Dynamically dysfunctional protein interactions in the development of Alzheimer’s disease

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Abstract—Alzheimer’s disease usually causes dementia in the old people and the symptom progression of the disease phenotype displays certain patterns. One possible reason is that the nerve cells in the brains of the patients degenerate at different stages. Here, we analyze the dynamics of disease progression based on its biomolecular network. We develop a novel computational method to integrate an ensemble protein network and the hippocampal gene expression data. Specifically, we construct the induced dynamical pathways which present particular characteristics at different disease stages from the control to disease samples. Based on the network-based method, we reveal that the active pathways tend to be more complicated during the development of disease. Also we find that the disease proteins performing important functions are always located in the cooperations of the identified pathways. These results also demonstrate that the network-based analysis can provide knowledge and evidences on the dynamics and pathological pathways of the complex Alzheimer’s disease.

Index Terms—Protein-protein interaction; differential gene; dysfunctional pathway; dynamics; systems biology; Alzheimer’s disease.

I. INTRODUCTION

Alzheimer’s disease (AD) is a debilitating neurodegenerative disorder affecting about 24 millions of elderly individuals worldwide [1]. Although several hypotheses have been proposed [2] and hundreds of putative AD susceptibility genes have been witnessed in the past decades [3], the genetics mechanism underlying the complex disease is still unclear. Existing studies reveal that the process of AD has certain patterns of symptom progression [2], [4]. For example, senile plaques (SP) and neurofibrillary tangles (NFT) are two common hallmarks in brains of AD patients [5], [6]. Moreover, many individuals with AD begin with loss of memory and learning ability, and then spread to loss control of other aspects of thinking, judgement, even behavior with the development of severity [4]. From the temporal perspective, there is clinical category systems in which disease severity correspond to the incipient, moderate and severe stages [4], [7].

Recently, the increasing availability of high-throughput experimental data has provided us new opportunity and challenge to analyze the complex disease [8] from systems perspective. For instance, DNA microarray technology presents the amount of data in the genome-wide measurements of human transcriptome [7], which can be exploited to bridge the gap between genotype and phenotype of human complex disease [9]. The kernel idea is that a cellular process is carried out through interactions among many genes and their products, which are coordinated to achieve specific tasks [10]. This activity is often organized into pathways or networks, i.e. genes in the same pathway are activated together and thus exhibit similar gene expression profiles. On the other hand, genes with similar expression profiles are more likely to encode interacting proteins to coordinate to achieve the particular function [10], [11]. In this study, we will identify the pathway of protein interactions and analyze the specific cooperations guiding by the information of its corresponding gene expression profiling.

One way to detect molecular pathways is to identify differential genes by statistical test and gene set expression enrichment study [12]–[14]. Another way is to approximate protein activity by gene expression significance, and then those differentially scored proteins and all their reported interactions are regarded as dysregulated pathways [15], [16]. However, since these methods focus on the gene level, they are inefficient to detect the specific pathways in protein interaction networks because some interactions would not truly be activated under specific conditions. The interactions between proteins are often both related to their differential expression and their coexpression with others. The known protein-protein interactions (PPI) should be carefully regarded to the expression of genes and the correlation between them [17]–[19], especially in the detailed analysis for dysfunctional disease pathway. Here, we refer to the pathways as specific linkages between two genes and thereafter proteins directly with the aim to identify the dysfunctional interactions responding to a particular stage during AD progression.

In this paper, we identify the key AD dysfunctional protein-protein interactions by developing a new network-based computational method. Specifically, we firstly collect AD-related proteins and reconstruct the interactions among them from an
II. METHODS

Figure 1 gives our schematic framework to identify dysregulated pathways. Basically, we weight the protein-protein interaction (PPI) network by integrating the corresponding gene expression profiles in different development of disease stages, and then identify the dysregulated pathways.

A. Data sources

We collect AD related genes from the AlzGene database (www.alzgene.org) and that in KEGG (www.genome.jp/kegg) AD pathway. The total 553 AD related genes are represented by NCBI Entrez Gene IDs and we extract their corresponding protein interactions from five available PPI databases, i.e. HPRD [20], BIND [21], BioGrid [22], IntAct [23] and MINT [24]. The total binary interactions contain 7533 nodes and 22345 edges. We filter these PPI between AD proteins by a Naive Bayesian rule to get the interactions that contained at least in three of the five databases. We use the data of gene expression profiling research on AD patients with normal controls in [7], which is downloaded from NCBI GEO (www.ncbi.nlm.nih.gov/geo) database (ID:GSE1297). The microarray assessed gene expression from hippocampus CA1 region from 31 individuals, comprising 9 controls, 7 with incipient AD, 8 with moderate AD and 7 with severe AD. We transform the absolute expression value by logarithm (base 2) calculation. Probesets are mapped to NCBI entrez genes using DAVID [25]. If there are multiple probesets that correspond to the same gene, we take the average value. The expression dataset contains 22283 probes and resulted to 13932 genes. Temporarily, the AD genes with no corresponding proteins in the integrated PPI network are not be included in the analysis as well as the AD proteins in the PPI network without a corresponding gene in the expression experiment. This ends up with an AD protein network with 235 nodes and 373 edges.

B. Mapping gene expression to PPI network

We use Welch’s upper-tailed t-test to determine the differential expression of node x and y respectively. corr(x, y) represents the p-value of their correlation. f is a general data integration method that can handle multiple data sources differing in statistical power, k is the number of p values have been combined. Here Fisher’s method is adopted and mathematically, Score ∼ χ²k [26]. This method has been widely used in statistical meta-analysis to un-weighted combine p values from several data sets [27]. In our paper, the critical value for the combined score is 12.59 (k=3, df=6, p < 0.05). The expressions for different specific-stages of AD are mapped respectively and we get different condition-based weighted AD protein networks.

C. Identifying pathways

Every edge in AD protein network is assigned a combined p-value score for every disease stage. Then we detect the induced active pathways in specific disease stages individually. Generally, identification of maximum scored subnetwork in a weighted graph is a NP-hard problem [11], [16], [28]. An alternative is to use a greedy edge expansion algorithm to detect most significantly dysfunctional pathway by growing from every locally maximal scored edge respectively, whose score (rank) is better than its adjacency (see Figure 1). Every growing new edge will get a probability by a hypergeometric distribution: p = \prod_{i=0}^{r-1} \frac{E-i}{E-i}. Here E is the number of edges in AD protein network, e is how many edges have already been included during extension. This value is used to measure the probability to get an edge with higher rank than r from the total E edges when we get the r-rank at present step. The probability equals to 1 when we choose all edges and...
there is a minimum with the increase of $e$ [29]. We choose this minimum as our cutoff to terminate the growth procedure. In this way, we choose the most significant subnetwork as the identified pathway. For an identical standard in different stages for comparison, we choose 12.59 as the threshold to filter the expansions.

III. RESULTS

A. Dysfunctional pathways

For the three stages during AD progression, we identify the active pathways which are formed due to the cooperations among proteins from the control to different stages of disease. Figure 2 shows the topology changes of activated subnetwork among AD proteins in the development process. Figures 2 (a), (b) and (c) correspond incipient, moderate and severe stages of the disease individually (larger figures showing the identifiers can be found in our website). Number of edges containing in three pathways and that of involved proteins are shown in Figures 3 (a) and (b), respectively. We found that more than half of integrated interactions (218/373) and proteins (116/235) in AD network have not been included in these activated pathways. The number of interactions and proteins involved in induced pathways will be increased during AD progression. The edge overlap among the three stages shows that a few (6/152) of the interactions are consistently induced in three development process and most (101/155) of the interactions are dynamically activated during the disease development. However, the overlap edges in two of three edges are relatively larger (54/155). Especially, most of the activated edges during the early stage are also contained in middle stage (20/45) and in last stage (18/45). Moreover, we find out the number of proteins activated in incipient stages are almost contained in moderate (35/48) and serious (32/48) stages. The overlap of edges and nodes in the dysregulated pathways provide evidences that the difference and conservation of AD progression mechanism. The result shows that the active pathways would have more and more edges during the disease progression during developing stages. In other words, there are more and more proteins will be activated to cooperate the dysfunction of AD. In phenotype, this would cause the development of dementia. The increasing tendency of complexity among proteins with their cooperations would imply the development of severity. For instance, the patients will lose their behavior abilities gradually.

B. Pathways underlying KEGG AD proteins

The identified dysfunctional pathways have certain implications to the development of dementia of patients in the popular hypotheses. We will investigate these details in the smaller KEGG AD gene set. KEGG provides a curated metabolic pathway related to AD, which is based on higher level of interactions. We will focus on the dysregulated pathways underlying the smaller set. Figure 4 shows the identified pathways underlying the KEGG AD proteins, where (a), (b), and (c) indicate the induced pathways in three stages respectively. Totally, KEGG contains 28 AD-related genes, in which 27 genes have gene expression data and contains 48 interactions in our integrated AD network. The active pathways with these non-active part are shown in Figure 4. In amyloid hypothesis, APP is cleaved by $\gamma$-secretase and then the concentration of $\alpha\beta$ peptide would cause senile plaques in the brain. Gene ID for $\gamma$-secretase complex is PSEN1 and PSEN2 (PS), PSENEN (PEN-2), NCSTN (NCT) and APH1A (APH-1). The induced pathways among these proteins imply there are more $\gamma$-secretase would be assembled in the disease. From Figure 4, we can find that the interaction between gamma-secretase and APP is always induced in three processes. PSEN1 and PSEN2 are two important genes encoding PS part of $\gamma$-secretase, which is known to the subcomponent responsible for the cutting of APP [5]. Interactions between PS and APP will be activated during incipient and moderate processes of AD progression, and the interaction between NCT and APP would be activated in the severe stage. Generally, $\gamma$-secretase will undergo significant post-translational modification before becoming active, while NCT is to promote the maturation and proper trafficking of the other proteins in the complex [30]. This indicates that $\alpha\beta$ peptide would be produced abundantly during disease progression, which is the main pathology of amyloid hypothesis. Direct interaction between $\beta$-secretase (BACE1 and BACE2) and APP would not be involved in three stages. However, it will be included in identified pathways connecting with APP through protein LRP1 in the moderate and through $\gamma$-secretase in the severe stage. The relay of $\beta$-secretase cooperating with APP sheds light on that the accelerated production of C99 peptide might accompany with activation of $\beta$-secretase with...
LRP (LRP1) and nicastrin. The pathway potentially increases the amount of C99, and the up-stream formation of Aβ peptide cleavage by γ-secretase. This implies that many C99 fragments will be produced when the disease steps into its serious stages, and then the former activation of γ-secretase with APP will have more possibility to produce Aβ in the cascade hypothesis of sequential cleavages [6]. APOE is another important protein related to AD by catalytic the resolution of the short peptide of APP [5]. It enhances proteolytic break-down of various forms of β-amyloid peptides. It is known that LRP is a receptor for apolipoprotein APOE4 import cholesterol into neurons [31]. We found that the pathway between APOE and APP, through LRP1, is activated in the moderate stage, which means that APOE will accelerate the speed of dissolution of Aβ peptide when it is accumulated too much in the process.

During AD progression, the induced pathways will contain 18 of the 48 ensemble interactions in KEGG AD proteins. Simultaneously, 15 of the 27 proteins are involved in these dysregulated interactions. The proteins APP and γ-secretase are always in the pathways during development. There are more implications from other included proteins during AD progression, such as GSK3B and MME. GSK3B is also known to be essential in the pathogenesis of Alzheimer’s disease by inducing hyperphosphorylation of Tau (MAPT) and is involved in Aβ-activated neuronal death in hippocampus region [32]. NFT is another pathological characteristic of AD caused by hyperphosphorylation of Tau [6]. It has been reported that Tau-dependent microtubule disassembly initiated by prefibrillar β-amyloid [33]. The activated GSK3B by APP might cause the acceleration of phosphorylation. MME is a major enzyme for degradation of β-amyloid [5]. The pathway between MME and APP, through APBB1, is activated during incipient and moderate stage. APBB1 is known to be in an adaptor Fe65 protein family which binds APP [5]. This implies that MME is activated during disease development for accumulating β-amyloid processed by γ-secretase.

C. Consistency of GO functions

To show the functional consistency in these dysregulated pathways, we identify GO function enrichments underlying the cooperations. Results are shown in Table I. In three different stages of AD, there are conserved GO functions in the identified pathways. From the top three significant GO functions of induced pathways, some common functions are activated in three stages. Functional significance of these induced pathways provides validation of identified pathways. The p-value are calculated by hypergeometric test [34].

IV. DISCUSSIONS

In this paper, we analyzed dysregulated protein interactions of AD-related proteins from the temporal perspective of disease development. We identified the dynamically dysfunctional pathways underlying AD proteins during AD progression, i.e. incipient, moderate and severe stages. We firstly integrated a comprehensive PPI network of AD proteins. From genomics-level expression profiling of AD patients, we mapped the information of differential and correlational expression information to the ensemble interactions. Then we detected induced pathways of protein interactions from control to disease. From different development stages, we reconstructed the dysfunctional pathways. We found that there are many interactions and proteins to cooperate together to perform certain dysfunctions. After detailed analysis of dysregulated pathways in AD key proteins, we identify the functional enrichments are conserved in identified pathways. The theoretical results show that the network-based approach can provide deep insight onto the essential pathological mechanism and also give implications on different hypotheses of AD pathologies at the transcriptome and interactome level.

A. Integrating transcriptome and interactome information

Proteins are functional units in the cell. The complexity of AD disease is caused by misfolding of Aβ and Tau proteins in the brain [6]. Proteins cooperate each other to perform dysfunctions during AD progression. We proposed a novel framework to integrate gene expression and protein interaction information to investigate dynamically pathways in AD related proteins. Recently, [35] and [36] conducted gene...
coexpression study of AD and found AD highly susceptibility genes are tend to coexpressed together constructing modules. The molecular network information of protein interaction has evidenced an application to prioritize AD candidate genes [37]. This evidence implies that both gene expression and protein interaction can provide useful information for AD study. In contrast, a novel scheme has been developed here to utilize expression information efficiently by differential features and close relationships with others simultaneously. We regarded the pathways as the dysregulated proteins with highly correlation between them. We mapped the gene expression in different stages of AD progression to integrated protein interactions. The identified pathways show the dynamics of cooperations among them. AD proteins have been shown to cooperate each other during the disease progression. These reconstructed processes of disease progression provide implications for potential AD mechanisms.

B. Identification of pathways

This work proposed a method to identify dysregulated pathways of protein interactions at different stages of AD progression. The detection is conducted by exploring edge information combined by the corresponding gene expression of two nodes and their correlation. The method is based on assumption that proteins in the same pathway are differential expressed and closed correlations. Dysfunction of AD might lie in cooperations among certain proteins. We regard the terminology of pathway as the cooperations in a protein set, which we identify it by their interactions directly. In contrast to the methods based on differential expressed genes and related all the reported interactions [9], [15], [16], [29], our method consider not only the differential genes, but also the cooperations among them. In fact, the methods use the identified distinct proteins, and all their interactions as functional pathway are failed to detect any specific information [28].

We identified dysregulated pathways based on gene expression information at protein level. These identified pathways in our method are those significant or relevant interactions in different stages in hippocampal CA1 regions. Proteins in the same pathway are differential expressed and with high correlations in specific AD stages. The gene expression is based on different individuals and measured by limited samples. The whole scenario of comprehensive interactions in AD brain with temporal and spatial processes is still challenging tasks for our detection.

From the available data so far of gene expressions for different stages in AD progression and protein-protein interactions, we integrated them to identify the dynamic dysregulated subnetworks. We observed that interactions among AD proteins become more and more complicated with the progression of disease severity. In other words, more and more proteins took part in the active pathways during AD progression. The risk proteins, such as APP, γ-secretase and β-secretase, are identified to perform significant interactions with other proteins. This dynamics provides us valuable information of the cooperations among them during disease development. The conservation of functional enrichments in these dysfunctional pathways gives further evidence of protein cooperations in the pathways. These results also imply that we should pay much attention to the complexity of disease from systematic perspective.

V. CONCLUSION

In this work, we proposed a network-based approach to analyze the dynamics of protein interactions among AD proteins in different stages during disease progression. The derived information of AD genes from the transcriptome in different stages provides valuable insight onto the interactome features. We identified active pathways involved in interactions among proteins which are both differential expression individuals and closely cooperate with others. The identified dysregulated pathways give more information on development mechanisms about AD progression. The proteins in the active subnetwork cooperate together to perform toxicity functions to a neuron cell. From these induced pathways, the cooperations among the AD proteins have been investigated in detail. There are changes and conservations in the protein interactions corresponding to AD severity. The reconstructed dynamics during AD development can be further explored for deciphering mechanisms at genotype level. The analysis gives a screen displaying the cooperations among AD proteins, and our method provides a novel framework to identify dysfunctional pathways which can be used for studying other complex disease.

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