Female-specific risk of Alzheimer's disease is associated with tau phosphorylation processes: A transcriptome-wide interaction analysis.

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Key Words

Alzheimer's disease, female risk, sexual dimorphism, gene expression, transcriptome, expression array, transcriptome, tau, phosphorylation, neurofibrillary tangles, NCL, KIF2A, transcriptome-wide interaction analysis, differential expression.

ABSTRACT

The levels of tau phosphorylation differ between sexes in Alzheimer's disease (AD). Transcriptome-wide associations of sex by disease interaction could indicate whether specific genes underlie sex differences in tau pathology, however, no such study has been reported yet. We report the first analysis of the effect of the interaction between disease status and sex on differential gene expression, meta-analyzing transcriptomic data from the three largest publicly available case-control studies (N=776) in brain to date. 121 genes, significantly associated with sex-AD interactions, were enriched in phosphoproteins (FDR=9.65×10⁻⁶). High and consistent associations were found for the over-expressions of NCL (FDR=0.0020), whose phosphorylated protein generates an epitope against neurofibrillary tangles, and KIF2A (FDR=0.01), a microtubule-associated motor protein gene. Transcriptome-wide interaction analyses suggest sex-modulated tau phosphorylation, at sites like Thr231, Ser199 or Ser202 that could increase the risk of women to AD and indicate sex-specific strategies for intervention and prevention.

1. Introduction

Two-thirds of Alzheimer's disease patients are women (Association et al., 2018). One contributor to the higher prevalence of AD in women is survival bias, as men at risk are more likely to die before developing AD than women at risk (Mielke et al., 2014). However, sex-biased, environmental or biological risk factors also contribute to the difference in AD risk, such as educational attainment or the APOE-e4 genotype among others (Stern et al., 1994; Altmann et al., 2014; Ferretti et al., 2018). Evidence indicates, in particular, important differences in tau cerebrospinal fluid in women with amyloidosis (Hohman et al., 2018), supported by recently observed differences in the production and the structure of neurofibrillary tangles between sexes (Buckley et al., 2019). Depletion of hormones, in particular in estrogen, a known risk factor of AD (Merlo et al., 2017), could play important roles in female tauopathy as hormones can influence tau phosphorylation at specific sites (Muñoz-Mayorga et al., 2018).

While genetic studies have shown that tau pathology is higher in female APOE-e4 carriers (Altmann et al., 2014), it is unknown which other genes could be linked to sex-specific-tauopathy processes. Recent studies have found sex, and AD by sex differences in gene expression in the brain in a few individuals (Shi et al., 2016; Mathys et al., 2019). However, there is no large study that identifies transcriptome-wide signatures of sex-AD interactions. To fill this gap, we performed a meta-analysis of transcriptomic data from the three largest, publicly available, transcriptomic studies to date. We aimed to find which genes showed a differential expression that was specific to female AD patients, and contrast the findings with relevant sex-biased risk factors of AD, such as processes involved in tau pathology.

2. Methods

2.1 Statistical Analyses

We performed transcriptomic-wide interaction analysis on large AD case-control studies. For each study, we tested the association between gene expression and the interaction between sex and AD status using the linear model

$$E_{ij} = \alpha_i + \beta_i (S_j \times D_j) + \sum_{r=1 \dots k} \gamma_{irj} C_{rj} + \varepsilon_{ij}$$

where E_{ij} is the gene expression at probe i for individual j with sex S_j and disease status D_j . C_{rj} are k covariates that include sex, disease status, age, post-mortem interval and batch effects. Coding sex as Female: 1 and Male: 0, and Alzheimer status as AD: 1 and control: 0, γ_i is the specific effect of AD-females on the transcription of probe i . For each common gene across studies, we meta-analyzed the associations with the sex-AD interaction, using an adaptive weighted combination of P-values, as implemented in the Bicoductor's library MetaDE. We adjusted the one-tailed testing of the combination by combining the P-values for each tail across the studies and selected the direction of the test with the most significant association. P-values were then corrected for multiple comparisons with false discovery rate (FDR). We also performed enrichment pathway analyses for the significant genes.

2.2 Expression Data

We identified three large case-control transcriptomic studies ($N > 70$) of AD in the GEO repository. These have accession numbers GSE15222, GSE44770 and GSE84422 (see Table 1). The three studies collected microarray transcriptomic data from cortical samples including frontal, temporal and visual cortices. The study GSE15222 contains transcriptomic data in cortical tissue of non-demented individuals and late-onset Alzheimer's disease (LOAD) patients. GSE44770/GSE44771 is a case/control transcriptomic study of LOAD based on autopsied tissues from the dorsolateral prefrontal cortex (DLPF) and visual cortex (VC). In GSE84422, 19 brain regions from 125 brains were used for transcriptomic analysis, with an average of 50 to 60 subjects per brain region. We selected individuals sampled in only one cortex among DPFC, PFC and VC. For individuals with multiple samples in the cortices, data for one cortex was randomly selected, such that only one individual had data for only one cortex.

The numbers of females and AD cases varied across studies, where GSE84422 had the largest ratios of AD-females. Overall, the studies comprised a total of 858 individuals, 384 (44.7%) females and 473 (55.1%) AD cases.

2.3 Software

We used Bioconductor and R packages to download and analyze the transcriptomic data of all four studies using a common pipeline. Quality controlled and normalized transcriptomic and phenotypic data were downloaded with the GEOquery package. Variables that represented underlying technical (batch) effects were estimated using surrogate variable analyses (SVA package), Differential expression analyses for the sex-AD interaction was performed using the limma package. Models were adjusted by sex, AD status, age, post mortem interval and the surrogate variables for each study. Meta-analyses were performed with the MetaDE package, and enrichment analyses with STRING using the Uniprot database. The entire reproducible code and analyses can be found in the supplementary material.

3. Results

3.1 Transcriptome-wide meta-analysis in cerebral cortex

We first analyzed the sex-specific transcriptome-wide signatures of AD in each study, testing the association between gene expression and the sex-AD interaction. P-values for the differential expression of 10,795 common genes were combined across the three studies and adjusted for multiple comparisons. We discarded genes in chromosome Y. We observed 70 statistically significant genes after FDR correction (Table S1-S2). Many of these genes have been previously linked with tau phosphorylation and AD.

The top three genes that significantly associated with the sex-AD interaction, from the meta-analysis (Tables S1-S2) were NCL (FDR = 0.0022), TARDBP (FDR = 0.0030), and KIF2A (FDR = 0.0052). In particular, NCL and KIF2A showed consistent nominal association in two different studies, where both up-regulated in AD females with respect to female controls but down-regulated for AD males with respect to male controls (Figure 1A). These genes are

linked to tau phosphorylation. NCL encodes the nucleolin protein, whose phosphorylation by Cdc2 kinase generates the TG-3 epitope against neurofibrillary tangles (Dranovsky et al., 2001). KIF2A encodes the microtubule-associated motor protein gene that is phosphorylated by tau tubulin kinase 2 (TTBK2) (Sato et al., 2006). TARDBP is also targeted by TTBK2 in frontotemporal lobar degeneration (Taylor et al., 2018), and its rare p.Ala90Val substitutions in exon 3 were associated to AD (Brouwers et al., 2010). Top associated genes also included DNAJB6 (FDR=0.0098), a chaperone molecule that inhibits amyloid-beta concentrations (Månsson et al., 2014), and PPP2R2A (FDR = 0.01), involved in site-specific regulation of tau phosphorylation (Yu et al., 2014). In summary, we observed numerous genes whose expression levels associated with the sex-AD interaction, many of which with direct links to AD through tau-phosphorylation processes.

3.2 Enrichment meta-analysis

We performed a gene enrichment analysis for the 128 genes that significantly deregulated in AD-females, to identify the molecular mechanisms that could underlie these associations (Table S3). We observed that the 128 genes were highly connected in a protein-protein interaction network ($P=1.6 \times 10^{-5}$) (Figure 1B). The network was strongly enriched with phosphoproteins (FDR =0.0016) and neuropathy (FDR=0.026), in line with the single gene associations we had found. We also observed significant enrichment with acetylation (FDR= 2.05×10^{-5}), Ubl conjugation (FDR= 3.4×10^{-4}), isopeptide bonds (FDR = 0.0012), mitochondrion (FDR=0.026) and nucleotide-binding (FDR=0.035), Figure 1C. We did not observe any significantly enriched pathway from Kyoto encyclopedia of genes and genomes (KEGG). We also performed a sensitivity analysis, removing the samples from the visual cortex; less affected by AD, and observed consistent results in genes and pathways (Tables S4-S5).

4. Discussion

Compelling evidence indicates that the presence and fate of neurofibrillary tangles by tau hyperphosphorylation in AD is different between men and women (Buckley et al., 2019). Differences in sex hormones could explain these differences (Muñoz-Mayorga et al., 2018). In

particular, menopause and oophorectomy could increase AD-risk by the removal protection by estrogen and progesterone against tau phosphorylation by GSK3 (Merlo et al., 2017). In this context, our analyses indicate specific processes and phosphorylation sites that could be involved in the sex differences in AD tauopathy. For instance, the epitope TG-3, generated by the phosphorylation of nucleolin (Dranovsky et al., 2001), is specific of tau phosphorylation at Thr231. Interestingly, pThr231 tau is found to be attenuated by 17betaestradiol (E2) (Shi et al., 2008) and to increase in the latter stages of AD (Braak stage V/VI) (Neddens et al., 2018), suggesting a sex-specific evolution of tau phosphorylation mediated by estrogen at Thr231 and giving mechanistic support to our observation of NCL over-expression in AD females. Notably, E2 also attenuates tau phosphorylation at Ser199, Ser202, targets of TTBK1 in addition to Ser135 of KIF2A (Sato et al., 2006), which can also be linked to neurofibrillary tangles by its microtubule functions (Homma et al., 2003), and that we also observed consistently overexpressed in AD females. While we remarkably also observed associations with PPP2R2A, which appears to precisely reduce tau phosphorylation at Ser199, Ser202/Thr205 and Thr231 (Yu et al., 2014), we noted that its over-expression unexpectedly correlated with AD females. It is important to note, however, that our results are transcriptome-wide hypothesis-free findings and, therefore, they require further validation of the concrete observations they reveal, together with their interrelation. Our results suggest that specific phosphorylation sites of tau should be further investigated for precision treatment and risk assessment of women. More generally, our results importantly provided consistent and numerous evidence on the sex differences in tau phosphorylation in AD etiology. AD is among several diseases that show sexual dimorphism in risk and progression. Deepening into the biological causes of these differences will help to design sex-specific treatments and prevention. We showed that, in the case of AD, interaction analyses between sex and phenotypes can provide relevant transcriptomic signatures of sexual dimorphism. Consequently, the analyses could also be informative in other sexually dimorphic traits and diseases.

Conflicts of interest:

All authors declare no conflict of interests.

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Table and Figure Legends

Study	Cortical region	N	Males (AD/control)	Females (AD/control)	Mean age	Mapped genes	Platform	PubmedID
GSE15222	Frontal/ Temporal/unreported	311	157 (62/95)	154 (70/84)	82.3	15612	Sentrix HumanRef-8	19361613
GSE44770/ GSE44771	Dorsolateral prefrontal/ Visual	390	255 (110/1145)	135 (104/31)	71.5	18121	Rosetta/Merck 44k 1.1	23622250
GSE84422	Dorsolateral Prefrontal/ Prefrontal/Visual	84	25 (8/17)	59 (10/49)	85.7	13516	Affymetrix U133 Plus 2.0	27799057

Table 1. Large case-control transcriptomic studies of AD identified in the GEO repository. The three studies in post mortem brain tissue were meta-analyzed. In all studies cortical regions were assayed.

Figure 1. A) Gene expression residuals, after adjusting by sex, disease state, age and surrogate variables, as a function of disease state and stratified by sex. Sex-AD interactions for *NCL* and *KIF2A* expression are significant in the meta-analysis, corrected for multiple comparisons, and consistent between GSE15222 and GSE44770/ GSE44771, with nominally

significant associations each. No significant associations were observed in GSE84422 (grey background). **B)** Protein-protein STRING network. The network was constructed for the genes with significant differential expression for the sex-AD interaction in post-mortem cortical tissue. The genes showed transcriptome-wide significance in the meta-analysis across three studies and were also significant in two studies. 68 connected genes are shown in blue, from a total of 76 significant genes within the protein phosphorylation pathway. **C)** Enrichment analyses for the UniProt database of biochemical pathways. Genes in phosphoprotein are colored in the network.