A Network-Based Perspective in Alzheimer's Disease: Current State and an Integrative Framework

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***Abstract*—A major rise in the prevalence and impact of Alzheimer's disease (AD) is projected in the coming decades, resulting from increasing life expectancy, thus leading to substantially increased healthcare costs. While brain disfunctions at the time of diagnosis are irreversible, it is widely accepted that AD pathology develops decades before clinical symptoms onset. If incipient processes can be detected early in the disease progression, prospective intervention for preventing or slowing the disease can be designed. Currently, there is no noninvasive biomarker available to detect and monitor early stages of disease progression. The complex etiology of AD warrants a systems-based approach supporting the integration of multimodal and multilevel data, while network-based modeling provides the scaffolding for methods revealing complex systems-level disruptions initiated by the disease. In this work, we review current state-of-the-art, focusing on network-based biomarkers at molecular and brain functional connectivity levels. Particular emphasis is placed on outlining recent trends, which highlight the functional importance of modular substructures in molecular and connectivity networks and their potential biomarker value. Our perspective is rooted in network medicine and summarizes the pipelines for identifying network-based biomarkers, as well as the benefits of integrating genotype and brain phenotype information for a comprehensively noninvasive approach in the early diagnosis of AD. Finally, we propose a framework for integrating knowledge from molecular and brain connectivity levels, which has the potential to enable noninvasive diagnosis, provide support for monitoring therapies, and help understand heretofore unexamined deep level relations between genotype and brain phenotype.**

***Index Terms*—Alzheimer's disease, omics, neuroimaging, network biomarkers, multilevel integration.**

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I. Alzheimer's Disease

A. Facts and Current Situation

ALZHEIMER's disease (AD) results in progressive loss of cognitive function and typically, by the time a patient is diagnosed, the disease has progressed for many years. Therefore, early detection is crucial for therapy design. Approximately 15% of the population >65 years old is affected by AD, incurring the highest healthcare costs among brain related conditions [1]. Current statistics of AD indicate a prevalence of 44 million and, with the increase in life expectancy, it is projected to quadruple by 2050 [2]. An estimated one out of nine people aged 65 and older, and one out of three people aged 85 and older suffer from the condition [1]. The economic and societal impact is reflected by the healthcare costs associated with AD, which are the highest among brain related medical conditions, being estimated in Europe at €106 billion and in the US at $236 billion [1], [3]. Additionally, the quality of life of patients and the welfare of families are dramatically affected [4].

In this context, global leaders have recently agreed that battling AD should be made a strategic priority, with the overarching goal of finding an effective way to treat or prevent the disease by 2025 [5]. One major hurdle in achieving this goal is the fact that, even in high income countries, only around 50% of people living with dementia receive proper diagnosis, whereas in low and middle-income countries less than 10% of cases are diagnosed [1]. From this perspective, as focus is shifted from the development of palliative treatments for populations in late disease stages to disease modifying therapies (DMTs) targeting early stage treatment, the design of reliable, accurate biomarkers and tools able to diagnose AD and monitor disease progression is expected to have a major scientific impact.

B. Current Therapies, Early Detection and the Need for a Paradigm Shift

AD is a multi-factorial and heterogeneous disease involving, besides a genetic factor, environmental, epigenetic and metabolic factors [6]. These factors, coupled with brain-related etiopathogenic mechanisms, result in a complex cognitive phenotype. Due to this inherent complexity of AD, most drug agents entering the AD drug-development pipeline have failed in the last three decades [7].

Current therapies of AD have extremely limited efficiency and therefore the development of DMTs is considered as alternative [8]. However, the development of reliable DMTs is limited by several factors, to name the most important: (i) the lack of reliable noninvasive biomarkers in early disease stages [9]; (ii) the absence of mechanistic understanding of the multifactorial nature of AD, having as result a large number of therapeutic interventions to fail in phase II and III of clinical trials [10]; (iii) the slow progressive nature of AD, implying extended monitoring time and consequently delaying the clinical assessment. In this context, it is not surprising that no new drug is expected to be available on the market by 2025 [5].

Current early AD diagnosis relies on topographical markers (volume changes in the brain) measured by magnetic resonance imaging (MRI) and hypometabolism of neocortical regions assessed by fluorodeoxyglucose (FDG)-positron emission tomography (PET), in conjunction with evidence of Aβ and tau pathology from Cerebrospinal Fluid (CSF), for a definitive diagnosis [2], [11]. The deposition of amyloid β (Aβ) in the extracellular space characterizes AD and can be measured efficiently through CSF samples, thus CSF-based approaches constitute a vast body of AD early diagnosis literature [12] Additionally, tau phosphorylated at Thr181 (P-tau181P) is considered as a well-established marker of AD [13]. Recently, also the mixture of Aβ1-42 and P-tau181P offered a molecular signature associated with AD [14]. However, a number of clinical studies pointed that up to 25% of patients clinically diagnosed with probable AD during their lifetime, did not have post-mortem evidence of AD pathology, such as Aβ plaques or tau pathology, thus further complicating diagnosis [15]

Under these circumstances, even if CSF-based analysis has contributed effectively in AD diagnosis, reflecting metabolic processes in the brain owing to direct contact between the brain and CSF, the lumbar puncture and collection of CSF is still an invasive approach with potential side effects [16]. In parallel, attempts to measure Aβ or tau from plasma as potential predictive markers of AD have so far not been successful [16]. Additionally, diagnosis based on PET is relatively expensive, has limited availability, and exposes subjects to radiation [17]. Thus, the major drawback of current approaches is that they do not support the DMT scenario, where the longitudinal evolution of disease as well as effects of treatment must be followed, increasing the need for noninvasive biomarkers. For more details about current AD diagnosis and biomarkers, extensive surveys published recently are recommended [16].

In this context, a paradigm shift is bound to occur in preclinical AD diagnosis and treatment: from invasive biomarkers, based on CSF and PET to blood-based biomarkers and more affordable neuroimaging techniques (MRI) and from palliative therapies to DMTs [8], [9], [18], [19]. Although a number of significant challenges are posed by the peculiarities of AD [9], initial outcomes are encouraging and significant progress is being made towards personalized medicine approaches based on omics technologies which have significantly impacted the management of other complex diseases [20].

In this review article, we provide a summary on recent research on the discovery of AD biomarkers. We focus on network-based molecular biomarkers derived from omics data, particularly from patients' blood samples and neuroimaging biomarkers derived from functional connectivity data in resting state, that is, the subjects not performing an explicit task. Special emphasis is placed on approaches identifying biomarkers as modular substructures of functional relevance within global molecular and connectivity networks. Since disruptive disease processes take place at different rates in different molecular pathways and brain areas, such approaches are more sensitive to small local perturbations Therefore, analysis focusing on network substructures may reveal changes otherwise undetected. The underlying rationale of investigating network-based biomarkers is that such biomarkers take into account the biological and functional context in which disease evolves, rather than focusing on specific events (e.g., hypometabolism, phosphorylation) or measurements (volumetric assessment of specific regions). Finally, a framework for integrating the network-based multi-level (molecular omics and brain connectivity) biomarkers is provided that can further improve diagnosis, help identifying heretofore unexplored relations between the genotype and phenotype and, consequently, facilitate the implementation of a new generation of biomarkers in AD.

II. Molecular and Brain Level Biomarkers – The Network Perspective

Integration of molecular level biomarkers from omics data analysis of blood samples with brain level biomarkers retrieved from MRI neuroimaging, holds the promise of advancing AD biomarker research. Both types of data (i) have high impact due to their noninvasive nature, (ii) offer support in terms of monitoring DMT outcomes, and (iii) have an increasing trend in experimental data availability and quality. Several recent approaches based on these two types of data have proposed novel biomarkers at either molecular level or brain level [18], [21], [22].

A. Molecular Level, Blood Omics-Based Biomarkers for AD

Research on blood based biomarkers has attracted increasing interest recently due to the noninvasive nature and wide availability of samples, in contrast with approaches based on CSF [18], [23], [24]. A number of studies have identified AD related blood-based biomarker panels in serum and plasma in the last decade [25], [26]. Among these, several blood transcriptome based approaches using expression profiles of gene panels yielded diagnostic value related to AD [27], [28]. Extensive reviews have been published recently highlighting the current state of AD analysis and biomarkers identification through blood-based samples [24], [29].

However, despite the plethora of approaches using blood-based samples and their encouraging results, as well as the obtained EU approval (CE marking), their accuracy remains relatively low. Generally, studies report a lack of reproducibility across cohorts for all types of blood-based markers and lack of functional context [9]. Recently, it has been suggested that the limited reproducibility of markers is due to the reductionist, single marker-based approach (even for gene panels, the methodology relies on individual gene-level statistical test used to identify differential expression).

Emerging approaches: Network-based biomarkers

In contrast, a holistic approach would conveniently link markers to pathophysiology by providing wider functional context. Generally, complex diseases, such as AD, have driven the need for systems-based approaches that elucidate the molecular mechanisms underlying the disorder, rather than the effect of individual genes [30]. The molecular basis of complex diseases is highly heterogeneous and affected by multiple factors simultaneously, such as genetic predisposition, multipart molecular mechanisms and effects of the environment and numerous other factors [31]. Hence, to account for these aspects, the research community has shifted towards systems-based approaches (see Table I). The recent work of Voyle *et al.* [32] adopts such a framework, by designing a pathway-based model using Random Forest modeling with recursive feature elimination. Their approach furthers the currently prevalent approaches based on gene panel biomarkers, by considering functional context and correlations between the genes' expressions, encoded in the form of network interactions between genes. The model is applied to AD diagnosis using gene expression data from blood-based samples.

The systems biology approaches to AD developed recently can be categorized into two types of networks: predefined Protein-Protein Interaction (PPI) networks and co-expressed gene networks usually built based on genes co-expression profiles. More specifically, based on PPIs the authors of [33] successfully identified 13 novel AD-related candidate genes through a classification approach. The information obtained from a list of AD-associated genes and not-related genes was incorporated within the PPI network, while various global topological features were used to predict disease. Similarly in [34], a network-based approach to identify novel AD-related genes was described using an integration of PPI and a list of AD-genes along with a strategy that combines local and global network analysis. Going beyond, a robust integration was implemented in [35], where the authors proposed the integration of transcriptome (gene expressions data – as node attributes) and proteome (edges in a PPI network). This combination can provide additional relevant information in the study of complex diseases like AD by identifying key genes, proteins and cellular pathways involved in disease processes.

The second category of AD systems biology approaches focuses on co-expressed gene networks using as edge attributes various similarity measures among gene expression profiles from a case under study and subsequently searching for topological overlap between networks [36], or modular structures related to AD [37]–[39]. However, most such studies are based on brain/postmortem samples and are thus not suitable for non-invasisve diagnosis and DMTs. Concluding, despite the wider use of systems-level approaches in complex neurodegenerative diseases [43], [44], approaches extracting network-based biomarkers in AD using omics data from blood samples are still in their infancy, though considered as the emerging paradigm in AD prognosis and early diagnosis [45].

Given the exponential growth of omics data and the natural view offered by molecular networks, one of the main challenges in AD remains the development of a System Biology framework integrating heterogeneous omics data by mapping to a molecular network [46]. This has led to an emerging field known as Network Medicine, at the confluence between Systems Medicine and Network Science. Network Medicine approaches hold the promise to improve our understanding of how changes in cellular processes can lead to complex diseases by providing the modeling tools needed to identify correlations between essential molecules and the discovery of phenotype-associated substructures (subnetworks or subpathways) in biological networks [47].

As part of the Network Medicine approaches, pathway analysis has been gaining ground in molecular-based network analysis over the past few years, since it can capture the complex mechanisms in biological processes and human diseases in a realistic manner [48]. The current trend of pathway analysis methods, called subpathway analysis, focuses on the identification of 'active subpathways' related to a case under study [49]. Subpathways are local areas of cellular networks that can be associated with specific biological processes, the deregulation of which can give rise to disease. Tools based on cellular subpathways are attractive due to the fact that they can explore deeper the biological significance of genotype-phenotype associations identified through full-genome sequencing [50]. Additionally, it was shown that disease associated genes aggregate in local neighborhoods within interactome [51]. Towards this direction, several groups proposed that key subpathway regions may better represent pathway dysregulations and be more relevant than whole pathways in interpreting the associated biological phenomena [52]. So far, a number of subpathway-based tools have been published in the recent years, offering new insights into uncovering the human diseases mechanisms [53].

One basic part of these approaches is to place genes into a biologically relevant context by mapping them to an organism-level molecular pathway interaction network. Databases such as KEGG [54], Reactome [55] and software packages such as CHRONOS [52], graphite [56] or SubpathwayMiner [57], contain robust frameworks and conversion tools for constructing and analyzing molecular pathway interaction networks. A subsequent part is the integration of multi-omics data, where heterogeneous data (transcriptomics, genomics, proteomics, metabolomics, epigenomics etc.) are combined and overlaid onto the pathway interaction networks to provide additional functional insights. The reference point that can integrate the multi-omics data – where a typical bioinformatics analysis cannot relate them - is network science. Networks (or graphs) can offer a platform for investigating complex systems by integrating various types of data and enabling downstream analysis [58]. One of the first attempts to construct an integrated network from heterogeneous various expressions data was made by Cheng *et al.* [59] while they presented a network framework with PPIs, RNA-Seq and Chip-Seq expression data and interactions among Transcription Factors and miRNAs. The specific network consisted of three types of nodes and four types of interactions in which the authors performed various topological analyses including network motifs identification. In [60] a robust methodology is described for integrating multi-source information on a single network.

TABLE I
OVERVIEW OF MOLECULAR OMICS APPROACHES USED TO INVESTIGATE NETWORK-BASED BIOMARKERS IN AD

Study	Data (Samples)	Graph	Methodology	Measures
Jamal et al. 2016 [33]	Gene List	PPI network	Classification framework based on an integrated network with topological properties, sequence features and FA.	SPL, CC, CLC, Degree, Eccentricity, NC, TC, Radiality, GO
Zanzoni, 2016 [34]	Gene List	PPI network	Network-based approach combining local and global analysis strategies	GO, FM
Ray et al., 2008 [38]	Single-cell GE (Entorhinal cortex/postmortem)	Co-expressed gene network	Network-based approach to identify modular structures embedded in GE	FM, Hub genes
Miller et al., 2010 [37]	GE (brain/postmortem)	Co-expressed gene network	Hierarchical clustering in modules of co-expressed genes	Module membership
Hallock, 2012 [35]	GE (hippocampal/ postmortem)	PPI network	Construction of a core network of AD followed by network structure and key nodes analysis	Degree, PLC, CC, CCL, SPL, Density, Diameter
Ray et al., 2010 [36]	GE (brain/postmortem)	Co-expressed gene network	Construction and analysis of co-expression networks involving their differential topology	Topological overlap
Zhang et al.,2013 [39]	GE (brain/postmortem)	Co-expression networks	Bayesian network approach for using co-expression modules	Modular differential connectivity measures
Bai et al., 2016 [40]	GE (brain/postmortem)	Co-expressed gene network	Network modules detection and enrichment analysis	Module-level connectivity
Voyle et al. 2015 [32]	GE (blood)	Molecular pathways	Random Forest modeling, pathway level scores used for classification	Singular value decomposition
Li et al., 2017 [41]	GE (blood)	PPI network	Local network analysis (gene-pairs), enrichment analysis	DEG, FA
Satoh, et al., 2015 [42]	miRNA-Seq data (blood)	Networks of miRNA-gene targets	Pathway analysis and enrichment analysis	FA
Han et al., 2013 [28]	Transcriptomics (blood)	Functional network (constructed)	Pathway network analysis and enrichment analysis	DEG, FA

SPL = shortest path length, CC = closeness centrality, CLC = clustering coefficient, NC = neighborhood connectivity, TC = topological coefficient, PLC = power-law coefficient, GO = Gene Ontology, FM = functional modules, GE = gene expressions, FA = functional annotation, DEG = differentially expressed genes

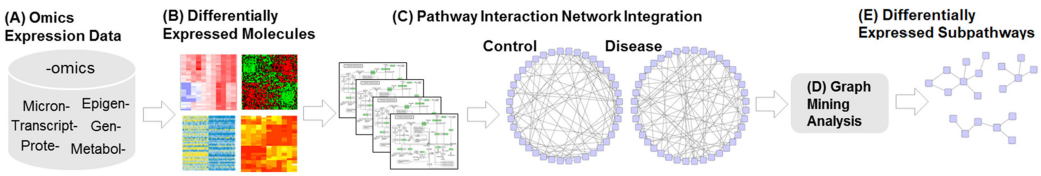

Fig. 1. Typical steps followed to identify network-based biomarkers through pathway analysis. Heterogeneous data from various omics experiments are first collected and analyzed (A) and differentially expressed molecules (genes, proteins, metabolites, etc.) between different conditions are identified (B). The resulting information is subsequently overlaid onto a pathway interaction network constructed from pathway databases (C). Subsequently, activated regions, or subpathways, of the pathway interaction network are identified through graph mining analysis (D) and network biomarkers are retrieved among the differentially expressed subpathways exhibiting statistically significant differential expression among conditions (E).

Further, differentially expressed subpathways (between healthy and disease conditions) can be identified and interrogated for significant enrichment with disease associated molecular entities, such as miRNA targets or methylated genes, via various statistical approaches based on hypergeometric test [61], randomized rotation test [62], or Fisher exact test [63]. The result of this statistical significance analysis are subpathways with increased biomarker value. The functional context provided by subpathways, as well as the thorough statistical testing, reduces the risk associated to biomarker relevance. Briefly, the typical analysis workflow in such a network-based paradigm is described in Fig. 1 and would consist of the following steps: (i) collection and analysis of heterogeneous data from various omics experiments, (Fig. 1A) (ii) identification of differentially expressed molecules (genes, proteins, metabolites etc.) between different conditions (e.g., control, disease), (Fig. 1B) (iii) overlaying the resulting information onto a pathway interaction network constructed from pathway databases (by means of nodes and edge properties), (Fig 1C) (iv) identification of activated regions, or subpathways, of the pathway interaction network through graph mining analysis (Fig. 1D) and (v) selection of potential network biomarkers in the form subpathways with statistically significant differential expression among conditions (Fig. 1E). Additionally, the activity of groups of molecules connected in subpathways can be summarized using scoring schemes which reflect their active/non-active status while considering topological relations between the subpathway molecules. Towards this direction, the works of [62] and [64] suggested such subpathway scores.

B. Brain Level, Volumetric Approaches, Connectivity From MRI

Given the complex nature of AD, the discovery of effective early AD biomarkers must go beyond the investigation of molecular level disruptions and be able to link them to changes in brain level, cognitive function, in order to monitor both disease progression and treatment effects in a coherent and integrative manner.

Measurements from magnetic resonance imaging (MRI), such as volumetric and morphometric analysis have been used extensively in AD diagnosis [65], [66]. However, MRI reflects structural changes such as atrophies in various regions, which are effects of disease progress into symptomatic stages. Additionally, atrophies are hallmarks of brain aging in general, and thus, differentiating AD specific structural changes from MRI may need to involve more elaborate investigations.

Resting-state functional magnetic resonance imaging (rs-fMRI) enables the assessment of functional connectivity between different brain regions with high spatial resolution. It does so by means of estimating correlations in the blood oxygen level dependent (BOLD) signals between brain regions, which are reflective of brain activations due to cognitive processes. By focusing on the brain function instead of structural changes, rs-fMRI connectivity methods are more likely to detect early cognitive decline as result of early manifestation of genetic effects. It is currently the most widely used neuroimaging modality to investigate the functional pathological changes in early AD, due to its noninvasive nature and convenient process of data acquisition [67]. Additionally, it allows monitoring disease progression in longitudinal studies, tracking cognitive decline by means of functional connectivity disruptions [68].

It was very recently suggested that brain networks may be viewed as 'intermediate phenotypes' between molecular systems and individual behavior, and that brain networks mediate the causal effect of genetics on behavior and vice versa [69]. The architecture of these networks affects higher order, cognitive functions, and thus network interactions provide insight into the biological mechanisms and their disruptions brought by disease [70]. Neural circuit connectivity and network activity were shown to be affected by amyloids deposits in AD [71]. Therefore, studying the connectivity in brain networks provides the opportunity to reveal patterns underlying disease propagation throughout the brain and could elucidate which network and subnetwork connectivity features are relevant biomarkers in prodromal AD.

Emerging approaches: Network-based biomarkers

Some studies (see Table II) investigated the direct use of correlation values from the functional connectivity matrix as potential biomarkers. Chen *et al.* [72], ranked the correlation coefficients by means of z-scores and top ranked coefficients were considered as discriminating biomarkers in a Fisher linear discriminant analysis (LDA) to classify a subject as AD or non-disease. In a multimodal approach, Dai *et al.* [73], used besides correlation values also structural MRI features, as well as frequency domain transformations of BOLD time series, and regional homogeneity features. Discriminative features from each modality were fed into ensembles of weighted LDA classifiers used to discriminate AD and normal cases. However, a major disadvantage of studies using direct connectivity values (or direct voxel-derived measures) is that they fail to account for the topological organization of brain networks. In this context, Dipasquale *et al.* [74] decomposed fMRI data into distinct networks maximally independent in the spatial domain, by using group-based independent component analysis (ICA) on the temporally concatenated fMRI data across subjects. The networks resulting from high-dimensional ICA decomposition were then investigated in terms of inter- and intra-network correlation differences between groups.

Recent studies found that healthy brains are characterized by optimal balance between local segregation and global integration, which can be reflected by network modularity and connectivity measures [75], [76]. Additionally, these studies suggested that network hubs (nodes with high degrees of connections) are preferentially affected in AD, thus affecting the connectivity networks' modularity. These observations have pointed researchers to characterize AD as a disconnection syndrome [75]. Overall, a number of local and global network-based connectivity measures, have been investigated as possible biomarkers to discriminate AD and healthy patients, such as node degrees, shortest path lengths, clustering coefficient, small-world, characteristic path length, various centrality measures and others. For a survey on commonly used connectivity measures we refer the reader to [77].

A typical example of such approaches is the classification based method in [78] for extracting network-based discriminating features among a pool of measures of functional segregation (clustering coefficient, local efficiency), functional integration (characteristic path length, global efficiency) and nodal measures (degree, participation coefficient and betweenness centrality). Feature selection and subsequent classification using a support vector machine (SVM) classifier showed that the connectivity measures can be efficiently used for AD diagnosis. Zhang *et al.* [79] took a slightly different approach, while still using connectivity measures (modularity, nodal and edge centrality) to detect group differences. Their high-order connectivity network was defined based on correlations among correlation profiles of each node. Supekar *et al.* [76] analyzed connectivity measures at regional, sub-network level, and indicated that clustering coefficients in the left and right hippocampus differed significantly between groups, suggesting connectivity asymmetry measures are potential biomarkers.

However, the multifactorial pathogenesis of AD, as well as the wide range of brain networks disruptions potentially induced by plaques and tangles formation, is at odds with the current implementation of neuroimaging-based biomarkers, which are single-dimensional. Thus, the redefinition of the biomarker as a network model that can be used to explain group differences in brain connectivity between healthy and disease subjects, presents the potential to capture additional relevant discriminative information. Under this definition, biomarkers are multidimensional network structures connecting several brain regions.

TABLE II
OVERVIEW OF FUNCTIONAL MRI CONNECTIVITY APPROACHES USED TO INVESTIGATE NETWORK-BASED BIOMARKERS IN AD

Study	Modality	Focus	Connectivity Methodology	Measures	Sample Size
Chen et al., 2011 [72]	fMRI	Global functional connectivity	Pearson correlation	Connectivity values	20 AD, 15 aMCI 20 HC
Dai et al., 2012 [73]	fMRI, sMRI	Global functional connectivity and structural morphology	Pearson correlation	Connectivity and morphology features	16 AD, 22 HC
Dipasquale et al., 2015 [74]	fMRI	Global intrinsic connectivity network	Pearson correlation on ICs	Intra-↓ and inter-↓ subnetwork connectivity	21 AD, 20 HC
Khazaee et al., 2015 [78]	fMRI	Global functional connectivity	Pearson correlation	PC, ND, BC, CC used as features in classification	20 AD, 20 HC
Zhang et al., 2016 [79]	fMRI	Global high-order functional connectivity (HOFC)	Correlation of connectivity profiles	M, NC and EC of the HOFC matrix	77 MCI, 89 HC
Supekar et al., 2008 [76]	fMRI	Global and regional functional connectivity	Wavelet correlation	CC ↓, SW ↓	21 AD, 18 HC
Chen et al., 2013 [85]	fMRI	Global functional connectivity	Cross-correlation coefficient	Symmetry index↓ subnetwork integrity↓	30 AD, 30 HC
Sun et al., 2015 [75]	fMRI, DTI	Global functional and structural connectivity	Pearson correlation	CC ↓, SW ↓, M ↓	12 AD, 15 aMCI 14 HC
John et al., 2017 [87]	sMRI	Global structural connectivity	Spearman correlation	Subnetwork based connectivity measures	100 AD, 135 HC
Dai et al., 2015 [88]	fMRI	Local and global seed-based connectivity	z-transform of Pearson correlation	Hub connectivity ↓	34 AD, 41 HC
Jie et al., 2014 [89]	fMRI	Global functional connectivity	z-transform of Pearson correlation	Discriminative subnetworks	12 MCI, 25 HC
Jie et al., 2018 [90]	fMRI	Global functional connectivity	Pearson correlation	Discriminative subnetworks	34 AD, 99 MCI 30 HC
Guo et al., 2017 [91]	fMRI	Global and regional functional hyper-network connectivity	Sparse linear regression model	Discriminative subnetworks and brain region features	38 AD, 28 HC
Cui, 2018 [92]	fMRI	Global functional connectivity	Pearson correlation	Discriminative subnetworks	21 AD, 25 MCI 22 HC

IC = independent component, PC = Participation coefficient, ND = nodal degree, BC = betweenness centrality, CC = clustering coefficient, M = modularity, NC = nodal centrality, EC = edge centrality, SW = small-worldnes, sMRI = structural MRI, DTI = diffusion tensor imaging, aMCI = amnestic mild cognitive impairment.

An increasing body of research is focusing on investigating subnetworks, as substructures of the brain network, thus providing useful information regarding mesoscale network organization [80]. It is well established that the human brain network has a modular organization, consisting of a number of subnetworks dedicated to different cognitive functions [81]. Given the peculiarities of AD-induced disruptions to the brain network, it is obvious that subnetwork-based approaches may yield more accurate disease markers that cannot be reflected in the individual properties of a node or an edge, or of the whole network [82]. Under this perspective, several subnetwork-based biomarker approaches have been proposed in AD [83], [84].

To exemplify, Chen *et al.* [85] estimated group level connectivity networks by averaging individual network correlations into a group representative network and subsequently performed community detection using the spectral algorithm [86] to identify subnetworks. Subnetwork integrity and symmetric connectivity was found to be disrupted in AD. In another study, Sun *et al.* [75] performed community detection using the spectral algorithm and further investigated changes in subnetwork structure in terms of connections density and hubness. In a related approach, John *et al.* [87] used community detection algorithms to identify group-level subnetworks in structural networks and a pool of connectivity measures were then used to assess significant differences.

A variation from the above approaches was used by Dai *et al.* [88], who performed community detection on a reduced connectivity network defined around seed regions of interest (ROIs) and examined differences among connectivity strengths between- and within- subnetworks. They concluded that long-range connectivity between subnetwork hubs and modular integrity disruptions could be used as potential biomarkers.

A different category of approaches are measuring direct topological similarity between connectivity networks using methods such as graph kernels, without the need of computing additional connectivity subnetwork-based measures. Examples of such approaches are those in [89], where authors define subnetworks by first thresholding connectivity matrices and then identify the most discriminative ROI connections by a feature extraction method based on graph kernel distance and SVM classifiers. A variation of this approach is proposed in [90], where multi-scale subnetworks are built around seed network nodes (by increasing neighborhood around seed) and similarity between subnetwork profiles of pairs of corresponding nodes in AD vs healthy networks is computed by graph kernels in an effort to identify most discriminative seeds (and subnetworks). While still based on the

idea of identifying discriminative subnetworks using topological dissimilarity measured via graph kernels, Guo *et al.* [91] took a different approach in defining subnetworks by means of hyper-network connectivity defined using a sparse linear regression model that estimates a region using linear combination of time series of other regions. Thus, a hyper-network can represent, through a connecting edge, higher-order relationships among multiple brain regions (i.e., subnetworks).

Recently, Cui *et al.* [92], merged the graph kernel approach with a prior step in which frequently occurring subnetworks are searched in connectivity networks at group level via the gSpan algorithm [93]. Discriminative subnetworks are then identified based on their frequency of occurrence in the AD and healthy group. Graph kernel PCA is then used as a feature extraction and a method for embedding the subnetworks into a vector representation for subsequent classification using SVM. Table II presents an overview of the described approaches.

A general pipeline for the analysis of rs-fMRI functional connectivity for identification of subnetworks with biomarker value can therefore be summarized in Figure 2. Typically, the steps involved consist of: i) extraction of BOLD signals from functional data scans, which includes region of interest (ROI) parcellation, according to anatomical atlases (Fig 2. A-C); ii) estimation of inter-regional connectivity based on correlation coefficients of the BOLD time-series signals (Fig. 2D). This step may involve various signal processing such as wavelet analysis, such that specific frequency components of the signals are emphasized [94]; iii) filtering of the correlation coefficients matrix to exclude spurious connectivity values (Fig. 2E); iv) estimation of the topological graphs reflecting functional connectivity between brain regions (Fig. 2F), and network community structure analysis to identify densely connected communities (subnetworks) representing group consistent connectivity network structure (consensus clustering) (Fig. 2G). Finally, v) comparison of connectivity structure between different patient groups (disease vs healthy) and identification of discriminative subnetworks with significant differences between the groups (Fig. 2H). The search for subnetworks which exhibit topology changes across different patient groups is typically accomplished by either (i) determining a group representative (consensus) connectivity network which is subsequently clustered using a community finding algorithm, e.g., the spectral or Louvain methods [95]; significant changes between subnetworks among different groups are then assessed using statistical measures, such as the normalized mutual information (NMI) [94], and subnetworks with lowest NMI are considered to be relevant for between group discimination. Or, alternatively by ii) employing graph mining methods, such as the gSpan algorithm [93], which search for frequent subnetworks present within individual connectivity networks of different patient groups. Then, among the retrieved frequent subnetworks in each group, are retained only those which carry group discriminative information. The procedure can follow a simple approach based on a discriminative score, in terms of difference in the frequency of occurrence between the AD group samples D_{AD} and control group samples D_{HC}, such as:

$$DS\ (s_i) = |f\ (s_i\,|D_{AD}\,) - f\ (s_i\,|D_{HC}\,)|$$

Where s_{i} is a subnetwork from $S = \{S_{\mathrm{AD}}, S_{\mathrm{HC}}\}$ with $S_{\mathrm{AD}} = \{s_{\mathrm{AD1}}, s_{\mathrm{AD2}}, \ldots, s_{\mathrm{ADm}}\}$ denoting the set of all subnetworks retrieved from AD group samples and $S_{\mathrm{HC}} = \{s_{\mathrm{HC1}}, s_{\mathrm{HC2}}, \ldots, s_{\mathrm{HCn}}\}$ denoting the set of all subnetworks retrieved control group samples [91]. The score allows ranking of subnetworks and the selection of only those with top discriminative value.

III. Integrating Omics and Brain Level - Towards the Future Big Challenge

It has become increasingly clear that combining information from different modalities, and at different biological and systems levels, has the potential to provide accurate assessment of disease stage and progression, a vital step in the development of DMTs [96]–[99]. This assessment is grounded on evidence that AD, like most neurodegenerative disorders evolves at the systems level and that biomarkers–molecular and neuroimaging - need to be considered from a holistic point of view.

System-level approaches, which integrate data from different levels, and thus have the potential to uncover a wider range of dysfunctions, are expected to drive the biomarker discovery process into a new generation of biomarkers. Additionally, they hold the promise of identifying currently unknown mechanisms of AD, as well as causal relations between the genotype and brain phenotype. Existing approaches mostly combine information from different imaging modalities, commonly voxel based intensities from MRI and PET [73], [98], [99]. Several multimodal approaches include also CSF markers, besides MRI and PET [96]–[99], MRI, PET and neuropsychological measures [100], or MRI, PET, CSF and APOE genotype [101].

However, the drawbacks of existing integrative approaches result from using markers obtained by invasive methods (CSF – lumbar puncture is needed for sample collection; PET- requires radioactive tracers), as well as the combination of low-level features (with limited information power – such as MRI voxel intensities) from individual modalities. In this context, integration of multimodal and multilevel network-based markers from molecular and brain connectivity levels has the potential to improve diagnosis in the early, preclinical stage of AD, as well as provide breakthrough insights to understanding the mechanisms and dynamics of the disease. Towards this direction, classification approaches based on deep learning techniques have gained increased attention recently, due to the excellent representational power characteristic of deep architectures [102]. Deep networks, such as the deep autoencoders, are able to discover latent feature representations from biomarkers identified at molecular and brain connectivity levels, thus enhancing significantly classification accuracy. In this context, we propose the use of a deep learning model based on the bimodal deep autoencoder which is able to identify relevant cross-level features [103] and use them to improve diagnosis.

Typically, the proposed framework consists of the following steps:

1a-2a. Construction of integrated pathway network and extrac-tion of differentially expressed subpathways from omics data following the pipeline described in Fig. 1.

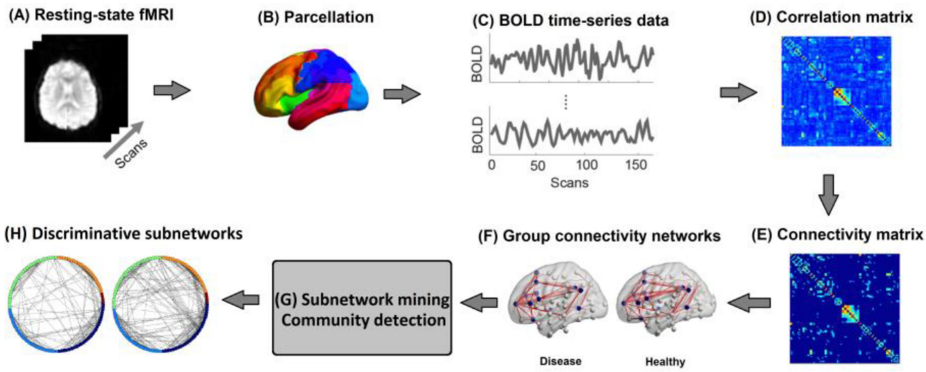

Fig. 2. Flowchart for identifying subnetwork biomarkers from rs-fMRI functional images. (A) Resting-state BOLD fMRI data is acquired from each individual and pre-processed; (B) Pre-processed signals are parcellated into regions of interest (ROI), according to anatomical atlases; C) Bold signal time series are estimated for each ROI by averaging over ROI voxels; (D) BOLD signals are used for estimation of inter-regional connectivity based on correlation coefficients, which are stored into a correlation matrix. (E) The correlation coefficients matrix is filtered to exclude spurious connectivity values between regions and the connectivity matrix is obtained; (F) Group connectivity networks reflecting functional connectivity between brain regions are determined and (G) network community structure analysis is performed to identify densely connected communities (subnetworks) representing consistent connectivity network structure (consensus); (H) Comparison of connectivity structure between patient groups (disease vs healthy) is performed for the identification of subnetworks with significant discriminative differences between the groups (figure shows four subnetworks with different colors using circle graphs). Figure adapted from [95].

1b-2b. Construction of brain functional connectivity networks and extraction of discriminative subnetworks following the pipeline described in Fig. 2, using fMRI scans.

3. Transformation of individual data samples into binary vector representations for each of the two levels, with features corresponding to the reference set of subpathways/sub-networks identified at steps 1–2), and values representing either activation, or presence (1) of the respective subpathway/subnetwork in the current data sample, or its inactivity, or absence (0). This can be achieved by means of a subpathway activation scoring scheme [62] at molecular level, and a subnetwork score, e.g., based on NMI, at brain connectivity level.

4. In the *pre-training* step, vectorized input data from each level will be fed into a level-specific (omics and brain connectivity) restricted Boltzmann machine (RBM) which will encode in the first hidden layer posteriors the dependencies between features within each level input. Subsequently, the posteriors of the first input layer will be used as input data for the next layer, where the new shared representation will be achieved by merging variables of the level-specific first hidden layer (Fig. 3C), an approach inspired by [103]. Further, one or more additional layers can be added by stacking autoencoders, which use as input previous' hidden layer variables, forming a deep hierarchy of stacked autoencoders. Following approaches in [103] and [105], pretraining can be done using an augmented dataset with additional samples which have only single level data (either subpathways from omics level or subnetworks from brain connectivity level). Features corresponding to the other level are zero-masked. This strategy enables learning of cross-level correlations, while at the same time learns a network model robust to input were only single level data is available [103]. The bimodal deep network presented so far is able to learn, in an unsupervised manner, existing latent higher-order correlations across levels. At this pre-training step, publicly available blood sample gene expression and other omics data, as well as MRI imaging data, of patients and controls enrolled in large scale multi-center longitudinal studies, such as the Alzheimer's Disease Neuroimaging Initiative (ADNI) [104] can be used.

5. Finally, in the *supervised training (or fine-tuning)* step, an output layer will be stacked on top of the bimodal deep network to represent the class labels of input data (ADNI labeled data could be used here as control and early AD, i.e., converted MCI patients for whom follow-up exams confirmed transition to AD). A linear classifier, softmax regression layer or a linear SVM, can be used for discriminating incoming test data (Fig. 3C). The new test data samples will be diagnosed after their transformation into binary vector representation following steps 1-3 above.

Notably, the advantage of using such bimodal deep network models is that they can be pre-learned in a greedy layer-wise manner, to obtain the optimal features in an unsupervised way. Subsequently, during training, the pre-learned network' parameters are fine-tuned based on supervised class information, using backpropagation. The pre-learning of parameters reduces the risk of falling into local maxima. Another advantage of the proposed framework compared to other deep learning-based approaches (such as convolutional neural networks), besides the fact that once pre-learned, the model is able to work with inputs where partial data from a modality is absent, is the possibility to enrich the pre-training dataset with unlabeled data from other available sources during unsupervised learning. This was shown to enhance the learning of more robust representations [105].

The rationale behind the integration framework we propose is two-fold: first, currently, the relation between connectivity markers and molecular pathology, especially in preclinical stages of AD is largely unknown. Since, it is widely accepted that pathological process in AD starts well before cognitive

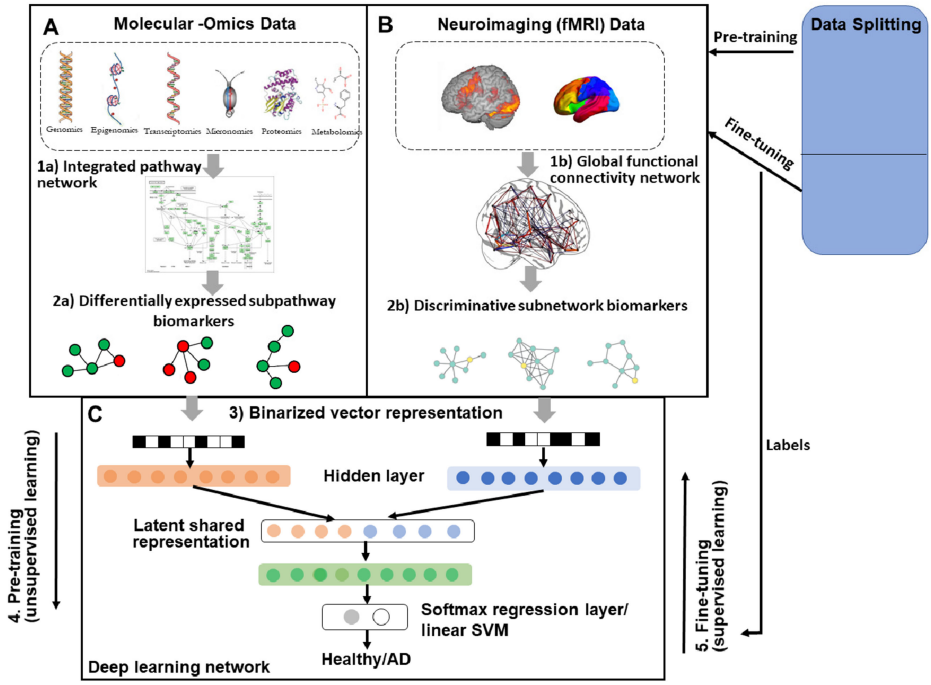

Fig. 3. The proposed framework for integrating network-based biomarkers from molecular omics level (A) and brain connectivity level (B). The data samples from each level are converted into binary vector representations using the reference set (from A and B) of differentially expressed subpathways and discriminative subnetworks through subpathway/subnetwork scoring schemes and subsequently fed into a bimodal deep network which extracts high-level shared features subsequently fed into linear classifiers (C).

deficits manifest, integrating omics level functional information has the potential of providing 'upstream' evidence, increasing diagnostic sensitivity. Second, the deep learning-based models better fit the intuitive notion of cross-level relation (between genotype and phenotype). Several recent studies based on similar deep learning approaches have demonstrated the ability to extract such high-level relations with diagnostic value, either from neuroimaging (PET and MRI) and genetic (single nucleotide polymorphism), or neuroimaging and CSF biological data [98], [106]. In our proposed framework, the shared representation, learned by the bimodal deep network may encode higher-level relations between input data features, e.g., cross-talk between omics level subpathways, cognition-related associations between brain connectivity subnetworks, or synergy between subpathways and subnetworks reflective of processes transcending the blood-brain barrier. However, the nature of deep networks architectures makes the direct examination of such representations a complex task, which is currently the focus of sustained research [102].

Challenges and Limitations

A common issue in omics data integration and analysis is the difference induced by non-biological variables or technical heterogeneity (e.g., different reagent lots, technicians and labs, etc.), known as batch effects. Robust approaches for batch effects removal need to be considered at the experimental and data preprocessing phases, in order to preserve biological heterogeneity and avoid erroneously skewed analysis. Recently, an increasing number of studies tackle these aspects in several directions such as: developing best practices and guidelines for experimental design (e.g., required technical and biological replicates) [107] and analysis pipelines for removal of non-biological variation [108].

In the context of our proposed framework, a number of recent studies have highlighted the ability of network-based approaches to mitigate effects and confounding variability. This is due to their inherent incorporation of biological constraints by the use of validated background knowledge and biological context from structured pathway databases [109]. Thus, when overlaying experimental data onto pathway interaction networks constructed from databases, additional validation steps can be implemented to check the agreement of new data with biological context, and prune spurious edges as in [52].

Similar challenges are posed by the reliability and reproducibility of MRI neuroimaging data. Several parameters may differ between MRI studies, such as scanner protocol, preprocessing and analysis pipelines, or subject related variability in rs-fMRI experiments. However, particularly in the case of AD, the emergence of multi-center international consortia and initiatives, such as the ADNI and the European Alzheimer's Disease Consortium (EADC) has fostered sustained efforts for developing field-wise consensus on the harmonization of experimental, assessment and biorepository protocols [24]. Additionally, recent works have demonstrated that implementation of specific processing pipelines, which incorporate biology-inspired filtering schemes of brain networks, as well as subsequent analysis pipelines result in increased reproducibility of results [110], [111]. Akin to the efforts pursued in the case of omics data, the neuroimaging research community has been taking significant steps in the implementation of standards and best practices [112].

Another significant challenge in the context of the proposed integration framework is the need of deep learning algorithms for sufficient and balanced sample for learning robust representations of data [102]. However, recent studies attempting multi-modal integration of low-level neuroimaging and CSF biological data and employing similar deep learning architectures have shown encouraging results using as few as 51 AD and 99 MCI patients' data [98]. The advantage of the proposed framework is that it can easily incorporate in the training phase publicly available data from datasets collected by standardized protocols under the above-mentioned AD initiatives (e.g., ADNI database includes omics and neuro-imaging data from a cohort of more than a thousand participants, longitudinally collected since its launch in 2004 [104], thus enabling 'data hungry' deep learning approaches).

Moreover, the availability of data collected longitudinally and from a wide range of modalities in studies such as ADNI, help overcome other limitations. Such a presumable limitation would be the need for training on same subject data from both levels, in order to extract meaningful cross-level correlations between omics and brain connectivity network biomarkers. Nevertheless, this limitation is additionally accounted in the proposed framework by the possibility of using partial data (when data from one level is unavailable) by employing the zero-masking strategy described above [103].

IV. Conclusion

Network-based biomarkers are attracting increasing interest lately due to their ability of facilitating comprehensive systems-level approaches and modeling of intrinsic functional relations at multiple biological levels [25]. In this context, the study of complex diseases, such as AD, benefits from the established methodologies of network science and graph theory which offer not only a conceptual framework but also practical toolkits and techniques able to tackle the challenges and limitations of existing biomarker discovery approaches.

The development of efficient frameworks for identifying non-invasive biomarkers in preclinical stages of AD is a crucial step in managing the disease. In this context, omics-based data collected from blood samples, as well as information obtained through neuroimaging techniques provide ideal candidates for discovery of noninvasive biomarkers. Moreover, the integration of information from genotype and brain phenotype levels, provides systems-level insight into disruptions brought by the disease, while the network- based modeling benefits from the functional context provided by the network interactions. At molecular level, pathway analysis methods can identify differentially expressed, dynamic substructures in biological networks. Such substructures, or subpathways, have highly coherent function, based on underlying topology, and enhanced capability to detect variations in response to specific biological context. At brain connectivity level, complex network analysis can identify discriminative modular substructures, which may serve as biomarkers. Such substructures, or subnetworks, often correspond to underlying brain functional specialization, since it is well established that brain networks have modular organization and, therefore, can provide valuable information about mesoscale network organization and serve as useful approach for identifying biomarkers [80], [81].

While in the present review we focus on diagnostic biomarkers in early AD, network-based biomarkers have the potential to find wider use in AD biomarker research. Specifically, given the noninvasive nature of considered data modalities, similar network-based approaches could be employed for the identification of prognostic biomarkers, as well as biomarkers for monitoring therapeutic efficacy. For this, the discussed methods should be adapted in order to be able to extract biomarkers reflective of network dynamical changes. Existing longitudinal studies such as ADNI, provide extensive data support for such approaches.

The recent high-profile failures of AD drugs in various phases of clinical trials [10] suggest there is a strong need for objective early stages biomarkers, able to provide support for DMTs. The network-based perspective presented in the current review holds the promise of facilitating the development of such biomarkers, while the integration of genotype-brain phenotype information leads the way to the personalized medicine solutions in preclinical AD research.

References

[1] Alzheimer's Association, "2017 Alzheimer's disease facts and figures," 2017.

[2] C. Reitz and R. Mayeux, "Alzheimer disease: Epidemiology, diagnostic criteria, risk factors and biomarkers," *Biochem. Pharmacology*, vol. 88, no. 4, pp. 640–651, 2014.

[3] M. DiLuca and J. Olesen, "The cost of brain diseases: A burden or a challenge?" *Neuron*, vol. 82, no. 6, pp. 1205–1208, 2014.

[4] A. Wimo, L. Jönsson, J. Bond, M. Prince, and B. Winblad, "The worldwide economic impact of dementia 2010," *Alzheimer's Dementia*, vol. 9, no. 1, pp. 1–11, 2013.

[5] G. Vradenburg, "A pivotal moment in Alzheimer's disease and dementia: How global unity of purpose and action can beat the disease by 2025," *Expert Rev. Neurotherapeutics*, vol. 15, no. 1, pp. 73–82, 2014.

[6] K. Iqbal and I. Grundke-Iqbal, "Alzheimer's disease, a multifactorial disorder seeking multitherapies," *Alzheimer's Dementia*, vol. 6, no. 5, pp. 420–424, 2010.

[7] L. S. Schneider *et al.*, "Clinical trials and late-stage drug development for Alzheimer's disease: An appraisal from 1984 to 2014," *J. Internal Med.*, vol. 275, no. 3, pp. 251–283, 2014.

[8] J. Cummings, G. Lee, T. Mortsdorf, A. Ritter, and K. Zhong, "Alzheimer's disease drug development pipeline: 2017," *Alzheimer's Dementia*, vol. 3, no. 3, pp. 367–384, 2017.

[9] H. M. Snyder *et al.*, "Developing novel blood-based biomarkers for Alzheimer's disease," *Alzheimer's Dementia*, vol. 10, no. 1, pp. 109–114, 2014.

[10] J. Godyn, J. Jonczyk, D. Panek, and B. Malawska, "Therapeutic strategies for Alzheimer's disease in clinical trials," *Pharmacological Rep.*, vol. 68, no. 1, pp. 127–138, 2016.

[11] V. Mantzavinosa, A. Alexiou, N. Greig, and M. Kamal, "Biomarkers for Alzheimer's disease diagnosis," *Curr. Alzheimer Res.*, vol. 14, no. 11, pp. 1149–1154, 2017.

[12] K. Blennow, N. Mattsson, M. Schöll, O. Hansson, and H. Zetterberg, "Amyloid biomarkers in Alzheimer's disease," *Trends Pharmacological Sci.*, vol. 36, no. 5, pp. 297–309, 2015.

[13] N. Mattsson *et al.*, "CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive impairment," *J. Amer. Med. Assoc.*, vol. 302, no. 4, pp. 385–393, 2009.

[14] G. De Meyer *et al.*, "Diagnosis-independent Alzheimer disease biomarker signature in cognitively normal elderly people," *Arch. Neurology*, vol. 67, no. 8, pp. 949–956, 2010.

[15] M. N. Sabbagh, L. F. Lue, D. Fayard, and J. Shi, "Increasing precision of clinical diagnosis of Alzheimer's disease using a combined algorithm incorporating clinical and novel biomarker data," *Neurology Therapy*, vol. 6, pp. 83–95, 2017.

[16] C. Humpel, "Identifying and validating biomarkers for Alzheimer's disease," *Trends Biotechnol.*, vol. 29, no. 1, pp. 26–32, 2011.

[17] E. Salmon, C. Bernard Ir, and R. Hustinx, "Pitfalls and limitations of PET/CT in brain imaging," *Semin. Nucl. Med.*, vol. 45, no. 6, pp. 541–551, 2015.

[18] K. Henriksen *et al.*, "The future of blood-based biomarkers for Alzheimer's disease," *Alzheimer's Dementia*, vol. 10, no. 1, pp. 115–131, 2014.

[19] P. Fehlbaum-Beurdeley *et al.*, "Toward an Alzheimer's disease diagnosis via high-resolution blood gene expression," *Alzheimer's Dementia*, vol. 6, no. 1, pp. 25–38, 2010.

[20] K.-H. Yu and M. Snyder, "Omics profiling in precision oncology," *Mol. Cellular Proteomics*, vol. 15, no. 8, pp. 2525–2536, 2016.

[21] K. Lunnon *et al.*, "A blood gene expression marker of early Alzheimer's disease," *J. Alzheimer's Dis.*, vol. 33, no. 3, pp. 737–753, 2013.

[22] D. A. Wiepert *et al.*, "A robust biomarker of large-scale network failure in Alzheimer's disease," *Alzheimer's Dementia, Diagnosis, Assess. Dis. Monit.*, vol. 6, pp. 152–161, 2017.

[23] H. M. Snyder *et al.*, "Developing novel blood-based biomarkers for Alzheimer's disease," *Alzheimer's Dementia*, vol. 10, no. 1, pp. 109–114, 2014.

[24] S. E. O'Bryant *et al.*, "Blood-based biomarkers in Alzheimer disease: Current state of the science and a novel collaborative paradigm for advancing from discovery to clinic," *Alzheimer's Dementia*, vol. 13, no. 1, pp. 45–58, 2017.

[25] E. Nagele, M. Han, C. DeMarshall, B. Belinka, and R. Nagele, "Diagnosis of Alzheimer's disease based on disease-specific autoantibody profiles in human sera," *PLoS One*, vol. 6, no. 8, 2011, Art. no. e23112.

[26] Y. Kitamura *et al.*, "Plasma protein profiling for potential biomarkers in the early diagnosis of Alzheimer's disease," *Neurological Res.*, vol. 39, no. 3, pp. 231–238, 2017.

[27] B. B. Booij *et al.*, "A gene expression pattern in blood for the early detection of Alzheimer's disease," *J. Alzheimer's Dis.*, vol. 23, no. 1, pp. 109–119, 2011.

[28] G. Han *et al.*, "Characteristic transformation of blood transcriptome in Alzheimer's disease," *J. Alzheimer's Dis.*, vol. 35, no. 2, pp. 373–386, 2013.

[29] B. Olsson *et al.*, "CSF and blood biomarkers for the diagnosis of Alzheimer's disease: A systematic review and meta-analysis," *Lancet Neurology*, vol. 15, pp. 673–684, 2016.

[30] N. Alcaraz, M. List, R. Batra, F. Vandin, H. J. Ditzel, and J. Baumbach, "De novo pathway-based biomarker identification," *Nucleic Acids Res.*, vol. 45, no. 16, 2017, Art. no. e151.

[31] A. Oulas, G. Minadakis, M. Zachariou, K. Sokratous, M. M. Bourdakou, and G. M. Spyrou, "Systems bioinformatics: Increasing precision of computational diagnostics and therapeutics through network-based approaches," *Briefings Bioinformat.*, bbx151. [Online]. Available: https://doi.org/10.1093/bib/bbx151

[32] N. Voyle *et al.*, "A pathway based classification method for analyzing gene expression for Alzheimer's disease diagnosis," *J. Alzheimer's Dis.*, vol. 49, no. 3, pp. 659–669, 2016.

[33] S. Jamal, S. Goyal, A. Shanker, and A. Grover, "Integrating network, sequence and functional features using machine learning approaches towards identification of novel Alzheimer genes," *BMC Genomics*, vol. 17, no. 1, 2016, Art. no. 807.

[34] A. Zanzoni, "A computational network biology approach to uncover novel genes related to Alzheimer's disease," *Methods Mol. Biol.*, vol. 1303, pp. 435–446, 2016.

[35] P. Hallock and M. A. Thomas, "Integrating the Alzheimer's disease proteome and transcriptome: A comprehensive network model of a complex disease," *OMICS*, vol. 16, nos. 1–2, pp. 37–49, 2012.

[36] M. Ray and W. Zhang, "Analysis of Alzheimer's disease severity across brain regions by topological analysis of gene co-expression networks," *BMC Syst. Biol.*, vol. 4, 2010, Art. no. 136.

[37] J. A. Miller, S. Horvath, and D. H. Geschwind, "Divergence of human and mouse brain transcriptome highlights Alzheimer disease pathways," *Proc. Nat. Acad. Sci. USA*, vol. 107, no. 28, pp. 12698–12703, 2010.

[38] M. Ray, J. Ruan, and W. Zhang, "Variations in the transcriptome of Alzheimer's disease reveal molecular networks involved in cardiovascular diseases," *Genome Biol.*, vol. 9, no. 10, 2008, Art. no. R148.

[39] B. Zhang *et al.*, "Integrated systems approach identifies genetic nodes and networks in late-onset Alzheimer's disease," *Cell*, vol. 153, no. 3, pp. 707–720, 2013.

[40] Z. Bai *et al.*, "AlzBase: An integrative database for gene dysregulation in Alzheimer's disease," *Mol. Neurobiol.*, vol. 53, no. 1, pp. 310–319, 2016.

[41] H. Li *et al.*, "Identification of molecular alterations in leukocytes from gene expression profiles of peripheral whole blood of Alzheimer's disease," *Sci. Rep.*, vol. 7, no. 1, 2017, Art. no. 14027.

[42] J. I. Satoh, Y. Kino, and S. Niida, "MicroRNA-Seq data analysis pipeline to identify blood biomarkers for Alzheimer's disease from public data," *Biomarker Insights*, vol. 2015, no. 10, pp. 21–31, 2015.

[43] N. N. Parikshak, M. J. Gandal, and D. H. Geschwind, "Systems biology and gene networks in neurodevelopmental and neurodegenerative disorders," *Nature Rev. Genetics*, vol. 16, no. 8, pp. 441–458, 2015.

[44] E. Horgusluoglu, K. Nudelman, K. Nho, and A. J. Saykin, "Adult neurogenesis and neurodegenerative diseases: A systems biology perspective," *Amer. J. Med. Genetics B, Neuropsychiatric Genetics*, vol. 174, no. 1, pp. 93–112, 2017.

[45] J. Yerbury, D. Bean, and G. Favrin, "Network approaches to the understanding of Alzheimer's disease: From model organisms to humans," *Methods Mol. Biol.*, vol. 1303, pp. 447–458, 2016.

[46] A. Alyass, M. Turcotte, and D. Meyre, "From big data analysis to personalized medicine for all: Challenges and opportunities," *BMC Med. Genomics*, vol. 8, no. 1, 2015, Art. no. 33.

[47] A.-L. Barabási, N. Gulbahce, and J. Loscalzo, "Network medicine: A network-based approach to human disease," *Nature Rev. Genetics*, vol. 12, no. 1, pp. 56–68, 2011.

[48] P. Khatri, M. Sirota, and A. J. Butte, "Ten years of pathway analysis: Current approaches and outstanding challenges," *PLoS Comput. Biol.*, vol. 8, no. 2, 2012, Art. no. e1002375.

[49] A. Bezerianos, A. Dragomir, and P. Balomenos, *Computational Methods for Processing and Analysis of Biological Pathways*. New York, NY, USA: Springer, 2017.

[50] P. Sebastian-Leon *et al.*, "Understanding disease mechanisms with models of signaling pathway activities," *BMC Syst. Biol.*, vol. 8, no. 1, 2014, Art. no. 121.

[51] M. Caldera, P. Buphamalai, F. Müller, and J. Menche, "Interactome-based approaches to human disease," *Curr. Opinion Syst. Biol.*, vol. 3, pp. 88–94, 2017.

[52] A. G. Vrahatis, K. Dimitrakopoulou, P. Balomenos, A. K. Tsakalidis, and A. Bezerianos, "CHRONOS: A time-varying method for microRNA-mediated subpathway enrichment analysis," *Bioinformatics*, vol. 32, no. 6, pp. 884–892, 2015.

[53] A. Amadoz, M. R. Hidalgo, C. Çubuk, J. Carbonell-Caballero, and J. Dopazo, "A comparison of mechanistic signaling pathway activity analysis methods," *Briefings Bioinformat.*, bby040. [Online]. Available: https://doi.org/10.1093/bib/bby040

[54] M. Kanehisa, Y. Sato, M. Kawashima, M. Furumichi, and M. Tanabe, "KEGG as a reference resource for gene and protein annotation," *Nucleic Acids Res.*, vol. 44, no. D1, pp. D457–D462, 2016.

[55] L. Matthews *et al.*, "Reactome knowledgebase of human biological pathways and processes," *Nucleic Acids Res.*, vol. 37, no. S1, pp. D619–D622, 2009.

[56] G. Sales, E. Calura, D. Cavalieri, and C. Romualdi, "Graphite - A bioconductor package to convert pathway topology to gene network," *BMC Bioinformat*, vol. 13, no. 1, 2012, Art. no. 20.

[57] C. Li *et al.*, "SubpathwayMiner: A software package for flexible identification of pathways," *Nucleic Acids Res.*, vol. 37, no. 19, 2009, Art. no. e131.

[58] F. Emmert-Streib, *Structural Analysis of Complex Networks*. New York, NY, USA: Springer, 2011.

[59] C. Cheng *et al.*, "Construction and analysis of an integrated regulatory network derived from high-throughput sequencing data," *PLoS Comput. Biol.*, vol. 7, no. 11, 2011, Art. no. e1002190.

[60] M. Zachariou, G. Minadakis, A. Oulas, S. Afxenti, and G. M. Spyrou, "Integrating multi-source information on a single network to detect disease-related clusters of molecular mechanisms," *J. Proteomics*, in Press. [Online]. Available: https://doi.org/10.1016/j.jprot.2018.03.009

[61] C. Li *et al.*, "Subpathway-GM: Identification of metabolic subpathways via joint power of interesting genes and metabolites and their topologies within pathways," *Nucleic Acids Res.*, vol. 41, no. 9, 2013, Art. no. e101.

[62] W. A. Haynes, R. Higdon, L. Stanberry, D. Collins, and E. Kolker, "Differential expression analysis for pathways," *PLoS Comput. Biol.*, vol. 9, no. 3, 2013, Art. no. e1002967.

[63] E. Y. Chen *et al.*, "Enrichr: Interactive and collaborative HTML5 gene list enrichment analysis tool," *BMC Bioinformat.*, vol. 14, 2013, Art. no. 128.

[64] A. G. Vrahatis, G. N. Dimitrakopoulos, A. K. Tsakalidis, and A. Bezerianos, "Identifying miRNA-mediated signaling subpathways by integrating paired miRNA/mRNA expression data with pathway topology," in *Proc. Annu. Int. Conf. IEEE Eng. Med. Biol. Soc.*, 2015, pp. 3997–4000.

[65] H. Matsuda, "MRI morphometry in Alzheimer's disease," *Ageing Res. Rev.*, vol. 30, pp. 17–24, 2016.

[66] R. Cuingnet *et al.*, "Automatic classification of patients with Alzheimer's disease from structural MRI: A comparison of ten methods using the ADNI database," *Neuroimage*, vol. 56, pp. 766–781, 2011.

[67] W. Li, M. Wang, W. Zhu, Y. Qin, and Y. Huang, "Simulating the evolution of functional brain networks in Alzheimer's disease: Exploring disease dynamics from the perspective of global activity," *Sci. Rep.*, vol. 6, 2016, Art. no. 34156.

[68] J. S. Damoiseaux, K. E. Prater, B. L. Miller, and M. D. Greicius, "Functional connectivity tracks clinical deterioration in Alzheimer's disease," *Neurobiol. Aging*, vol. 33, no. 4, pp. 828.e19–828.e30, 2012.

[69] D. S. Bassett and O. Sporns, "Network neuroscience," *Nature Neurosci.*, vol. 20, no. 3, pp. 353–364, 2017.

[70] A. Fornito, A. Zalesky, and M. Breakspear, "The connectomics of brain disorders," *Nature Rev. Neurosci.*, vol. 16, no. 3, pp. 159–172, 2015.

[71] R. G. Canter, J. Penney, and L.-H. Tsai, "The road to restoring neural circuits for the treatment of Alzheimer's disease," *Nature*, vol. 539, no. 7628, pp. 187–196, 2016.

[72] G. Chen *et al.*, "Classification of Alzheimer disease, mild cognitive impairment, and normal cognitive status with large-scale network analysis based on resting-state functional MR imaging," *Radiology*, vol. 259, no. 1, pp. 213–221, 2011.

[73] Z. Dai *et al.*, "Discriminative analysis of early Alzheimer's disease using multi-modal imaging and multi-level characterization with multi-classifier (M3)," *Neuroimage*, vol. 59, no. 3, pp. 2187–2195, 2012.

[74] O. Dipasquale, L. Griffanti, M. Clerici, R. Nemni, G. Baselli, and F. Baglio, "High-dimensional ICA analysis detects within-network functional connectivity damage of default-mode and sensory-motor networks in Alzheimer's disease," *Frontiers Hum. Neurosci.*, vol. 9, 2015, Art. no. 43.

[75] Y. Sun *et al.*, "Disrupted functional brain connectivity and its association to structural connectivity in amnestic mild cognitive impairment and Alzheimer's disease," *PLoS One*, vol. 9, no. 5, 2014, Art. no. e96505.

[76] K. Supekar, V. Menon, D. Rubin, M. Musen, and M. D. Greicius, "Network analysis of intrinsic functional brain connectivity in Alzheimer's disease," *PLoS Comput. Biol.*, vol. 4, no. 6, 2008, Art. no. e1000100.

[77] B. M. Tijms *et al.*, "Alzheimer's disease: Connecting findings from graph theoretical studies of brain networks," *Neurobiol. Aging*, vol. 34, no. 8, pp. 2023–2036, 2013.

[78] A. Khazaee, A. Ebrahimzadeh, and A. Babajani-Feremi, "Identifying patients with Alzheimer's disease using resting-state fMRI and graph theory," *Clin. Neurophysiol.*, vol. 126, no. 11, pp. 2132–2141, 2015.

[79] H. Zhang *et al.*, "Topographical information-based high-order functional connectivity and its application in abnormality detection for mild cognitive impairment," *J. Alzheimer's Dis.*, vol. 54, no. 3, pp. 1095–1112, 2016.

[80] O. Sporns and R. F. Betzel, "Modular brain networks," *Annu. Rev. Psychol.*, vol. 67, no. 1, pp. 613–640, 2016.

[81] J. D. Power *et al.*, "Functional network organization of the human brain," *Neuron*, vol. 72, no. 4, pp. 665–678, 2011.

[82] G. Atluri *et al.*, "Complex biomarker discovery in neuroimaging data: Finding a needle in a haystack," *NeuroImage, Clin.*, vol. 3, pp. 123–131, 2013.

[83] J. Gomez-Ramirez and J. Wu, "Network-based biomarkers in Alzheimer's disease: Review and future directions," *Frontiers Aging Neurosci.*, vol. 6, 2014, Art. no. 12.

[84] Q. Ruan, G. D'Onofrio, D. Sancarlo, Z. Bao, A. Greco, and Z. Yu, "Potential neuroimaging biomarkers of pathologic brain changes in mild cognitive impairment and Alzheimer's disease: A systematic review," *BMC Geriatrics*, vol. 16, no. 1, 2016, Art. no. 104.

[85] G. Chen *et al.*, "Modular reorganization of brain resting state networks and its independent validation in Alzheimer's disease patients," *Frontiers Hum. Neurosci.*, vol. 7, 2013, Art. no. 456.

[86] M. Newman and M. Girvan, "Finding and evaluating community structure in networks," *Phys. Rev. E*, vol. 69, no. 2, 2004, Art. no. 026113.

[87] M. John, T. Ikuta, and J. Ferbinteanu, "Graph analysis of structural brain networks in Alzheimer's disease: Beyond small world properties," *Brain Struct. Funct.*, vol. 222, no. 2, pp. 923–942, 2017.

[88] Z. Dai *et al.*, "Identifying and mapping connectivity patterns of brain network hubs in Alzheimer's disease," *Cerebral Cortex*, vol. 25, no. 10, pp. 3723–3742, 2015.

[89] B. Jie, D. Zhang, C. Y. Wee, and D. Shen, "Topological graph kernel on multiple thresholded functional connectivity networks for mild cognitive impairment classification," *Hum. Brain Mapping*, vol. 35, no. 7, pp. 2876–2897, 2014.

[90] B. Jie, M. Liu, D. Zhang, and D. Shen, "Sub-network kernels for measuring similarity of brain connectivity networks in disease diagnosis," *IEEE Trans. Image Process.*, vol. 27, no. 5, pp. 2340–2353, May 2018.

[91] H. Guo, F. Zhang, J. Chen, Y. Xu, and J. Xiang, "Machine learning classification combining multiple features of a hyper-network of fMRI data in Alzheimer's disease," *Frontiers Neurosci.*, vol. 11, 2017, Art. no. 615.

[92] X. Cui *et al.*, "Classification of Alzheimer's disease, mild cognitive impairment, and normal controls with subnetwork selection and graph kernel principal component analysis based on minimum spanning tree brain functional network," *Frontiers Comput. Neurosci.*, vol. 12, 2018, Art. no. 31.

[93] X. Yan and J. Han, "gSpan: Graph-based substructure pattern mining," in *Proc. IEEE Int. Conf. Data Mining*, 2002, pp. 721–724.

[94] Z. Zhang, Q. K. Telesford, C. Giusti, K. O. Lim, and D. S. Bassett, "Choosing wavelet methods, filters, and lengths for functional brain network construction," *PLoS One*, vol. 11, no. 6, 2016, Art. no. e0157243.

[95] F. Taya, J. de Souza, N. V. Thakor, and A. Bezerianos, "Comparison method for community detection on brain networks from neuroimaging data," *Appl. Netw. Sci.*, vol. 1, no. 1, 2016, Art. no. 8.

[96] D. Zhang, Y. Wang, L. Zhou, H. Yuan, D. Shen, and Alzheimer's Disease Neuroimaging, "Multimodal classification of Alzheimer's disease and mild cognitive impairment," *Neuroimage*, vol. 55, no. 3, pp. 856–867, 2011.

[97] H. Il Suk, S. W. Lee, and D. Shen, "Hierarchical feature representation and multimodal fusion with deep learning for AD/MCI diagnosis," *Neuroimage*, vol. 101, pp. 569–582, 2014.

[98] H. Il Suk and D. Shen, "Deep learning-based feature representation for AD/MCI classification," *Med. Image Comput. Comput. Assist. Intervention*, vol. 16, pp. 583–590, 2013.

[99] T. Tong, K. Gray, Q. Gao, L. Chen, and D. Rueckert, "Multi-modal classification of Alzheimer's disease using nonlinear graph fusion," *Pattern Recog.*, vol. 63, pp. 171–181, 2017.

[100] Y. Cui *et al.*, "Identification of conversion from mild cognitive impairment to Alzheimer's disease using multivariate predictors," *PLoS One*, vol. 6, no. 7, 2011, Art. no. e21896.

[101] J. Young *et al.*, "Accurate multimodal probabilistic prediction of conversion to Alzheimer's disease in patients with mild cognitive impairment," *Neuroimage, Clin.*, vol. 2, no. 1, pp. 735–745, 2013.

[102] S. Min, B. Lee, and S. Yoon, "Deep learning in bioinformatics," *Briefings Bioinformat.*, vol. 18, no. 5, pp. 851–869, 2017.

[103] J. Ngiam, A. Khosla, M. Kim, J. Nam, H. Lee, and A. Y. Ng, "Multimodal deep learning," in *Proc. 28th Int. Conf. Mach. Learn.*, 2011, pp. 689–696.

[104] M. W. Weiner *et al.*, "The Alzheimer's disease neuroimaging initiative 3: Continued innovation for clinical trial improvement," *Alzheimer's Dementia*, vol. 13, no. 5, pp. 561–571, 2017.

[105] S. Liu *et al.*, "Multimodal neuroimaging feature learning for multiclass diagnosis of Alzheimer's disease," *IEEE Trans. Biomed. Eng.*, vol. 62, no. 4, pp. 1132–1140, Apr. 2015.

[106] T. Zhou, K. H. Thung, X. Zhu, and D. Shen, "Feature learning and fusion of multimodality neuroimaging and genetic data for multi-status dementia diagnosis," in *Proc. Int. Workshop Mach. Learn. Med. Imag.*, 2017, vol. 10541, pp. 132–140.

[107] K. Robasky, N. E. Lewis, and G. M. Church, "The role of replicates for error mitigation in next-generation sequencing," *Nature Rev. Genetics*, vol. 15, no. 1, pp. 56–62, 2014.

[108] A. Conesa *et al.*, "A survey of best practices for RNA-Seq data analysis," *Genome Biol.*, vol. 17, no. 1, pp. 13–19, 2016.

[109] W. W. Bin Goh, W. Wang, and L. Wong, "Why batch effects matter in omics data, and how to avoid them," *Trends Biotechnol.*, vol. 35, no. 6, pp. 498–507, 2017.

[110] S. I. Dimitriadis *et al.*, "Improving the reliability of network metrics in structural brain networks by integrating different network weighting strategies into a single graph," *Frontiers Neurosci.*, vol. 11, 2017, Art. no. 694.

[111] A. Abrol *et al.*, "Replicability of time-varying connectivity patterns in large resting state fMRI samples," *Neuroimage*, vol. 163, pp. 160–176, 2017.

[112] "Fostering reproducible fMRI research," *Nature Neurosci.*, vol. 20, no. 3, 2017, Art. no. 298.