.B., Gosai, S.J., Gregory, B.D., Torres, C., et al. (2018). Dysregulation of the epigenetic landscape of normal aging in Alzheimer's disease. Nat. Neurosci. *21*, 497–505. https://doi.org/10.1038/s41593-018-0101-9.

77. Dang, W., Steffen, K.K., Perry, R., Dorsey, J.A., Johnson, F.B., Shilatifard, A., Kaeberlein, M., Kennedy, B.K., and Berger, S.L. (2009). Histone H4 lysine 16 acetylation regulates cellular lifespan. Nature *459*, 802–807. https://doi.org/10.1038/nature08085.

78. Marzi, S.J., Leung, S.K., Ribarska, T., Hannon, E., Smith, A.R., Pishva, E., Poschmann, J., Moore, K., Troakes, C., Al-Sarraj, S., et al. (2018). A histone acetylome-wide association study of Alzheimer's disease identifies disease-associated H3K27ac differences in the entorhinal cortex. Nat. Neurosci. *21*, 1618–1627. https://doi.org/10.1038/s41593-018-0253-7.

79. Klein, H.U., McCabe, C., Gjoneska, E., Sullivan, S.E., Kaskow, B.J., Tang, A., Smith, R.V., Xu, J., Pfenning, A.R., Bernstein, B.E., et al. (2019). Epigenome-wide study uncovers large-scale changes in histone acetylation driven by tau pathology in aging and Alzheimer's human brains. Nat. Neurosci. *22*, 37–46. https://doi.org/10.1038/s41593-018-0291-1.

80. Frost, B., Hemberg, M., Lewis, J., and Feany, M.B. (2014). Tau promotes neurodegeneration through global chromatin relaxation. Nat. Neurosci. *17*, 357–366. https://doi.org/10.1038/nn.3639.

81. Nativio, R., Lan, Y., Donahue, G., Sidoli, S., Berson, A., Srinivasan, A.R., Shcherbakova, O., Amlie-Wolf, A., Nie, J., Cui, X., et al. (2020). An integrated multi-omics approach identifies epigenetic alterations associated with Alzheimer's disease. Nat. Genet. *52*, 1024–1035. https://doi.org/10.1038/s41588-020-0696-0.

82. Zhu, C., Zhang, Y., Li, Y.E., Lucero, J., Behrens, M.M., and Ren, B. (2021). Joint profiling of histone modifications and transcriptome in single cells from mouse brain. Nat. Methods *18*, 283–292. https://doi.org/10.1038/s41592-021-01060-3.

83. Bendl, J., Hauberg, M.E., Girdhar, K., Im, E., Vicari, J.M., Rahman, S., Fernando, M.B., Townsley, K.G., Dong, P., Misir, R., et al. (2022). The three-dimensional landscape of cortical chromatin accessibility in Alzheimer's disease. Nat. Neurosci. *25*, 1366–1378. https://doi.org/10.1038/s41593-022-01166-7.

84. Anderson, A.G., Rogers, B.B., Loupe, J.M., Rodriguez-Nunez, I., Roberts, S.C., White, L.M., Brazell, J.N., Bunney, W.E., Bunney, B.G., Watson, S.J., et al. (2023). Single nucleus multiomics identifies ZEB1 and MAFB as candidate regulators of Alzheimer's disease-specific cis-regulatory elements. Cell Genom. *3*, 100263. https://doi.org/10.1016/j.xgen.2023.100263.

85. Morabito, S., Miyoshi, E., Michael, N., Shahin, S., Martini, A.C., Head, E., Silva, J., Leavy, K., Perez-Rosendahl, M., and Swarup, V. (2021). Single-nucleus chromatin accessibility and transcriptomic characterization of Alzheimer's disease. Nat. Genet. *53*, 1143–1155. https://doi.org/10.1038/s41588-021-00894-z.

86. Kosoy, R., Fullard, J.F., Zeng, B., Bendl, J., Dong, P., Rahman, S., Kleopoulos, S.P., Shao, Z., Girdhar, K., Humphrey, J., et al. (2022). Genetics of the human microglia regulome refines Alzheimer's disease risk loci. Nat. Genet. *54*, 1145–1154. https://doi.org/10.1038/s41588-022-01149-1.

87. Zhong, S., Zhang, S., Fan, X., Wu, Q., Yan, L., Dong, J., Zhang, H., Li, L., Sun, L., Pan, N., et al. (2018). A single-cell RNA-seq survey of the developmental landscape of the human prefrontal cortex. Nature *555*, 524–528. https://doi.org/10.1038/nature25980.

88. Sun, N., Akay, L.A., Murdock, M.H., Park, Y., Galiana-Melendez, F., Bubnys, A., Galani, K., Mathys, H., Jiang, X., Ng, A.P., et al. (2023). Single-nucleus multiregion transcriptomic analysis of brain vasculature in Alzheimer's disease. Nat. Neurosci. *26*, 970–982. https://doi.org/10.1038/s41593-023-01334-3.

89. Montagne, A., Nation, D.A., Sagare, A.P., Barisano, G., Sweeney, M.D., Chakhoyan, A., Pachicano, M., Joe, E., Nelson, A.R., D'Orazio, L.M., et al. (2020). APOE4 leads to blood-brain barrier dysfunction predicting cognitive decline. Nature *581*, 71–76. https://doi.org/10.1038/s41586-020-2247-3.

90. Zeng, H., Huang, J., Zhou, H., Meilandt, W.J., Dejanovic, B., Zhou, Y., Bohlen, C.J., Lee, S.H., Ren, J., Liu, A., et al. (2023). Integrative in situ mapping of single-cell transcriptional states and tissue histopathology in a mouse model of Alzheimer's disease. Nat. Neurosci. *26*, 430–446. https://doi.org/10.1038/s41593-022-01251-x.

91. Hansen, D.V., Hanson, J.E., and Sheng, M. (2018). Microglia in Alzheimer's disease. J. Cell Biol. *217*, 459–472. https://doi.org/10.1083/jcb.201709069.

92. Cain, A., Taga, M., McCabe, C., Green, G.S., Hekselman, I., White, C.C., Lee, D.I., Gaur, P., Rozenblatt-Rosen, O., Zhang, F., et al. (2023). Multicellular communities are perturbed in the aging human brain and Alzheimer's disease. Nat. Neurosci. *26*, 1267–1280. https://doi.org/10.1038/s41593-023-01356-x.

93. Mathys, H., Peng, Z., Boix, C.A., Victor, M.B., Leary, N., Babu, S., Abdelhady, G., Jiang, X., Ng, A.P., Ghafari, K., et al. (2023). Single-cell atlas reveals correlates of high cognitive function, dementia, and resilience to Alzheimer's disease pathology. Cell *186*, 4365–4385.e27. https://doi.org/10.1016/j.cell.2023.08.039.

94. Consens, M.E., Chen, Y., Menon, V., Wang, Y., Schneider, J.A., De Jager, P.L., Bennett, D.A., Tripathy, S.J., and Felsky, D. (2022). Bulk and Single-Nucleus Transcriptomics Highlight Intra-Telencephalic and Somatostatin Neurons in Alzheimer's Disease. Front. Mol. Neurosci. *15*, 903175. https://doi.org/10.3389/fnmol.2022.903175.

95. Horsfield, J.A. (2023). Full circle: a brief history of cohesin and the regulation of gene expression. FEBS J. *290*, 1670–1687. https://doi.org/10.1111/febs.16362.

96. Olah, M., Menon, V., Habib, N., Taga, M.F., Ma, Y., Yung, C.J., Cimpean, M., Khairallah, A., Coronas-Samano, G., Sankowski, R., et al. (2020). Single cell RNA sequencing of human microglia uncovers a subset associated with Alzheimer's disease. Nat. Commun. *11*, 6129. https://doi.org/10.1038/s41467-020-19737-2.

97. Sun, N., Victor, M.B., Park, Y.P., Xiong, X., Scannail, A.N., Leary, N., Prosper, S., Viswanathan, S., Luna, X., Boix, C.A., et al. (2023). Human microglial state dynamics in Alzheimer's disease progression. Cell *186*, 4386–4403.e29. https://doi.org/10.1016/j.cell.2023.08.037.

98. Keren-Shaul, H., Spinrad, A., Weiner, A., Matcovitch-Natan, O., Dvir-Szternfeld, R., Ulland, T.K., David, E., Baruch, K., Lara-Astaiso, D., Toth, B., et al. (2017). A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease. Cell *169*, 1276–1290.e17. https://doi.org/10.1016/j.cell.2017.05.018.

99. Otero-Garcia, M., Mahajani, S.U., Wakhloo, D., Tang, W., Xue, Y.Q., Morabito, S., Pan, J., Oberhauser, J., Madira, A.E., Shakouri, T., et al. (2022). Molecular signatures underlying neurofibrillary tangle susceptibility in Alzheimer's disease. Neuron *110*, 2929–2948.e8. https://doi.org/10.1016/j.neuron.2022.06.021.

100. Costa-Mattioli, M., and Walter, P. (2020). The integrated stress response: From mechanism to disease. Science *368*, eaat5314. https://doi.org/10.1126/science.aat5314.

101. Gouveia Roque, C., Chung, K.M., McCurdy, E.P., Jagannathan, R., Randolph, L.K., Herline-Killian, K., Baleriola, J., and Hengst, U. (2023). CREB3L2-ATF4 heterodimerization defines a transcriptional hub of Alzheimer's disease gene expression linked to neuropathology. Sci. Adv. *9*, eadd2671. https://doi.org/10.1126/sciadv.add2671.

102. Niu, M., Cao, W., Wang, Y., Zhu, Q., Luo, J., Wang, B., Zheng, H., Weitz, D.A., and Zong, C. (2023). Droplet-based transcriptome profiling of individual synapses. Nat. Biotechnol. *41*, 1332–1344. https://doi.org/10.1038/s41587-022-01635-1.

103. Hobson, B.D., and Herzog, E. (2023). Methodological concerns and lack of evidence for single-synapse RNA-seq. Nat. Biotechnol. *41*, 1221–1224. https://doi.org/10.1038/s41587-023-01877-7.

104. Rao, A., Barkley, D., França, G.S., and Yanai, I. (2021). Exploring tissue architecture using spatial transcriptomics. Nature *596*, 211–220. https://doi.org/10.1038/s41586-021-03634-9.

105. Chen, W.T., Lu, A., Craessaerts, K., Pavie, B., Sala Frigerio, C., Corthout, N., Qian, X., Laláková, J., Kühnemund, M., Voytyuk, I., et al. (2020). Spatial Transcriptomics and In Situ Sequencing to Study Alzheimer's Disease. Cell *182*, 976–991.e19. https://doi.org/10.1016/j.cell.2020.06.038.

106. Kametani, F., and Hasegawa, M. (2018). Reconsideration of Amyloid Hypothesis and Tau Hypothesis in Alzheimer's Disease. Front. Neurosci. *12*, 25. https://doi.org/10.3389/fnins.2018.00025.

107. Chen, S., Chang, Y., Li, L., Acosta, D., Li, Y., Guo, Q., Wang, C., Turkes, E., Morrison, C., Julian, D., et al. (2022). Spatially resolved transcriptomics reveals genes associated with the vulnerability of middle temporal gyrus in Alzheimer's disease. Acta Neuropathol. Commun. *10*, 188. https://doi.org/10.1186/s40478-022-01494-6.

108. Wang, X., Allen, W.E., Wright, M.A., Sylwestrak, E.L., Samusik, N., Vesuna, S., Evans, K., Liu, C., Ramakrishnan, C., Liu, J., et al. (2018). Three-dimensional intact-tissue sequencing of single-cell transcriptional states. Science *361*, eaat5691. https://doi.org/10.1126/science.aat5691.

109. Sadick, J.S., O'Dea, M.R., Hasel, P., Dykstra, T., Faustin, A., and Liddelow, S.A. (2022). Astrocytes and oligodendrocytes undergo subtype-specific transcriptional changes in Alzheimer's disease. Neuron *110*, 1788–1805.e1710. https://doi.org/10.1016/j.neuron.2022.03.008.

110. Timp, W., and Timp, G. (2020). Beyond mass spectrometry, the next step in proteomics. Sci. Adv. *6*, eaax8978. https://doi.org/10.1126/sciadv.aax8978.

111. Aebersold, R., Agar, J.N., Amster, I.J., Baker, M.S., Bertozzi, C.R., Boja, E.S., Costello, C.E., Cravatt, B.F., Fenselau, C., Garcia, B.A., et al. (2018). How many human proteoforms are there? Nat. Chem. Biol. *14*, 206–214. https://doi.org/10.1038/nchembio.2576.

112. Liu, Y., Beyer, A., and Aebersold, R. (2016). On the Dependency of Cellular Protein Levels on mRNA Abundance. Cell *165*, 535–550. https://doi.org/10.1016/j.cell.2016.03.014.

113. Tasaki, S., Xu, J., Avey, D.R., Johnson, L., Petyuk, V.A., Dawe, R.J., Bennett, D.A., Wang, Y., and Gaiteri, C. (2022). Inferring protein expression changes from mRNA in Alzheimer's dementia using deep neural networks. Nat. Commun. *13*, 655. https://doi.org/10.1038/s41467-022-28280-1.

114. Higginbotham, L., Ping, L., Dammer, E.B., Duong, D.M., Zhou, M., Gearing, M., Hurst, C., Glass, J.D., Factor, S.A., Johnson, E.C.B., et al. (2020). Integrated proteomics reveals brain-based cerebrospinal fluid biomarkers in asymptomatic and symptomatic Alzheimer's disease. Sci. Adv. *6*, eaaz9360. https://doi.org/10.1126/sciadv.aaz9360.

115. Johnson, E.C.B., Carter, E.K., Dammer, E.B., Duong, D.M., Gerasimov, E.S., Liu, Y., Liu, J., Betarbet, R., Ping, L., Yin, L., et al. (2022). Large-scale deep multi-layer analysis of Alzheimer's disease brain reveals

strong proteomic disease-related changes not observed at the RNA level. Nat. Neurosci. *25*, 213–225. https://doi.org/10.1038/s41593-021-00999-y.

116. DeTure, M.A., and Dickson, D.W. (2019). The neuropathological diagnosis of Alzheimer's disease. Mol. Neurodegener. *14*, 32. https://doi.org/10.1186/s13024-019-0333-5.

117. Johnson, E.C.B., Dammer, E.B., Duong, D.M., Ping, L., Zhou, M., Yin, L., Higginbotham, L.A., Guajardo, A., White, B., Troncoso, J.C., et al. (2020). Large-scale proteomic analysis of Alzheimer's disease brain and cerebrospinal fluid reveals early changes in energy metabolism associated with microglia and astrocyte activation. Nat. Med. *26*, 769–780. https://doi.org/10.1038/s41591-020-0815-6.

118. Rayaprolu, S., Higginbotham, L., Bagchi, P., Watson, C.M., Zhang, T., Levey, A.I., Rangaraju, S., and Seyfried, N.T. (2021). Systems-based proteomics to resolve the biology of Alzheimer's disease beyond amyloid and tau. Neuropsychopharmacology *46*, 98–115. https://doi.org/10.1038/s41386-020-00840-3.

119. Bai, B., Wang, X., Li, Y., Chen, P.C., Yu, K., Dey, K.K., Yarbro, J.M., Han, X., Lutz, B.M., Rao, S., et al. (2020). Deep Multilayer Brain Proteomics Identifies Molecular Networks in Alzheimer's Disease Progression. Neuron *105*, 975–991.e7. https://doi.org/10.1016/j.neuron.2019.12.015.

120. Huang, Z., Merrihew, G.E., Larson, E.B., Park, J., Plubell, D., Fox, E.J., Montine, K.S., Latimer, C.S., Dirk Keene, C., Zou, J.Y., et al. (2023). Brain proteomic analysis implicates actin filament processes and injury response in resilience to Alzheimer's disease. Nat. Commun. *14*, 2747. https://doi.org/10.1038/s41467-023-38376-x.

121. Saka, S.K., Wang, Y., Kishi, J.Y., Zhu, A., Zeng, Y., Xie, W., Kirli, K., Yapp, C., Cicconet, M., Beliveau, B.J., et al. (2019). Immuno-SABER enables highly multiplexed and amplified protein imaging in tissues. Nat. Biotechnol. *37*, 1080–1090. https://doi.org/10.1038/s41587-019-0207-y.

122. Goltsev, Y., Samusik, N., Kennedy-Darling, J., Bhate, S., Hale, M., Vazquez, G., Black, S., and Nolan, G.P. (2018). Deep Profiling of Mouse Splenic Architecture with CODEX Multiplexed Imaging. Cell *174*, 968–981.e15. https://doi.org/10.1016/j.cell.2018.07.010.

123. Mostafavi, S., Gaiteri, C., Sullivan, S.E., White, C.C., Tasaki, S., Xu, J., Taga, M., Klein, H.U., Patrick, E., Komashko, V., et al. (2018). A molecular network of the aging human brain provides insights into the pathology and cognitive decline of Alzheimer's disease. Nat. Neurosci. *21*, 811–819. https://doi.org/10.1038/s41593-018-0154-9.

124. Gabitto, M.I., Travaglini, K.J., Rachleff, V.M., Kaplan, E.S., Long, B., Ariza, J., Ding, Y., Mahoney, J.T., Dee, N., Goldy, J., et al. (2023). Integrated multimodal cell atlas of Alzheimer's disease. Preprint at bioRxiv. https://doi.org/10.1101/2023.05.08.539485.

125. Iturria-Medina, Y., Adewale, Q., Khan, A.F., Ducharme, S., Rosa-Neto, P., O'Donnell, K., Petyuk, V.A., Gauthier, S., De Jager, P.L., Breitner, J., and Bennett, D.A. (2022). Unified epigenomic, transcriptomic, proteomic, and metabolomic taxonomy of Alzheimer's disease progression and heterogeneity. Sci. Adv. *8*, eabo6764. https://doi.org/10.1126/sciadv.abo6764.

126. Yang, M., Matan-Lithwick, S., Wang, Y., De Jager, P.L., Bennett, D.A., and Felsky, D. (2023). Multi-omic integration via similarity network fusion to detect molecular subtypes of ageing. Brain Commun. *5*, fcad110. https://doi.org/10.1093/braincomms/fcad110.

127. Wu, J., Chen, Y., Wang, P., Caselli, R.J., Thompson, P.M., Wang, J., and Wang, Y. (2021). Integrating Transcriptomics, Genomics, and Imaging in Alzheimer's Disease: A Federated Model. Front. Radiol. *1*, 777030. https://doi.org/10.3389/fradi.2021.777030.

128. Kapasi, A., DeCarli, C., and Schneider, J.A. (2017). Impact of multiple pathologies on the threshold for clinically overt dementia. Acta Neuropathol. *134*, 171–186. https://doi.org/10.1007/s00401-017-1717-7.

129. Guo, Y., You, J., Zhang, Y., Liu, W.S., Huang, Y.Y., Zhang, Y.R., Zhang, W., Dong, Q., Feng, J.F., Cheng, W., and Yu, J.T. (2024). Plasma proteomic profiles predict future dementia in healthy adults. Nat. Aging *4*, 247–260. https://doi.org/10.1038/s43587-023-00565-0.

130. Blennow, K., Hampel, H., Weiner, M., and Zetterberg, H. (2010). Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. Nat. Rev. Neurol. *6*, 131–144. https://doi.org/10.1038/nrneurol.2010.4.

131. Jain, G., Stuendl, A., Rao, P., Berulava, T., Pena Centeno, T., Kaurani, L., Burkhardt, S., Delalle, I., Kornhuber, J., Hüll, M., et al. (2019). A combined miRNA-piRNA signature to detect Alzheimer's disease. Transl. Psychiatry *9*, 250. https://doi.org/10.1038/s41398-019-0579-2.

132. Wingo, A.P., Wang, M., Liu, J., Breen, M.S., Yang, H.S., Tang, B., Schneider, J.A., Seyfried, N.T., Lah, J.J., Levey, A.I., et al. (2022). Brain microRNAs are associated with variation in cognitive trajectory in advanced age. Transl. Psychiatry *12*, 47. https://doi.org/10.1038/s41398-022-01806-3.

133. King, E.A., Davis, J.W., and Degner, J.F. (2019). Are drug targets with genetic support twice as likely to be approved? Revised estimates of the impact of genetic support for drug mechanisms on the probability of drug approval. PLoS Genet. *15*, e1008489. https://doi.org/10.1371/journal.pgen.1008489.

134. van Bokhoven, P., de Wilde, A., Vermunt, L., Leferink, P.S., Heetveld, S., Cummings, J., Scheltens, P., and Vijverberg, E.G.B. (2021). The Alzheimer's disease drug development landscape. Alzheimer's Res. Ther. *13*, 186. https://doi.org/10.1186/s13195-021-00927-z.

135. Mummery, C.J., Börjesson-Hanson, A., Blackburn, D.J., Vijverberg, E.G.B., De Deyn, P.P., Ducharme, S., Jonsson, M., Schneider, A., Rinne, J.O., Ludolph, A.C., et al. (2023). Tau-targeting antisense oligonucleotide MAPT(Rx) in mild Alzheimer's disease: a phase 1b, randomized, placebo-controlled trial. Nat. Med. *29*, 1437–1447. https://doi.org/10.1038/s41591-023-02326-3.

136. Restifo, L.L. (2022). Unraveling the Gordian knot: genetics and the troubled road to effective therapeutics for Alzheimer's disease. Genetics *220*, iyab185. https://doi.org/10.1093/genetics/iyab185.

137. Lamb, J., Crawford, E.D., Peck, D., Modell, J.W., Blat, I.C., Wrobel, M.J., Lerner, J., Brunet, J.P., Subramanian, A., Ross, K.N., et al. (2006). The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. Science *313*, 1929–1935. https://doi.org/10.1126/science.1132939.

138. Subramanian, A., Narayan, R., Corsello, S.M., Peck, D.D., Natoli, T.E., Lu, X., Gould, J., Davis, J.F., Tubelli, A.A., Asiedu, J.K., et al. (2017). A Next Generation Connectivity Map: L1000 Platform and the First 1,000,000 Profiles. Cell *171*, 1437–1452.e17. https://doi.org/10.1016/j.cell.2017.10.049.

139. Williams, G., Gatt, A., Clarke, E., Corcoran, J., Doherty, P., Chambers, D., and Ballard, C. (2019). Drug repurposing for Alzheimer's disease based on transcriptional profiling of human iPSC-derived cortical neurons. Transl. Psychiatry *9*, 220. https://doi.org/10.1038/s41398-019-0555-x.

140. Rodriguez, S., Hug, C., Todorov, P., Moret, N., Boswell, S.A., Evans, K., Zhou, G., Johnson, N.T., Hyman, B.T., Sorger, P.K., et al. (2021). Machine learning identifies candidates for drug repurposing in Alzheimer's disease. Nat. Commun. *12*, 1033. https://doi.org/10.1038/s41467-021-21330-0.

141. Cummings, J., Zhou, Y., Lee, G., Zhong, K., Fonseca, J., and Cheng, F. (2023). Alzheimer's disease drug development pipeline: 2023. Alzheimers Dement. *9*, e12385. https://doi.org/10.1002/trc2.12385.

142. De Cecco, M., Ito, T., Petrashen, A.P., Elias, A.E., Skvir, N.J., Criscione, S.W., Caligiana, A., Brocculi, G., Adney, E.M., Boeke, J.D., et al. (2019). L1 drives IFN in senescent cells and promotes age-associated inflammation. Nature *566*, 73–78. https://doi.org/10.1038/s41586-018-0784-9.

143. Xiong, J., Kang, S.S., Wang, Z., Liu, X., Kuo, T.C., Korkmaz, F., Padilla, A., Miyashita, S., Chan, P., Zhang, Z., et al. (2022). FSH blockade improves cognition in mice with Alzheimer's disease. Nature *603*, 470–476. https://doi.org/10.1038/s41586-022-04463-0.

144. Yan, Y., Wang, X., Chaput, D., Shin, M.K., Koh, Y., Gan, L., Pieper, A.A., Woo, J.A.A., and Kang, D.E. (2022). X-linked ubiquitin-specific peptidase 11 increases tauopathy vulnerability in women. Cell *185*, 3913–3930.e19. https://doi.org/10.1016/j.cell.2022.09.002.

145. Hu, Y., Zhang, H., Liu, B., Gao, S., Wang, T., Han, Z., International Genomics of Alzheimer's Project IGAP; Ji, X., and Liu, G. (2020). rs34331204 regulates TSPAN13 expression and contributes to Alzheimer's disease with sex differences. Brain *143*, e95. https://doi.org/10.1093/brain/awaa302.

146. Kunkle, B.W., Schmidt, M., Klein, H.U., Naj, A.C., Hamilton-Nelson, K.L., Larson, E.B., Evans, D.A., De Jager, P.L., Crane, P.K., Buxbaum, J.D., et al. (2021). Novel Alzheimer Disease Risk Loci and Pathways in African American Individuals Using the African Genome Resources Panel: A Meta-analysis. JAMA Neurol. *78*, 102–113. https://doi.org/10.1001/jamaneurol.2020.3536.

147. Conniff, K.R., Grill, J.D., and Gillen, D.L. (2024). Retention of American Indian and Alaska Native participants in the National Alzheimer's Coordinating Center Uniform Data Set. Alzheimers Dement. *20*, 1601–1613. https://doi.org/10.1002/alz.13573.

148. O'Bryant, S.E., Johnson, L.A., Barber, R.C., Braskie, M.N., Christian, B., Hall, J.R., Hazra, N., King, K., Kothapalli, D., Large, S., et al. (2021). The Health & Aging Brain among Latino Elders (HABLE) study methods and participant characteristics. Alzheimers Dement. *13*, e12202. https://doi.org/10.1002/dad2.12202.

149. Tang, M.X., Cross, P., Andrews, H., Jacobs, D.M., Small, S., Bell, K., Merchant, C., Lantigua, R., Costa, R., Stern, Y., and Mayeux, R. (2001). Incidence of AD in African-Americans, Caribbean Hispanics, and Caucasians in northern Manhattan. Neurology *56*, 49–56. https://doi.org/10.1212/wnl.56.1.49.

150. Ho, P.C., Yu, W.H., Tee, B.L., Lee, W.P., Li, C., Gu, Y., Yokoyama, J.S., Reyes-Dumeyer, D., Choi, Y.B., Yang, H.S., et al. (2024). Asian Cohort for Alzheimer's Disease (ACAD) pilot study on genetic and non-genetic risk factors for Alzheimer's disease among Asian Americans and Canadians. Alzheimers Dement. *20*, 2058–2071. https://doi.org/10.1002/alz.13611.