

Supplementary Data

Mitochondrial Dysfunction and Immune Activation are Detectable in Early Alzheimer's Disease Blood

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GLOSSARY FOR WGCNA ANALYSIS

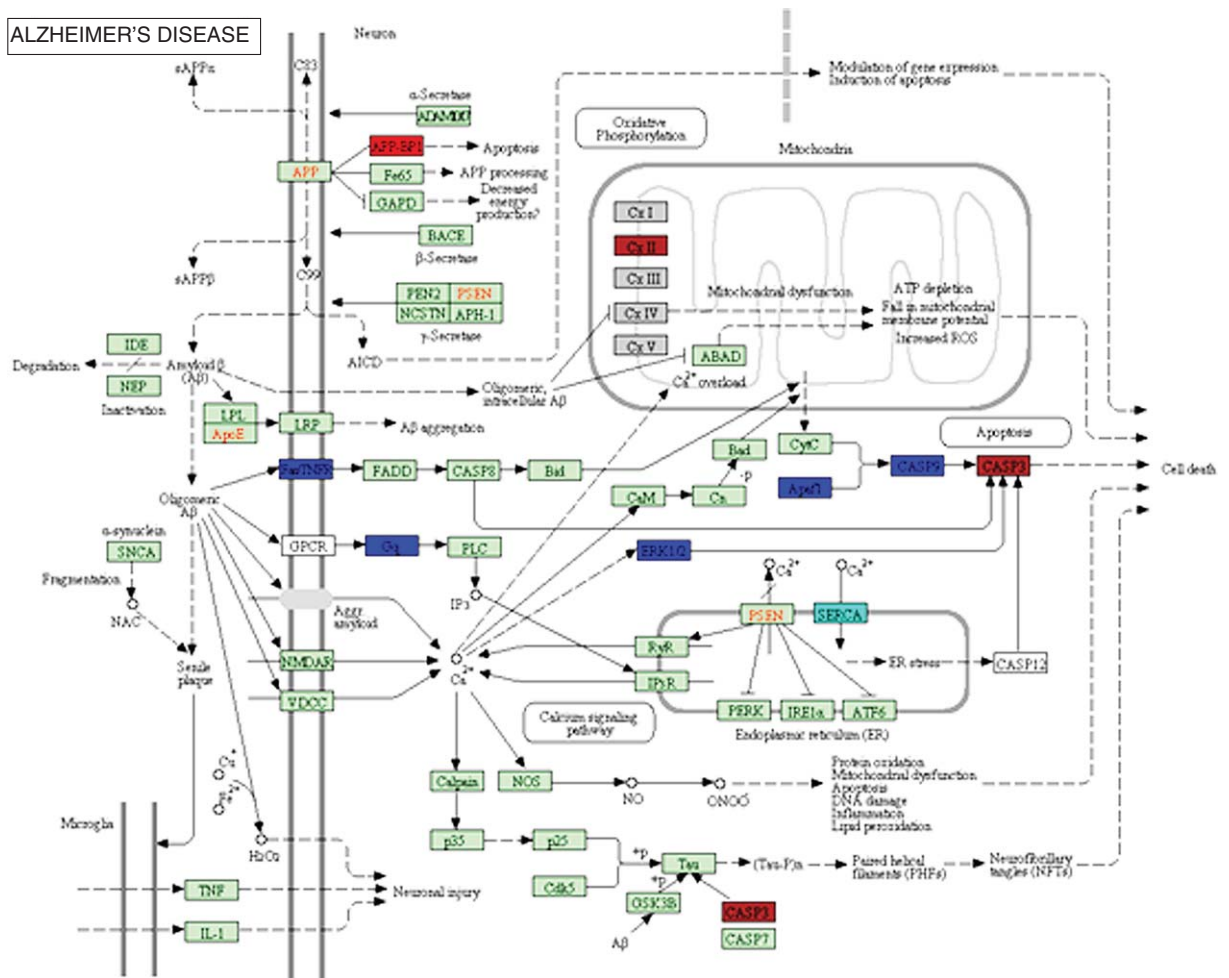
We have used gene expression data consisting of individual probes from microarrays to perform a Weighted Gene Co-expression Network Analysis (WGCNA). A gene co-expression network is a

graphical representation of the relationship between genes according to the similarity of their expression profiles and thus potentially their biological relatedness. Within the network, a node represents a probe and an edge exists between two probes if they exhibit similar expression patterns across the samples, i.e., they are co-expressed. The following terms and definitions are used to represent different features of the network and associated analyses. For further details we refer readers to the glossary provided at the WGCNA web site: <http://www.genetics.ucla.edu/labs/horvath/CoexpressionNetwork/Rpackages/WGCNA/Tutorials/Simulated-00-Background.pdf>.

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Supplementary Figure 1. Over represented KEGG pathways ($p < 5 \times 10^{-4}$) identified by DAVID analysis (<http://david.abcc.ncifcrf.gov/>) [4] using probes with higher-than-median module membership and trait significance for each module (i.e., probes highlighted in green in Fig. 5 and listed in column Q, supplementary Tables 2–7). KEGG pathways with significantly over-represented modules include: Alzheimer's disease ($p = 1.8 \times 10^{-4}$; black module), Oxidative Phosphorylation ($p = 3.9 \times 10^{-5}$; black module), Ribosome ($p = 6.6 \times 10^{-5}$; black and $p = 1.7 \times 10^{-7}$; red module), Leukocyte Transendothelial Migration ($p = 1.2 \times 10^{-4}$; blue module). In each KEGG pathway probes with higher-than-median module membership and trait significance are indicated by a star with the color of the star indicating their assigned module, except the black module which is represented by grey.

Probe: A probe assesses the expression levels of a particular gene within a given sample.

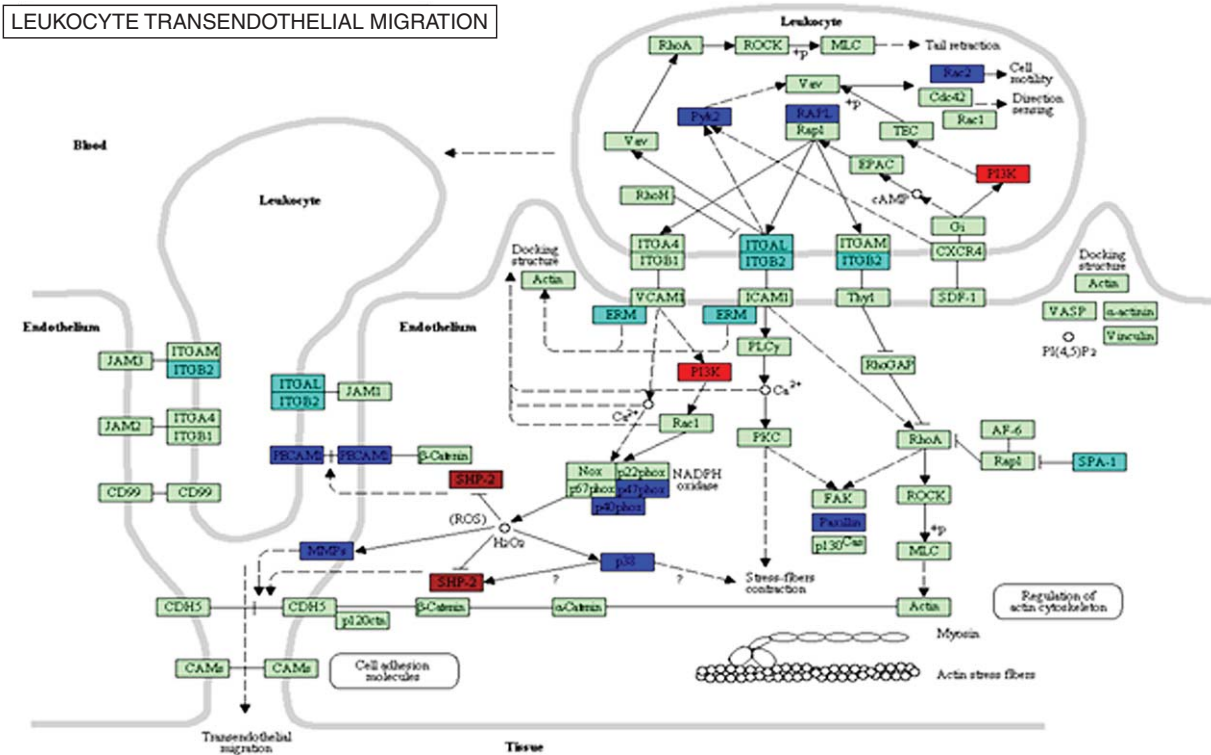
Connectivity: In its simplest form, the connectivity of a probe is computed as the number of neighbors it is connected to in a co-expression network, or:

$$\text{Connectivity}_i = k_i = \sum_{j \neq i} a_{ij}$$

In a weighted network, connectivity can be measured by different parameters, including topological overlap (see below). Probes with high connectivity

values share a similar profile of gene expression with a relatively large number of other probes.

Topological Overlap (TO): Topological Overlap provides the score/weight for the edges in the co-expression network. To calculate the topological overlap for a pair of probes, their connections with all other probes in the network are compared. If the two probes show similar patterns of correlation with other probes, then they have a high topological overlap. Several studies have shown that probes showing high topological overlap are more likely to be functionally related than probes that do not. For two nodes



Supplementary Figure 1. (continued)

i and j , the topological overlap of the two nodes (t_{ij}) is computed as follows:

$$t_{ij} = (I_{ij} + a_{ij}) / (\min\{k_i, k_j\} - 1 - a_{ij}) \quad \text{if } i \neq j$$

$$= 1 \quad \text{if } i = j$$

Where I_{ij} , k_i and k_j are the connectivity measures of nodes i and j as defined earlier.

Topological Overlap Matrix (TOM): The Topological Overlap Matrix describes the pairwise TO between all probes in the network [1]. The numbers in the matrix measure similarity amongst the probes in the network. In this work, the TOM was used to define edges between probe pairs.

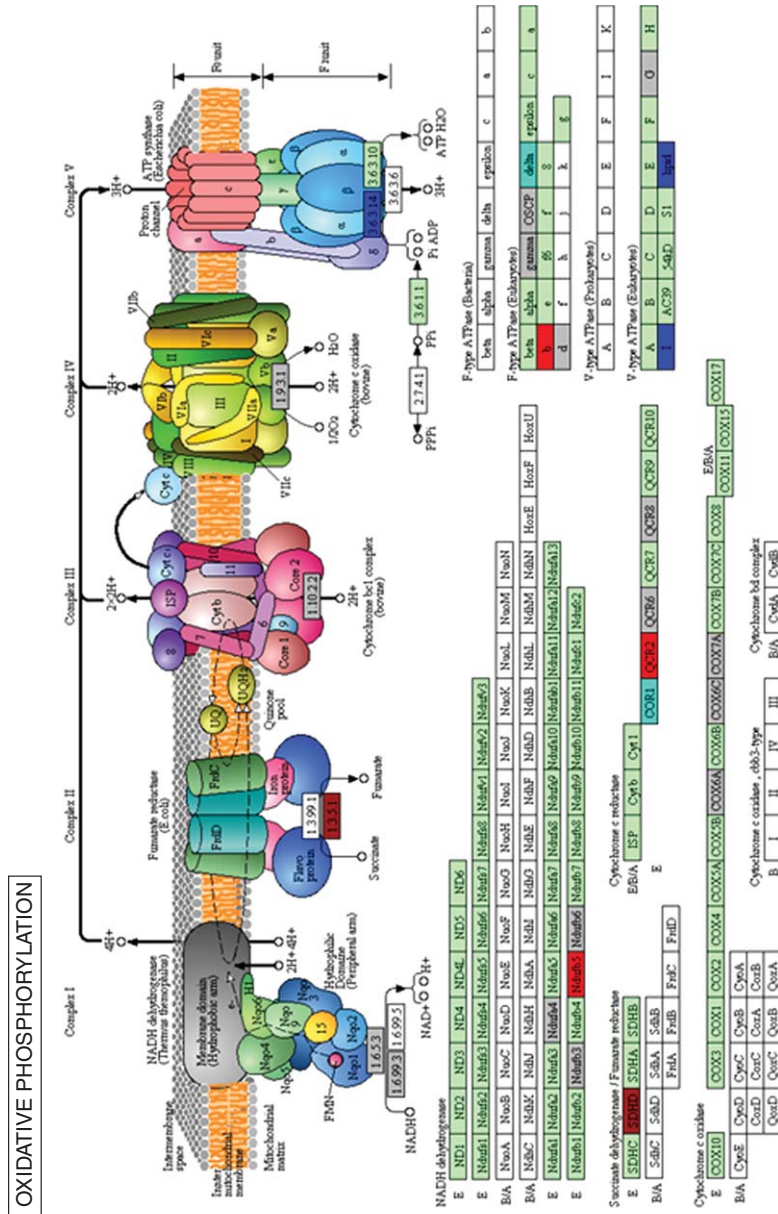
Modules: Modules are sub-networks of the larger network, comprising probes with similar expression patterns across samples. Probes belonging to the same module are thought to be functionally related, e.g., represent genes encoding a pathway or a protein complex or related biological function and are therefore considered biologically important [2]. The biological characteristics and behavior of modules may reveal far more than only considering individual genes in isolation. Computationally, a network module is

comprised of a set of probes which are closely connected according to a suitably defined measure of interconnectivity (TOM) and the set of samples from which the expression data is derived.

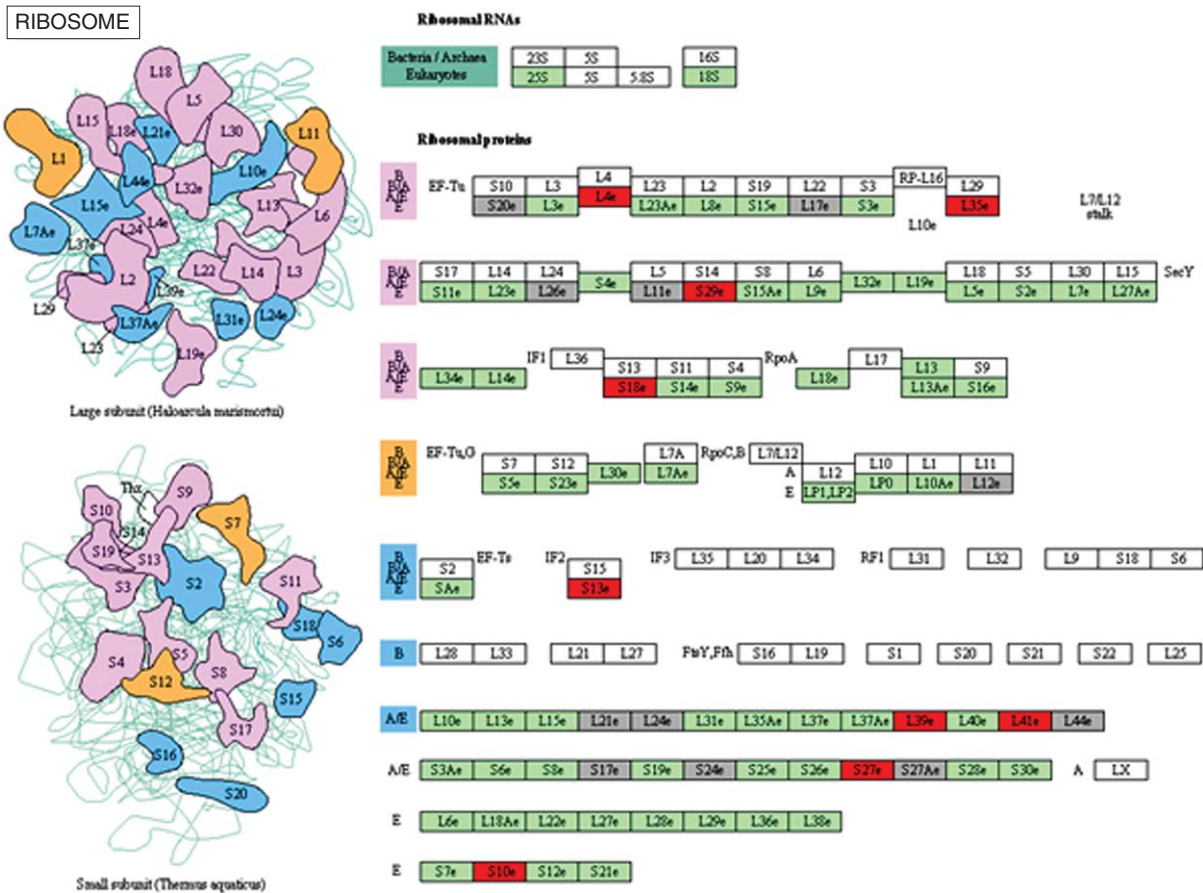
Module Eigengene (ME): A Module Eigengene is the expression profile chosen to represent that of the module. Module Eigengenes are important for establishing whether there are correlations between modules and clinical traits and each other. Mathematically, an eigengene is computed as the first eigenvector of the adjacency matrix of the module and represents the first-principal component of the genes within the module [2].

Module Membership (MM): Module Membership is a measure of the extent to which a probe conforms to the characteristics of the module it is assigned to. It is measured by the correlation between the expression profile of a probe and the Module Eigengene (ME) of the corresponding module to which the probe belongs.

Gene Significance (GS): A Gene Significance measure of a gene is used to assess the biological significance of a particular probe and therefore gene, with respect to a trait (e.g., disease severity). GS is defined as the correlation coefficient resulting from



Supplementary Figure 1. (continued)



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correlating the outcome of the trait in question with the expression profile of the gene [3]. GS can take positive or negative values depending on the correlation relationship (a positive GS results from a positive correlation while a negative GS results from a negative correlation). A GS value of zero indicates no significance while higher absolute values indicate a higher significance of the gene to the trait [3].

Weighted Co-expression Network: A Weighted Co-expression network is a network in which the edges are annotated with numbers (weights) denoting the extent to which two nodes (probes) are similar. In this case the weights represent the topological overlap between nodes, i.e., the numbers represent the strength of the correlation of the expression profiles of the nodes connected via the edge.

Signed Weighted Co-expression Network: Signed weighted co-expression network is a variant of weighted co-expression networks which attaches a sign to the weights assigned to its edges. The sign designates

the direction of expression change among the expression profiles. Signed networks are thought to be more biologically relevant than unsigned networks whereby the modules are created based on absolute measures of correlation, i.e., genes assigned to the same module can have opposite directions of change in their gene expression profiles.

Table 1: Differential gene expression in blood samples from AD, MCI, and control subjects. A total of 2,908 significant differences were identified between the three groups (FDR corrected $p < 0.01$). Positive or negative fold-change indicates increased or decreased expression in MCI and/or AD with respect to control blood or AD with respect to MCI blood ($p < 0.001$ in post-hoc T-test).

Tables 2–7: A list of probes assigned to the disease-associated modules red, black, pink, brown, blue, and turquoise, respectively. Probe level associations with the diagnostic traits control, MCI-MCI, MCI-AD, AD, ALL AD, and disease severity are indicated. Gene

significance (GS) of a gene describes the strength and sign of the correlation between the probe and the trait in question, while the module membership score (MM) quantifies the extent to which a gene conforms to the characteristics of a module. The combination of MM and GS identifies genes which play important roles in a given network module and their significance for the clinical trait in question. Probes with higher-than-median module membership and trait significance for each module are indicated in column Q.

Table 8: Compiled gene lists comprising top Alzheimer's GWAS genes, other candidate genes thought to be associated with Alzheimer's, OXPHOS genes, MRP genes, and immune genes. Genes are annotated with their module membership.

Table 9: Test for over-representation of MCI-associated (A) and AD-associated (B) gene expression changes in specific blood cell populations in blood samples from AD patients or normal elderly controls. A total of 19,161 probes were used in the analysis (see methods) of which some had significantly altered expression in MCI ($n = 1,999$ with $FDR < 0.01$) and/or AD ($n = 1,319$ with $FDR < 0.01$). These were mapped to a set of probes previously reported to be enriched in particular blood cell types by Watkins et al. [5] using RNA from blood analyzed with the same arrays. Over-representation of cell-type enriched transcripts was examined using Chi-square or the Fisher's exact test if the number of probes was less than 10 (*). To increase confidence in our results, we also tested whether more cell lineage probes attained a given p -value than would be expected by chance by randomly selecting 1,319 or 1,999 of the 19,161 used in the analysis and repeating the analysis for each cell-type enriched probe list for 10,000 permutations. We further tested for over- rather than under-representation of significantly altered probes in particular blood cells in AD blood by a hypergeometric probability test.

Table 10: We tested the blood modules (column A) for enrichment using a large pre-defined collection of brain-related gene sets (column C) [6–27]. Classification categories and functional annotation for each test dataset were defined by the individual study (column G). Significance was computed using a hypergeometric test. Each dataset is identifiable by the publication first describing each study (column B).

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