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Alzheimer's brains show inter-related changes in RNA and lipid metabolism

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ABSTRACT

Alzheimer's disease (AD) involves changes in both lipid and RNA metabolism, but it remained unknown if these differences associate with AD's cognition and/or post-mortem neuropathology indices. Here, we report RNA-sequencing evidence of inter-related associations between lipid processing, cognition level, and AD neuropathology. In two unrelated cohorts, we identified pathway-enriched facilitation of lipid processing and alternative splicing genes, including the neuronal-enriched NOVA1 and hnRNPA1. Specifically, this association emerged in temporal lobe tissue samples from donors where postmortem evidence demonstrated AD neuropathology, but who presented normal cognition proximate to death. The observed changes further associated with modified ATP synthesis and mitochondrial transcripts, indicating metabolic relevance; accordingly, mass-spectrometry-derived lipidomic profiles distinguished between individuals with and without cognitive impairment prior to death. In spite of the limited group sizes, tissues from persons with both cognitive impairment and AD pathology showed elevation in several drug-targeted genes of other brain, vascular and autoimmune disorders, accompanied by pathology-related increases in distinct lipid processing transcripts, and in the RNA metabolism genes hnRNPH2, TARDBP, CLP1 and EWSR1. To further detect 3′-polyadenylation variants, we employed multiple cDNA primer pairs. This identified variants that showed limited differences in scope and length between the tested cohorts, yet enabled superior clustering of demented and non-demented AD brains versus controls compared to total mRNA expression values. Our findings indicate inter-related cognition-associated differences in AD's lipid processing, alternative splicing and 3′-polyadenylation, calling for pursuing the underlying psychological and therapeutics implications.

1. Introduction

Multiple recent studies seek causative links between Alzheimer's disease (AD)'s pathology, dementia and metabolic functions. Principally, neuronal numbers are lower in demented compared to non-demented individuals with AD Braak neuropathology (Andrade-Moraes et al., 2013; Braak and Braak, 1995), and individuals with inheritably impaired phospholipid metabolism are particularly susceptible for cognitive decline (Lacour et al., 2017). This agrees with the recently reported global sharing of physical and mental health parameters (Hagenaars et al., 2016). It also supports the co-ignition of the AD metabolic decline by β-amyloid fibril formation and tau hyper-phosphorylation (Pascoal et al., 2016), which includes altered phosphatidylcholine metabolism (Whiley et al., 2014). That AD neuropathology may be attenuated by the epigenetic SIRT1 pathway (Shah et al., 2016), and that phosphatidylcholine protects neurons from β-amyloid toxicity (Ko et al., 2016) further suggests complex inter-relationships between lipid metabolism, neuronal death and cognitive deterioration in AD.

The role and composition of brain lipids has recently emerged as a major criterion in aging and AD studies, especially for β-amyloid toxicity. Brain lipids are brain region- and species-specific, and their levels and composition are modified with aging in a human-characteristic pattern (Fu et al., 2011). Neuronal lipids regulate the location and function

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of synaptic membrane proteins and relay signals from the membrane to intracellular sites or to other cells, as well as impairments in depression and anxiety disorders (Muller et al., 2015). New technologies facilitate these discoveries. Thus, time of flight secondary ion mass spectrometry (TOF-SIMS) revealed cortical cholesterol overload in the AD cortex (Lazar et al., 2013), and Nonlinear microscopy enables visualization of plaque-associated lipids in AD brain tissues (Kiskis et al., 2015), where they may invert inert β-amyloid fibrils into neurotoxic ones (Martins et al., 2008). However, it remained unknown whether lipid differences are associated with AD's cognitive deterioration.

RNA processing impairments are causally involved in various neurodegenerative events (Kim et al., 2013). Therefore, we hypothesized that they may be associated with AD's progression and lipid alterations. Pre-mRNA-processing activities, including capping, alternative splicing and 3′-alternative polyadenylation (APA) are continuously engaged in extensive crosstalk with one another (Elkon et al., 2013; Proudfoot, 2011). Specifically, alternative splicing is a major regulator of gene expression in health and disease (Di Giammartino et al., 2011). Also, impaired alternative splicing associates with decreased dendritic spine density, mal-functioning of entorhinal–hippocampal neural circuits and suppressed learning capability in mice (Berson et al., 2012). About 54% of the human genes also possess multiple APA sites (Tian et al., 2005), which can either selectively affect the 3′-untranslated region (3′-UTR) and/or change the coding region. The brain preferentially uses distal poly(A) sites (Di Giammartino et al., 2011), and functional microRNA (miRNA) recognition sites often reside within, and near both ends of the 3′-UTR (Grimson et al., 2007). This suggests *co*-evolution of poly(A) sites (Lee et al., 2008), miRNAs that target them (Barbash et al., 2014) and mRNA isoforms with modified repertoire of cis-regulatory elements. Supporting this notion, evolutionarily younger genes are less likely to include APA signals. For example, the longer APA variants of brain-derived neurotrophic factor (BDNF) and calmodulin-activated protein kinase 2 (Camk2a) show elevated dendritic location (An et al., 2008; Bulleit et al., 1988; Timmusk et al., 1993). Thus, both alternative splicing and APA may affect neuronal properties in more than one manner.

We, and others found both alternative splicing (Berson et al., 2012; Kolisnyk et al., 2013) and miRNA alterations (Lau et al., 2013) in AD brains with dementia. These changes may modify mRNA translation, stability and localization. However, in-depth RNA processing studies in AD are still limited, and none of the genomics, transcriptomics or proteomics resources of the AD brain cover global APA or lipidomic parameters. Therefore, it is still largely unclear whether or how RNA processing impairments are linked to the characteristic histopathology and/or cognitive and phospholipid metabolism decline, which are the major hallmarks of AD. To identify transcript differences that are separately or jointly associated with AD's dementia, neuropathology or phospholipid metabolism, we initiated an in-depth RNA-sequencing based study of temporal gyrus tissues from two independent groups of persons with or without prior evidence of cognitive impairments. We subjected polyadenylated RNA transcripts to RNA sequencing using either QuantSeq 3′-sequencing (for the first cohort), or multiple 3′ primer variants-derived cDNA libraries (for the second cohort), to identify RNA metabolism and APA modifications; profiled lipids in these tissues by mass spectrometry; and examined associations between these diverse changes and the donors' cognitive and pathology states.

2. Results

2.1. RNA-Seq discriminates between brains of demented and non-demented Alzheimer patients

To address transcript changes that associate with AD pathology and/or cognitive status prior to death, we studied brain samples from 3 groups of 12 clinically diagnosed male participants each from the Rush Memory and Aging Project (MAP; N = 36). Participants who presented no cognitive impairment (NCI), mild cognitive impairment (MCI) or AD dementia were included in each of these groups, which included 4 cases each with low (Braak 1–2), moderate (Braak 3–4) and high (Braak 5–6) levels of AD (Fig. 1a). Likewise, each of the groups with a given Braak staging level was composed of 4 samples with NCI, MCI or AD dementia. Individual brain RNA samples from these MAP groups were subjected to Illumina RNA sequencing and bioinformatics analysis (see Supplementary Tables 1 and 2 for full sample data, Fig. 1a, b for descriptions of the studied groups and workflow, and Methods for definitions).

The second group, received from the Netherland Brain Bank (NBB) was composed of 24 temporal lobe samples from demented male patients diagnosed with AD, and non-demented individuals without or with pathologic AD (Controls; 'non-demented with pathology', NDWP; AD). RNA preparations from the NBB samples were reverse transcribed into full-length 3′-tagged cDNA and segregated through a SQUARE selective exponential amplification using one universal 5′-primer and 12 selective 3′-primers, each of which hybridizes at the junction of the mRNA body and poly(A) tail. An additional group of AD diagnosed patients with early Braak 3–4 neuropathology and cognitive decline was added to the NBB groups. Sequencing files from both groups were processed and analyzed for differential expression and functional enrichment, and for the NBB groups also for APA detection (Fig. 1d). Observations were validated by high-throughput RT-PCR, enzyme activity tests and comparison to previous databases (Fig. 1e).

RNA transcript differences in MAP groups (details in Supplementary Table 2) were analyzed for association with the level of AD pathology and/or clinical diagnosis. In spite of the modest group sizes and the individual heterogeneity between samples, global transcript differences showed significant associations with either cognition or AD pathology (Supplementary Fig. 1), and the association of transcript modifications with cognitive impairment was stronger than the association to AD pathology. Gene Ontology analysis by selecting transcript groups with ANOVA P value <0.05 revealed enriched pathways that associated with cognitive impairment or with pathology (not corrected for multiple comparisons; DAVID (https://david.ncifcrf.gov/) (Supplementary Tables 3 and 4, Fig. 2a). In MAP AD tissues, the cognition-related brain pathways included RNA processing and lipid synthesis. In the 24 sample NBB groups (Fig. 2b), the deeper sequencing protocol highlighted distinct significant differences in additional, non-overlapping AD-related pathways, including alternative splicing and nuclear activities, which differed in NDWP tissues. We conclude that in both cohorts, modified transcript groups vary more distinctively throughout cognitive decline than with progressing pathology and show association with RNA processing.

To validate our NBB results by an independent technology we selected 80 transcripts with high and medium expression levels in the three NBB groups and AD samples from patients with cognitive impairment and early yet discernible neuropathology (Braak stages 3 and 4), similar to that of the pathologic AD without dementia groups, for quantitation by microfluidic RT-PCR (Fluidigm, US) (Supplementary Fig. 2; Primer sequences in Supplementary Table 5). Out of the 80 tested transcripts, 65 showed linear RT-PCR calibration curves in the examined expression range with a lower than 1% ratio of technical noise to biological signal (Supplementary Figs. 3 and 4). Transcript levels showed significant correlation coefficients and P values with the Fluidigm RT-PCR results for 85.5% of tested transcripts that yielded linear curves out of the 65 (Supplementary Fig. 5). Selected comparisons included global expression of CAMK2A ($P < 2 * 10^{-8}$) and the distal and proximal SOD1 APA variants ($P < 0.001$) (Supplementary Fig. 6). Importantly, the Fluidigm analysis of the distal SOD1 variant showed no significant correlation with the proximal SOD1 variant, and vice versa, pointing at the high fidelity and resolution power of the results. Deep 3′-segregated RNA sequencing thus demonstrated valid levels of both global differences and identified APA variants of brain transcripts.

Impaired cholinergic transmission has long been reported in AD (Giacobini and Gold, 2013), and cholinergic neurons are the primary producers of acetylcholinesterase (AChE) (Soreq and Seidman, 2001).