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Rare Variants and Transcriptomics in Alzheimer disease

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Abstract

Alzheimer disease (AD) is the most common dementia in the elderly, still without effective treatment. Early-onset AD (EOAD) is caused by mutations in the genes *APP*, *PSEN1* and *PSEN2*. Genome-wide association studies have identified >20 late-onset AD (LOAD) susceptibility genes with common variants of small risk, with the exception of *APOE*. We review rare susceptibility variants in LOAD with larger effects that have been recently identified in the EOAD gene *APP* and the newly discovered AD genes *TREM2* and *PLD3*. Human genetic studies now consistently support the amyloid hypothesis of AD for both EOAD and LOAD. Moreover, they identified biological processes that overlap with human transcriptomics studies in AD across different tissues, such as inflammation, cytoskeletal organization, synaptic functions, etc. Transcriptomic profiles of pre-symptomatic AD-associated variant carriers already reflect specific molecular mechanisms reminiscent to those of AD patients. This might provide an avenue for personalized medicine.

Keywords

transcriptomics; Alzheimer disease; dementia; elderly; APP; PLD3; PSEN1; PSEN2; TREM2; personalized medicine; late-onset Alzheimer disease (LOAD)

Introduction

Alzheimer Disease

Alzheimer disease (AD) is the most common form of dementia in the elderly, accounting for up to 75% of all dementia patients. Currently, there are more than 25 million people worldwide with AD and their numbers are anticipated to double every 20 years due to the

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Conflict of Interest

C Humphries declares no conflicts of interest.
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Human and Animal Rights and Informed Consent

All studies by the authors involving animal and/or human subjects were performed after approval by the appropriate institutional review boards. When required, written informed consent was obtained from all participants.

expected demographic shift towards higher age. Disease onset of AD is usually over 70 years of age. However, age-specific prevalence of about 4% at age 65 increases exponentially with age and exceeds 20% at age 90 [1]. AD is clinically characterized by a slow but progressive impairment in memory, executive function, language, and other areas of cognition leading to a loss of social and occupational functions [2]. The pathological hallmarks of AD are extracellular amyloid β (Aβ) plaques and intracellular neurofibrillary tangles (NFT) that accumulate over time in the aging brain. According to the predominant amyloid hypothesis of AD, aggregation of Aβ initiates a pathogenic cascade that eventually leads to inflammation, loss of neurons and synapses and brain atrophy [3, 4].

Genetics of Alzheimer Disease

The heritability of late-onset AD (LOAD) has been estimated to be between 58% and 79% and can be regarded as the proportion of disease vulnerability explained by heritable genetic factors. The remaining risk for LOAD has been attributed to environmental factors, for instance, exposure to vascular risk (e.g. obesity) and possibly beneficial psychosocial factors such as high education and physical exercise [1, 5]. Besides LOAD, there are early-onset forms of AD (EOAD) with disease onset prior to age 60 that represent up to 1% of all AD cases at an average prevalence of about 65 EOAD cases per 100,000 individuals [6, 7]. Approximately 60% of EOAD cases have several relatives also affected by AD and 13% of EOAD cases occur in families which are in concordance with autosomal-dominant inheritance of EOAD over at least three generations (ADEOAD) [6].

In the 1990's, genetic linkage and subsequent positional cloning studies in large, multi-generational ADEOAD families led to the discovery of the first causative AD mutations in the genes encoding the amyloid precursor protein (*APP*) [8, 9], presenilin 1 (*PSEN1*) [10, 11] and presenilin 2 (*PSEN2*) [12, 13]. Meanwhile, around 24 mutations in *APP*, 185 in *PSEN1* and 13 in *PSEN2* have been reported to be pathogenic for AD [14] (see [15] for review on clinical aspects of genetic variants in AD). Most of these extremely rare, familial variants are single amino acid substitutions and show dominant, fully penetrant co-segregation with ADEOAD. The majority of pathogenic variants in full-length APP are located near the N-terminal β-secretase and the C-terminal γ-secretase proteolytic cleavage site of amyloidogenic Aβ fragments of APP that are endogenously produced by cells [15]. The presenilin proteins are the catalytic units of γ-secretase complexes that are involved in the proteolytic cleavage of APP to produce Aβ fragments. Mutations in the presenilins most often alter the proteins' catalytic properties in a way that increase the absolute or relative production of most amyloidogenic $A\beta_{42}$ fragments [16]. Thus, genetic and biochemical evidence for the predominant amyloid hypothesis of AD is convincing [17].

Early genetic linkage and genetic association studies also led to the identification of the two most prominent genetic risk variants for LOAD in exon 4 of the apolipoprotein E (*APOE*) gene; both in terms of their high population frequencies and large effect on LOAD risk [18, 19]. Depending on the population, the *APOE ε4* LOAD risk allele typically occurs in about 15–20% of individuals. Heterozygous carriers (*ε3/ε4*) for the *APOE ε4* risk allele are 3-fold and homozygous carriers (*ε4/ε4*) up to 15-fold more likely to suffer from LOAD compared to individuals with the predominant *APOE ε3/ε3* genotype. Homozygous *ε4* carriers reach

close to complete penetrance at age 90 and older. Moreover, the *APOE ε4* risk allele is also associated with EOAD, an earlier disease onset of AD and the rarer *APOE ε2* allele is protective for AD [20, 21]. Multiple functions of the APOE protein have been suggested in the pathogenesis of AD. There is strong evidence for differential effects of APOE isoforms on Aβ aggregation and clearance. Additionally proposed mechanisms of APOE with regard to AD involve neurotoxicity, tau phosphorylation, synaptic plasticity and neuroinflammation [22]. The advent in high content genotype chip array technology enabled genome-wide association studies (GWAS) in large cohorts of unrelated LOAD cases and unaffected aged controls. To date, large GWAS consortia in LOAD have identified >20 non-*APOE* loci that show association with LOAD [23, 24]. Unlike *APOE*, these loci confer low individual, but reproducible risk to LOAD with odds ratios (OR) between 1.08 and 1.30. Several of these LOAD susceptibility genes can be functionally linked to the pathways of APP and protein tau, are enriched for immune response and inflammation and involve cell migration, lipid transport, endocytosis, hippocampal synaptic function, cytoskeletal function, axonal transport, regulation of gene expression and post-translational modification of proteins, and microglial and myeloid cell function. The population-attributable fraction (PAF) of *APOE* on risk for LOAD is at about 30%, while each single GWAS-identified locus contributes with individual PAFs between 1.0–8.0% to risk or protection of LOAD [24].

The genetics of sporadic LOAD is consistent with the amyloid hypothesis of AD, but seem to go far beyond the immediate APP pathway and are therefore complex. Moreover, the yet established susceptibility loci for LOAD do not completely explain the overall heritability of LOAD.

Rare variants and insights from transcriptomics

GWAS successfully identified many susceptibility loci in LOAD, which is important for the better understanding of LOAD etiology. However, with the exception of *APOE*, GWAS-identified associations confer small risk to LOAD and GWAS loci defining SNPs most often merely represent genetic markers correlated to nearby functional risk variants that remain to be revealed. Since the discovery of rare disease susceptibility variants is challenging due to their low abundance, resulting in low statistical power to detect association with disease in a genome-wide screening approach, most LOAD GWAS so far only considered common variants with minor allele frequencies (MAF) above 5% in the general population. Therefore, missing heritability in AD can be explained by low-frequency (5%>MAF>1%) and rare variants (MAF<1%) [25]. The first rare susceptibility variants for LOAD have been identified in the last two years and they show intermediate to large effects, some even comparable to APOE [26]. In the first part of this review, we focus on rare variant associations with LOAD that reached study-wide significance with independent replication in a chronological order of publication. In contrast to GWAS-identified common risk variants, pathogenic ADEOAD mutations and emerging rare susceptibility variants in LOAD are most often protein coding and therefore the actual functional variants. This opens an avenue of functional studies that can assess the molecular consequences of disease-associated variants in AD. The second part of this review concentrates on whole-transcriptomics studies from human post-mortem brain and peripheral blood cells. We compare biological processes with differential gene expression in AD to pathways revealed

by genetic studies and summarize first studies on transcriptional profiles typical to AD risk allele carriers.

Genetics of rare variants in Alzheimer disease

Rare variants in previously identified AD genes

The rare APP p.A673T variant is protective from LOAD—Next generation sequencing methods now allow for rapid and deep sequencing of many human genomes. Cruchaga et al. sequenced the coding region of ADEOAD genes in 440 probands of LOAD families and showed an overall overrepresentation of rare protein sequence changing variants in the genes *APP*, *PSEN1 and 2* when compared to 12,500 population controls, while describing known EAOD mutations and novel, likely pathogenic variants in *APP* (table 1) [27].

Johnsson et al. took advantage of whole-genome sequence data from 1,795 Icelander to search for low-frequency variants in the *APP* gene [28]. The genotypes of variants found at least twice were then imputed (*in silico* genotyped) into 71,700 Icelanders that had high-density SNP array genotypes available in order to test for association with LOAD. The rare *APP p.A673T* substitution (rs63750847) was found to confer a large protective effect from LOAD (OR=4.2–7.5, depending on age and cognitive status of control groups) and also showed reduced cognitive decline among elderly subjects without a diagnosis of AD. The population frequencies of *APP p.A673T* in Northern Europe are around 0.4% (MAF), whereas the protective allele seems to be even rarer in the US (MAF<0.01%). Functionally, *p.A673T* is located adjacent to the aspartyl protease β-site in APP and results in a reduced β-cleavage efficiency of the aspartyl protease β-site cleaving enzyme 1 (BACE1) and thereby in an about 40% reduction of Aβ fragments, as the authors showed *in vitro*. Of importance, the strong protective effect of *APP p.A673T* serves as a proof of principle that reducing β-cleavage of APP may protect from AD.

Discovery of new LOAD genes through rare variants

Rare variants in TREM2, e.g. p.R47H confer risk to LOAD—Two independent groups reported back-to-back a highly significant association with LOAD for the rare substitution *p.R47H* (rs75932628, MAF=0.3%) in the gene encoding the triggering receptor expressed on myeloid cells 2 (*TREM2*) [26, 29]. This variant already reached genome-wide significant association (p<5e-08) with LOAD in the large Icelandic study samples of Jonsson et al. described above, with subsequent replication in several LOAD cohorts of European descent. Interestingly, the rare allele of *p.R47H* confers similarly high risk to LOAD (overall OR=2.90) as the common *APOE ε4* allele and comparably reduces age of LOAD onset also by about three years per risk allele copy. In reminiscence of Jonsson et al.'s finding that the protective *APP p.A673T* substitution reduces cognitive decline in elderly controls, *TREM2 p.R47H* accelerates cognitive decline in aged controls [26]. Guerreiro et al. [29] independently found highly significant association of *p.R47H* in large European LOAD GWA study samples, while also showing an overall significant accumulation of rare variants in exon 2 of the *TREM2* gene in LOAD cases versus controls including additional variants such as *p.D87N*. Interestingly, they found that three variants

(*Q33X, Y38C, and T66M*) in the recessive state cause the rare Nasu-Hakola disease and related forms of early-onset dementia distinct from AD [29]. TREM2 is a membrane protein that forms a receptor signaling complex with the TYRO protein tyrosine kinase binding protein (TYROBP) and is involved in macrophage activation and inflammation. In the brain, *TREM2* is mainly expressed in microglia of white matter. *TREM2* expression and microglial phagocytosis of cell debris and amyloid concomitantly increases with the accumulation of Aβ plaques in transgenic mice models of AD that carry pathogenic ADEAOD mutations in a human copy of the *APP* gene [26, 29]. Thus, loss-of-function variants in *TREM2* may interfere with Aβ clearance and anti-inflammatory responses and thereby increasing the risk for AD.

Rare variants in PLD3, e.g. p.V232M confer risk to LOAD—Cruchaga et al. [30] applied whole-exome sequencing in multiple multiplex families with a high burden of LOAD and identified the rare missense variant *p.V232M* (rs145999145, MAF=0.4%) in the phospholipase D3 (*PLD3*) gene that co-segregated with disease in two independent families. Subsequent genotyping of *p.V232M* in several large LOAD case-control cohorts of European descent (>11,000 individuals) confirmed the LOAD risk conferring character of the variant allele with high significance and intermediate effect size (OR=2.1). This case-control association resulted in a large effect when only familial LOAD cases were compared against controls (OR=3.4). Moreover, the risk allele also showed association with an earlier onset of LOAD. The authors then sequenced the coding region of *PLD3* in more than 2,000 LOAD cases and as many controls of European descent and found a genome-wide significant gene-based association with LOAD and several rare variants in the *PLD3* gene (OR=2.6). Nominally significant single-variant associations with intermediate to large effect could be shown for *PLD3 p.M6R* and the synonymous splice site variant *p.A442A*. This gene-based association of rare *PLD3* variants being overrepresented in LOAD cases was replicated in an African American case-control cohort as well as the single-variant association for *p.A442A. PLD3* expression is high in several AD-relevant brain regions in healthy controls, but reduced in neurons of LOAD patients. *PLD3* overexpression and knockdown experiments in cell cultures revealed that high *PLD3* expression correlates with lower extracellular Aβ levels and that PLD3 protein can be co-immunoprecipitated with APP. Thus, PLD3 protein is likely protective against AD through its role in APP trafficking [30].

Transcriptomics in Alzheimer disease

EOAD and LOAD have similar clinical manifestations and pathological features [31]. This suggests that similar cellular and biological processes are disrupted in both forms of Alzheimer disease. This notion is also supported by genetics of AD, most recently complemented by rare variant associations as mentioned above. Genome-wide gene transcription is a measurable intermediate proxy of how the genomic sequence gives rise to the altered protein formation that eventually triggers disease and reflects disease progression and cellular coping mechanisms to pathological changes. There are two primary means of measuring genome-wide transcription: Microarray technology and massively parallel RNA sequencing (RNA-seq). While microarrays use hybridization to measure known transcripts, the recent advent of RNA-seq allows for measurement of known and novel transcripts,

including alternatively spliced transcripts. Both of these technologies have been used to shed light on gene transcriptional changes related to AD pathology.

Profiling in Post-Mortem Brain Tissue

Since 2005, at least twenty-five studies have been published examining postmortem human brain tissue. These studies primarily focused their efforts on tissue from the frontal cortex, hippocampus, and temporal lobe because these regions are most affected by AD pathology, essentially Aβ plaques and NFTs [32–50]. These studies differ in their findings on which individual genes are significantly altered between AD patients and healthy controls; however, several biological processes are consistently indicated in AD. The most recent and largest studies describe several hundreds of differentially expressed genes in AD after correction for multiple testing [50, 51]. Although post-mortem studies might also reflect pathological changes that might rather be consequence than cause of the disease, identified biological processes do overlap with recent genetic findings (table 2).

Processes with increased expression—Overall gene expression in post-mortem brain tissue of AD patients is generally lowered compared to controls. However, gene expression in following biological processes is up-regulated in brains of AD subjects. Inflammation has been associated with AD since the 1980s. Several studies demonstrated that genes related to inflammation have increased expression in AD across several brain regions [32, 43, 46, 49, 50, 52, 53]. It is still unknown whether inflammation is the culprit, the result, or a secondary response of AD; however, it is important to note that five (*CR1*, *CD33*, *HLA-DRB5-DRB1*, *INPP5D*, *MEF2C*) of the 20 LOAD GWAS-identified genes are involved in inflammation [24] (table 2). Increased calcium signaling was also observed across many transcriptome studies [36, 48, 49, 54]. Studies in neurons and mice expressing human *APP* and presenilin genes harboring ADEOAD mutations also show altered calcium signaling and calcium storage in the endoplasmic reticulum (ER) as well as synaptic dysfunction and loss of dendritic spines [54]. Further, cellular processes involved in mitochondrial and metabolic functions were shown to have increased gene expression in several transcriptome studies [42, 44, 49]. Mitochondrial function is impaired by APP, Aβ and presenilins [55]. Moreover, PET scans report a decrease in resting-state brain glucose metabolism and metabolic failure in AD brains [56]. The expression of genes related to cytoskeletal architecture is also increased in AD; this is consistent with the tau hypothesis of AD [57]. Microtubules are a major component of the cytoskeleton and the formation of NFTs in AD increasingly depletes microtubules by hyper phosphorylated and misfolded tau [58]. Another cytoskeletal process increased in AD patients is the formation of cofilin-actin rods along axons and dendrites, which results in cellular disruption. Cofilin-actin rods are known to form in response to heat shock, osmotic pressure, and ATP rundown within the hippocampus and frontal cortex of AD patients [59].

Processes with decreased expression—Synaptic related processes are decreased in AD [36, 48, 49, 54]. While Aβ plaques and oligomers indirectly destroy synapses, aggregation of NFTs in neurons results in apoptosis, and inadvertently destroys synapses [60, 61]. Normal neurons remain in the G_0 phase; however, most AD neurons re-enter the cell cycle into the G_1 phase. This departure from the normal cell cycle in neurons results in

axonal defects. These findings are congruent with the decrease in synaptic related processes and the finding that negative regulation of cell cycle processes in AD is decreased [50, 62]. Moreover, signal transduction is also decreased in AD [63], particularly insulin signaling [64–66]. Lastly, genes involved in myelination are also decreased in LOAD [50, 67]. This finding corresponds to studies demonstrating that brain regions with the most myelination are the most vulnerable to AD pathology and that Aβ plaques form retroactively to the developmental progression of myelination in the brain [68].

Profiling in Peripheral Tissue

Studies have attempted to find expression profiles specific to AD in peripheral blood leukocytes to serve as biomarkers. Apoptosis is increased in peripheral blood cells (PBCs) of patients with AD [69]. Chemokine and cytokine signaling processes, which are both heavily involved in inflammation, also show increased expression in AD [53, 70, 71]. This is in line with a general increase in inflammation in response to apoptosis [72, 73]. Moreover, increased expression of inflammatory genes in PBCs has been associated with dementia, and is thought to be triggered by progressing AD pathology [74]. Another response to inflammation observed in AD is increased expression of TGF-β [75–77]. Decreasing the expression of TGF-β within innate immune cells mitigates AD symptoms in mice, such as an increase in spatial memory and Aβ phagocytosis [78].

Most profiles that examined peripheral blood leukocytes in AD observed overall decreases in expression. In contrast to transcriptional profiles in brain tissue, peripheral blood leukocytes profiles show decreased expression of genes involved in cell structure related processes in AD [75, 79]. This finding has been explained by increased apoptosis observed in peripheral blood of AD patients. Similar to brain transcriptome studies, cellular signaling, lipid rafts, and cholesterol related processes are decreased in AD [77]. Two proteins responsible for lipid transport, APOE and APOJ (alias CLU) are genetically associated with AD, and the LOAD associated alleles of both *APOE* and *CLU* result in a decrease of lipid transportation [24, 51]. Moreover, lipid transport is reduced in patients with AD [76]. Additionally, AD patients had decreased expression of ATP-binding cassette transporters (ABC transporters), transporters which utilize ATP and carry out different processes within the cell. Seven ABC transporters have been directly linked to AD through functional studies or GWAS including *ABCA7* [80]. The cellular processes altered in the blood parallel those disrupted in the brain. PBC gene expression profiling in AD points to processes that are disrupted across the body and can potentially serve as a biomarker for AD.

Single-Cell profiling

Aβ plaques are extracellular, whereas NFTs are intracellular deposits typical to AD pathology. According to the tau hypothesis of AD, abnormal hyper-phosphorylation of the microtubule-associated protein tau (*MAPT*) leads to neurotoxic aggregates of tau, the formation of intracellular NFTs, the disintegration of microtubules, the collapse of neuronal transport and finally cell death [57]. To understand the transcriptional responses of neurons affected by intracellular NFTs, several studies applied single-cell transcriptomic profiling. Similar to post-mortem brain transcriptomics, there was an overall decrease of gene expression within neurons of AD patients with versus without NFTs [35, 62, 81]. Genes

involved in cell cycle, cell signaling, cytoskeleton, mitochondria, and metabolism were decreased in AD [35, 62, 81]. In addition, most cellular processes with increased gene expression in post-mortem brain studies also show increased expression within neurons affected by NFTs, such as inflammation and mitochondrial dysfunction [35, 62, 81]. Intriguingly, an increase in vesicle-mediated transport in singular neurons was observed prior to NFT development [62]. Defects in axonal transport were also observed in neurons prior to NFT formation. This increase in vesicle-mediated transport might be explained by tau oligomer toxicity prior to NFT formation, but might also be mediated by concomitant Aβ toxicity or neuroinflammation.

Impact of AD-related variants on transcriptional profiles

To understand how human genetic variants impact AD pathogenesis, transcriptomics have been applied by either utilizing humanized transgenic cell and animal models or AD patient-derived cells with and without variant allele status. Nagasaka et al. demonstrated that single causative ADEAOD mutations (*APP p.K595N/M596L*, *p.E693G* and *PSEN1 p.H163Y*) significantly impact transcriptional profiles [82]. The authors compared transcriptomic profiles of cultured fibroblasts from AD patients carrying an ADEOAD mutation with profiles from unaffected siblings that were non-carriers. While the levels of *APP* and *PSEN1* were comparable between fibroblasts of mutation carriers and non-carriers, up to 200 genes were differentially expressed between the groups, but showed similar profiles among AD affected mutation carriers.

Transcriptional profiles of *APOE ε4* AD risk allele carriers differed greatly when compared to non-risk allele carriers [49, 52]. Xu et al [49] compared hippocampal gene expression of AD patients with the *APOE ε4/ε4* genotype versus patients with the *APOE ε3/ε3* genotype and found increased gene expression in processes such as cell growth, protein modification and RNA binding/editing; whereas gene expression was lowered in stress response, ER-Golgi transport, and mitochondrial oxidative phosphorylation. Expression differences were also observed between *APOE ε4* carriers and non-*APOE ε4* carriers when examining expression profiles in subjects with mild cognitive impairment (MCI) [52]. Similar to the Xu et al. study, genes involved in MHC class II protein complex, cell–matrix adhesion and cell growth had increased expression in *APOE ε4* carriers, whereas genes involved in processes such as mitochondrial electron transport, microtubule, synaptic and nucleosome assembly were down regulated [52].

Using post-mortem brain tissue, Rhinn et al [51] constructed gene expression networks to examine how APOE alleles influence gene network interactions in AD patients and healthy controls with different *APOE* risk genotypes. Interestingly, transcriptional profiles of non-demented *APOE ε4* AD risk allele carriers already most resembled that of subjects with a diagnosis of LOAD when compared to patients with neurological diseases other than AD. Transfection of N2a-APP cells with human *APOE ε4* alleles increased $A\beta_{40}$ and $A\beta_{42}$ levels, but did not with *APOE ε3* or *ε2* alleles. Moreover, their analyses identified six genes (*RNF219*, *SV2A*, *HDLBP*, *ROGDI*, *CALU* and *PTK2B*) that exclusively interacted with the *APOE ε4* allele, but not with the other *APOE* alleles. Importantly, knockdown of these genes

in *APOE ε4* allele transfected cells resulted in decreased $A\beta_{40}$ and $A\beta_{42}$ levels and had no effect on Aβ levels in cells transfected with alternative *APOE* alleles.

Conclusions

Three different study approaches so far led to the successful identification of rare variants in LOAD: (1) large-scale sequencing of autosomal-dominant early-onset Alzheimer disease (ADEOAD) genes in a case-control design with subsequent association testing in several even larger case-control cohorts followed by functional studies, (2) an analogous unbiased, genome-wide sequencing approach, and (3) a combined approach of sequencing and co-segregation analyses in several families enriched for LOAD also in conjunction with subsequent large association and molecular studies. Importantly and for the first time, strategy 1 showed genetic association between LOAD and a variant in the ADEOAD gene *APP*. Noteworthy, the strong protective effect of the newly discovered rare *APP p.A673T* variant serves as a proof of principle that reducing β-cleavage of APP may protect from AD. Strategy 2 and 3 led to the identification of completely novel LOAD susceptibility genes (*TREM2, PLD3*) that were not implicated by common GWAS variants. Given the complex nature of LOAD, it is likely that additional, yet unknown rare variant associations also with intermediate to large risk to or protection from LOAD will be revealed in the near future. Furthermore, biological processes defined by genes with causative mutations, rare and common susceptibility variants in AD overlap with processes indicated by human whole-transcriptomics studies in AD examining post-mortem brain, peripheral blood cells (PBC) and single neurons. Genes commonly up regulated in AD involve processes such as mitochondrial function, inflammation, calcium signaling and cytoskeletal organization, whereas gene expression in synaptic functions and signal transduction is reduced in AD. PBC transcriptional profiles reflect those obtained from brain tissues of AD patients. Thus, PBC profiling could become a practical biomarker for AD diagnosis and disease progression monitoring [83]. Moreover, transcriptome profiles of pre-symptomatic and AD affected pathogenic AD mutation or *APOE* risk allele carriers both reflect transcriptional changes reminiscent to those of LOAD patients. As more rare variants will be related to AD, transcriptional profiling in AD variant carriers will likely give variant specific insight into molecular mechanisms involved in LOAD pathogenesis which might provide an avenue for personalized medicine.

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• Of importance;

•• Of major importance

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Table 1

Overview of rare variants associated with late-onset Alzheimer disease (LOAD)

Variants in bold achieved study-wide significance and were replicated in an independent study sample. Variants in italic obtained nominal significant association in one study and remaining variants are likely pathogenic according to co-segregation in LOAD families. Minor allele frequencies (MAF) are reported as were given by the cited studies and reflect frequencies in populations of European descent.

Gene	Amino acid change	dbSNP rs#	Odds ratio	MAF (%)	PolyPhen2 prediction	Reference
APP	**A673T**	**rs63750847**	**4.2***	**0.4**	**benign**	[28]
	N660Y	-	-	<0.01	probably damaging	[27]
PLD3	**V232M**	**rs145999145**	**2.1**	**0.4**	**probably damaging**	
	M6R	-	*7.7*	*<0.1*	*benign*	[30]
	A442A (ss)	-	*2.3*	*1.6*	-	
TREM2	**R47H**	**rs75932628**	**2.9**	**0.3**	**probably damaging**	[26, 29]
	D87N	*rs142232675*	-	*0.1*	*probably damaging*	[29]
	Q33Ter~	rs104894002	-	0.01	-	[29]
	Y38C~	-	-	<0.08	probably damaging	[29]
	T66M~	rs201258663	-	<0.03	probably damaging	[29]

*protective from AD

~cause frontotemporal dementia–like syndrome or Nasu-Hakola disease in homozygous state

ss variant affects splice site

Table 2

Overview of biological processes altered in Alzheimer disease (AD) from transcriptome studies since 2005

Listed beside each process are studied tissues, direction of change and genes involved in the same process implicated to AD by Genome-Wide Association Studies (GWAS).

Processes	Tissues	Direction	GWAS genes	References
Inflammation/Immune System	**1** Brain Tissue **2** PBCs **3** Single-cell	Increased	*BIN1* *CD33* *CR1* *EPHA1* *HLA-DRB1* *INPP5D* *MEF2C* *MS4A6A*	**1** [32, 43, 46, 49, 50, 52, 53] **2** [53, 70, 71]. **3** [35, 45, 62, 70, 75, 81]
Calcium Signaling	Brain Tissue	Increased		[36, 48, 49, 54]
Mitochondrial and Metabolic functions	**1** Brain Tissue **2** PBCs **3** Single-Cell	Increased		**1** [42, 44, 49] **2** [38] **3** [35, 62, 81]
Cytoskeleton related processes	**1** Post-Mortem Brain **2** PBCs **3** Single-Cell	Increased	*CASS4* *CD2AP* *CELF1* *NME8* *SORL1*	**1** [32, 49, 52] **2** [53, 75, 79].
Cell-cycle processes	**1** Brain Tissue **3** Single-cell	Decreased		**1** [50, 62] **3** [35, 62, 81]
Synaptic Transmission	**1** Brain Tissue **2** PBCs **3** Single-cell	Decreased	*PICALM* *CASS4*	**1** [32, 36–44, 52, 62] **2** [53] **3** [75]
Signal Transduction	**1** Brain Tissue **2** PBCs **3** Single-cell	Decreased	*SORL1* *CD33* *CD2AP*	**1** [42,48,49,52,63–66]. **2** [75, 79]. **3** [35, 62, 81]
Cholesterol Related Processes	**1** Brain Tissue **2** PBCs **3** Single-cell	Decreased	*ABCA7* *APOE* *CLU* *SORL1*	**1** [42] **2** [35] **3** [76, 77]

Processes	Tissues	Direction	GWAS genes	References
TGFβ related signaling	PBCs	Increased		[70–77]
ABC transporters	PBCs	Decreased	*ABCA7*	[76, 80]
Myelination	Brain tissue	Decreased	*SLC24A4*	[50, 67]
Vesicle-mediated transport	**1** Brain Tissue **2** Single-Cell	Increased	*CASS4* *CELF1* *NME8*	**1** [42] **2** [62]