

Sex Differences in Alzheimer's Disease: Insights From the Multiomics Landscape

Lei Guo, Margaret B. Zhong, Larry Zhang, Bin Zhang, and Dongming Cai

ABSTRACT

Alzheimer's disease (AD) has complex etiologies, and the impact of sex on AD varies over the course of disease development. The literature provides some evidence of sex-specific contributions to AD. However, molecular mechanisms of sex-biased differences in AD remain elusive. Multiomics data in tandem with systems biology approaches offer a new avenue to dissect sex-stratified molecular mechanisms of AD and to develop sex-specific diagnostic and therapeutic strategies for AD. Single-cell transcriptomic datasets and cell deconvolution of bulk tissue transcriptomic data provide additional insights into brain cell type-specific impact on sex-biased differences in AD. In this review, we summarize the impact of sex chromosomes and sex hormones on AD, the impact of sex-biased differences during AD development, and the interplay between sex and a major AD genetic risk factor, the *APOE* ϵ 4 genotype, through the multiomics landscape. Several sex-biased molecular pathways such as neuroinflammation and bioenergetic metabolism have been identified. The importance of sex chromosome and sex hormones, as well as the associated pathways in AD pathogenesis, is further strengthened by findings from omics studies. Future research efforts should integrate the multiomics data from different brain regions and different cell types using systems biology approaches, and leverage the knowledge into a holistic examination of sex differences in AD. Advances in systems biology technologies and increasingly available large-scale multiomics datasets will facilitate future studies dissecting such complex signaling mechanisms to better understand AD pathogenesis in both sexes, with the ultimate goals of developing efficacious sex- and *APOE*-stratified preventive and therapeutic interventions for AD.

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Alzheimer's disease (AD) is a multifactorial disorder with heterogeneous etiologies. Evidence from basic and clinical studies support sex-specific differences contributing to its complexity. Approximately two-thirds of patients with AD are female (1). Higher AD prevalence in females could be secondary to longer life expectancy or higher dementia incidence in females than in males. Literature reports regarding AD incidents among males and females are conflicting, with some suggesting no differences (2). On the other hand, sex differences in AD have been observed in clinical, neuroimaging, and pathology studies (Supplement). Surprisingly, sex-specific analyses were only performed occasionally during AD clinical trial phases, and the importance of considering sex as a critical modulator of patient responses to treatments was vastly underappreciated (3–6). While it is critical to take into consideration the sex differences in clinical trial design, current challenges reside in the limited knowledge of molecular mechanisms underlying sex-biased differences in AD.

Several mechanisms have been proposed for sex-biased differences in AD, including physiological differences during development, and sex-specific changes during aging and AD development. In recent years, large-scale omics analysis in tandem with systems biology studies has improved our understanding of molecular mechanisms of AD stratified by sex. Multiomics analysis of human AD samples, AD mouse models, and human brain cells derived from inducible pluripotent stem

cells of subjects with AD allows researchers to systematically unmask the complexity of sex-mixed samples and reveal sex-biased genomic, genetic, and epigenetic landscapes in AD. Unlike traditional hypothesis-driven studies, multiomics studies enable discovery of novel molecular changes, pathways, and targets common or specific to each sex group in AD development and progression. More importantly, recent advances in single-cell omics studies facilitate in-depth dissection of cell type-specific contributions to disease mechanisms.

While several aspects of sex differences in AD have been discussed extensively in the literature (7–11), we will discuss current studies of sex chromosomes and sex hormones, multiomics analyses of sex-biased differences in AD, and the molecular architecture underlying the interplay between sex and genetic risk factors of AD.

IMPACT OF SEX CHROMOSOMES IN AD

Mounting evidence has suggested that sex chromosomes contribute to the heterogeneity in AD. For example, the molecular cytogenetic analysis showed that somatically acquired X chromosome aneuploidy may contribute to brain aging and neurodegenerative processes. The frequency of X chromosome loss is over 100 times higher in older women (>65 years of age) than in younger women (<16 years of age) (12). The rates of X chromosome aneuploidy were increased twofold in

neural cells of the hippocampus and cerebrum of subjects with AD (13). Furthermore, premature centromere division (PCD), a genetic mechanism associated with increased aneuploidy, is found to be tightly correlated with aging and AD. The average frequency of PCD on the X chromosome in frontal cortical neurons of patients with AD is almost three times higher than that of control subjects (14). Significantly higher percentages of PCD were observed on chromosomes of peripheral lymphocytes of both men and women of advanced age (15) and on the X chromosome of patients with AD (16).

On the other hand, an extra X chromosome may account for protective effects against AD, probably by augmenting the expression of genes that elude X inactivation. Approximately 15% of X-linked genes escape this inactivation, resulting in an increased expression of X-linked genes in females compared with males (17). For example, the loss-of-function mutations in *KDM6A*, a gene encoding a histone demethylase (18), are associated with intellectual disability in humans (19), while deletion of *KDM6A* in CD4⁺ T cells ameliorated clinical disease and reduced neuropathology in autoimmune disorders like multiple sclerosis, indicating the cell type-specific roles of *KDM6A* in human diseases (20). Consistently, a recent mouse study showed that adding a second X chromosome to hAPP mice confers resilience to AD-related vulnerability in mice, probably through elevating *KDM6A* expression (21), and that *Kdm6a*^{-/-} mice manifested synaptic plasticity and memory deficits (22).

Another example of X-inactivation escapees associated with sex differences in developing AD is *PCDH11X*, a gene involved in cell-cell recognition essential for central nervous system function (23,24). It was suggested that *PCDH11X* escapes X inactivation through epigenetic mechanisms (23). A study investigated 2356 patients with AD and 2384 control subjects and found a single nucleotide polymorphism (rs5984894) in the *PCDH11X* gene associated with higher risks of developing AD in women (25). However, two subsequent genome-wide association studies failed to confirm the association between *PCDH11X* polymorphisms and AD (26,27). Moreover, many genes involved in immune processes are located on the X chromosome (28). Compared with males, females demonstrate higher diversity in immune responses due to random inactivation of one of the two X chromosomes and incomplete X inactivation (29). Future studies are needed to understand the regulation of neuroinflammation by X chromosomes.

Furthermore, loss of chromosome Y (LOY) is the most commonly acquired mutation in aging men (30). Dumanski *et al.* (31) investigated the susceptibility to AD in men with LOY in over 3000 subjects. It was found that male subjects with AD had higher degrees of LOY mosaicism, and men with higher levels of LOY had a greater risk of developing AD. It was speculated that LOY might lead to dysregulated immune system function contributing to AD pathogenesis. Moreover, a study of human inducible pluripotent stem cell-derived neurons from a patient with familial AD with presenilin 1 E280A mutation demonstrated that LOY in these neurons exacerbated toxic effects of A β ₄₂ leading to impaired neuronal differentiation and premature cell death (32). Consistently, a recent study of five transcriptomic datasets examined the degrees of extreme downregulation of chromosome Y and

observed a significant interaction between age and extreme downregulation of chromosome Y associated with AD, supporting the role of extreme downregulation of chromosome Y in age-related increased risks of AD in men (33).

Taken together, these lines of studies support the importance of sex chromosomes in contributing to sex-specific vulnerabilities and/or resilience in brain aging and neurodegenerative processes. The long-overlooked roles of sex chromosomes and linked genes in AD pathogenesis may indicate new directions of future studies to understand molecular mechanisms of sex differences in AD.

IMPACT OF SEX HORMONES IN AD

The roles of sex hormones in brain development as well as in aging and AD processes have been well recognized (34–37). Evidence from animal and human studies support functional roles of sex hormones such as estrogens, progesterone, and androgens in cognition and behavior (34,38). With many neuroprotective effects implicated, age-related decreases in sex hormone levels were associated with increased risks of cognitive decline and AD (34,37). For example, reduced exposure to estrogens across the lifetime is associated with increased risks of developing AD in females, whereas age-related decline in both peripheral and brain testosterone levels is associated with increased vulnerabilities of developing AD in males (36). Moreover, changes in sex hormone receptors and downstream signaling pathways during aging have been reported (37). For example, the expression of nonfunctional splicing variants of estrogen receptor alpha in the hippocampus was increased during aging and AD (39), with higher expression levels in female elderly subjects than in males (40,41). In addition, studies identified polymorphisms of estrogen receptors associated with cognitive decline (42) and AD development in females, particularly in *APOE* ϵ 4 (*APOE4*) carriers (43). These data suggest decreased brain responsiveness to sex hormones during aging and AD development.

On the other hand, clinical trial results of sex hormone therapy in AD are rather controversial (34,44–50). Despite earlier studies implicating protective effects of estrogen replacement against AD in females, large clinical studies failed to demonstrate any beneficial effects (51). It was proposed that initiation of hormone replacement in the critical window of perimenopause may lessen the risks of dementia, whereas it may elevate the risks if initiated years after menopause (52). Besides treatment timing, decreased responsiveness at brain receptors and downstream signaling pathways may contribute to the ineffectiveness of hormone therapy. Together, these studies suggest the complexity of sex hormones' involvement in AD.

BULK TISSUE OMICS STUDIES OF SEX DIFFERENCES IN AD

Large-scale bulk tissue omics studies in the recent years have advanced our knowledge of the molecular architecture of sex differences in AD. As shown in Figure 1, we searched published studies of sex differences in AD on PubMed and selected 134 publications for further analysis. Among them, gene association, gene expression, and protein expression studies account for the majority of selected studies showing

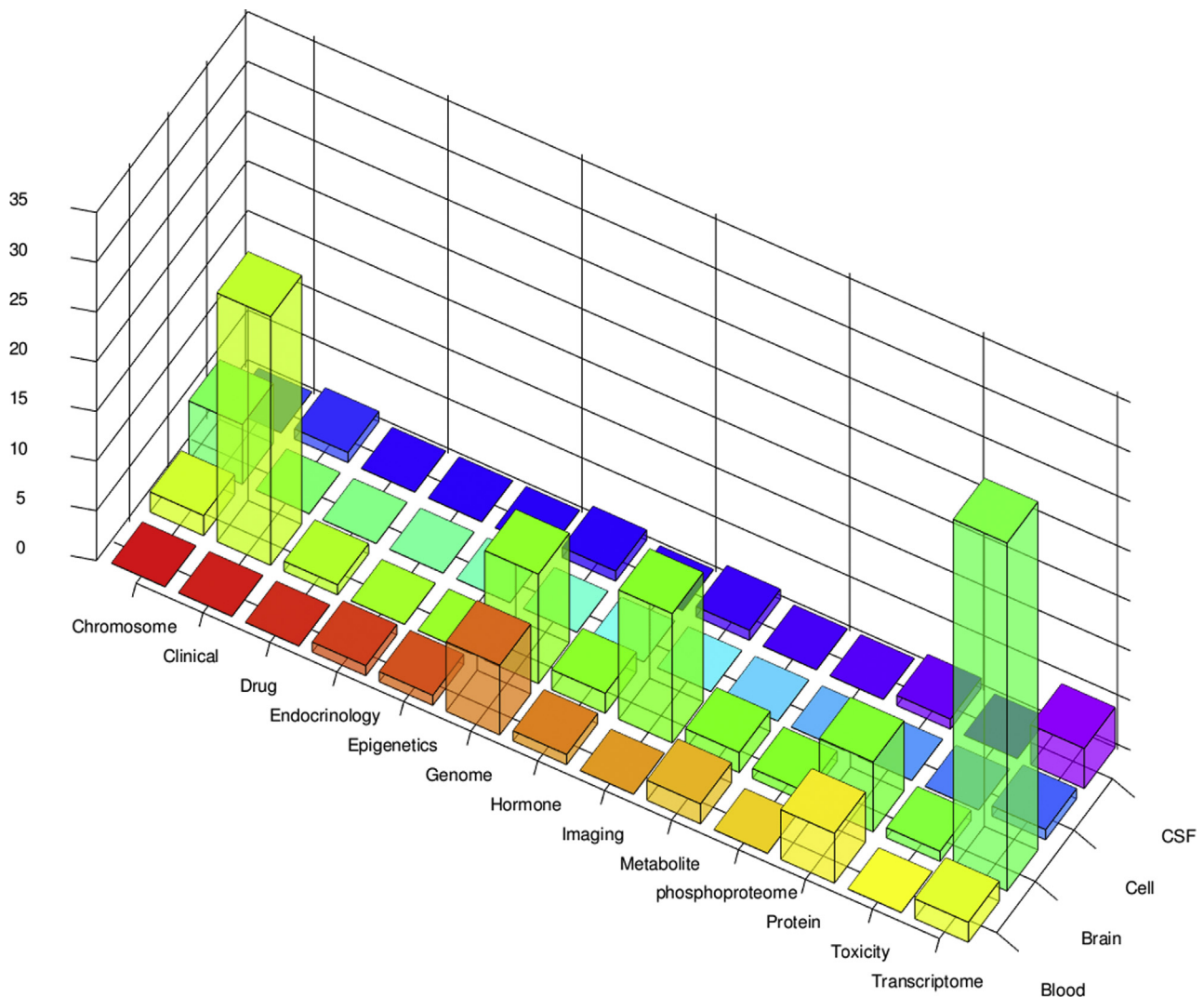


Figure 1. A summary of multiomics studies published in the literature. To explore the studies focused on sex differences in Alzheimer's disease, we searched on PubMed with the key words *Alzheimer*, *sex*, *gender*, *men*, *women*, *male*, and *female*. The search resulted in over 800 publications. We then manually scanned these studies and selected 134 publications covering multiple areas for further analysis. Among them, gene association studies, gene expression studies, and protein expression studies are in the majority (82%) (Table S1). Over 75% of the selected omics studies showed female-specific changes at molecular levels in the brain, blood, or cerebrospinal fluid (CSF), while only about 20% showed male-specific changes.

female-specific changes at molecular levels in the brain, blood, or cerebrospinal fluid (CSF) samples, with only about 20% of studies demonstrating male-specific changes. The reviewed and discussed omics studies in the following section are summarized in Table 1.

Transcriptome-wide sex differences in AD have been characterized in multiple studies. A recent study of 1053 postmortem brain samples across 19 brain regions constructed sex-specific gene coexpression networks for each brain region and found three regions with the most prominent differences between females and males in terms of network topology: the superior parietal lobule, dorsolateral prefrontal cortex, and occipital visual cortex. There were very few commonly shared differentially expressed genes (DEGs) (AD vs. control), except sex hormone genes such as estrogen-related receptor beta differentially

expressed in four brain regions (53). A meta-analysis study of protein-protein interaction network revealed important roles for sex steroids in hippocampal neuronal degeneration with androgen and estrogen receptors identified as key drivers of disrupted homeostatic processes of AD neurons (54). The identification of sex hormones through omics studies further strengthens their importance in sex-biased differences in AD. Future studies with multiomics analyses will facilitate a better understanding of the effects of sex hormones and associated pathways on sex-biased vulnerability and treatment responsiveness in AD.

Besides sex hormones, many female-specific pathways have been identified as contributing to AD susceptibilities. A cross-tissue meta-analysis revealed a female-specific immune signature in both brain and blood (55). With network-based analysis and cell type deconvolution approaches, this study

Table 1. Detailed Information of Large-Scale Omics Studies Cited, Discussed, or Reviewed in the Article

Study	Year	Organism	Tissue	Experiment	Sample Size	Focus
Impact of Sex Chromosomes in AD						
Yurov <i>et al.</i> (13)	2014	Human	Brain	Genetic abnormalities	20	Sex chromosome
Spremo-Potparević <i>et al.</i> (14)	2008	Human	Chromosome	Genetic abnormalities	10	Sex chromosome
Wojda <i>et al.</i> (15)	2006	Human	Blood	Genetic abnormalities	123	Sex chromosome
Spremo-Potparevic <i>et al.</i> (16)	2004	Human	Chromosome	Cytogenetic analysis	23	Sex chromosome
Carrel and Willard (17)	2005	Human	Human cells	Chromosomal expression	40 fibroblast lines with 931 X-linked transcripts	Sex chromosome
Greenfield <i>et al.</i> (18)	1998	Human Mice	Brain	Chromosomal expression	Human and mouse cDNA clones	Sex chromosome
Miyake <i>et al.</i> (19)	2013	Human	Brain	Genetic variation	32	Genomic (<i>KDM6A</i>)
Itoh <i>et al.</i> (20)	2019	Mice	Brain	Gene expression	34	Histone demethylase (<i>KDM6A</i>)
Davis <i>et al.</i> (21)	2020	Human Mice	Chromosome	Chromosomal modification	6065 (human) 10–15 per group (mice)	Sex chromosome
Tang <i>et al.</i> (22)	2017	Mice	Brain	Gene expression	70	Histone demethylase
Lopes <i>et al.</i> (23)	2006	Human	Brain	Gene expression	58	Gene expression on autism
Carrasquillo <i>et al.</i> (25)	2009	Human	Brain	Genetic variation	2099	Genomic (<i>PCDH11X</i>)
Beecham <i>et al.</i> (26)	2010	Human	Brain	Gene expression	1739	<i>PCDH11X</i> gene on AD
Miar <i>et al.</i> (27)	2011	Human	Brain	Gene expression	770	SNP and <i>PCDH11X</i> on AD
Dumanski <i>et al.</i> (31)	2016	Human	Brain	Chromosomal expression	1611	Sex chromosome
Mendivil-Perez <i>et al.</i> (32)	2019	Human	Stem cells	Chromosomal modification	2	Sex chromosome
Caceres <i>et al.</i> (33)	2020	Human	Brain/blood	Chromosomal expression	1018	Sex chromosome
Impact of Sex Hormones in AD						
Ishunina <i>et al.</i> (39)	2007	Human	Hippocampal tissue	Gene expression	27	ER and splicing variants
Yaffe <i>et al.</i> (42)	2009	Human	Blood	SNP	1343 females 1184 males	ER polymorphisms
Ryan <i>et al.</i> (43)	2014	Human	Blood	SNP	6959	ER polymorphisms <i>APOE4</i> association
Gleason <i>et al.</i> (44)	2015	Human		Hormone therapy Clinical trial	727	Efficacy
Henderson <i>et al.</i> (45)	2016	Human		Hormone therapy Clinical trial	567	Efficacy regarding timing Perimenopausal vs after menopause
Shumaker <i>et al.</i> (48)	2004	Human		Hormone therapy Clinical trial	1464 estrogen vs. 1483 placebo; 3693 estrogen + progesterone vs. 3786 placebo	Efficacy
Shumaker <i>et al.</i> (49)	2003	Human		Hormone therapy Clinical trial	2229 estrogen + progesterone vs. 2303 placebo	Efficacy
Zandi <i>et al.</i> (50)	2002	Human		Prospective clinical studies	1357 men 1889 women	HRT Incidence of AD
Bulk Tissue Omics Studies						
Sun <i>et al.</i> (53)	2019	Human	Brain	Transcriptomics	1053 19 brain regions 125 subjects	Sex-biased gene topology in different brain regions
Winkler and Fox (54)	2013	Human	Hippocampal neurons	Transcriptomics	17 AD and 21 control cases	Sex steroid pathways

Table 1. Continued

Study	Year	Organism	Tissue	Experiment	Sample Size	Focus
Paranjpe <i>et al.</i> (55)	2020	Human	Brain/blood	Transcriptomics	1084 (brain) 645 (blood)	Female-specific immune signature
Brooks and Mias (56)	2019	Human	Brain/blood	Microarray	2088	Female-specific increase in CXCR4
Sanfilippo <i>et al.</i> (58)	2019	Human	Brain	Microarray	992 subjects with AD and 1290 control subjects	Female-specific increase in <i>CHI3L1</i>
Deming <i>et al.</i> (59)	2018	Human	Brain/CSF	GWAS	3036	Sex-specific GWAS loci in AD
Cáceres and González (60)	2020	Human	Brain	Transcriptomics	785	Sex-biased tau phosphorylation
Fukamoto <i>et al.</i> (63)	2010	Human	Tissue	Gene expression	1182	BDNF SNPs
Bangasser <i>et al.</i> (65)	2017	Rat	Brain tissue	Protein receptor expression	40	CRF expression on AD
Zhao <i>et al.</i> (66)	2016	Mouse	Brain	Targeted array of qPCR of 182 genes	40	Hypometabolism phenotypes in AD
Aberg <i>et al.</i> (67)	2015	Human	Brain/CSF	Protein expression	80	Insulin growth factor in CSF AD
Piccio <i>et al.</i> (68)	2016	Human	Brain/CSF	Protein expression	107	sTREM2 in AD CSF
Viswanathan <i>et al.</i> (69)	2009	Human	Blood	SNPs	1161	<i>GRN</i> polymorphisms in AD
Boks <i>et al.</i> (70)	2009	Human	Blood	DNA methylation	188	Methylation modified by age and sex
Ei-Maarri <i>et al.</i> (71)	2007	Human	Brain	Epigenomics	192	Sex-biased epigenetics in AD
Mano <i>et al.</i> (72)	2017	Human	Brain/tissue	Methylation microarray	60	Epigenetics in tau and AD
Mahady <i>et al.</i> (73)	2019	Human	Brain/tissue	Protein, IHC	61	HDAC2 dysregulation in AD brain
Cao <i>et al.</i> (74)	2019	Human	Brain	Microarray	214	Long ncRNAs in AD
Single-Cell Omics Studies						
Mathys <i>et al.</i> (75)	2019	Human/mice	Cell/tissue	Transcriptomics	48	Brain cell types in AD
Pinheiro <i>et al.</i> (77)	2011	Human/mice	Brain	Transcriptomics	48	MicroRNA and X chromosome
Villa <i>et al.</i> (79)	2018	Mice	Brain/tissue	Transcriptomics	24	Sex features on microglia in mice
Sarvari <i>et al.</i> (81)	2012	Human/rat	Brain	Transcriptomics	39	Menopause and macrophage
Guneykaya <i>et al.</i> (82)	2018	Mice	Brain	Transcriptomics	6	Microglia in male female brains
Hanamsagar <i>et al.</i> (83)	2018	Mice	Brain	Transcriptomics	20	Microglia on AD sex differences
Keren-Shaul <i>et al.</i> (84)	2017	Mice	Brain/tissue	Transcriptomics	12	Microglia in AD
Stephen <i>et al.</i> (85)	2019	Mice	Brain/tissue	Transcriptomics	25	Sex and AD with microglia
Kodama L <i>et al.</i> (86)	2020	Mice	Brain/tissue	Transcriptomics	87	Sex effect on microglia and AD
The Interplay of Sex and Genetic Risk Factors in AD						
Hsu <i>et al.</i> (91)	2019	Human	Brain	Transcription factors	100	<i>APOE</i> and conversion of MCI to AD
Shang <i>et al.</i> (92)	2020	Mice	Brain/blood	Transcriptomics Metabolomics	40	<i>APOE</i> on metabolome

AD, Alzheimer's disease; *APOE4*, *APOE* ϵ 4; cDNA, complementary DNA; CRF, corticotropin-releasing factor; CSF, cerebrospinal fluid; ER, estrogen receptor; GWAS, genome-wide association study; HRT, hormone replacement therapy; IHC, immunohistochemistry; MCI, mild cognitive impairment; ncRNA, noncoding RNA; qPCR, quantitative polymerase chain reaction; SNP, single nucleotide polymorphism.

analyzed gene expression profiles derived from brain samples of 1084 patients with AD and age-matched control subjects, and whole blood samples of 645 patients with AD and control subjects. More DEGs and gene coexpression modules were identified in women than in men. Many upregulated DEGs in females were enriched in innate and adaptive immune systems, whereas downregulated DEGs were enriched in neurological signaling pathways such as synaptic vesicle exocytosis and autophagy. The blood sample analysis discovered female-specific immune cell-type changes in AD, i.e., increases in neutrophils and naïve B cells and decreases in M2 macrophages, memory B cells, and CD8⁺ T cells. This study also showed that machine learning predictive models performed better with female-only data, suggesting that molecular changes in females might better model AD-related changes than might those in males. Another meta-analysis examined gene expression changes in AD with eight publicly available microarray datasets from 2088 brain and blood samples and identified a female-specific increase of CXCR4 in AD samples compared with control samples (56). CXCR4 is a chemokine receptor involved in inflammatory signaling pathways, the dysregulation of which is associated with neurodegenerative diseases (57). Female-specific neuroinflammation in AD was also embodied in the female-specific expression pattern of chitinase-3-like 1, a protein related to inflammatory processes and neurodegeneration (58).

Female-biased changes in AD have also been observed in many other pathways associated with the development of amyloid and tau pathology. A genome-wide association study of 1527 male and 1509 female brain and CSF samples from the Religious Orders Study and Rush Memory and Aging Project (ROSMAP) cohort identified a strong association of serpin family B members *SERPINB1*, *SERPINB6*, and *SERPINB9* with amyloidosis, and *OSTN* and *CLDN16* with tau pathology in females but not in males (59). A transcriptome-wide meta-analysis of cerebral cortex samples discovered two female-specific AD risk genes associated with tau phosphorylation processes (60), *NCL* and *KIF2A*. Their expression levels were upregulated in females with AD when compared with control females but downregulated in males with AD in contrast to control males. Other female-specific genetic associations in AD such as neurotrophin signaling pathway and polymorphisms in *SERPINB1* have been observed (59,61,62). A meta-analysis of 657 patients with AD and 525 control subjects in a Japanese population identified a significant allelic association between Val66Met of the brain-derived neurotrophic factor gene (*BDNF*) and AD in women (63). A follow-up study of 4711 patients with AD and 4537 control subjects from 16 research centers around the world further confirmed the sex differences in *BDNF* allelic association with the Met66 allele conferring female-specific AD susceptibility.

Consistently, many female-biased changes in AD human studies as described above have been recapitulated in animal studies. A mouse study shows that expression changes of inflammatory mediators (5xFAD vs. control) in the brain were more robust in females than males, with significant expression changes of cytokines (interleukin 1 β , interleukin 6) and chemokines (*CCL2* and *CXCL10*) only observed in female brains when compared with control brains (64). Moreover, a study of corticotropin-releasing factor overexpression mice using

phosphoproteomic approaches found that cortical phosphopeptides of female corticotropin-releasing factor overexpression mice were overrepresented in tau pathology-related signaling pathways, suggesting a sex-biased corticotropin-releasing factor signaling in tau phosphorylation of AD (65). A mouse study of brain aging also revealed an overall decreased bioenergetic metabolism during early disease transition in female brains, suggesting hypometabolic phenotypes at the onset of AD (66).

Interestingly, some male-specific risk genes are associated with AD, such as *GRN*, *TREM2*, and *IGF-2* (67–69). For example, a Finnish study of 512 patients with AD and 649 control subjects identified male-specific single nucleotide polymorphisms of *GRN* associated with AD (69). Moreover, in a small cohort of 60 participants, CSF IGF-2 and IGFBP-2 levels were found higher in males with AD when compared with levels of mild cognitive impairment and/or control male counterparts, with no differences seen between groups of female subjects (67). A significant correlation of CSF IGF-2 levels with CSF phosphorylated tau and total tau levels was identified in male subjects as well (67). In addition, significantly higher levels of soluble *TREM2* in CSF have been reported in subjects with AD and subjects with frontotemporal dementia than those in normal aging control subjects in a cohort of 180 samples (68). Intriguingly, levels of CSF soluble *TREM2* were higher in male than in female participants, suggesting a possibility of sex-biased effects of *TREM2* on microglial activation and neurodegeneration in AD (68).

Evidence also indicates the impact of sex on epigenomic signatures of AD (70,71). For example, Mano *et al.* (72) showed hypomethylated CpG islands in the promoter region of a gene *aurora kinase C* in male subjects with AD, particularly in *APOE4* carriers, but the same CpG islands were hypermethylated in female subjects with AD. In addition, studies demonstrated reduced histone deacetylase 2 levels in female subjects with normal aging, female subjects with mild cognitive impairment, and female subjects with AD when compared with their male counterparts (73). Moreover, the expression of long noncoding RNAs was found to be different between male and female subjects with AD (74).

While most current studies focus on one or two modalities of omics analysis, little effort was devoted to integrating these studies into a holistic examination at multiscale levels, partially owing to limited data availability as well as technical challenges in developing more integrative systems biology approaches. With increasingly available large-scale omics data and fast-paced advances in integrative multiomics approaches, we will gain more mechanistic insights into AD pathogenesis and sex-biased differences in AD, which will guide future development of sex-stratified preventive and therapeutic interventions.

SINGLE-CELL OMICS STUDIES OF CELL TYPE-SPECIFIC CONTRIBUTIONS TO SEX DIFFERENCES IN AD

It has been increasingly recognized that the complexity of AD pathogenesis may reside in the heterogeneity of disease-associated changes in different brain cell types. While bulk

tissue multiomics profiling highlights downregulation of neuronal activities and upregulation of immune responses in AD, the single-cell RNA sequencing enables in-depth dissection of cell type-specific contributions to disease mechanisms during AD development and progression.

A recent seminal study by Mathys *et al.* (75) performed a single-nucleus transcriptomic analysis of the prefrontal cortex of 48 subjects with AD of the ROSMAP (Religious Orders Study and Rush Memory and Aging Project) cohort. It was found that DEGs in neurons were downregulated, whereas most DEGs in non-neuronal cells were upregulated. Cell type-specific DEGs were mostly seen in early AD stages, whereas upregulated DEGs in late AD stage were commonly shared across cell types. More importantly, robust sex-specific differences in association with AD were identified among female and male brain cells. It was demonstrated that AD pathology-associated cell subpopulations were enriched in female cells with higher levels of marker gene expression, whereas no-pathology subpopulations were enriched in male cells. The most dramatic differences in sex-specific transcriptional responses were observed in neurons and oligodendrocytes, with global transcriptional activation in male oligodendrocytes and global downregulation of gene activities in female excitatory and inhibitory neurons that were associated with increased AD pathology, respectively (75). This study provides evidence of sex-dimorphic changes in AD, suggesting greater transcriptional dysregulation accounting for higher disease burden in female subjects with AD.

The importance of microglia in sex-biased differences in AD has also been increasingly recognized. Understanding sex differences in microglial responses to disease states may help unveil sex-biased AD susceptibility. It is well known that the X chromosome contains the largest numbers of immune-related genes and micro-RNAs involved in immune regulation (76–78). In addition, microglia express steroid hormone receptors that are important in microglial sexual differentiation. Once differentiated, microglia maintain transcriptional profiles even in the absence of hormones during adulthood (79). Hormone depletion in female aging brains led to profound transcriptomic changes with heightened inflammatory responses (80,81), which may contribute to sex differences in developing AD.

A recent study performed transcriptomic and proteomic analyses of microglia from five different brain regions of male and female C57BL/6J mice (82). The baseline phagocytosis was similar, but the expression of major histocompatibility class I and II genes was higher in male cortical microglia than in females, suggesting a higher antigen-presenting capacity. More pronounced differences were seen in the hippocampus, with 1109 DEGs identified between male and female microglia, with only 55 DEGs in cortical microglia. The proteomic analysis indicated that proteins highly expressed in male microglia were involved in toll-like receptor pathways (immune responses after challenges), whereas proteins highly expressed in female microglia were involved in interferon-related processes (82). Another study of transcriptomic profiles of purified male and female hippocampal microglia from embryonic day 18 to postnatal day 60 mice reported that the increases of microglial genes occurred later in males than females, and that microglial maturation accelerated by acute immune stimuli were observed only in males (83).

A study of an AD mouse model identified a stepwise microglia activation with downregulation of microglia checkpoints followed by TREM2-dependent activation (84). However, how sex and *APOE* genotype impact these processes remains to be elucidated. A recent study examined microglial interaction with amyloid plaques in EFAD mouse models and reported significantly lower microglial coverage of amyloid plaques in female mouse brains with the *APOE4* genotype (85). The microglial expression of TREM2 around amyloid plaques was also significantly reduced by *APOE4* genotype and female sex. These studies support the regulatory roles of *APOE* and sex in a TREM2-dependent microglial activation (85).

Furthermore, a study of a microRNA (miRNA) sequencing dataset of microglia identified a unique role of microglial miRNAs in mediating sex-specific responses to tau pathology (86). Using microglia-specific knockout mice of Dicer with the depletion of the miRNA-processing enzyme and Dicer-dependent miRNAs, it was reported that the loss of mature miRNAs led to more dramatic changes in the male microglial transcriptome than in female counterparts with DEGs enriched in immune system pathways suggestive of a heightened inflammatory state. The studies of miRNA and messenger RNA profiles of male and female PS19 mouse models further noted greater changes induced by tau pathology in male microglia than female cells. The male microglia were enriched with genes involved in immune modulation and phagocytosis, characteristic of disease-associated microglia signature, whereas female microglia were enriched with homeostatic microglial genes and human AD genes (86). This study suggests that loss of miRNAs led to sex-dimorphic changes in microglial transcriptome and tauopathy-related phenotypes.

While single-cell or single-nucleus omics studies enable investigations of cell type-specific contributions to AD, future efforts are needed to integrate single-cell and bulk tissue omics data to better understand sex differences in AD and to determine the interactions among different brain cell types during AD development and progression.

THE INTERPLAY OF SEX AND GENETIC RISK FACTORS IN AD

Stratifying genetic association analysis by sex enables the identification of novel AD genetic risk loci and the determination of sex-dependent polygenic risks (87). For example, *BIN1* and *MS4A6A* were found to contribute to AD progression significantly more in females than in males (88). Among many genetic loci that manifest sex-specific effects on AD, the interplay between sex and the *APOE* allele in AD has been extensively explored (89,90). However, only limited multiomics studies have been performed to understand the interplay between *APOE* and sex in AD.

A small cohort of 100 RNA sequencing data derived from four human brain regions examined *APOE4* genotype-associated gene expression patterns. Many more transcription factors differentially expressed in *APOE4*⁺ females versus *APOE3*⁺ females than those in *APOE4*⁺ males versus *APOE3*⁺ males, with *PSEN2* expressed highest in the temporal cortex of *APOE4*⁺ females and *CNTNAP2* in *APOE4*⁺ males (91). A separate study performed transcriptomic and metabolomic analyses of 16-month-old human *APOE* mice (92). There were

more DEGs between sexes identified with the *APOE4* genotype when compared with the *APOE3* genotype. Metabolomic analysis showed elevated circulating acylcarnitine levels in *APOE4* females. The study also demonstrated that sex and *APOE* genotype in combination modulate metabolism, bioenergetics, and neuroinflammation processes, and *APOE4* females present with robust gene expression involved in lipid metabolism, antigen presentation, and interferon response genes (92). These observations need to be validated in human samples.

Interestingly, a hypothetic framework proposed by Dumitrescu *et al.* (93) suggests that during the development and progression of AD, genetic regulators of amyloid processes may be largely shared in both sexes, but among amyloid-positive subjects, a sex-specific genetic architecture may emerge with substantial *APOE* contributions to AD in females, including the sex-biased association between *APOE* and tau (94,95). This hypothesis needs to be further tested.

SUMMARY

To better understand the molecular and cellular mechanisms underlying sex-biased differences in AD, multiomics data have been made publicly available and systems biology approaches have been employed to model disease processes. Advances in single-cell and single-nucleus transcriptomic studies enable a better understanding of cell type-specific impact on sex-biased differences in AD. In this review, we discussed current studies of sex chromosomes and sex hormones, as well as multiomics analyses of sex-biased differences in AD processes. It should be noted that to better understand the varying susceptibilities of AD between males and females, it is important to examine sex-biased neurological changes in gene expression profiles and underlying molecular pathways during development and aging (Supplement). Leveraging the knowledge from these processes enables a better understanding of sex-biased differences in neurodegenerative processes.

Several sex-biased signatures in AD have been identified through omics studies, e.g., genes and pathways involved in neuroinflammation and bioenergetic metabolism. The importance of genes and pathways associated with sex chromosomes and sex hormones in AD have been further strengthened by omics studies. The major limitations are that most current analyses focused on one or two modalities of omics data, with little effort devoted to integrating multiomics and multiple studies into a holistic picture of AD. While single-cell or single-nucleus omics studies facilitate in-depth understanding of cell type-specific contributions to AD, future efforts are needed to integrate single-cell and bulk tissue omics data to better understand sex differences in AD and to determine the interactions among different brain cell types during AD development. More importantly, there are many overlooked aspects in studying sex-biased differences in AD such as the roles of sex chromosomes, and the intrinsic differences in male and female brains during development and aging. As summarized in Figure 2, future research should integrate the multiomics data from different brain regions and different cell types into multiscale network analysis and leverage the knowledge from holistic models of sex-biased differences in development and adulthood, aging, and AD. Advances in

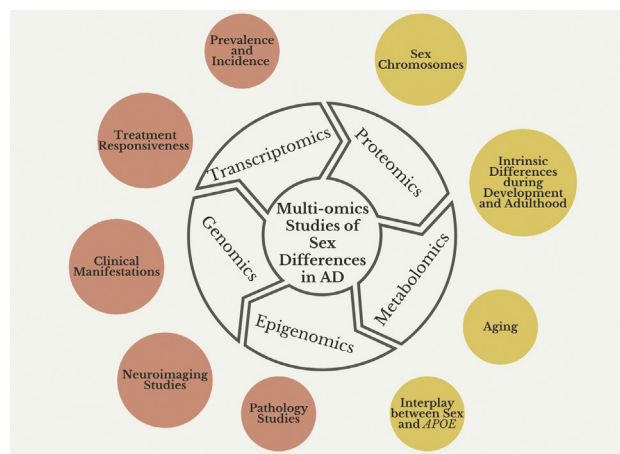


Figure 2. A holistic overview of sex differences in Alzheimer's disease (AD). Future studies need to take into considerations of different aspects of sex differences in AD including prevalence/incidence, clinical manifestations, neuroimaging studies, and pathology studies as well as treatment responsiveness. The integration of multiomics studies such as transcriptomics, proteomics, metabolomics, epigenomics, and genomics data will facilitate a better understanding of sex differences in AD. More importantly, leveraging the knowledge from studies of sex chromosomes, intrinsic differences in development and adulthood such as sex hormones, aging processes, and the interplay between sex and other genetic risk factors like *APOE* will enable a holistic examination of sex differences in AD, guiding future development of efficacious sex- and *APOE*-stratified preventive and therapeutic interventions for AD.

systems biology technologies and increasingly available large-scale multiomics data will facilitate future studies' dissecting such complex signaling mechanisms to better understand AD pathogenesis in both sexes, with the ultimate goals of developing efficacious sex- and *APOE*-stratified preventive and therapeutic interventions for AD.

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ARTICLE INFORMATION

From the Department of Genetics and Genomic Sciences (LG, BZ), Mount Sinai Center for Transformative Disease Modeling (LG, BZ), Department of Neurology (LZ, DC), Alzheimer Disease Research Center (DC), and Ronald M. Loeb Center for Alzheimer's Disease (DC), Icahn School of Medicine at Mount Sinai; and the Department of Neuroscience (MBZ), Barnard College of Columbia University, New York; and the Research and Development Service (LZ, DC), James J. Peters VA Medical Center, Bronx, New York.

Address correspondence to Dongming Cai, M.D., Ph.D., at dongming.cai@mssm.edu, or Bin Zhang, Ph.D., at bin.zhang@mssm.edu.

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