# Methods

## Functional enrichment of differentially expressed genes

To identify functional enrichment among differentially expressed genes we performed gene set enrichment analysis (GSEA) using the fgsea R package (v 1.26.0)1. The gene probe names were first changed to official gene symbols and then to ENTREZ ids using Bioconductor’s Genome wide annotation for Human (org.Hs.eg.db\_3.17.0)2. Gene ontology terms and their assigned genes were similarly pulled from this package3. KEGG pathways and their assigned genes were accessed using the KEGGREST R package (v 1.40.0)4,5. All probes with ENTREZ ids were included in the analysis and were ranked by the t-statistic calculated by limma before being input into fgsea. The resulting p-values were corrected for multiple comparisons using the Benjamini-Hochberg method6.

## WGCNA

We used the Weighted Gene Co-expression Network Analysis (WGCNA) R package (v 1.72-1) to identify patterns of co-expression among genes and groups them into modules based on their expression patterns7. Before performing WGCNA, the expression values the extra cellular domain and tyrosine kinase domain of NRTK1, 2, and 3 were averaged to represent expression of the NRTK1, 2, and 3 genes, respectively. The empiricalBayesLM function in the WGCNA package was used to adjust the gene expression for PMI. A signed network was built and modules detected using the blockwiseModules function with the following parameters:

* power = 22,
* TOMType = "signed",
* networkType = "signed",
* minModuleSize = 10,
* reassignThreshold = 0,
* mergeCutHeight = 0.25,
* pamRespectsDendro = FALSE.

Module-eigengene to trait Pearson correlations were calculated using WGCNA’s cor function for diagnosis (NCI, MCI, AD), neuron type (TOC, p75), age at death, sex (male, female), education in years, MMSE, GCS, APOE genotype (e2, e3, e4), PMI in hours, CERAD, BRAAK, and NIAREAGAN. Categorical variables were one-hot encoded, and correlations were calculated for each category individually. The corPvalueStudent function in the WGCNA package was used to calculate p-values, and the p-values were corrected for multiple comparisons using the Benjamini-Hochberg method6.

Module-eigengene to categorical trait associations were also tested using the Wilcoxon Rank Sum test (neuron type and sex) or the Kruskal-Wallis test (diagnosis and APOE genotype) as implemented in the rstatix package (v 0.7.2)8. We also used PERMANOVA (Permutational Multivariate Analysis of Variance) as implemented in the vegan package (v 2.6-10) to test for associations between categorical traits and the multivariate gene expression profiles of resident module genes 9,10. PERMDISP (Permutational Multivariate Analysis of Dispersion), as implemented in the vegan package, was used in conjunction with PERMANOVA to assess the homogeneity of group dispersions 11. In the univariate and multivariate tests, the p-values were corrected for multiple comparisons using the Benjamini-Hochberg method.

Candidate hub genes were identified by ranking genes within their resident modules by Kme and Kwithin calculated using the signedKM() and intramodularConnectivity.fromExpr() functions, respectively. Hub genes were also cross-referenced with genes differentially expressed between neuron types.

Over-representation analysis was used to test for enrichment of genes annotated to gene ontology terms and genes associated with KEGG pathways among module genes. As above, the gene probe names were first changed to official gene symbols and then to ENTREZ ids using Bioconductor’s Genome wide annotation for Human (org.Hs.eg.db\_3.17.0)2. The analysis was performed with the gprofiler2 package (v 0.2.2)12 which uses the hypergeometric test followed by correction for multiple testing with the package’s custom algorithm. The 861 array genes included in the WGCNA analysis were used as a custom background set.

Enrichment of module genes among differentially expressed genes was assessed with gene set enrichment analysis (GSEA) using the fgsea R package (v 1.26.0)1. As above, all probes with ENTREZ ids were included in the analysis and were ranked by the t-statistic calculated by limma before being input into fgsea. The resulting p-values were corrected for multiple comparisons using the Benjamini-Hochberg method6.

## References

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