

# Sex, ApoE4 and Alzheimer's disease: rethinking drug discovery in the era of precision medicine

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Alzheimer's disease (AD) is the most common cause of dementia and presents with an insidious onset and long prodromal period. Despite billions spent on clinical trials and decades of research, there are currently no disease modifying therapies approved for AD.

Two of the most well-appreciated risk factors for AD include female sex and the presence of the apolipoprotein  $\epsilon 4$  allele (APOE4; Riedel et al., 2016). In this Perspective, we highlight how greater disease subtyping through consideration of sex and APOE4 has the potential to elucidate new disease mechanisms, biomarkers and therapeutic strategies in AD (Figure 1).

While a number of genetic variants have been associated with AD, APOE4 is the most common genetic risk factor for late onset AD. In the brain, apolipoprotein E (APOE) is produced by astrocytes and functions to transport cholesterol to neurons via APOE receptor signaling. In humans, APOE exists as three major isoforms, APOE2, APOE3, and APOE4, encoded by the  $\epsilon 2$ ,  $\epsilon 3$ , and  $\epsilon 4$  alleles, respectively. The  $\epsilon 2$  allele confers protection against AD while the  $\epsilon 4$  allele increases genetic risk for developing AD in a dose-dependent manner. For example, a meta-analysis among White cohorts demonstrated that a single copy of  $\epsilon 4$  confers a 3 to 4-fold increased risk of developing AD (Farrer et al., 1997). Meanwhile, two copies of  $\epsilon 4$  is associated with a 15-fold increased risk of AD. In contrast, the  $\epsilon 2$  allele is associated with a 0.6-fold lower risk of AD. The role of APOE4 as a regulator of neuroinflammation is increasingly well appreciated and thought to partly underlie APOE4-mediated risk in AD. For example, mice with an extra copy of the APOE4 allele exhibit a heightened immune response to liposaccharide compared mice with the APOE  $\epsilon 3$  allele, suggesting a role of APOE4 in regulating both adaptive and innate immune activity (Lynch et al., 2003). Meanwhile young and middle aged APOE4 carriers have increased plasma levels of multiple proinflammatory cytokines, suggesting an elevated immune response that declines in later life (Ringman et al., 2012). Another mechanism underlying APOE4-mediated risk in AD might be through metal toxicity. Recent evidence also suggests APOE4 sequesters zinc, copper and iron inside neurons, leading to tau hyperphosphorylation and amyloid-beta aggregation (Xu et al., 2014).

In addition to APOE4, sex is another major risk factor in AD. After the age of 65, female sex is associated a two-fold increased lifetime risk of AD (Riedel et al., 2016). Interestingly, studies examining sex differences in cerebrospinal fluid (CSF) tau, CSF A $\beta$ , brain atrophy have produced inconsistent results. Some have hypothesized that the increased lifetime risk of AD among

females may result from an increased life expectancy for females (Mielke, 2018). Alternatively, some studies have provided support for the age related loss of sex steroid hormones including estrogen, which is known to protect against AD pathogenesis (Pike et al., 2009). Despite the therapeutic potential of a better understanding of these molecular mechanisms, sex differences in AD remain poorly understood.

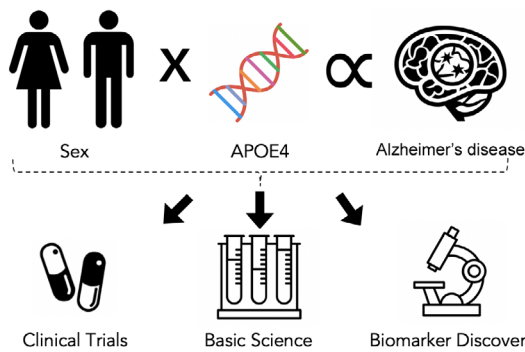
Recent epidemiological, imaging, and molecular studies have revealed that the effect of the APOE4 isoform on AD risk is stronger in females than in males. Altmann et al. (2014) applied Cox-proportional hazards models to examine the APOE4 interaction in conversion risk, from healthy aging to mild cognitive impairment (MCI) or AD and from MCI to AD in > 8000 patients. The authors found that in both conversions, the hazard ratio (HR) for women was higher (healthy to MCI or AD HR = 2.16 for women, 1.64 for men; MCI to AD HR = 1.81 for women, 1.27 for men). Notably, the interaction term of ApoE4-sex was significant in the healthy aging to diseased conversion ( $P = 0.01$ ), but not in the MCI to AD conversion. The interaction term was also significant in sub-analyses restricted to ApoE3/3 and ApoE3/4 genotypes for both conversions. Using data from the Alzheimer's Disease Neuroimaging Initiative, Altmann et al. (2014) also identified a significant ApoE4-sex interaction in CSF (cerebrospinal fluid) biomarker levels of total tau ( $P = 0.009$ ) and tau/amyloid beta ratio ( $P = 0.02$ ) among MCI patients. Sampedro et al. (2015) assessed the presence of an APOE4-sex interaction on core CSF biomarkers, brain metabolism, and structure (evaluated from imaging data) in healthy elderly control individuals for patients from the Alzheimer's Disease Neuroimaging Initiative. While the authors found no APOE4-sex interaction among CSF biomarkers, the interaction term in the context of brain metabolism and structure was significant (Sampedro et al., 2015). Both female and male APOE4 carriers presented with widespread brain hypometabolism and cortical thinning compared to non-carriers. However, female APOE4 carriers demonstrated a greater degree of hypometabolism and atrophy compared to female non-carriers, whereas male APOE4 carriers showed only small clusters of hypometabolism and regions of cortical thickening compared to their non-carrier counterparts. Their findings show that the effect of APOE4 on brain metabolism and structure is modified by sex (Sampedro et al., 2015).

While there is substantive evidence to support an APOE4-sex interaction from epidemiological, molecular, and imaging assays, the underlying mechanism is not well-established. However, data from recent transcriptomic and

metabolomics studies may provide potential mechanistic explanations. For example, Zhao et al. (2020) generated brain transcriptomic and blood metabolomic data from APOE2, APOE3, and APOE4 targeted-replacement mice. Age, sex, and APOE4 status exhibited a significant interaction effect on genes expression within the unfolded protein response pathway. The unfolded protein response pathway is responsible for protecting cells from the harmful effects of misfolded proteins when the cellular environment overwhelm the ability of the endoplasmic reticulum to fold and assemble secretory proteins. Because AD is characterized by the accumulation of misfolded amyloid and tau proteins it is plausible that an increased vulnerability to the pathological effects of misfolded proteins may underlie APOE-by-sex interaction effects in AD. Alternately, a human serum metabolomics study conducted by Arnold et al. (2020) found that acylcarnitine C10 was positively correlated with CSF p-tau in female APOE4 carriers but not in female ApoE4 non-carriers or males. Similarly, among female APOE4 carriers specifically, proline levels were negatively correlated with reduced brain glucose uptake measured by [<sup>18</sup>F]-fluorodeoxyglucose-positron emission tomography (FDG-PET). These data suggest a defect in glucogenic energy metabolism and beta oxidation in female APOE4 carriers. While these are promising preliminary studies, much work remains to be done in order to fully untangle the complex molecular mechanisms underlying APOE and sex differences in AD.

We envision a future in which, much like the precision immunotherapy revolution in oncology, greater disease subtyping will remarkably alter the AD therapeutic landscape. We highlight three clear actions, including redesigning clinical trials, integrating precise evaluation of neuroimaging endpoints, and leveraging large scale multiomics data, to foster APOE genotype- and sex-driven discovery of new AD therapeutics, biomarkers, and disease mechanisms.

First, a greater appreciation of the role of sex and APOE in AD has the capability to remarkably transform clinical trials in AD. The persistent failure of clinical trials may stem from underlying disease heterogeneity. Differences in disease etiology may render specific therapies to have varying degrees of efficacy in different populations. A promising step forward in disease subtyping in AD is the creation of the A/T/N (A/N/T stands for  $\beta$ -amyloid pathology/tau pathology/other nonspecific biomarkers of neurodegeneration) disease classification scheme in which individuals are characterized by their amyloid (measured by amyloid PET or CSF A $\beta$ 42), tau (CSF phosphorylated tau, or tau PET), neurodegenerative status (FDG-PET, structural MRI, or CSF total tau). In the future, patients should be enrolled in trials based on a combination of cognitive assessments and their place on the A/T/N scheme. By gating patients on A/T/N cluster, pharmaceutical interventions may be assessed in specific disease subtypes, accounting for heterogenous disease etiologies within AD. In addition to the A/T/N framework, clinical trials should be performed, and results interpreted, within specific sex and APOE-subgroups. For example, in trials aimed cognitively normal or MCI cohorts, individuals



**Figure 1 | Greater disease subtyping through consideration of sex and APOE4 has the potential to remarkably transform the AD clinical trial landscape and basic science research paradigm.**

In this Perspective, we highlight implications in clinical trial design, neuroimaging evaluation protocol, and usage of large-scale multiomics data to improve subtype-specific biomarker and therapeutic discovery for AD. AD: Alzheimer's disease; APOE4: apolipoprotein ε4 allele.

most likely to develop AD could be enriched for selecting high risk sex APOE groups. A number of emerging trials have included post-hoc analyses, analyzing results by APOE genotype (Kennedy et al., 2014). While these are promising steps, post hoc analysis stratified by sex and APOE genotype should become a routine analysis in AD clinical trials.

Neuroimaging techniques, including amyloid-PET and MRI, are increasingly used as endpoints and selection criteria in AD clinical trials. Our group has recently identified an APOE-by-sex interaction effect on tau deposition in the brains of patients with MCI (Liu et al., 2019) in which the APOE4 allele exerts a greater effect on regional tau deposition in females compared to males. Interestingly, females may remain at the same level of cognitive impairment compared to males, in spite of a higher tau burden, suggesting a greater resilience to tau-mediated neuropathy. In light of this and other studies showing APOE4-by-sex interaction effects on brain FDG metabolism and brain structure (Sampedro et al., 2015), future trials using an imaging based endpoint or recruitment criteria should redefine thresholds based on sex and APOE4 carrier status.

Technological advancements and the increasing availability of large-scale multi-omics datasets present a unique opportunity to create subtype-specific transcriptomic, metabolomics and genomic profiles for biomarker discovery, therapeutic discovery, and mechanistic studies in AD. While APOE genotype and sex have previously been shown to modify serum and brain transcriptomic, genomic, and metabolomic profiles in AD (Paranjpe et al., 2020), their interaction in these data has not been studied. One can imagine a paradigm in which the APOE4 allele leads to radically different pathophysiological mechanisms in males and females with AD. By generating APOE and sex- stratified multiomics profiles in AD, we can begin to elucidate subtype specific molecular candidates for therapeutic intervention and biomarker development. Numerous large scale multiomics data consortia are already freely accessible to researchers including the Alzheimer's Disease Neuroimaging Initiative and Accelerating Medicines Partnership. Another powerful and cost-effective drug development approach is repurposing existing FDA-approved medications. Powerful new computational

techniques including transcriptome and genomic-guided drug repurposing as well as literature-based drug repurposing have set the stage for researchers to identify APOE genotype- and sex-specific repurposed drugs in AD (Paranjpe et al., 2019).

In summary, the past several decades has seen billions of dollars in R&D spend with few therapeutic successes. With a growing global elderly population, there exists a substantial need to implement creative strategies to usher in a new generation of pharmaceutical approaches to AD. We are cautiously optimistic that a greater focus on disease subtyping through the inclusion of sex and the APOE genotype has the potential to elucidate new therapies and unlock the power of precision medicine in AD.

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**Date of submission:** July 10, 2020

**Date of decision:** November 5, 2020

**Date of acceptance:** December 1, 2020

**Date of web publication:** January 25, 2021

<https://doi.org/10.4103/1673-5374.306070>

**How to cite this article:** Paranjpe MD, Wang JK, Zhou Y (2021) Sex, ApoE4 and Alzheimer's disease: rethinking drug discovery in the era of precision medicine. *Neural Regen Res* 16(9):1764-1765.

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**Open peer reviewer:** Sandeep Kumar Singh, PhD, Indian Scientific Education and Technology Foundation, India.

**Additional file:** Open peer review report 1.

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C-Editors: Zhao M, Wang L; T-Editor: Jia Y