# Gene network analysis reveals insights into the function of APP interacting mitochondrial protein NIPSNAP1

Sarani Ghoshal[1], Robert W. Williams[2], Ramin Homayouni[1]

[1]Department of Biological Sciences, University of Memphis, Memphis, TN-38152

[2]Department of Anatomy and Neurobiology, University of Tennessee Health Science Centre, Memphis, TN-38163

## Introduction

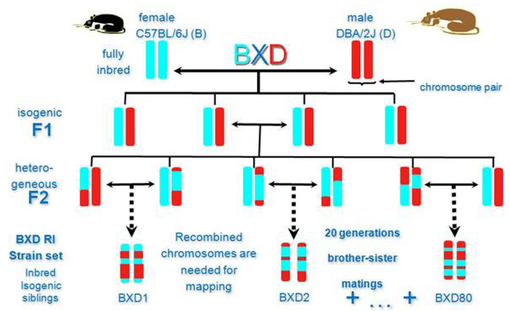
NIPSNAP1 (4-Nitrophenyl Phosphatase domain and non-neuronal SNAP25-like protein homolog 1) is a novel mitochondrial protein that interacts with the cytoplasmic domain of amyloid precursor protein (Tummala et al., 2010). In brain, NIPSNAP1 is specifically localized in neurons (Nautiyal et al., 2010).

Though NIPSNAP1 is evolutionarily conserved, its cellular function is still unknown. NIPSNAP1 is associated with the Branch Chain Amino Acid metabolon and shown to specifically interact with the E2 subunit of pyruvate dehydrogenase complex (Islam et al., 2010; Nautiyal et al., 2010.) In addition, phenylketonuric (PKU) mice show altered levels of NIPSNAP1 in the brain (Nautiyal et al., 2010)

In this study, we used a bioinformatics and systems genetics approach to gain insights into the function of NIPSNAP1. Our central hypothesis is that transcripts whose levels are tightly correlated across different genotypes and in different brain structures cooperate in similar cellular functions.

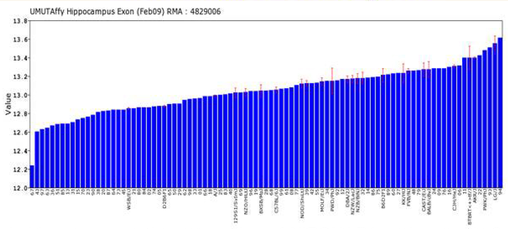
Gene expression data for a variety of tissues from recombinant inbred mouse strains were analyzed using the tools available in the web-resource GeneNetwork (www.genenetwork.org).

## BXD Recombinant inbred (RI) strains




For nearly 40 years, phenotypic data have been collected for a large panel of C57BL/6J x DBA/2J (BXD) recombinant inbred mouse strains. In the last few years, gene expression profiles have also been collected at various labs using different microarray technologies. These data are made available through GeneNetwork, which provides a tremendous resource for researchers to perform QTL analysis as well as discover relationships between genes and phenotypes.

## NIPSNAP1 expression varies across RI strains and brain regions




In hippocampus, the expression of NIPSNAP1 varies nearly 2-fold across different BXD strains. Using GeneNetwork, one can identify transcripts whose levels tightly correlate with NIPSNAP1 levels.

## Summary of Data sets in GeneNetwork

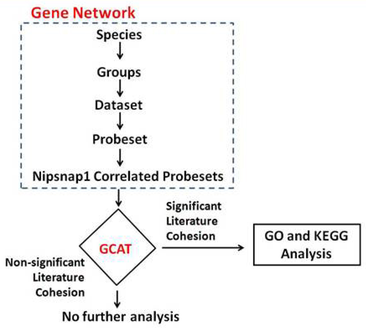
The datasets that we examined in GeneNetwork (GN) included:

- 17 RI strains
- 4 microarray platforms
- 10 different brain regions
- 62 total microarray datasets for brain

Challenges:

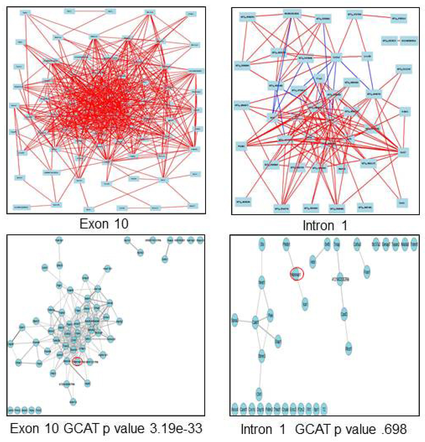
- Platform and probe variability
- Lab variability
- Tissue variability
- Statistical false discovery rate (>50,000 probes)

## Analysis Strategy



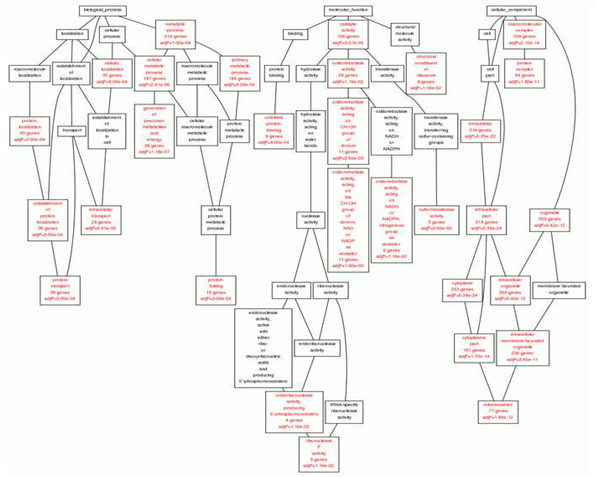

We used a novel literature mining tool called GeneSet Cohesion Analysis Tool (Xu et al., 2011) to determine the functional cohesion of NIPSNAP1 correlated gene sets and to narrow down the choice of datasets and probes. GCAT determines the functional coherence of gene sets by performing latent semantic analysis of Medline abstracts.

## Comparison of gene correlation networks for NIPSNAP1 exon 10 and intron 1 probes




Gene expression correlation network (top) and GCAT derived literature correlation network (bottom) for the top 100 transcripts correlated with either NIPSNAP1 exon 10 or intron 1 (control) probesets.

## GO analysis: NIPSNAP1 correlated genes are involved in mitochondrial metabolism

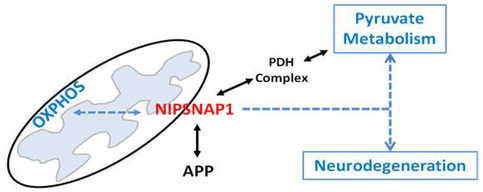


| Gene Ontology | Category | Exon 10 # of genes | Exon 10 Adj p value |
|---|---|---|---|
| Biological process | Protein transport | 36 | 3.00e-04 |
| | Intracellular transport | 28 | 5.51e-05 |
| | Generation of precursor metabolites and energy | 26 | 1.18e-07 |
| | Protein folding | 13 | 3.00e-04 |
| Molecular function | Unfolded protein binding | 9 | 8.00e-04 |
| | Ribonuclease P activity | 3 | 1.16e-02 |
| | Oxidoreductase activity, acting on NADH or NADPH, nitrogenous group as acceptor | 2 | 1.16e-02 |
| | Oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor | 11 | 1.90e-03 |
| | Sulfer transferase activity | 3 | 3.5e-03 |
| | Structural constituent of ribosome | 8 | 1.16e-02 |
| Cellular component | Mitochondrion | 77 | 1.46e-12 |
| | Protein complex | 88 | 1.80e-11 |

## KEGG analysis : NIPSNAP1 correlates with genes involved in Neurodegeneration

| KEGG Pathways (Exon 10) | # of genes | Adj p value |
|---|---|---|
| Proteasome | 12 | 5.52e-09 |
| Oxidative phosphorylation | 15 | 1.46e-06 |
| Metabolic pathways | 52 | 7.52e-06 |
| Huntington's disease | 16 | 8.74e-06 |
| Parkinson's disease | 12 | 6.76e-05 |
| Citrate cycle (TCA cycle) | 7 | 6.76e-05 |
| Alzheimer's disease | 13 | .0003 |
| Pyrulvate metabolism | 6 | .0024 |
| Amino and nucleotide sugar metabolism | 6 | .0043 |
| Propanoate metabolism | 4 | .0292 |

## Summary Diagram




We have shown previously that NIPSNAP1 interacts with the cytoplasmic domain of APP (Tummala et al., 2010) and others have shown that NIPSNAP1 interacts with pyruvate dehydrogenase complex (PDH; Nautiyal, et al., 2010). Here, using a systems genetics approach, we have found that NIPSNAP1 transcript is correlated with a number of genes involved in oxidative phosphorylation, pyruvate metabolism, and proteasome /neurodegenerative pathways.

## Summary and Conclusions

A large number (932) of transcripts were correlated (r>±0.639, p<2.6e-13) with NIPSNAP1 exon 10 expression levels in the hippocampus across BXD RI strains (n=74).

The literature derived cohesion of this gene set was highly significant (p<3.2E-33).

A significant number (77 genes; FDR adjusted p<1.46e-12) of NIPSNAP1 correlated genes were localized in the mitochondria. This is expected with the fact that NIPSNAP1 is localized to the mitochondria.

A significant number of NIPSNAP1 correlated genes are involved in mitochondrial metabolism: OXPHOS (adj p<1.46e-06), citrate cycle (adj p<6.76e-05), and pyruvate metabolism (adj p<2.4e-03).

Additionally, a significant number of NIPSNAP1 correlated genes are associated with the proteasome (p<5.52e-09), particularly genes linked to Huntington's (adj p<8.74e-06), Parkinson's (adj p<6.76e-05) and Alzheimer's (adj p<3.0e-04) disease pathways.

Taken together, our data suggest that NIPSNAP1 plays a role in regulation of energy metabolism and may in some way affect proteasome function and neurodegeneration.

## References

Islam, M.M., Nautiyal, M., Wynn, R.M., Mobley, J.A., Chuang, D.T., Hutson, S.M. (2010) The Branched Chain Amino Acid (BCAA) Metabolon: Interaction of Glutamate Dehydrogenase with the Mitochondrial Branched Chain Aminotransferase (BCATm). J. Biol. Chem., 285, 265–76.

Nautiyal, M., Sweatt, A.J., MacKenzie, J.A., Mark Payne, R., Szucs, S., Matalon, R., Wallin, R., and Hutson, S.M. Neuronal localization of the mitochondrial protein NIPSNAP1 in rat nervous system. Eur J Neurosci *32*, 560-569.

Tummala H, Li X, Homayouni R . Interaction of a novel mitochondrial protein, 4-nitro phenyl phosphatase domain and non-neuronal SNAP25-like protein homolog 1(NIPSNAP1), with the amyloid precursor protein family. Eur J Neurosci. 2010 Jun;31(11):1926-34.

Xu L, Furlotte N, Lin Y, Heinrich K, Berry MW, George EO, Homayouni R. Functional cohesion of gene sets determined by latent semantic indexing of PubMed abstracts. PLoS One. 2011 Apr 14;6(4):e18851.

## Acknowledgement

This work is supported by University of Memphis FedEx Institute of Technology and Assisi Foundation of Memphis.