CHAPTER ONE

Alzheimer's Disease: Genomics and Beyond

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Abstract

Alzheimer's disease (AD) is a major form of senile dementia. Despite the critical roles of Aβ and tau in AD pathology, drugs targeting Aβ or tau have so far reached limited success. The advent of genomic technologies has made it possible to gain a more complete picture regarding the molecular network underlying the disease progression which may lead to discoveries of novel treatment targets. In this review, we will discuss recent progresses in AD research focusing on genome, transcriptome, epigenome, and related subjects. Advancements have been made in the finding of novel genetic risk factors,

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new hypothesis for disease mechanism, candidate biomarkers for early diagnosis, and potential drug targets. As an integration effort, we have curated relevant data in a database named AlzBase.

1. INTRODUCTION

Alzheimer's disease (AD) affects a large population in the senior community, likely 10 million in China alone (Han et al., 2014; Lei, 2010). The pathological hallmarks of AD include extracellular deposit of Aβ amyloid plaques derived from *APP* and intraneuronal neurofibrillary tangles (NFTs) from hyperphosphorylation of tau. Much of the efforts in AD research have been devoted to molecular pathways centered at Aβ or tau. The vast majority of novel treatment strategies are also targeting either Aβ or tau. Nevertheless, promising results from animal models have not translated well in human clinical trials (Callaway, 2012). Thus, revolutionary ideas outside of the hallmarks are desperately needed.

Technology developments in genomics have provided a variety of tools to investigate AD at the whole system level (Fig. 1). Earlier genetic linkage

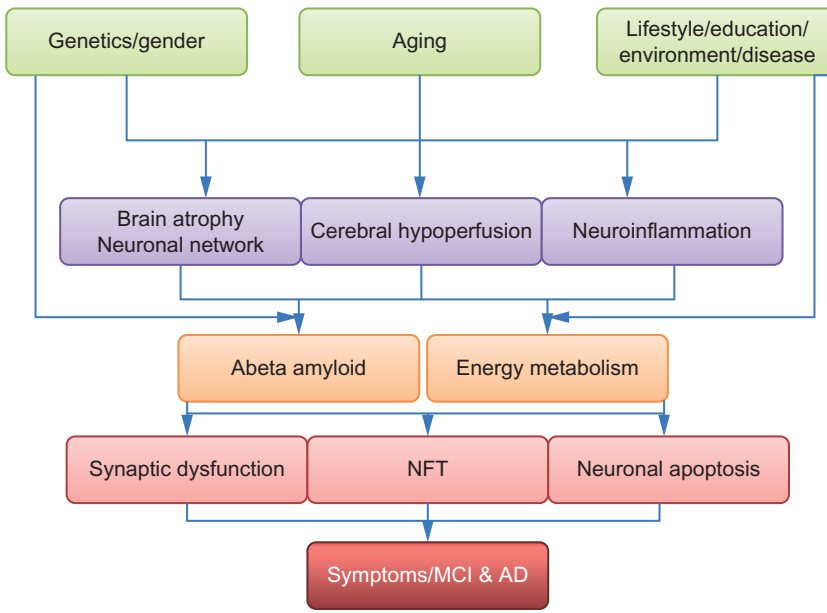

Figure 1 A framework of key factors and events in AD pathogenesis.

or association studies in both early-onset AD (EOAD) and late-onset AD (LOAD) have gradually evolved into genome-wide association studies (GWASs) and more recently developed into whole-exome sequencing (WES) and whole-genome sequencing (WGS; Bettens, Sleegers, & Van Broeckhoven, 2013; Guerreiro, Bras, & Hardy, 2013). Transcriptome studies are still in the process of transiting from microarray to RNA-seq (Sekar et al., 2015). Several types of epigenetic and epigenomic studies have been conducted on AD, including DNA methylation, histone modification, and microRNA (Lunnon & Mill, 2013). As a valuable supplement to the traditional studies on human subjects and animal models, induced pluripotency stem cell (iPSC) technology has been increasingly used to bridge the gap in translational medicine (Israel et al., 2012). In this review, we will survey representative investigations applying genomics technologies to the study of AD and relevant processes such as aging and neurological disorders.

2. GWASs ON THE PRIMARY PHENOTYPE OF AD

The quality of GWASs depends heavily on the sample size. In the most recent large-scale GWAS of AD (Lambert et al., 2013), over 74K samples were included in the meta-analysis (all of European ancestry), including ~54K samples in stage 1 (previously published datasets) and ~20K samples in stage 2 (new datasets). Over 7M imputed single-nucleotide polymorphisms (SNPs) were used in the meta-analysis of stage 1 datasets. In stage 2, only SNPs showing moderate significance in stage 1 ($P < 1 \times 10^{-3}$) were genotyped (~11K SNPs). In the meta-analysis of stage 1 datasets, the significance of *APOE* and nine other previously identified genes was confirmed (Table 1), including *CR1*, *BIN1*, *CLU*, *PICALM*, *ABCA7*, *CD2AP*, *EPHA1*, *MS4A4E*, and *CD33*. Additionally, five new loci reached genome-wide significance in the discovery datasets, namely, *SORL1*, *PTK2B*, *DSG2*, *HLA-DRB5*, and *SLC24A4*. When combining the discovery and replication datasets, seven new loci reached genome-wide significance, including *INPP5D*, *MEF2C*, *NME8*, *FERMT2*, *ZCWPW1*, *CELF1*, and *CASS4*. Among these 22 loci, *CD33* and *DSG2* did not reach genome-wide significance in the combined datasets. These genes are involved in immune response, lipid metabolism, synaptic function, and Aβ/tau-related pathways.

Besides testing the significance at the SNP level, gene-level test may reveal new genes affecting AD susceptibility. In a follow-up work of the above-described study (Escott-Price et al., 2014), gene-level analysis

Table 1 Key Genes from Genetic Studies of Late-Onset AD

Genes	Full Name	Relevant Functions
CR1	Complement component (3b/4b) receptor 1 (Knops blood group)	Complement pathway; clearance of amyloid
BIN1	Bridging integrator 1	Synaptic vesicle endocytosis
CLU	Clusterin	Stress response; lipid metabolism
PICALM	Phosphatidylinositol-binding clathrin assembly protein	Synaptic vesicle; lipid metabolism
ABCA7	ATP-binding cassette, subfamily A (ABC1), member 7	Lipid metabolism; immune
CD2AP	CD2-associated protein	Regulation of actin cytoskeleton; endocytosis
EPHA1	EPH receptor A1	Signaling in nervous system development; immune
MS4A4E	Membrane-spanning four domains, subfamily A, member 4E	Immune; signaling
CD33	CD33 molecule	Immune; signaling
SORL1	Sortilin-related receptor, L(DLR class) A repeats containing	Endocytosis and sorting
PTK2B	Protein tyrosine kinase 2 beta	Synaptic transmission
DSG2	Desmoglein 2	Cell adhesion
HLA-DRB5	Major histocompatibility complex, class II, DR beta 5	Immune response
SLC24A4	Solute carrier family 24 (sodium/potassium/calcium exchanger), member 4	Ion transport
INPP5D	Inositol polyphosphate-5-phosphatase, 145 kDa	Blood cell; immune
MEF2C	Myocyte enhancer factor 2C	Transcription regulation; brain development
NME8	NME/NM23 family member 8	Cytoskeleton
FERMT2	Fermitin family member 2	Cell–matrix adhesion
ZCWPW1	Zinc finger, CW type with PWWP domain 1	Epigenetic regulation; blood trait

Table 1 Key Genes from Genetic Studies of Late-Onset AD—cont'd

Genes	Full Name	Relevant Functions
CELF1	CUGBP, Elav-like family member 1	mRNA processing; cytoskeleton
CASS4	Cas scaffolding protein family member 4	Cell adhesion; signaling
TP53INP1	Tumor protein p53-inducible nuclear protein 1	Autophagic cell death
IGHV1-67	Immunoglobulin heavy variable 1–67 (pseudogene)	Immune response
PDE1A	Phosphodiesterase 1A, calmodulin dependent	Calcium signaling
RYR3	Ryanodine receptor 3	Calcium signaling

identified two genes with no previous reports, *TP53INP1* and *IGHV1-67*. The three most significant SNPs in *TP53INP1* represented at least two partially independent signals, while the two most significant SNPs in *IGHV1-67* represented the same signal. As for the gene function, *TP53INP1* is involved in apoptosis and *IGHV1-67* is involved in immune response.

We have adopted a different strategy in our reanalysis of public GWAS datasets. We considered up to four SNPs when evaluating intragenic epistasis using a generalized multidimension reduction algorithm (Sun et al., 2014). When considering 4-order intragenic epistasis, 10 genes were found to have consistent strong signal across the four publicly available GWAS datasets for AD. Among these 10 genes, *PDE1A* and *RYR3* may deserve further attention due to their involvement in calcium signaling which is known to be dysregulated in AD brains (Demuro, Parker, & Stutzmann, 2010).

3. GWASs ON THE SECONDARY PHENOTYPE OF AD

Age at onset (AAO) is a major secondary phenotype studied on AD. In the first GWAS of AAO of AD, several SNPs at the *APOE* region reached genome-wide significance, while *DCSH2* was a newly found risk gene affecting AAO of AD (Kamboh et al., 2012). In a study focused on the top 10 risk loci, it was found that the risk allele of *PICALM* may lead to

slightly earlier onset of AD, while the strong effect of *APOE* e4 allele on AAO was confirmed (Thambisetty, An, & Tanaka, 2013). In another study focused on the *TOMM40* intron 6 poly-T length, no association was found with the AAO in LOAD, but it may affect the neuropathology in AD (Li et al., 2013).

Cognition and cognitive decline in AD and general population is another area of interest. In a longitudinal study of nondemented elderlies between age 79 and 87, *APOE* e4 was found to be associated with cognitive decline regarding verbal memory and abstract reasoning (Schiepers et al., 2012). In a GWAS on 3511 unrelated adults, *APOE* e4 was found to be the only independent genome-wide significant signal associated with cognitive aging (Davies et al., 2014). In another longitudinal study over 54 months on healthy elderlies, the effect of *APOE* e4 on the rate of cognitive decline was found to be dependent on the presence of amyloid in the brain (Lim et al., 2014). In two studies on the top 10 risk loci for AD, collective effect of the risk loci on cognitive aging independent of *APOE* was found to be marginal (Carrasquillo et al., 2015; Verhaaren et al., 2013). Genome-wide polygenic risk score was also found to exert no effect on cognitive aging in a study on over 3000 nondemented old people (Harris et al., 2013). In two separate GWASs, *SPON1* was found to be the only gene affecting both cognitive decline (Sherva et al., 2014) and brain wiring (Jahanshad et al., 2013).

Another relevant phenotype is hippocampal volume (HV) or cortical thickness. An early GWAS on brain atrophy identified *ZNF292* as a novel genetic factor affecting entorhinal cortical thickness (Furney et al., 2011). In two large-scale studies on the general population, several novel loci were found to be associated with HV, including *TESC*, *MFRB3-WIF1*, and *HRK-FBXW8* (Bis et al., 2012; Stein et al., 2012). In a study on AD cases and relevant controls, four genes were found to be associated with HV, including *APOE*, *F5/SELP*, *LHFP*, and *GCFC2* (Melville et al., 2012). In a related study, two novel genes (*CARD10* and *PARP1*) were found to affect hippocampal degeneration and HV in *APOE* e3/e3 individuals (Nho et al., 2013).

Amyloid plaque burden is also of intense interest in the AD research community. In the first GWAS of amyloid plaque in 555 live patients, *APOE* and *BCHE* reached genome-wide significance with independent as well as additive effect on plaque load (Ramanan et al., 2014). In a study combining the target gene and GWAS on both deceased subjects and live patients, four target genes (*APOE*, *CR1*, *ABCA7*, and *CD2AP*) were associated with plaque burden. In addition, the GWAS revealed a novel risk locus near *APP* (Shulman et al., 2013).

4. CNV STUDIES

Copy number variation (CNV) is suggested to be a part of the missing heritability in AD. A small-scale genome-wide CNV studies were conducted on ~700 subjects. *CHRNA7* was found to be one of the genes with suggestive significance (Heinzen et al., 2010). A similar study on Caribbean Hispanics revealed nominal association at Chr15q11.2 (Ghani et al., 2012) encompassing five genes. In a study on the AD neuroimaging initiative samples, significant higher burden of CNV-region deletions was found in mild cognitive impairment (MCI) and AD cases compared to controls (Guffanti et al., 2013). In two separate studies on the effect of CNV on the AAO of AD, CNVs in *CPNE4* and *CHRFAM7A* were found to be potential risk for AD (Szigeti et al., 2014, 2013).

5. WES AND WGS STUDIES

The genome-wide significant SNPs from GWASs can only explain a small portion of the AD heritability. Some of the genetic risks are hidden in the rare coding variants which are not assessed in GWASs. The fast lowering of sequencing cost made it possible to conduct WES or even WGS studies on AD. One such study focused on *TREM2*, a gene known to be associated with an autosomal recessive form of early-onset dementia (Guerreiro et al., 2012). The study started with sequencing *TREM2* in ~2000 samples (approximately half AD patients and half controls). It was found that exon 2 of this gene had significantly more variants in patients compared to controls. The most significant variant rs75932628 (R47H mutation) was confirmed to be significant in GWAS datasets through SNP imputation. The significance of this variant was further validated through direct genotyping on additional ~60K samples. The functional relevance of *TREM2* to AD was demonstrated by the elevated expression in a mouse model of AD compared to control.

Another study started with WES of 14 selected LOAD families (Cruchaga et al., 2014). The WES combined with genotyping revealed only one rare variant (*PLD3*, V232M) segregating the disease status in two independent families. The significance of this variant was confirmed in independent datasets with ~11K samples. Furthermore, *PLD3* gene was resequenced in ~4400 European samples and ~300 African-American samples and the gene-level significance was validated. To evaluate the function

of *PLD3* in AD, overexpression and knockdown experiments were performed. It was found that *PLD3* level was strongly correlated with the level of extracellular Aβ42 and Aβ40.

6. FUNCTIONAL GENOMICS STUDIES

Compared to the well-accepted results on the odds ratio of the above-described genes from GWAS or WES studies, little consensus has been reached regarding the functional relevance of those genes and more specifically the SNP variations. Based on the examination of 73 cases and 39 controls, it was found that the expression level for several of the top 10 genes demonstrated association with various clinical assessment of AD including clinical dementia rating score, disease progression rate, disease status, and Braak stage (Karch et al., 2012). However, none of the examined SNPs showed association with gene expression level, although a few suggestive associations with secondary phenotypes were observed. In a study focused on the loss-of-function effect on Aβ production, it was shown that most of the top LOAD genes did not alter Aβ production except for *CLU* and *CD2AP* (Bali, Gheinani, Zurbriggen, & Rajendran, 2012). More importantly, none of the top LOAD genes affected Aβ42/40 ratio in the RNAi experiments. In an expression quantitative trait loci (eQTL) study, none of the top loci showed a regulatory role on gene expression (Holton et al., 2013). In the meantime, the brain-wide regional difference in gene expression showed no correlation with the difference in regional vulnerability.

7. BRAIN TRANSCRIPTOME STUDIES

Brain transcriptome as an endophenotype provides the most comprehensive information regarding the dynamic change of molecular network during disease development. Through gene expression profiling from various aspects of AD development, the systematic picture is becoming increasingly clear. In a pioneering study, hippocampus tissues from 31 subjects at four stages of AD development were examined, including normal elderly controls and AD patients at the incipient, moderate, and severe stages (Blalock et al., 2004). In addition to transcriptome itself, the correlation between gene expression and mini-mental state examination or NFT scores was evaluated. The major finding was the upregulation of transcriptional and

tumor suppressor response at the incipient stage, accompanied by the down-regulation of protein folding/metabolism/transport and energy metabolism.

Detailed Braak staging was adopted in a more recent work on the prefrontal cortex (PFC). Brain samples from Braak stage 0–6 were examined. Significant genes were clustered based on the temporal profiles of gene expression. Among the four important gene clusters, two displayed up-down feature and the other two displayed down-up feature, both with the turning point at Braak stage 2. Parallel measurement of intraneuronal Aβ showed decreased Aβ level at Braak stages 4–6. It was suggested that these two events were connected and the change of gene expression at the early stage may be a coping mechanism against the increase of soluble Aβ level in the PFC. Further dissection of *APOE* genotype revealed the potential role of *APOE* in accelerating gene expression change.

The development of AD pathology is not homogeneous across the brain. To examine the brain region-specific response to AD development, six relevant brain regions were selected for in-depth evaluation in a well-designed study employing laser-capture microdissection technology (Liang et al., 2008). For direct comparison with healthy elderly controls, only nontangle-bearing neurons were selected for gene expression profiling. The selected brain regions include hippocampus, entorhinal cortex, middle temporal gyrus, posterior cingulate cortex, superior frontal gyrus, and primary visual cortex (VCX). The brain-wide comparison revealed that different brain regions had distinctive response to AD pathology using the healthy controls as reference.

Due to the high quality of this dataset, we conducted reanalysis using a protein–protein interaction (PPI) network approach (Liang et al., 2012). First, we identified the perturbed network in each of the six brain regions. Subsequently, we found that there was high level of overlap among the perturbed networks in the six brain regions. This indicated that the molecular response to the AD pathology was similar in different brain regions, although the degree of perturbation may vary from one brain region to another. We further selected the top 10% most connected genes from each of the six perturbed networks. Interestingly, these 136 hub genes themselves formed a highly connected network. We demonstrated that the hub network was highly relevant to AD mechanism using several independent validations. Drug target enrichment analysis on the hub network further supported the potential application of the network in drug discovery for AD.

Small sample size is the major limitation for most brain transcriptome studies including AD. The first large-scale AD transcriptome study

employed 364 brain samples (approximately half for cases and half for controls; Webster et al., 2009). In addition to the case–control comparison, the study focused on the genetic control of gene expression by concurrent genotyping and gene expression profiling of the samples. The analysis revealed that the expression of 9% of the transcripts may be controlled by SNPs and 5% of the transcripts had LOAD-specific SNP-transcript correlation.

In a recent large-scale brain transcriptome study of AD, over 1600 brain tissues from three brain regions were examined (Zhang et al., 2013). The brain regions included PFC, cerebellum (CB), and primary VCX. Not surprisingly, the PFC region displayed much higher level of perturbation than the other two brain regions. Through integrated network analysis of genome and transcriptome, the immune and microglia module was found to have a significant role in AD pathogenesis. *TYROBP* was identified as a driver gene of this module and the causal relationship was validated by overexpressing *TYROBP* and measuring downstream effect.

In order to investigate gene dysregulation pattern during AD progression, we selected four datasets representing four stages of AD development. We found that downregulation of energy metabolism occurred at the early stage where dementia symptom was not yet manifested (Sun, Feng, Liang, Duan, & Lei, 2012). Downregulation of energy metabolism has been considered as a consequence of mitochondrial damage due to oxidative stress. However, the downregulation already occurred in tangle-free neurons at the nondementia stage where no apparent signals for oxidative stress were detected. Therefore, we proposed that the downregulation of energy metabolism is a protective response of neurons under the environment with deficiency of energy supply. Energy conservation might be the only strategy for the neurons to survive.

In order to examine the consistency among the brain transcriptome studies, we have also conducted a comprehensive survey on all of the publicly available datasets for AD transcriptome (Feng et al., 2014). We first demonstrated that gene dysregulation in the AD brains was highly reproducible. Then, we selected 100 genes representing the gene dysregulation in the AD brains. We validated the robust dysregulation of this set of genes with independent datasets. Among these 100 genes, 12 genes displayed consistent up- or downregulation along the trajectory of AD development, suggesting higher relevance to the disease progression. In addition, we also found an interesting scenario of gene expression cushion in the less vulnerable brain regions.

8. EARLY-ONSET AD

While most transcriptome studies focused on LOAD, one study examined monogenic and sporadic EOAD (Antonell et al., 2013). When compared to the control group, ~3000 dysregulated genes were found for both types of EOAD. The functional categories were also similar, including calcium signaling, neuroactive ligand–receptor interaction, axon guidance, and long-term potentiation. Direct comparison between sporadic and monogenic EOAD revealed little difference. It was suggested that these two types of EOAD converge to a similar final stage of the disease. We shall note that the dysregulated functional categories are also similar to those in LOAD, although the affected brain regions may be different.

Several studies have been conducted on the genetics of EOAD. In an earlier study on the AAO of familiar AD carrying *PSEN2* mutations, three loci were found to have effect on AAO, including 1q23.3, 17p13.2, and 7q33 (Marchani et al., 2010). In a study on familiar AD subjects in Caribbean Hispanics, 12 mutations were found in the 5 selected genes (*APP*, *PSEN1*, *PSEN2*, *MAPT*, and *GRN*), among which 4 were novel mutations (Lee et al., 2014). In a genome-wide rare CNV study of 261 families with EOAD, 10 novel private CNVs were found in gene-rich regions (Hooli et al., 2014).

9. EPIGENOMICS STUDIES IN THE BRAIN

Much of the epigenomics studies of AD are focused on DNA methylation (5-mC) and hydroxymethylation (5-hmC). However, inconsistent results have been reported in the literature. In an earlier study by Rogers and coworkers (Mastroeni et al., 2010), two markers for DNA methylation and eight key methylation factors were examined in entorhinal cortex layer II. Lower level for all 10 factors was observed in AD patients compared to controls. A more recent study by van den Hove and coworkers demonstrated robust decrease of 5-mC and 5-hmC in the hippocampus of AD patients and similarly in the AD twin examined in the same study (Chouliaras et al., 2013). In addition, the methylation level showed significant negative correlation with amyloid plaque load. Another study by Lunnon and coworkers reported significant decrease of 5-hmC level in both entorhinal cortex and CB of AD patients (Condliffe et al., 2014).

On the other hand, the study by Balazs and coworkers reported no significant change of global levels of 5-mC and 5-hmC in the entorhinal cortex

of AD patients (Lashley et al., 2014). A study by Lovell and coworkers reported increase of 5-mC and 5-hmC in hippocampus/parahippocampal gyrus of preclinical and late-stage AD patients (Bradley-Whitman & Lovell, 2013). Recently, the study by Dragunow and coworkers demonstrated global increase of 5-mC and 5-hmC in middle frontal gyrus and middle temporal gyrus of AD patients (Coppieters et al., 2014). An earlier study by Rapoport and coworkers also reported global hypermethylation in the frontal cortex of AD patients (Rao, Keleshian, Klein, & Rapoport, 2012).

The study by Rozek and coworkers focused on the locus-specific change of methylation in frontal cortex by methylation array experiment (Bakulski et al., 2012). A total of 948 CpG sites were reported to be potentially associated with AD phenotype, among which a CpG site at the promoter region of *TMEM59* demonstrated the highest discrimination power.

Several studies are focused on the deregulation of microRNA levels in the brain and the potential regulatory mechanism. The most consistent conclusion is the deregulation of miR-132 family in AD patients. Lau et al. found 35 deregulated microRNAs in the hippocampus and 41 deregulated microRNAs in the PFC of AD patients (Lau et al., 2013). The strongly deregulated miR-132-3p in both brain regions was further validated and the deregulation started at Braak stage 3. Functionally, miR-132-3p may regulate tau pathway through *FOXO1a*. In another study by Hebert et al. using sequencing technology, miR-132-3p was also found to be significantly decreased in the temporal cortex of AD patients (Hebert, Wang, Zhu, & Nelson, 2013). In yet another study, Wong et al. found downregulation of miR-132 and miR-212 in hippocampus and temporal cortex of AD patients (Wong et al., 2013). They further demonstrated that miR-132/212 may regulate neuronal apoptosis through AKT signaling pathway.

Deregulation of other microRNAs has also been reported. An earlier study by Hebert et al. demonstrated lower miR-29 cluster in AD patients, which may lead to the increase of *BACE1* and Aβ level (Hebert et al., 2008). In a study by Wang et al. with more disease stages, it was demonstrated that the decrease of miR-107 was found in early AD which may also regulate Aβ level through the increase of *BACE1* (Wang et al., 2008). In a study by Cui et al. on the hippocampus and neocortex, increased level of miR-146a was found in AD which may together with *NFkB* play a critical role in regulating the *IRAK* level (Cui, Li, Zhao, Bhattacharjee, & Lukiw, 2010).

It is encouraging to see consistent observations on some of the studies on DNA methylation and microRNA in the brain. However, due to the

limited number of such studies and small sample size in those studies, caution shall be taken when interpreting the results.

10. AD, BRAIN AGING, AND LONGEVITY

Aging is the primary risk factor for AD; thus, studies on brain aging are highly relevant to AD. An earlier study focused on frontal cortex spanning age 26–106. Downregulation of synaptic transmission and mitochondrial function was observed starting at 40, followed by the upregulation of stress response and DNA repair (Lu et al., 2004). In addition, DNA damage was found in the promoter regions of the downregulated genes. Another study assessed four distinctive brain regions at age 20–99. Superior frontal gyrus was found to be the most significantly dysregulated brain region and the most prominent change was observed from 60 to 70 (Berchtold et al., 2008). In addition, clear gender difference was found during the aging process, including the total number of affected genes and the exact nature of the affected functional categories.

Genetic studies have also been conducted on aging and longevity. In an earlier meta-analysis of aging-related GWAS datasets, no genome-wide significant SNPs were found, although 22 SNPs demonstrated moderate prediction power on the risk of death or event-free survival (Walter et al., 2011). A study on people with exceptional longevity revealed a panel of genetic signature with 281 SNPs for longevity (Sebastiani et al., 2012). In a genome-wide linkage study of human longevity, *APOE* was identified as a major contributor to longevity (Beekman et al., 2013). Three additional loci were found in 19p13.3-11, 17q21-22, and 14q11.2. An epigenomic scan revealed 490 differentially methylated regions correlated with chronological age (Bell et al., 2012). As a reference source, LongevityMap has curated genes associated with aging and longevity from genetic studies (Budovsky et al., 2013).

11. AD AND RELEVANT DISEASES

Due to the similarities in symptoms and disease pathology, investigations have been conducted to unveil the common molecular mechanism between AD and related diseases. A study on AD and age-related macular degeneration revealed common genetic risks in *ABCA7* and *ZCWPW1* (Logue et al., 2014). In a study between schizophrenia (SZ) and general cognitive ability, a polygenic risk score for SZ was found to affect cognitive

ability (Lencz et al., 2014). Results from other investigations, however, mostly turned out to be negative. A recent combined GWAS revealed no significant SNP conferring risk for both AD and Parkinson's disease (PD; Moskvina et al., 2013). A study on Korean population also showed no significant effect of the AD top loci on the susceptibility of PD (Chung et al., 2013). An investigation on the genetic link between type 2 diabetes (T2D) and AD also revealed no significant effect for the T2D top loci on AD susceptibility (Proitsi et al., 2014).

12. SEEKING PERIPHERAL BIOMARKERS

Due to the difficulty in accessing the brain with noninvasive approaches, peripheral biomarkers have been pursued for the early diagnosis of AD. An earlier study on blood transcriptome revealed lower expression of cytoskeletal integrity and DNA repair among other functions (Maes et al., 2007). A later study identified a 170-probe signature with high sensitivity and specificity distinguishing AD and elderly controls. Most of the signature genes were involved in immune function and lipid metabolism (Fehlbaum-Beurdeley et al., 2010). In another study, a 96-gene set was derived and displayed good sensitivity and specificity (Booij et al., 2011; Rye et al., 2011). The major functions included nucleic acid metabolism, protein metabolism, apoptosis, and cell cycle. In a study on both AD and MCI, lipid metabolism was among the dysregulated functions in AD and *ABCB1* displayed good correlation with disease progression (Chen et al., 2011). In another study with similar design, increased expression of immune response and decreased expression of mitochondria were identified in AD patients as well as in the early stage of AD (MCI; Lunnon et al., 2012).

Since peripheral blood circulates through the whole body, the specificity is a major concern when deriving biomarkers from the blood. We collected blood transcriptome datasets from investigations on various diseases including neurological disorders, cancer, diabetes, and infectious diseases. We found that the concurrent downregulation of core metabolism and upregulation of environmental response is a unique feature for AD (Han et al., 2013). We also validated this finding using a small Chinese cohort of AD, MCI, and elderly control. In addition, we found that the degree of perturbation in oxidative phosphorylation went up as the disease progressed. Some of the critical genes were further discussed in our collaborative investigation using samples from the United States (Bai et al., 2014).

In addition to the expression level of genes, microRNA expression in the peripheral blood has also been investigated. In a study focused on MCI, miR-132 and miR-134 families were found to have discrimination power (Sheinerman et al., 2012); further validation using large independent samples demonstrated 96% and 87% overall accuracy for these two microRNA families, respectively (Sheinerman, Tsivinsky, Abdullah, Crawford, & Umansky, 2013). In a study focused on AD, six microRNAs were found to have good discrimination power, among which miR-342-3p displayed the best performance (Tan et al., 2014). In another similar study, seven microRNAs including let-7d-5p were able to separate AD from normal controls with high accuracy (Kumar et al., 2013). In another study with both AD and MCI, a panel of 12 microRNAs was found to have good accuracy separating AD from controls as well as other neurological disorders (Leidinger et al., 2013). Despite the progress, one of the main concerns in this field is the inconsistency among independent studies which shall be taken into account in the future.

13. ANIMAL STUDIES OF AD

Animal models especially mouse models have been widely used in the investigation of the molecular mechanism of AD. In one such study on the 5XFAD mouse model, cardiovascular disease-related genes were found to be downregulated in the frontal cortex, while mitochondrial genes were downregulated in CB (Kim, Moon, Yu, Mook-Jung, & Kim, 2012). In another study, two types of transgenic mice (5XFAD and Tg4-42) were both compared with wild-type mice, and a set of 36 common differentially expressed genes were found which may underlie the common molecular pathways leading to the plaque pathology (Bouter et al., 2014). In a rare study on *Microcebus murinus*, both old animals and AD-like animals were compared to young animals (Abdel Rassoul et al., 2010). Opposite trend of gene dysregulation was found in certain functional categories especially metabolic pathways, which was suggested as a compensatory mechanism.

Epigenetic studies have also been conducted on mouse models. In a DNA methylation study, differentially methylated regions were found in two types of mouse models for AD (Sanchez-Mut et al., 2013). The hypermethylation of three genes (*TBXA2R*, *SORBS3*, and *SPTBN4*) was consistent with the downregulation of the three genes in human AD. In a study on both aging and AD, global decrease of 5-mC was observed during the aging process, while increase of 5-hmC was observed in 3xTgAD

mouse compared to wild-type control (Cadena-del-Castillo et al., 2014). In a study on histone modification, the level of H3/H4 acetylation started to increase at 4 months of age in 3xTgAD mouse, which is a distinctive feature compared to wild-type mice (Walker, LaFerla, Oddo, & Brewer, 2013). In a microRNA study, the upregulation of microRNA in 3xTgAD mouse converged to the regulation of synaptic function, and miR-325 was identified as a key player in both AD pathology development and response to environmental enrichment (Barak et al., 2015). In another microRNA study, upregulation of miR-181 was found in 12-month-old 3xTgAD mouse (Rodriguez-Ortiz, Baglietto-Vargas, Martinez-Coria, LaFerla, & Kitazawa, 2014). Concurrent decrease of *c-Fos* and *SIRT1* was found which might be regulated by miR-181. The level of miR-206 was found to be higher in an embryonic *APP/PS1* transgenic mouse covering several tissues including hippocampal tissue, cerebrospinal fluid, and plasma (Tian, Cao, & Zhang, 2014). This increase of miR-206 was proposed as a key regulator of *BDNF* which was lower in the transgenic model. In another study, miR-342-5p was found to be upregulated in three transgenic models including the *APP/PS1* mice. The upregulation was linked to several genes including *ankG* which is involved in axon guidance (Sun, Wu, Gu, & Zhang, 2014).

14. iPSC TECHNOLOGY IN AD RESEARCH

The iPSC technology has been applied to the understanding of the functional consequence of critical mutations using isogenic human stem cell. Several works have been conducted on the *PSEN1* mutations. In one such study, *PS1* mutation was found to affect the γ-secretase function but not other functions of *PS1*. Therefore, the mutational effect is a mixture of loss of and gain of functions (Woodruff et al., 2013). In another study, Aβ42/Aβ40 ratio was found to be higher in the *PS1* mutant NPC. The study further revealed 14 genes dysregulated in the *PS1* mutant NPC, among which 5 genes showed consistent feature in LOAD (Sproul et al., 2014). In another study on drug response, higher Aβ42/Aβ40 ratio was also observed in the mutant *PS1* neurons compared to the wild type. More importantly, a novel drug response signature was observed when treating the cell culture with a GSM, suggesting the value of using human-derived neurons (Liu et al., 2014). A study focused on the protective effect of A673T of *APP* from an earlier finding. The authors demonstrated that the mutation led to reduced cleavage of *APP* by *BACE1* as well as reduced aggregation of Aβ42 (Maloney et al., 2014).

15. INTEGRATING MULTIOMICS INFORMATION FOR AD

The fast accumulation of data from systematic studies of AD has created a daunting task for data integration. Different types of omics studies are not isolated; the integration requires great effort in order to reach the clearer picture of the jigsaw puzzle. We have attempted to bridge the gap between genetic and transcriptome studies by evaluating the transcriptional regulation in different chromosome regions. We found that chr19p displayed the most significant dysregulation in three stages of AD development (Wang et al., 2014). This was especially interesting considering the fact that a strong signal was observed in chr19p in previous genetic studies. In addition, *APOE*, the most consistent genetic risk for LOAD, resides on the same chromosome. Therefore, we proposed several critical genes such as *C3* and *KANK2* on chr19p.

Recently, we collected omics data related to AD and constructed a database named AlzBase as a reference for gene dysregulation in AD and relevant processes (Fig. 2). The core data were the gene dysregulation in brain

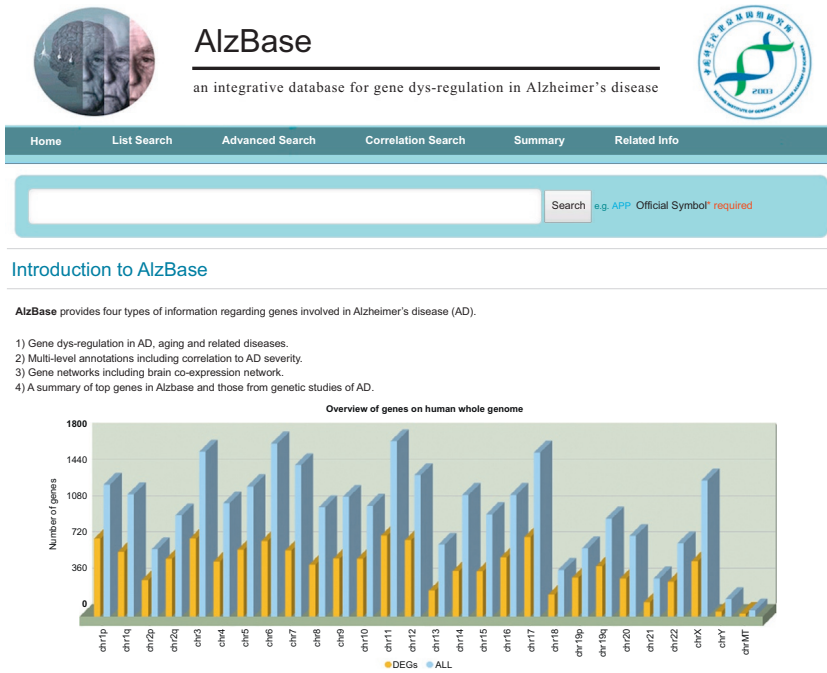

Figure 2 The homepage of AlzBase—an integrative genomics database for AD.

transcriptome datasets for AD. Genes correlated with AD progression were also curated. This was further extended to gene dysregulation in brain aging, other neurological diseases, and T2D. Other data included results from blood transcriptome studies, GWASs, eQTL studies, and microRNAs and drugs relevant to AD. To further illustrate the relationship among the critical genes, we provided several types of networks including coexpression network, PPI network, mutual information network, and cocitation network. The first version of AlzBase (http://alz.big.ac.cn/alzBase/) is available online and we plan to update the database to keep up with the development in this field (Bai et al., 2015).

16. CONCLUDING REMARKS

The field of AD research has advanced tremendously while embracing the fast-growing omics technologies. The development of sequencing technology is making personal genome increasingly accessible to the general public as well as patients with specific diseases. It will also make the dynamic monitoring of the blood transcriptome a more realistic goal in the near future. The development of iPSC technology including 3D neuron culture will make the disease model more human-like to avoid costly failures in traditional drug development employing mouse models. On another note, neuroimaging, which is not covered in this review, will certainly play an important role in visualizing the molecular details of the dysfunctional network. With the increasing use of these technologies, we expect to see exciting advancements in the field of AD research.

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