Integrated WGCNA with an application to chronic fatigue syndrome

Methodology article Open Access Highly accessed

Integrated weighted gene co-expression network analysis with an application to chronic fatigue syndrome

Angela P Presson, Eric M Sobel, Jeanette C Papp, Charlyn J Suarez, Toni Whistler, Mangalathu S Rajeevan, Suzanne D Vernon, Steve Horvath

BMC Systems Biology 2008, **2**:95 (6 November 2008)

[Abstract] [Provisional PDF] [PubMed] [Related articles]

Angela Presson, Steve Horvath
Department of Biostatistics
Department of Human Genetics
University of California, Los Angeles

UCLA Biostatistics

Chronic Fatigue Syndrome

6 Months or more of medically unexplained severe fatigue + 4 of the following symptoms:

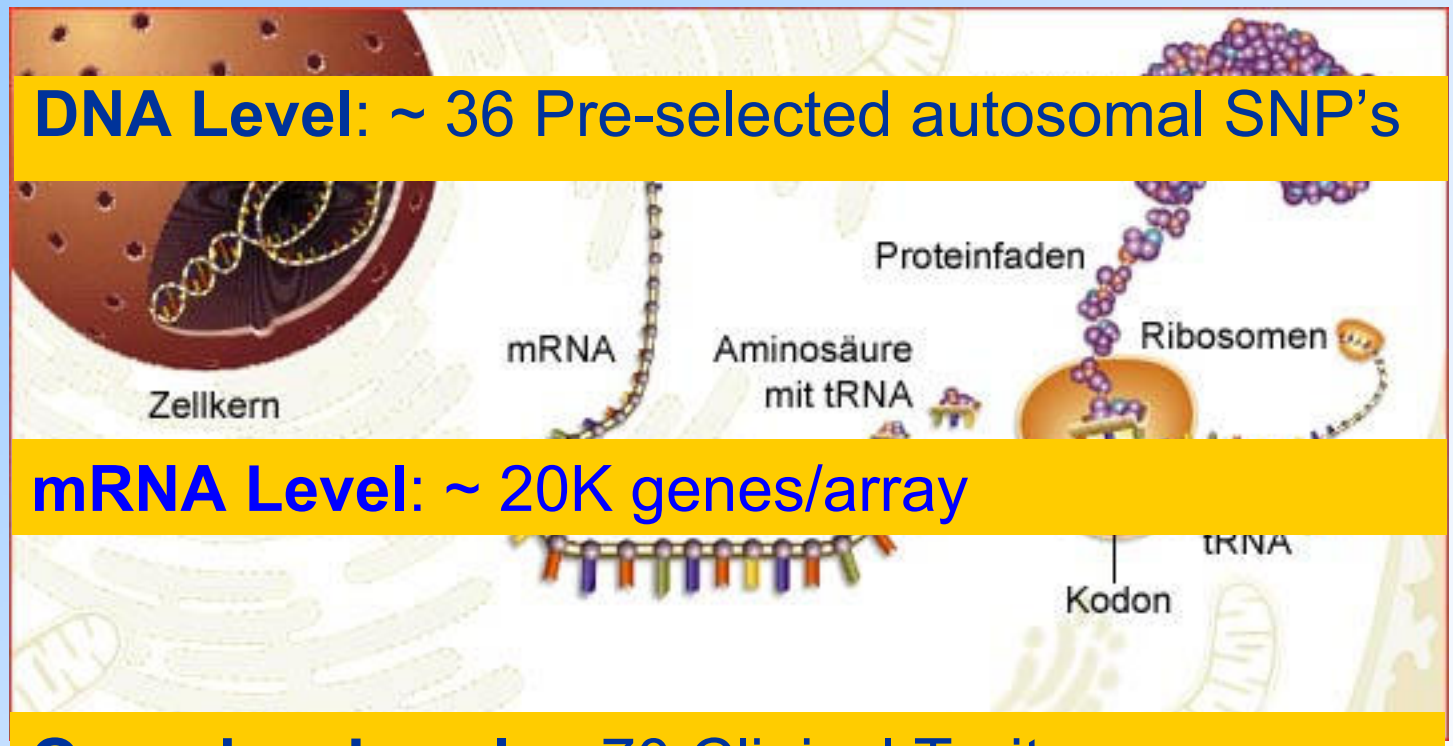
Post exertional fatigue lasting > 24 hrs
Unrefreshing sleep
Difficulty concentrating or remembering
Headaches unusual in frequency or duration
Muscle pain
Joint pain
Sore throat
Tender lymph nodes
(Fukuda et al. 1994)

Outline

1. Demonstrate a weighted gene co-expression network analysis (WGCNA)
   a. Screen for ~20 candidate genes to consider for follow up analysis using:
      i. SNP
      ii. Severity
      iii. Module connectivity
   b. Check for biological relevance of module and candidate genes using gene ontology software.
2. Conduct a standard microarray analysis using the false discovery rate and ignoring the SNP data.
   Identify ~20 candidate genes and annotate.
3. Compare standard analysis with WGCNA.

Chronic Fatigue data set

164 Samples with the following data:



Analyzed the following subset of data:
1.) 127 fatigued samples
2.) 8966 genes with high mean and variance

Selecting a clinical trait

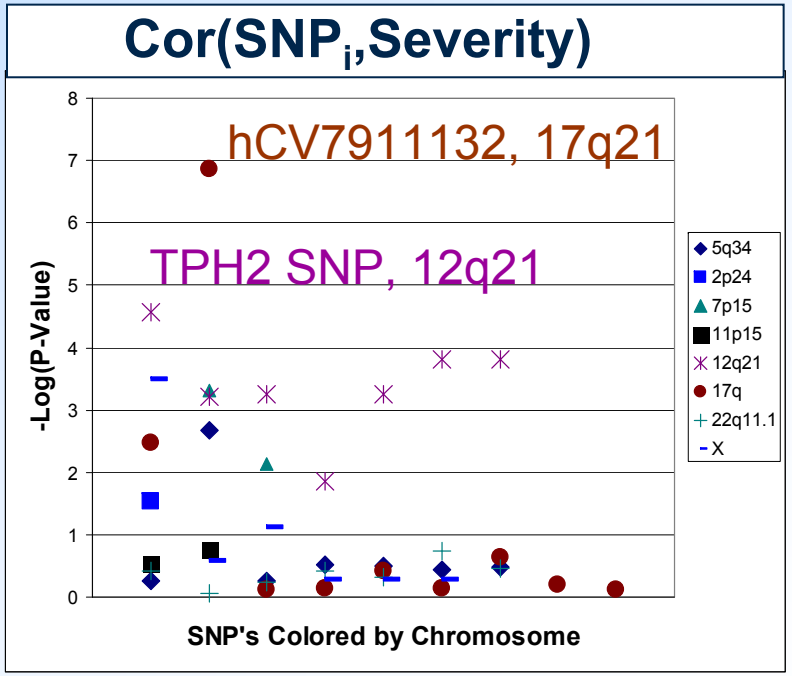
- Scores from diagnostic procedures used to evaluate quality of life:
  - Medical Outcomes Survey Short Form (SF-36)
  - Multidimensional Fatigue Inventory (MFI)
  - CDC Symptom Inventory Case Definition scales
- Reeves et al. (2005) clustered these 14 scores from 118 patients and identified three clusters of CFS severity: high, moderate and low.

➢ Out of 70 clinical scores, we chose to use this CFS severity trait.

Selecting a SNP marker

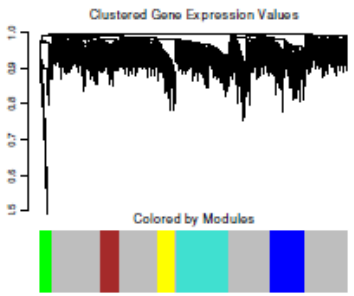
- CDC provided 36 autosomal SNPs from 8 candidate CFS genes: TPH2, POMC, NR3C1, CRHR2, TH, SLC6A4, CRHR1, COMT (Smith et al. 2006)

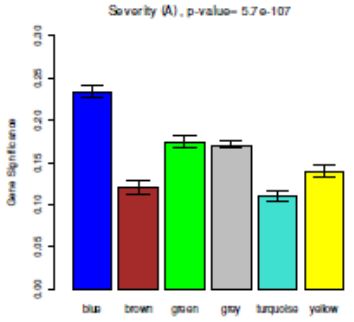
- Selected "*TPH2* SNP": rs10784941 (12q21) from the *TPH2* gene because:
  - Previously found to be associated with chronic fatigue (Goertzel et al. 2006)
  - Had significant correlation with CFS severity (p-value = 0.0099).

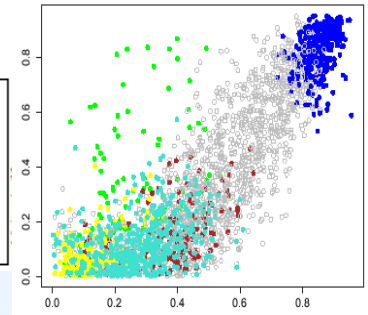


a. IWGCNA steps

1. Construct a co-expression network and modules
2. Find clinical trait related modules
3. Prioritize module genes using disease-related SNP
4. Use the integrated model to screen for genes







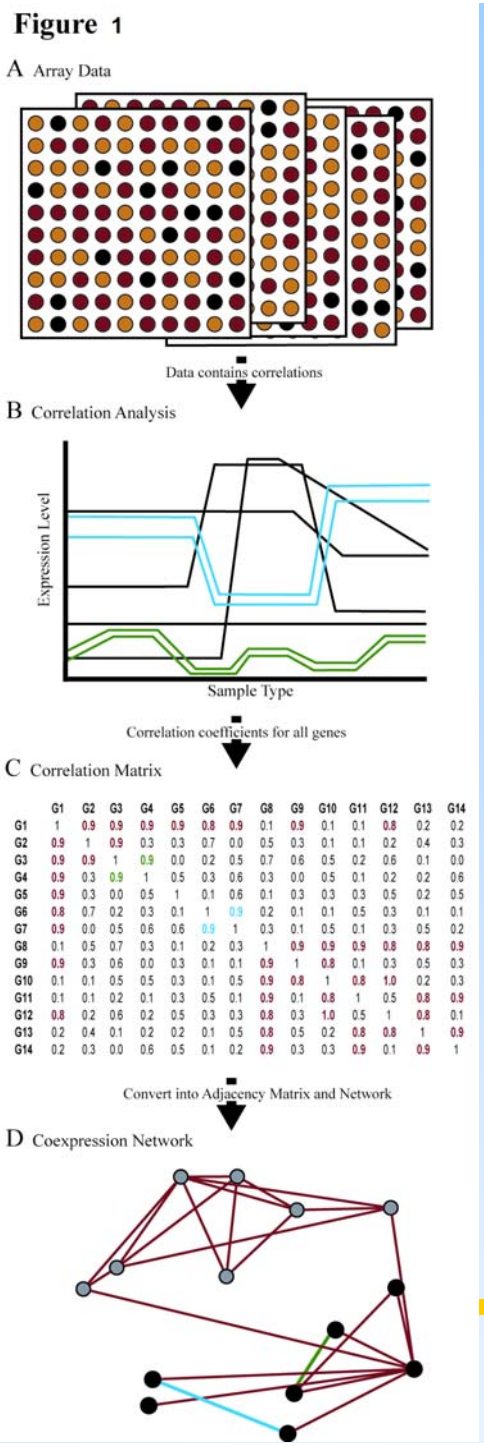
b. CFS Patients analyzed in IWGCNA steps

1. Full data set: 127 CFS patients
2. Primary data set: 87 patient subset with CFS severity scores

Exclude heterogeneous female samples

3-5. 76 samples with CFS severity scores

UCLA Biostatistics

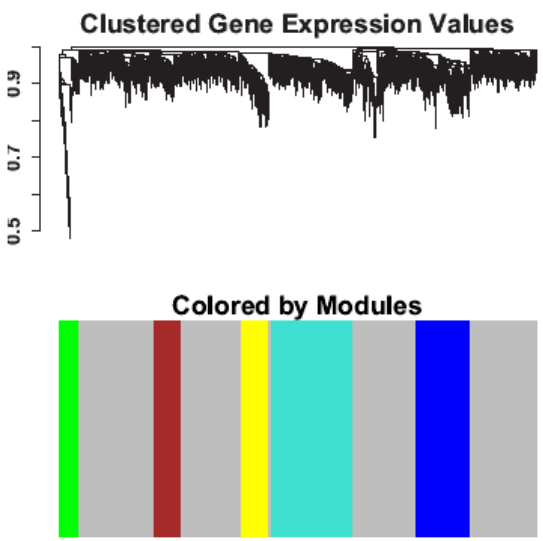


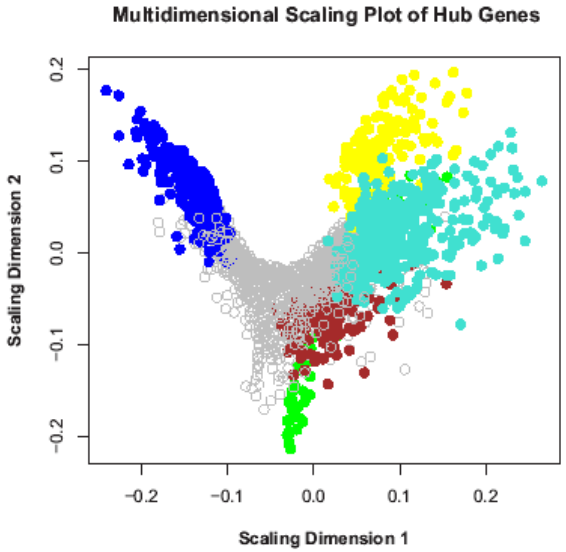
Constructing a weighted gene co-expression network

1. Construct a Pearson correlation matrix from microarray data:
   x_i and $x_j \rightarrow r(x_i,x_j)$
2. Transform via an adjacency function:
   - Step function: $a_{ij} = I_{r(xi,\, xj) > \tau} \rightarrow$ Unweighted network
   - Power function: $a_{ij} = r(x_i, x_j)^{\beta} \rightarrow$ Weighted network

UCLA Biostatistics

Five modules identified using hierarchical clustering





a) Gene Network

b) MDS view

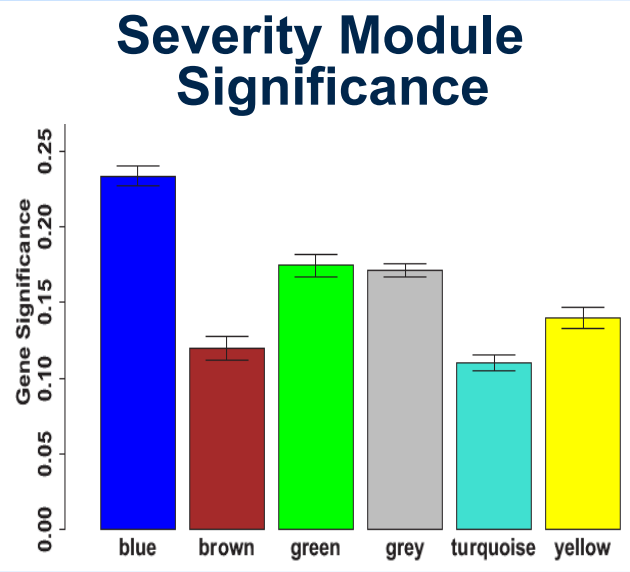
- Grey colors indicate genes outside of any module.
- MDS plot indicates separation of blue, green, brown, turquoise and yellow modules.

The blue module relates to severity

GS.severity(i) = |*cor(x(i), severity)*|, where *GS* = "Gene Significance" and *x(i)* is the gene expression profile of the i^{th} gene. Can also define:

Module.Significance(k) = *E(GS.severity(i)* genes in module *k)*

Blue module (299 genes) has highest Module Significance



UCLA Biostatistics

Correlate gene expression data with *TPH2* SNP

- Integration of WGCNA with genetic marker data: IWGCNA

$$GS.SNP(i) = |cor(x(i), TPH2\ SNP)$$

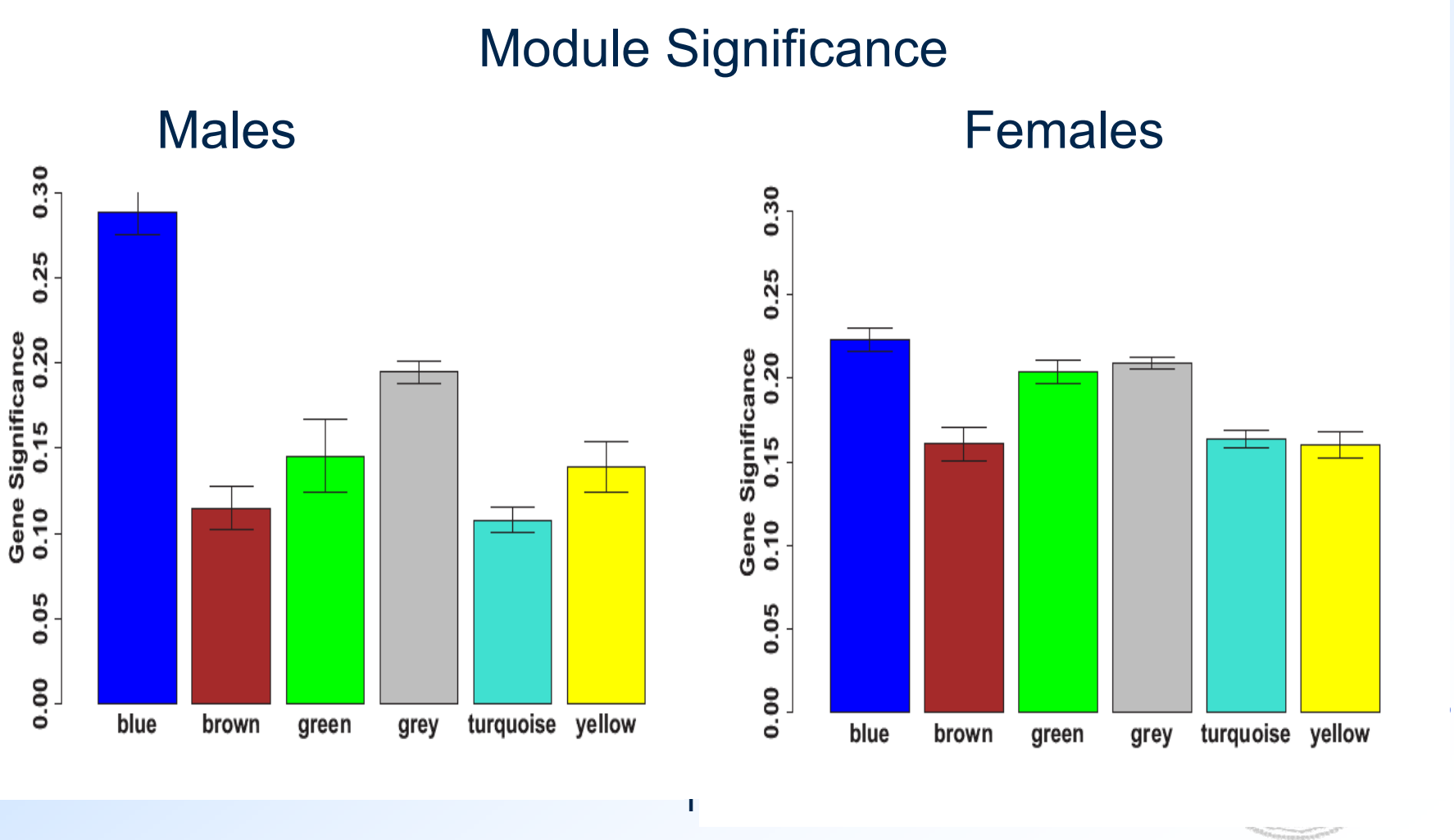
where $x(i)$ is the i-th gene expression

- Additive SNP marker coding: AA = 2, AB = 1, BB = 0
- Absolute value of the correlation ensures that this is equivalent to AA = 0, AB = 1, BB = 2
- Dominant or recessive coding is more appropriate for most Mendelian diseases

Why Consider Gender Differences?

- We chose to investigate sex differences for the following reasons:
  1. CFS is 4x more prevalent in women. (Reyes et al. 2003)
  2. Possible that prevalence difference due to genetic differences between genders.
  3. Women outnumber men 3:1 in this data set (98 females, 29 males).
- If no gender difference, analyze male and female arrays together.
- If gender differences, exclude some female samples with expression patterns that differ most from module eigengene.

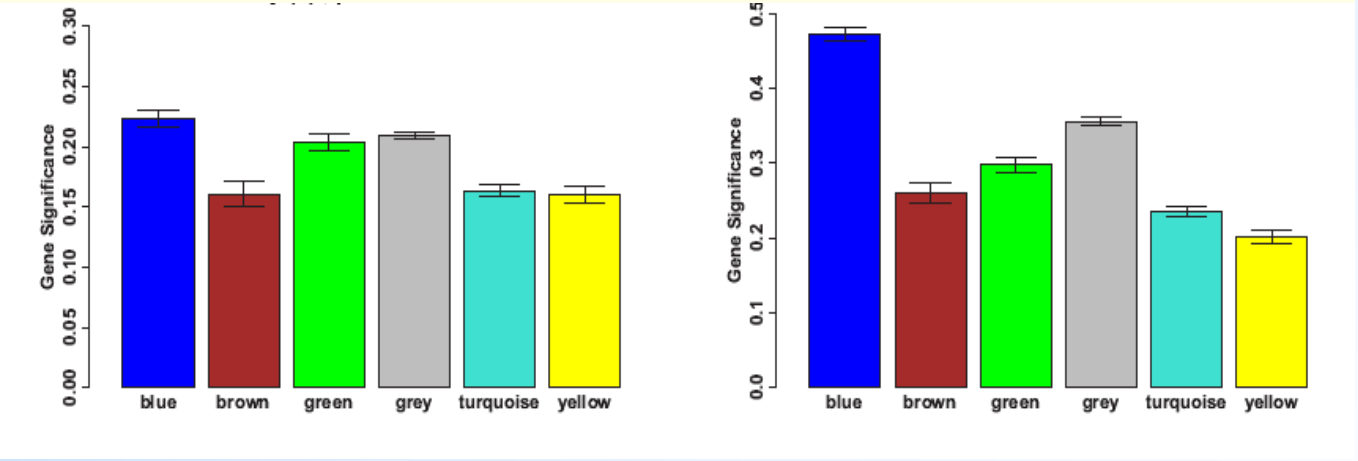
The blue module is related to severity in males, several modules relate in females



Homogenization of Female Samples

- Based on the idea that blue module is related to severity. Uses first principal component of blue module: “module eigengene” (ME) summary measure.
- ME_{blue} > median(ME_{blue}) and high severity (severity > 1) OR ME_{blue} < median(ME_{blue}) and low severity (severity < 3).
- Reduced female samples from 64 to 53.

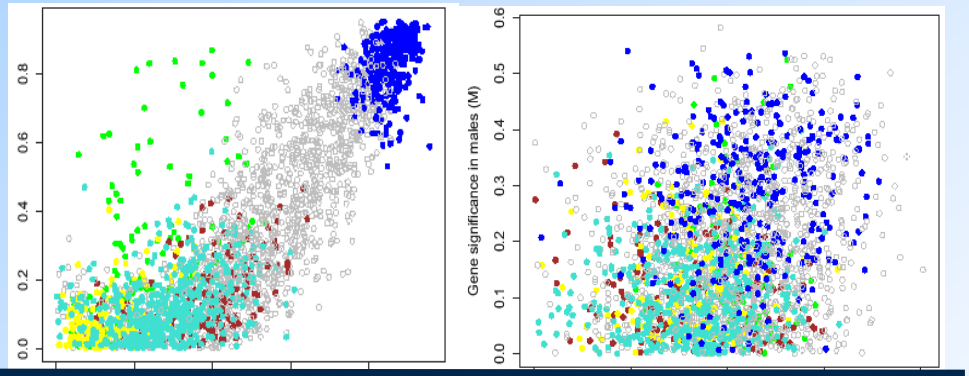
Severity - All Females **Severity - Homogenized Females**



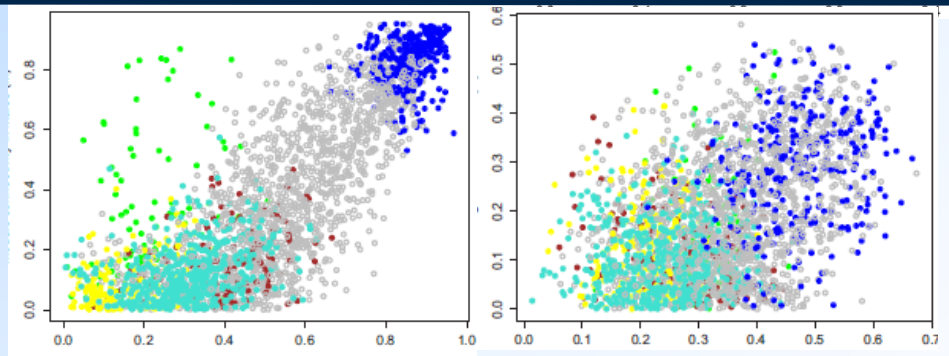
- Increased the module significance from 0.22 (p-value = 0.074) to 0.47 (p-value = 0.00016).

Gender-stratified network views

M vs. F : k_{ME} & $GS_{severity}$



M vs. FH : k_{ME} & $GS_{severity}$



1. Calculate connectivity for a gene x(i): $k_{ME}(i) = |cor(MEblue,x(i))|$
2. Blue module connectivity (membership) is highly preserved between genders
3. Less preservation for GS.severity

➢ Due to GS.severity gender difference, it is useful to impose screening criteria in both males and females separately.

Gene screening procedure

Screening criteria imposed in both males and homogenized females:

1) High connectivity within blue module (k_{ME} in top 2/3rd's)
2) Association with severity trait ($GS_{severity}$ > .2 in males and $GS_{severity}$ > .35 in homogenized females)
3) Association with TPH2 SNP (top 50%)

⇒ **20 Genes met these criteria**

IWGCNA Candidate Genes

- 12/16 genes were a) verified as interacting and b) estimated to function in a hematological disease pathway by Ingenuity Pathways Analysis (IPA) software
- **Viral function**, **hematological disease** and **connective tissue** are consistent with previous findings.

Gene Name and Genbank Accession	Full gene name. Entrez Gene and/or GeneRIFs description. Chromosome Location	Ingenuity Pathways Gene Annotation		c) Causality	
		a) 16 out of 20 Candidate Genes	b) 212 out of 299 Module Genes	LEO score[13]	Rank in Module
FOXN1 (NM_003593)	Forkhead box N1. Mutations result in a severely compromised immune system, T-cell immunodeficiency, skin disorder congenital alopecia. 17q11-q12	Hematological Disease[1] Rank = 1, p-value ≈ 10^{-32}	Cell Function[5] Rank = 8, p-value ≈ 10^{-16}	0.82	6
PRDX3 (AF118073)	Peroxiredoxin 3. Antioxidant function, regulates abundance of H_20_2, which promotes apoptosis. 10q25-q26	Hematological Disease[1]	Endocrine Disorders/ Inflammation[6] Rank = 6, p-value ≈ 10^{-20}	0.77	8
SUCLA2 (AK001458)	Succinate-CoA ligase, ADP-forming, beta subunit. Defects associated with encephalomyopathy. 13q12.2-q13.3	Hematological Disease[1]	Cell Cycle[7] Rank = 5, p-value ≈ 10^{-22}	0.77	9
TFB2M (AK026314)	Transcription factor B2, mitochondrial. 1q44	Hematological Disease[1]	Cell Cycle[7]	0.69	18
MED8 (BC010019)	Mediator complex subunit 8	Hematological Disease[1]	Amino Acid Met.[8] Rank = 1, p-value ≈ 10^{-45}	0.82	7
SNURF (AF101044)	SNRPN upstream reading frame. Alternative splicing/deletion leads to Angelman syndrome or Prader-Willi. 15q12	Hematological Disease[1]	Amino Acid Met.[8]	0.53	36
DCTN2 (NM_006400)	Dynactin 2 (p50). Required in peroxisome biogenesis. 12q13.2-q13.3	Hematological Disease[1]	Amino Acid Met.[8]	0.30	66
PGK1 (AB062432)	Phosphoglycerate kinase 1. Glycolysis. Xq13	Hematological Disease[1]	Amino Acid Met.[8]	-0.28	132
PRKCH (BC001000)	Protein kinase C, eta. Regulates keratinocyte differentiation. 14q22-q23	Hematological Disease[1]	Connective Tissue[9] Rank = 2, p-value ≈ 10^{-44}	-0.13	116

RYK (NM_002958)	RYK receptor-like tyrosine kinase. May play a role in the development of cleft lip and/or palate. 3q22	Hematological Disease[1]	Connective Tissue[9]
VAMP5 (AF077197)	Vesicle-associated membrane protein 5 (myobrevin). Associated with myogenesis. 2p11.2	Hematological Disease[1]	Connective Tissue[10] Rank = 7, p-value ≈ 10^{-18}
PBLD (AK027673)	Phenazine biosynthesis-like protein domain containing. 10pter-q25.3	Hematological Disease[1]	Connective Tissue[10]
NPAL2 (AK024017)	NIPA-like domain containing 2. 8q22.2	Digestive System[2] Rank = 2, p-value ≈ 10^{-3}	Viral Function[11] Rank = 3, p-value ≈ 10^{-32}
CD302 (BC020646)	C-type lectin receptor involved in cell adhesion and migration, as well as endocytosis and phagocytosis. 2q24.2	Carbohydrate Metabolism[3] Rank = 2, p-value ≈ 10^{-3}	Viral Function[11]
PPP1R14C (AF407165)	Protein phosphatase 1, regulatory (inhibitor) subunit 14C. Enriched in brain, heart and skeletal muscle. 6q24.3-q25.3	Cancer[4] Rank = 2, p-value ≈ 10^{-3}	Cell Proliferation[12] Rank = 9, p-value ≈ 10^{-14}
TMEM50A (AF081282)	Transmembrane protein 50A. May contribute to RH haplotype selection. 1p36.11	NA, Rank = 2	NA, Rank = 14
CRNKL1 (AF111802)	Crooked neck pre-mRNA splicing factor-like 1. 20p11.2	NA	NA
LTV1 (AK027815)	Protein coding. 6q24.2	NA	NA
AF090939	Discontinued record.	NA	NA
XM13557	Unmapped.	NA	NA

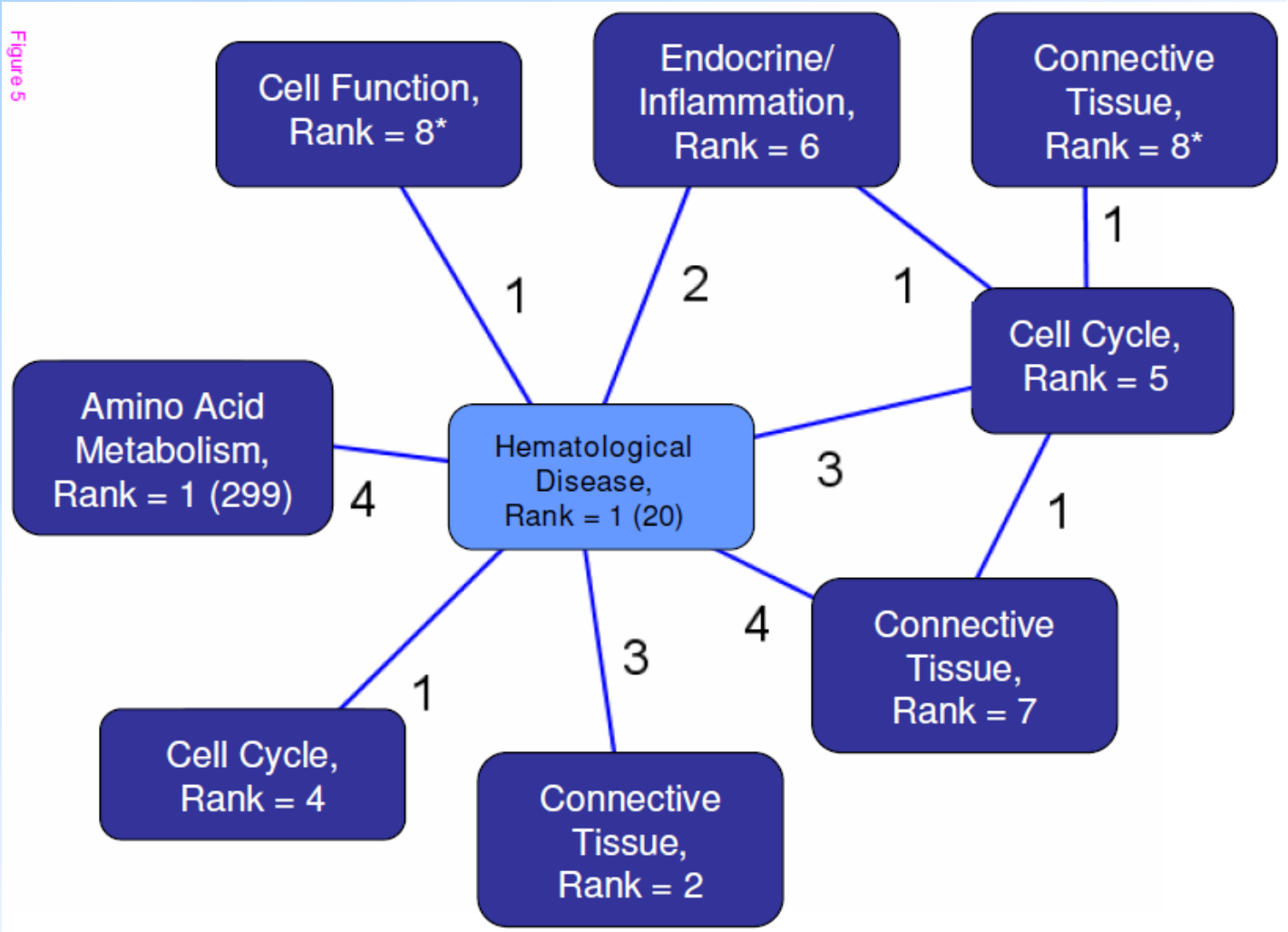
[1]Cell Cycle, Cancer, Hematological Disease
[2]Digestive System D&F, Hepatic System D&F, Organ Dev.
[3]Carbohydrate Metabolism, Gene Expression, Genetic Disorder
[4]Cancer, Cellular Movement, Skeletal and Muscular Disorders
[5]Cell Fun. and Main., Small Molecule Biochem., Molecular Transport
[6]Endocrine System Disorders, Infectious Disease, Inflammatory Disease
[7]Cell Assembly and Org., Cell Cycle, DNA Replication/Recomb./Repair
[8]Post-Translational Modification, Amino Acid Metabolism, Molecular Transport
[9]Organ Morphology, Cell Morphology, Connective Tissue D&F
[10]Gene Expression, Cellular Development, Connective Tissue D&F
[11]Viral Function, Cell. Assembly and Org., Cell Fun. and Maintenance
[12]Post-Translational Modification, Cancer, Cellular Growth/Proliferation
[13]LEO.NB.SingleMarker scores (converted to fold changes).

Ingenuity pathways analysis results for IWGCNA genes

Light blue = 20 candidate genes

Dark blue = 299 module genes

Centrality of candidate gene pathway reflects use of connectivity in gene screening strategy.



Repeat IPA with *TPH2* gene:

Does including *TPH2* SNP in screening procedure result in genes that interact with *TPH2*?

IPA #1: Without TPH2

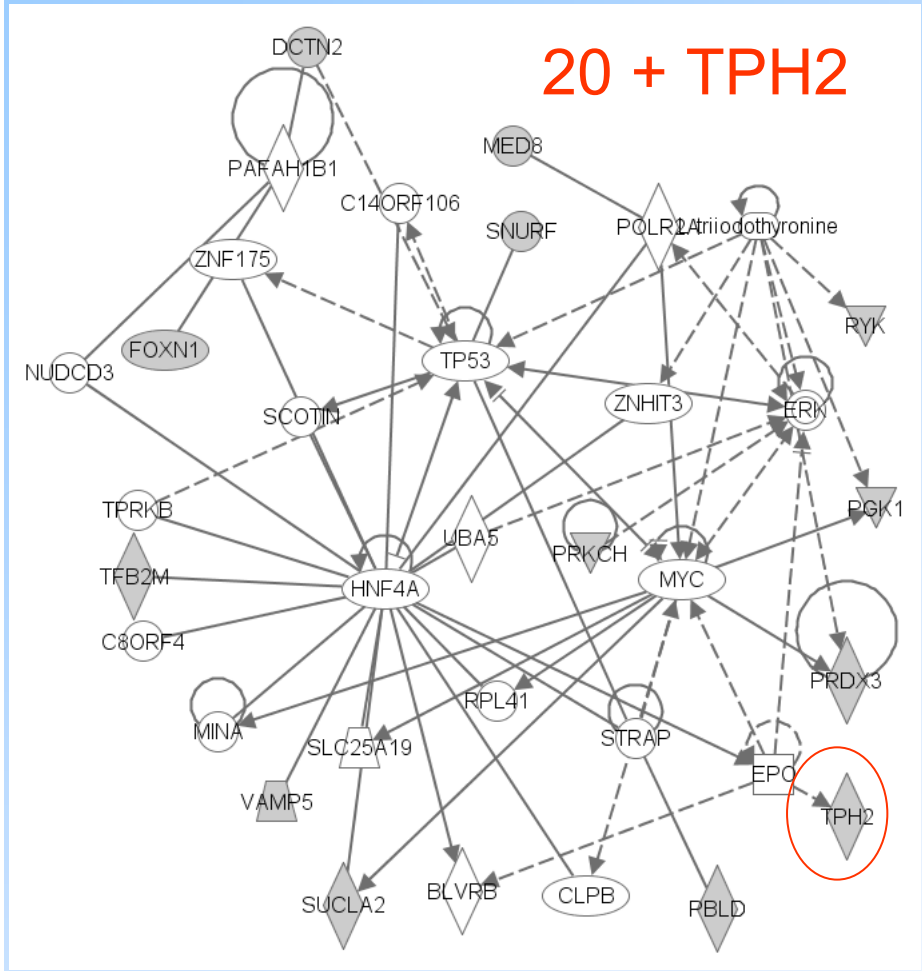
ID	Molecules in Network	Score	Focus Molecules	Top Functions
1	C14ORF106, C8ORF4, CLPB, **DCTN2,** ERK, **FOXN1,** HNF4A, HSPD1 (includes EG:3329), L-triiodothyronine, **MED8,** MGST3, MINA, MYC, NUDCD3, PAFAH1B1, **PBLD, PGK1, PRDX3, PRKCH,** PTK7, RPL41, **RYK,** SCOTIN, SLC25A19, **SNURF,** STRAP, **SUCLA2, TFB2M,** TP53, TPRKB, UBA5, **VAMP5,** YY2 (includes EG:404281), ZNF175, ZNHIT3	32	12	Cell Cycle, Cancer, Hematological Disease
2	C7ORF43, **TMEM50A**	3	1	
3	**NPAL2,** ONECUT1	3	1	Digestive System
4	**CD302,** HNF1A	3	1	Carbohydrate
5	PP1, PP1/PP2A, **PPP1R14C,** PRKCG	3	1	Cancer, Cellular

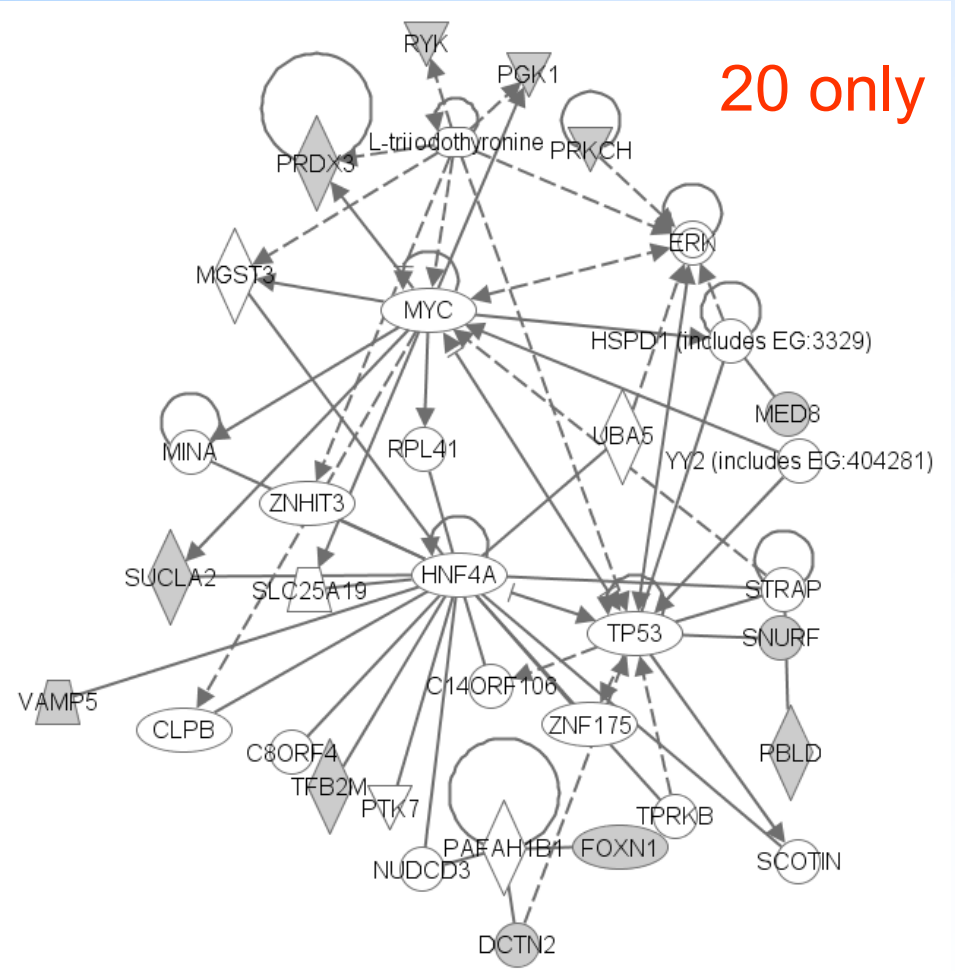
IPA #2: With TPH2

ID	Molecules in Network	Score	Focus Molecules	Top Functions
1	BLVRB, C14ORF106, C8ORF4, CLPB, **DCTN2,** EPO, ERK, **FOXN1,** HNF4A, L-triiodothyronine, **MED8,** MINA, MYC, NUDCD3, PAFAH1B1, **PBLD, PGK1,** POLR2A, **PRDX3, PRKCH,** RPL41, **RYK,** SCOTIN, SLC25A19, **SNURF,** STRAP, **SUCLA2, TFB2M,** TP53, **TPH2,** TPRKB, UBA5, **VAMP5,** ZNF175, ZNHIT3	35	13	Cell Cycle, Cancer, Hematological Disease

- Yes, it is part of the large pathway.
- The p-value improves slightly and the functions stay the same.

Pathways are very similar





UCLA Biostatistics

Results from previous CFS studies

1. Associated with other conditions: fibromyalgia, connective tissue disease and mitochondrial deficiency (Bains 2008; Hench 1989)
2. Affects the endocrine, muscular and immune systems and some cases may be triggered by viruses (Lloyd et al. 1991; Holmes et al. 1987; Torpy and Chrousos 1996; Kaushik et al. 1987)
3. Evidence for immune and hypothalamic-pituitary-adrenal (HPA) axis abnormalities have been observed at the symptom, molecular and genetic level of CFS patients (Klimas and Koneru 2007)
4. Higher cytotoxic T-cell counts and impaired T-cell function in CFS patients (Rasmussen et al. 1994; Patarca 2001)
5. Evidence for higher rates of immune cell apoptosis in CFS patients, specifically neutrophils and peripheral blood lymphocytes (Vojdani et al. 1997; Kennedy et al. 2004)

Outline

1. Demonstrate a weighted gene co-expression network analysis (WGCNA)
   a. Screen for ~20 candidate genes to consider for follow up analysis using:
      i. SNP
      ii. Severity
      iii. Module connectivity
   b. Check for biological relevance of module and candidate genes using gene ontology software.
2. Conduct a standard microarray analysis using the false discovery rate and ignoring the SNP data.
   - Identify ~20 candidate genes and annotate.
3. Compare standard analysis with WGCNA.

Standard analysis results in 29 candidate genes

- Starting from 8966 most varying genes, computed p-values for Pearson correlation test of gene expression profiles with severity.
- For each p-value, we computed the corresponding local false discovery rate (q-value) using the qvalue package in R.
- **346** genes achieved minimum fdr = 0.081; and 241 eligible for IPA network construction. Top 3 IPA pathways:

  1. **Viral Function, Molecular Transport, RNA Trafficking (p-value ~ 10[−52], focus molecules = 29)**
  2. Connective Tissue Development and Function, Cell Signaling, Molecular Transport (p-value ~ 10[−31], focus molecules = 20)
  3. Cell Morphology, Cellular Assembly and Organization, Cancer (p-value ~ 10[−29],focus molecules = 19)

➢ Selected 29 genes from Viral Function pathway as candidate genes for standard analysis.

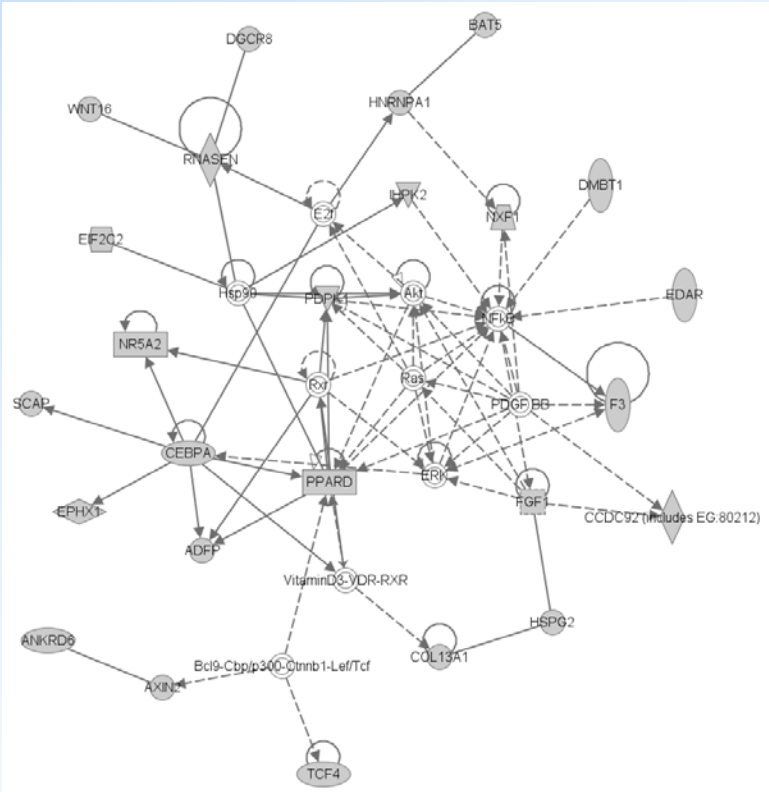
IPA of 29 standard analysis genes with and without *TPH2*

- Analysis of 29 genes alone results in 2 networks.

networks, click Merge Networks.

	ID	Molecules in Network	Score	Focus Molecules	Top Functions
☐	1	**ADFP,** Akt, **ANKRD6, AXIN2, BAT5,** Bcl9-Cbp/p300-Ctnnb1-Lef/Tcf, **CCDC92 (includes EG:80212), CEBPA, COL13A1, DGCR8, DMBT1,** E2f, **EDAR, EIF2C2, EPHX1,** ERK, **F3, FGF1, HNRNPA1,** Hsp90, **HSPG2, IHPK2,** NFkB, **NR5A2, NXF1,** PDGF BB, **PDPK1, PPARD,** Ras, **RNASEN,** Rxr, **SCAP, TCF4,** VitaminD3-VDR-RXR, **WNT16**	71	25	Viral Function, Molecular Transport, RNA Trafficking
☐	2	ADARB2, ALDH18A1, ALPP, ARID5B, BAK1, BCKDK, BCL2A1, CHD2, **DFFA,** DHX30, **FAIM,** FAM120C, HIST2H2BE, KIAA1553, NFkB, PPP1R10, PRR6, RBAK, RREB1, RSBN1, SART1, SPTBN1, STOM, WDR33, ZFP106, **ZMYND11,** ZNF174, ZNF317, ZNF362, ZNF462, ZNF592, **ZNF687,** ZNF768, ZNHIT4, ZSCAN12	7	4	Amino Acid Metabolism, Hepatic System Disease, Liver Cholestasis

	ID	Molecules in Network	Score	Focus Molecules	Top Functions
☐	1	**ADFP,** Akt, **ANKRD6, AXIN2, BAT5,** Bcl9-Cbp/p300-Ctnnb1-Lef/Tcf, **CCDC92 (includes EG:80212), CEBPA, COL13A1, DGCR8, DMBT1,** E2f, **EDAR, EIF2C2, EPHX1,** ERK, **F3, FGF1, HNRNPA1,** Hsp90, **HSPG2, IHPK2,** NFkB, **NR5A2, NXF1,** PDGF BB, **PDPK1, PPARD,** Ras, **RNASEN,** Rxr, **SCAP, TCF4,** VitaminD3-VDR-RXR, **WNT16**	71	25	Viral Function, Molecular Transport, RNA Trafficking
☐	2	ADARB2, ALDH18A1, ALPP, ARID5B, BAK1, BCKDK, BCL2A1, CHD2, **DFFA,** DHX30, **FAIM,** FAM120C, HIST2H2BE, KIAA1553, NFkB, PPP1R10, PRR6, RBAK, RREB1, RSBN1, SART1, SPTBN1, STOM, WDR33, ZFP106, **ZMYND11,** ZNF174, ZNF317, ZNF362, ZNF462, ZNF592, **ZNF687,** ZNF768, ZNHIT4, ZSCAN12	7	4	Amino Acid Metabolism, Hepatic System Disease, Liver Cholestasis
☐	3	EPO, TPH, **TPH2,** Tryptophan 5-monooxygenase	2	1	Behavior, Cardiac



- TPH2 is not involved in either network.

Outline

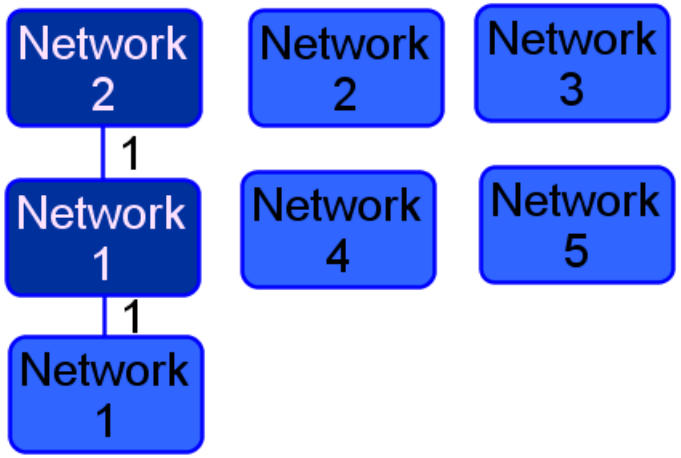
1. Demonstrate a weighted gene co-expression network analysis (WGCNA)
   a. Screen for ~20 candidate genes to consider for follow up analysis using:
      i. SNP
      ii. Severity
      iii. Module connectivity
   b. Check for biological relevance of module and candidate genes using gene ontology software.
2. Conduct a standard microarray analysis using the false discovery rate and ignoring the SNP data.
   - Identify ~20 candidate genes and annotate.
3. Compare standard analysis with WGCNA.

IPA network comparison between 20 IWGCNA and 29 standard analysis genes

Light blue = 20 IWGCNA genes

Dark blue = 29 standard genes

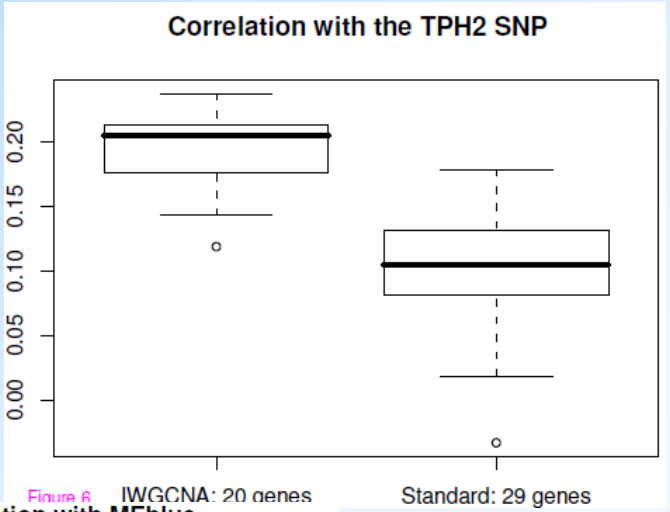
- No overlap between these two lists.
- But, overlap between **Hematological Disease** and **Viral Function** networks.

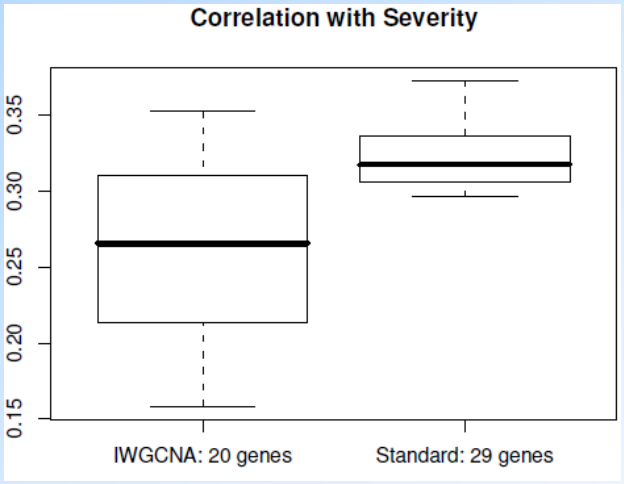


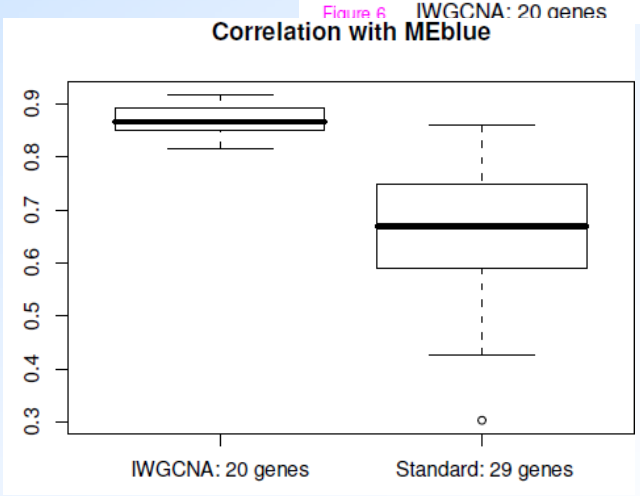
UCLA Biostatistics

Correlation results: IWGCNA vs. standard analysis

- r(IWGCNA,TPH2 SNP) > r(Std,TPH2 SNP)
- r(IWGCNA,MEblue) > r(Std,MEblue)
- r(Std,Severity) > r(IWGCNA, Severity)



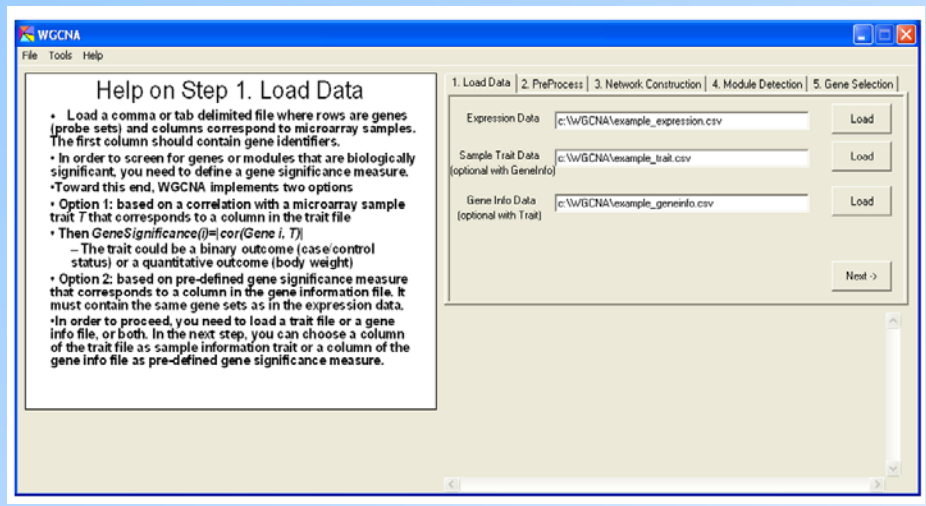




Conclusions

1. Weighted gene co-expression networks:
   a) Useful for selecting patient samples with similar gene expression profiles.
   b) Can be easily integrated with genetic marker, clinical, and other types of data.
2. Both IWGCNA and a standard analysis of CFS microarray data identify clinically interesting pathways and genes.
3. While the 20 and 29 cg lists do not overlap, IPA finds overlap between networks.
4. Integrating genotypes from a SNP marker with WGCNA identifies candidate genes that:
   - Functionally interact with the SNP-containing gene
   -
5. Whereas a standard analysis excluding SNP data does not find expression correlations with the SNP genotypes nor does the SNP-containing gene interact with these candidate genes.

WGCNA Software: stand alone and R package



http://www.genetics.ucla.edu/labs/horvath/CoexpressionNetwork

Acknowledgements

UCLA Human Genetics/Biostatistics

- Steve Horvath
- Eric Sobel
- Jeanette Papp
- Jason Aten
- Charlyn Suarez

Centers for Disease Control

- Suzanne Vernon
- Toni Whistler

References

1. Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A: The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. Ann Intern Med 1994, 121(12):953–9.
2. Reyes M, Nisenbaum R, Hoaglin DC, Unger ER, Emmons C, Randall B, Stewart JA, Abbey S, Jones JF, Gantz N, Minden S, Reeves WC: Prevalence and incidence of chronic fatigue syndrome in Wichita, Kansas. Arch Intern Med 2003, 163(13):1530-1536.
3. Smith AK, White PD, Aslakson E, Vollmer-Conna U, Rajeevan MS: Polymorphisms in genes regulating the HPA axis associated with empirically delineated classes of unexplained chronic fatigue. Pharmacogenomics 2006, 7(3):387–94.
4. Goertzel BN, Pennachin C, de Souza Coelho L, Gurbaxani B, Maloney EM, Jones JF: Combinations of single nucleotide polymorphisms in neuroendocrine effector and receptor genes predict chronic fatigue syndrome. Pharmacogenomics 2006, 7(3):475–83.
5. Bains W: Treating Chronic Fatigue states as a disease of the regulation of energy metabolism. Med Hypotheses 2008.
6. Hench PK: Evaluation and differential diagnosis of fibromyalgia. Approach to diagnosis and management. Rheum Dis Clin North Am 1989, 15:19–29.
7. Lloyd AR, Gandevia SC, Hales JP: Muscle performance, voluntary activation, twitch properties and perceived effort in normal subjects and patients with the chronic fatigue syndrome. Brain 1991,114 (Pt 1A):85–98.

References II

8. Torpy DJ, Chrousos GP: The three-way interactions between the hypothalamic-pituitary-adrenal and gonadal axes and the immune system. Baillieres Clin Rheumatol 1996, 10(2):181–98.
9. Kaushik N, Fear D, Richards SC, McDermott CR, Nuwaysir EF, Kellam P, Harrison TJ, Wilkinson RJ, Tyrrell DA, Holgate ST, Kerr JR: Gene expression in peripheral blood mononuclear cells from patients with chronic fatigue syndrome. J Clin Pathol 2005, 58(8):826–32.
10. Holmes GP, Kaplan JE, Stewart JA, Hunt B, Pinsky PF, Schonberger LB: A cluster of patients with a chronic mononucleosis-like syndrome. Is Epstein-Barr virus the cause? JAMA 1987,257(17):2297–302.
11. Klimas NG, Koneru AO: Chronic fatigue syndrome: inflammation, immune function, and neuroendocrine interactions. Curr Rheumatol Rep 2007, 9(6):482–7.
12. Rasmussen AK, Nielsen H, Andersen V, Barington T, Bendtzen K, Hansen MB, Nielsen L, Pedersen BK, Wiik A: Chronic fatigue syndrome–a controlled cross sectional study. J Rheumatol 1994, 21(8):1527–31.
13. Patarca R: Cytokines and chronic fatigue syndrome. Ann N Y Acad Sci 2001, 933:185–200.
14. Vojdani A, Ghoneum M, Choppa PC, Magtoto L, Lapp CW: Elevated apoptotic cell population in patients with chronic fatigue syndrome: the pivotal role of protein kinase RNA. J Intern Med 1997, 242(6):465–478.
15. Kennedy G, Spence V, Underwood C, Belch JJ: Increased neutrophil apoptosis in chronic fatigue syndrome. J Clin Pathol 2004, 57(8):891–3.