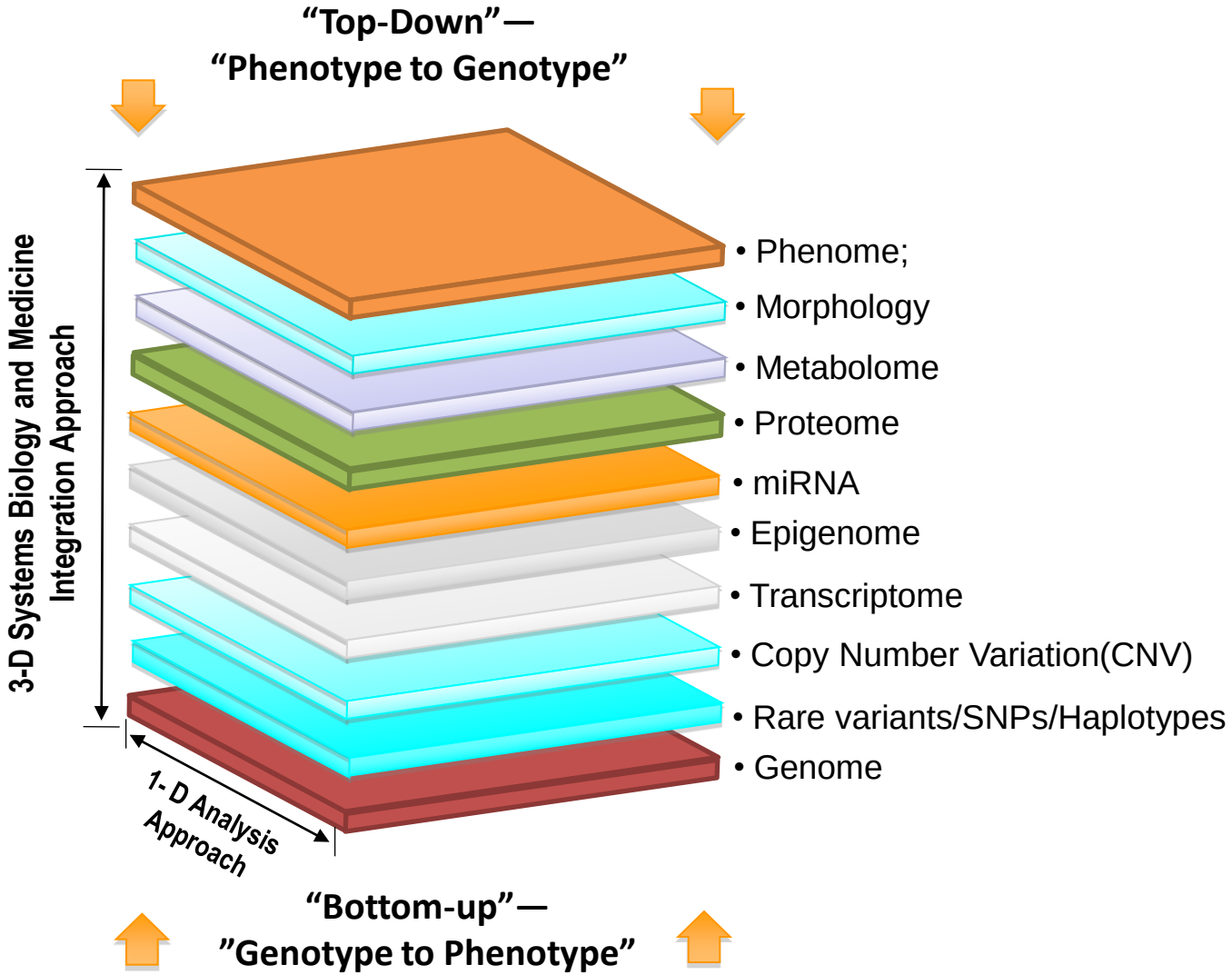
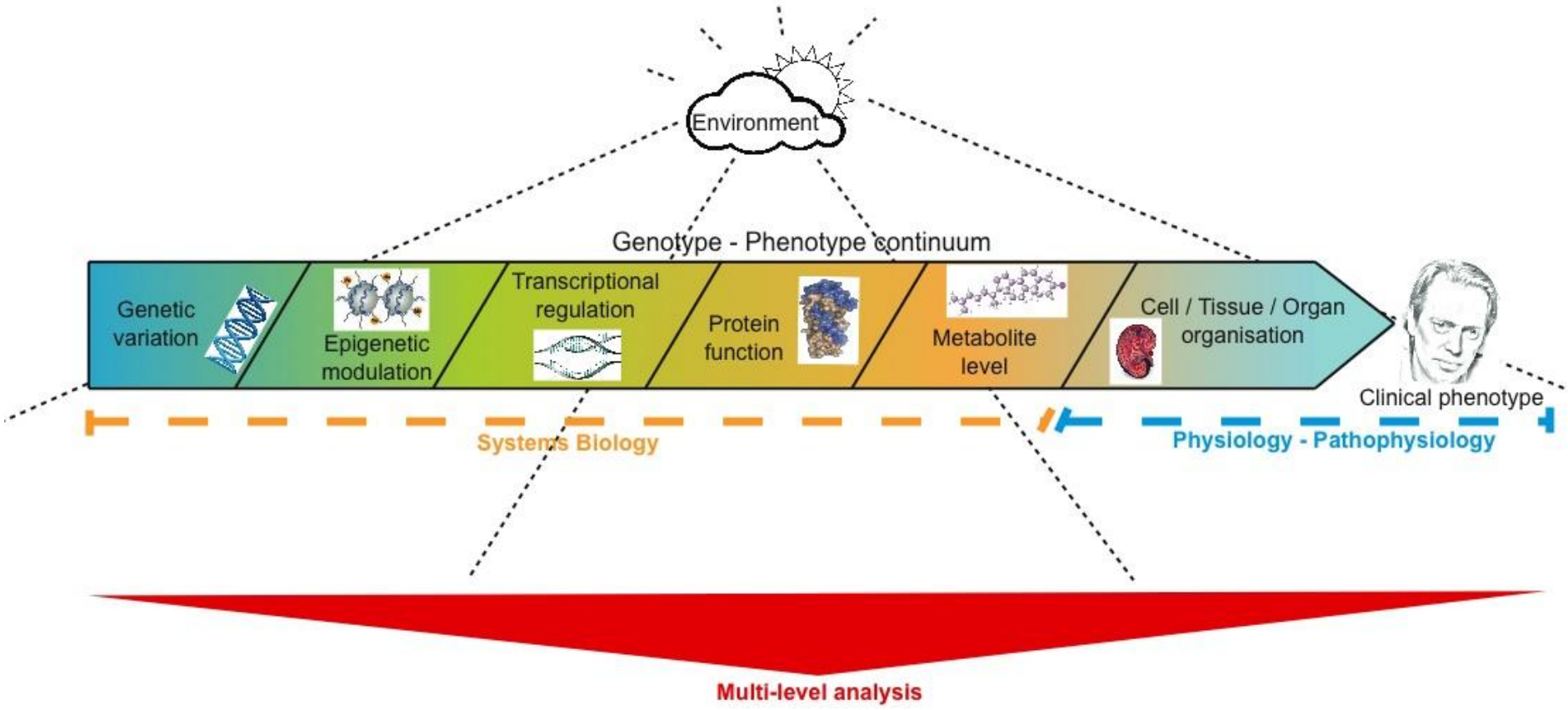
Systems Biology Approaches to Diabetic Complications: Current State

Multiscale Systems Integration in Diabetic Complications

From 1-D Analysis to 3-D Integrated Analysis



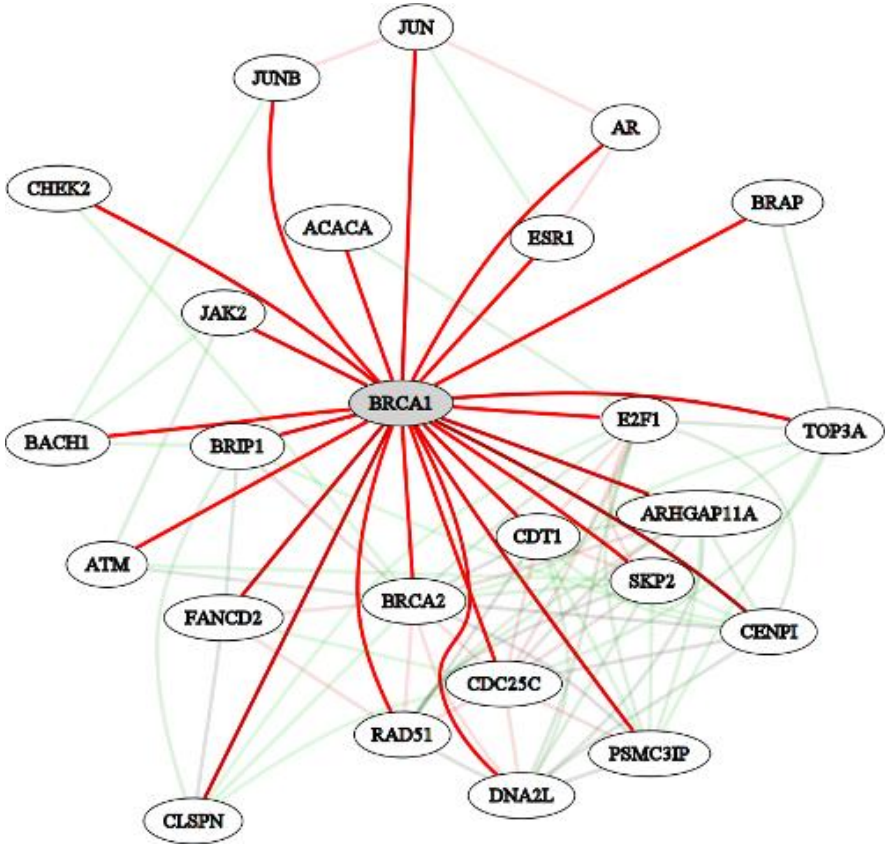
Integrative systems biology
Genotype – Phenotype continuum



Current Approaches

- “Simple” transcriptomic, proteomic, etc. analysis of human/model organism tissues in diabetes +/- intervention
- Bayesian integration approach to make specific predictions of functional relationships between genes
- Combined ‘omics: e.g., GWAS/transcriptomics, transcriptomics/metabolomics/lipidomics
- Cross-species and cross-strain ‘omics

Bayesian integration approach to make specific predictions of functional relationships



- BRCA1 and “response to DNA damage stimulus.”
- HEFalMp. The datasets used for this integration encompassed more than 30,000 experiments from more than 15,000 publications and included more than 27,000,000,000 data points
- The context specific functional relationship network from HEFalMp for BRCA1 in the context of “Response to DNA damage.” Red edges indicate high confidence links and green edges indicate lower confidence links.

Combined GWAS/transcriptomics CKDGen Identification of SNPs for Reduced Renal Function

- Meta-analysis of 20 GWAS population based cohorts of Caucasians in the USA and Europe
  - ~67.000 individuals where ~5800 were CKD
  - GFR (mls/min/1.73m^2, mean 87 +/-7)
- Independent replication in cohorts of European ancestry
  - ~23.000 individuals where ~1300 were CKD
- Reduced renal function was estimated by
  - serum creatinine (eGFRcrea),
  - serum cystatin c (eGFRcys) and
  - CKD (eGFRcrea< 60 ml/min/1.73 m2)

Kötten Nature Genetics 2010

Loci Linked to Renal Function

SNP ID	Chr	position (b36)	Genes Within 60 kb
Known Loci			
Rs17319721	4	77587871	**SHROOM3**;FLJ25770
Rs10109414	8	23807096	STC1
Rs12917707	16	20275191	UMOD;FLJ20581,GP2,PDILT
Novel Loci			
rs267734	1	149218101	ANXA9;FAM63A,PRUNE,BNIPL,LASS2,SETDB1
rs1260326	2	27584444	**GCKR**;IFT172,FNDC4
rs13538	2	73721836	**NAT8**;NAT8B,ALMS1
rs347685	3	143289827	TFDP2
rs11959928	5	39432889	**DAB2**;C9
rs6420094	5	176750242	**SLC34A1**;GRK6,RGS14,LMAN2,PRR7,F12,PFN3
rs881858	6	43914587	VEGFA
rs7805747	7	151038734	**PRKAG2**
rs4744712	9	70624527	**PIP5K1B**;FAM122A
rs10794720	10	1146165	**WDR37**
rs653178	12	110492139	**ATXN2**
rs626277	13	71245697	**DACH1**
rs491567	15	51733885	**WDR72**
rs1394125	15	73946038	**UBE2Q2**;FBXO22
rs12460876	19	38048731	**SLC7A9**;CCDC123,ECAT8

Correlation of mRNA levels of CKDGen associated genes with GFR

- Across all diseases and by individual disease type
- Glomeruli and Tubulo-Interstitium:
  - 296 Caucasian CKD patients:
    - FSGS, MCD, MGN, DN, IgAN, SLE, HN, RPGN,
    - GFR range 6–193 ml/min/1.73m^2, average: 65±37

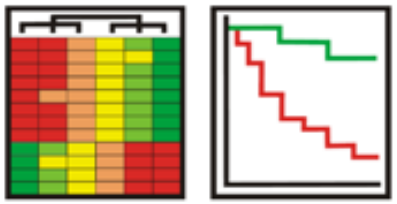
42 Candidate Genes for 18 SNPs

↓

30 Genes Contained in ERCB for 16 SNPs

↓

Correlation of gene expression to GFR

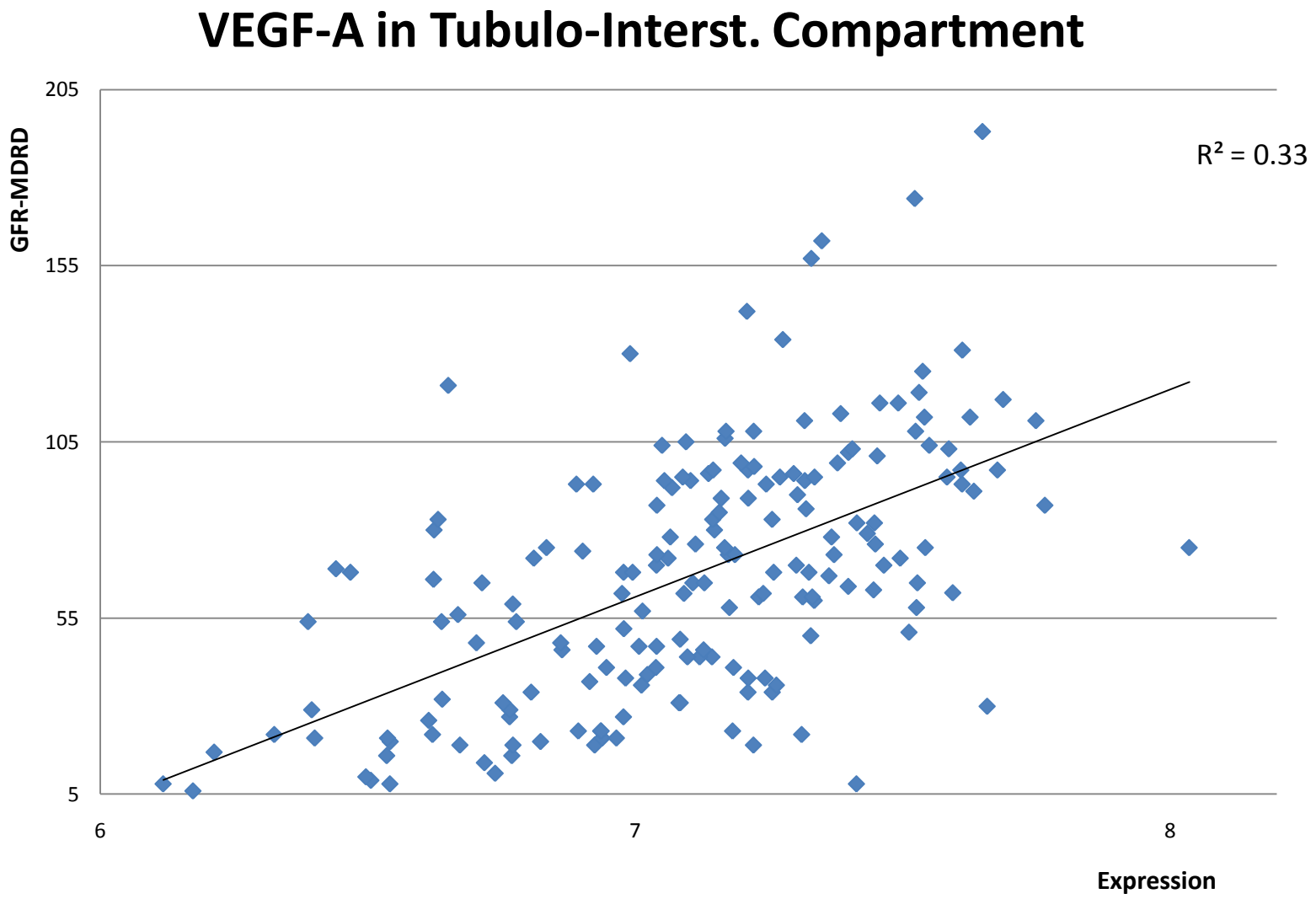


Genename	GFR r	GFR r
SLC34A1	0.44	0.52
ANXA9	0.38	0.54
NAT8B	0.37	0.53
DACH1	0.33	0.14
DAB2	0.28	0.35
SLC7A9	0.28	0.45
VEGFA	0.28	0.59
UMOD	0.27	0.32
ACSM5(FLJ20581)	0.25	0.46
PRUNE	0.25	0.24
NAT8	0.23	0.42
LASS2	0.22	0.37
GP2	0.17	0.32
TFDP2	0.17	0.52
ATXN2	0.12	0.06
ALMS1	0.06	-0.32
FBXO22	0.02	0.32
SETDB1	0.01	0.06
FAM63A	0.00	-0.19
WDR37	0.00	0.13
PRKAG2	-0.07	0.24
GCKR	-0.09	0.21
PIP5K1B	-0.10	0.28
LMAN2	-0.11	-0.37
RGS14	-0.13	-0.12
FNDC4	-0.15	0.12
F12	-0.19	-0.37
STC1	-0.19	0.14
GRK6	-0.20	-0.06
PRR7	-0.23	0.07

Correlation of transcripts with GFR in ERCB

FDR < 0.01
Correlation to GFR

Correlation of transcripts with GFR in ERCB



Combined transcriptomics/proteomics

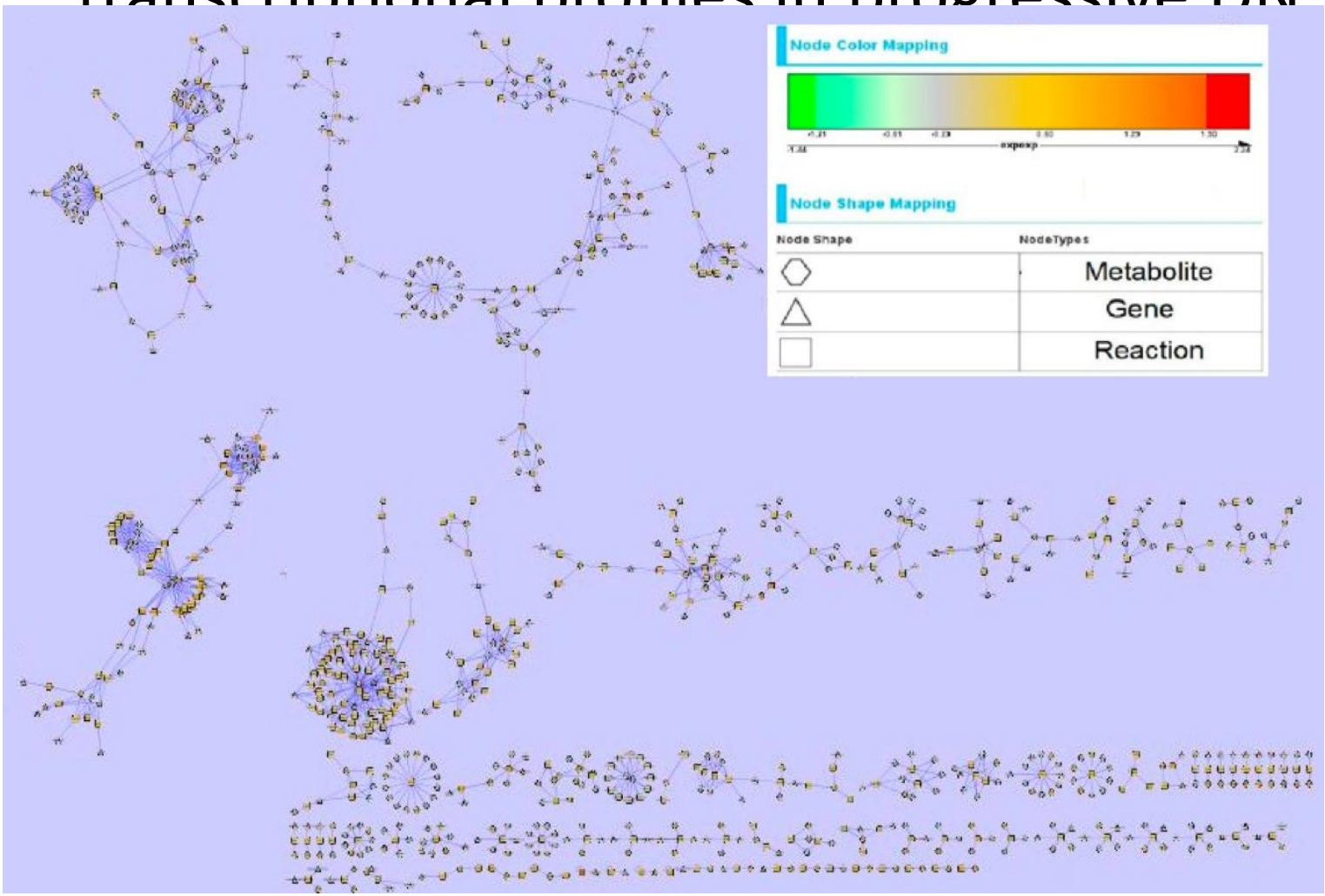
TABLE 2
Comparison of gene expression and mitochondrial protein abundance of selected OXPHOS and FAO proteins in liver, brain, heart, and kidney tissue obtained from wild-type and Akita mice, presented as fold change relative with wild type

	Gene	Protein
Liver		
Oxidative phosphorylation		
NADH dehydrogenase (ubiquinone) flavoprotein 1	1.29	1.37†
NADH dehydrogenase (ubiquinone) 1 α subcomplex, 9	1.51†	1.56†
Ubiquinol-cytochrome c reductase core protein 1	1.31†	1.10
Cytochrome c oxidase subunit IV isoform 1	1.30	1.32†
ATP synthase coupling factor 6 (ATPase subunit F6)	1.29	1.25
FAO		
Acyl-coenzyme A dehydrogenase, medium chain	1.52†	0.96
Acyl-coenzyme A dehydrogenase, long chain	1.48†	0.89
Acyl-coenzyme A acyltransferase 2	1.32†	0.75*
Brain		
Oxidative phosphorylation		
NADH dehydrogenase (ubiquinone) flavoprotein 1	1.07	0.99
NADH dehydrogenase (ubiquinone) 1 α subcomplex, 9	0.83	0.90
Ubiquinol-cytochrome c reductase core protein 1	0.94	0.99
Cytochrome c oxidase subunit IV isoform 1	0.95	1.01
ATP synthase coupling factor 6 (ATPase subunit F6)	0.93	1.16
FAO		
Acyl-coenzyme A dehydrogenase, medium chain	1.30†	1.12
Acyl-coenzyme A dehydrogenase, long chain	1.15†	1.25†
Acyl-coenzyme A acyltransferase 2	1.03	1.27†
Heart		
Oxidative phosphorylation		
NADH dehydrogenase (ubiquinone) flavoprotein 1	0.79*	0.87
NADH dehydrogenase (ubiquinone) 1 α subcomplex, 9	0.76*	0.93
Ubiquinol-cytochrome c reductase core protein 1	0.84*	0.88
Cytochrome c oxidase subunit IV isoform 1	0.88	1.11
ATP synthase coupling factor 6 (ATPase subunit F6)	0.52*	1.04
FAO		
Acyl-coenzyme A dehydrogenase, medium chain	0.71*	1.03
Acyl-coenzyme A dehydrogenase, long chain	0.90	1.14†
Acyl-coenzyme A acyltransferase 2	1.17	1.41†
Kidney		
Oxidative phosphorylation		
NADH dehydrogenase (ubiquinone) flavoprotein 1	1.04	1.09
NADH dehydrogenase (ubiquinone) 1 α subcomplex, 9	1.04	1.01
Ubiquinol-cytochrome c reductase core protein 1	0.93	1.05
Cytochrome c oxidase subunit IV isoform 1	0.99	1.09
ATP synthase coupling factor 6 (ATPase subunit F6)	1.15	1.03
FAO		
Acyl-coenzyme A dehydrogenase, medium chain	0.96	0.94
Acyl-coenzyme A dehydrogenase, long chain	0.96	1.18†
Acyl-coenzyme A acyltransferase 2	1.26	1.69†

*Significantly downregulated gene/protein. †Significantly upregulated gene/protein. Symbols indicate a significant difference ($P < 0.05$) compared with wild type.

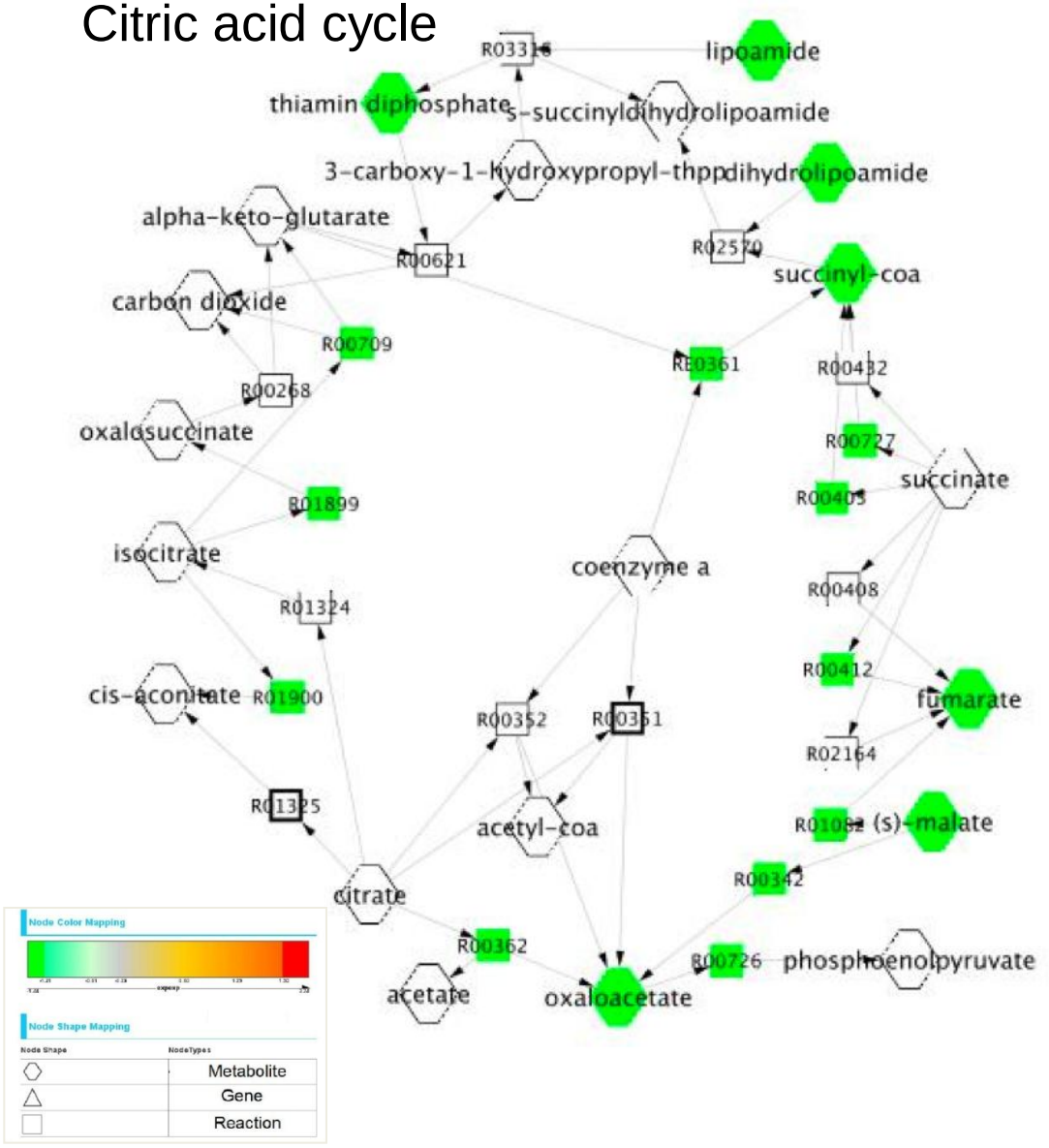
Bugger et al., Diabetes *58:*1986–1997, 2009

Combined transcriptomics/metabolomics: Mapping metabolite pathways from transcriptional profiles in progressive DN



Genome scale human metabolic network reconstruction was used for human- specific metabolic reactions. Progressive DN-associated transcripts (FDR <1%) were mapped into Entrez Gene for identification in *H. sapiens* Recon1.

Citric acid cycle



METSCAPE:

Mapping metabolite pathways from transcriptional profiles of progressive DN

Concordant transcriptional regulation of key metabolic pathways

=> Experimental validation and iterative optimization

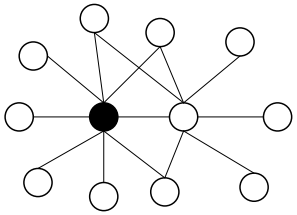
=> Definition of underlying transcriptional regulation

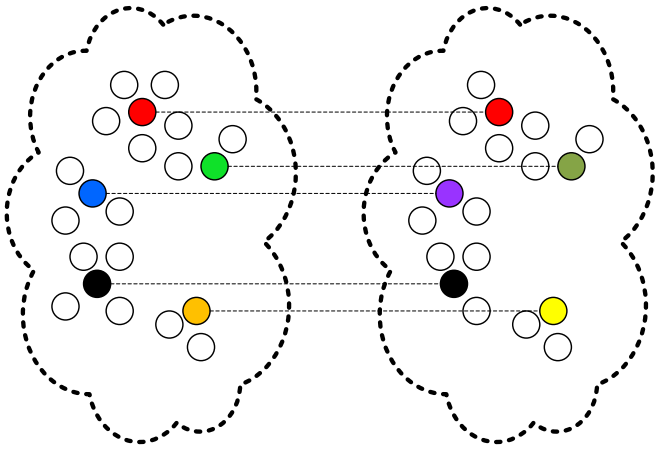
Metscape based pathway specific network of specific reactions related to citric acid cycle flux and shows a repression of 11 of 22 modeled reactions in progressive DN

Cross-species and cross-strain ‘omics: Defining transcriptional responses shared between human and mouse DN on a network level

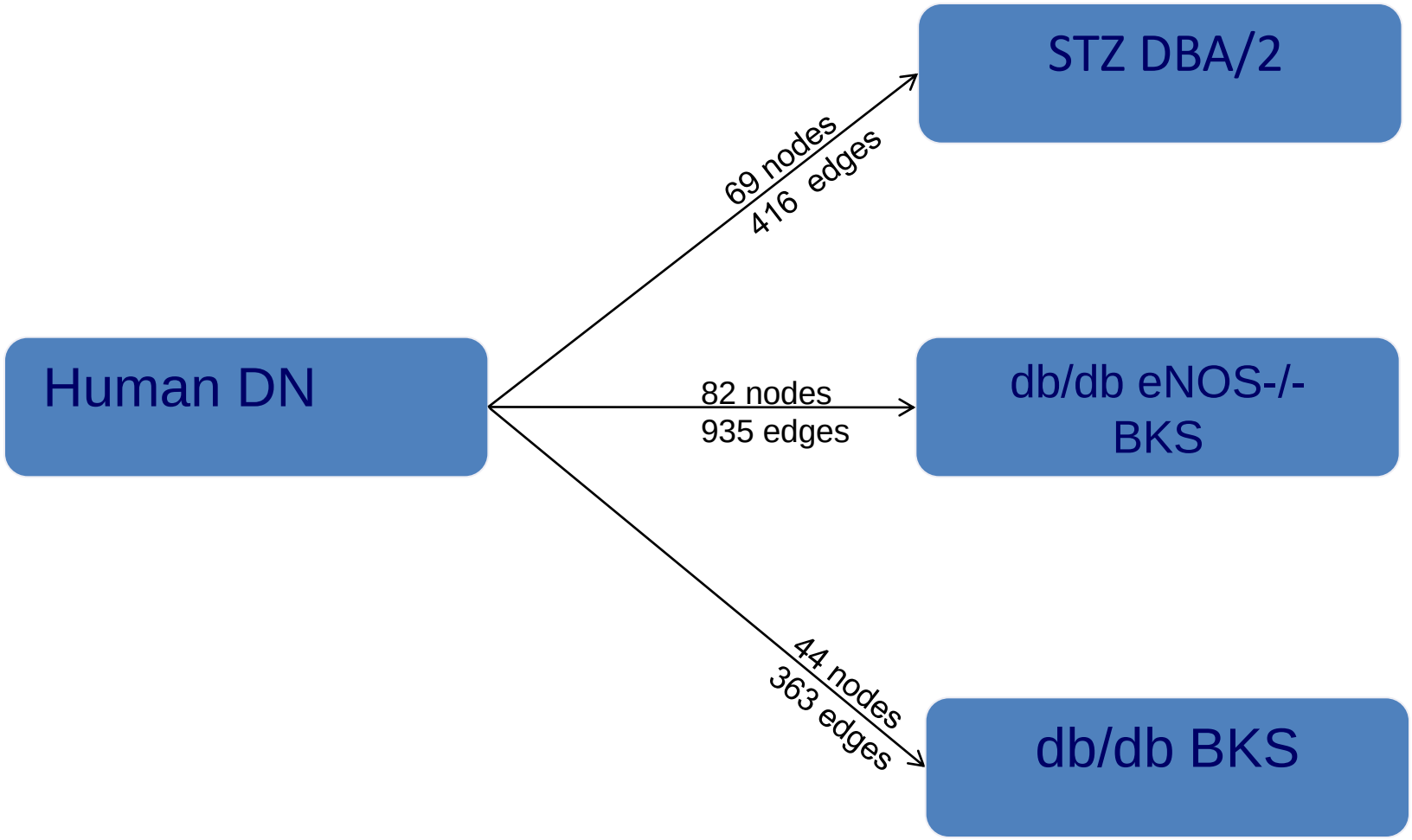
- Compare complex regulatory networks for conserved network structures
  - Subopitmal graph matching tools required

- TALE Indexing Technique (Y.Tian et al., 2008)
  - Index neighborhoods of nodes which characterize the local graph structure around each node
  - An hybrid index structure for efficient search of matching database neighborhoods
- The Novel Matching Paradigm
  - Distinguish nodes by their relative importance in the graph structure
  - Match the important nodes in the query graph
  - Extend the matches progressively by enclosing nearby nodes of already matched nodes



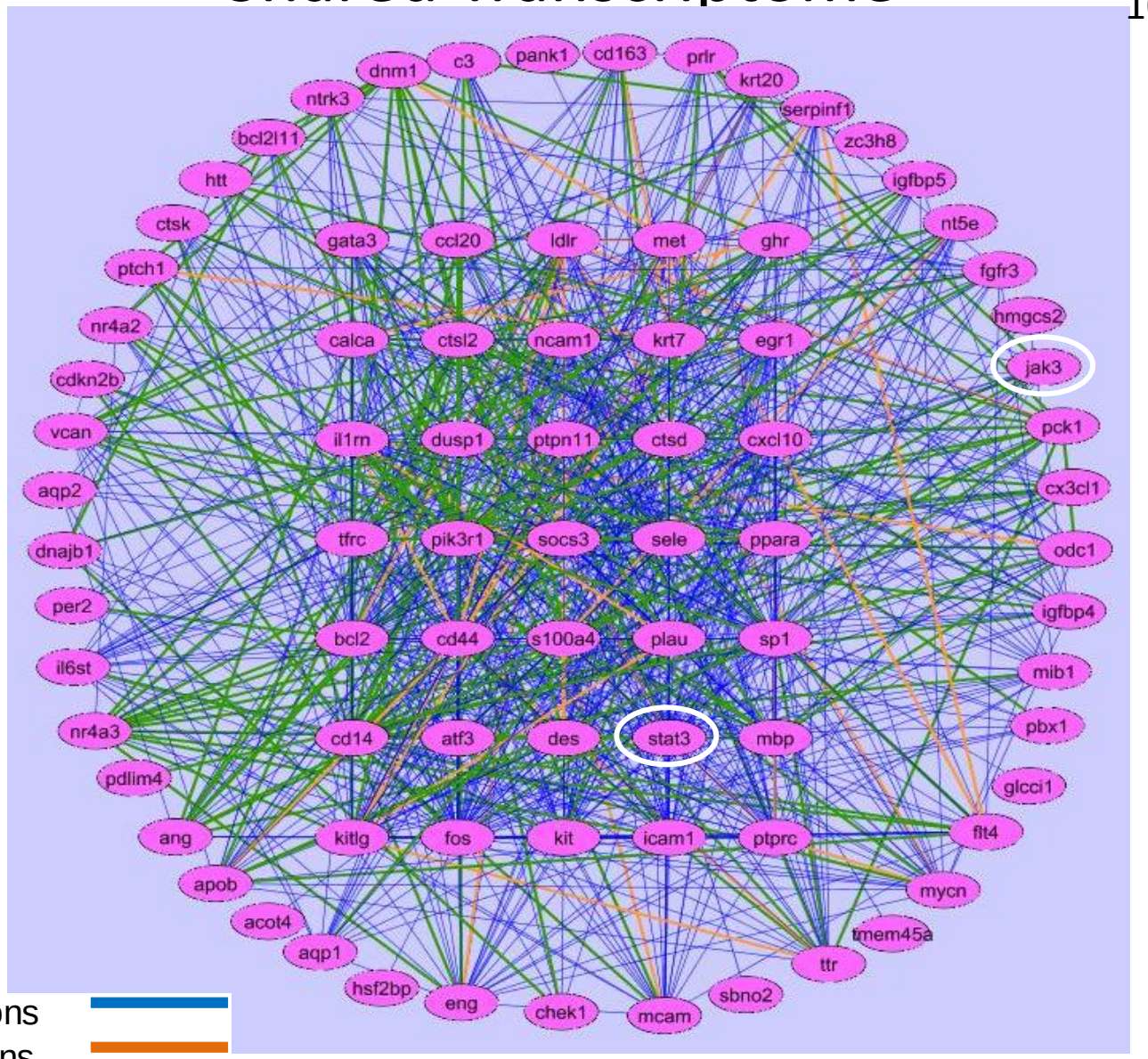


TALE-overlay



TALE: BKS db/db eNOS -/- and Human DN Shared Transcriptome

15% - imp. Nodes
10 % - mismatch

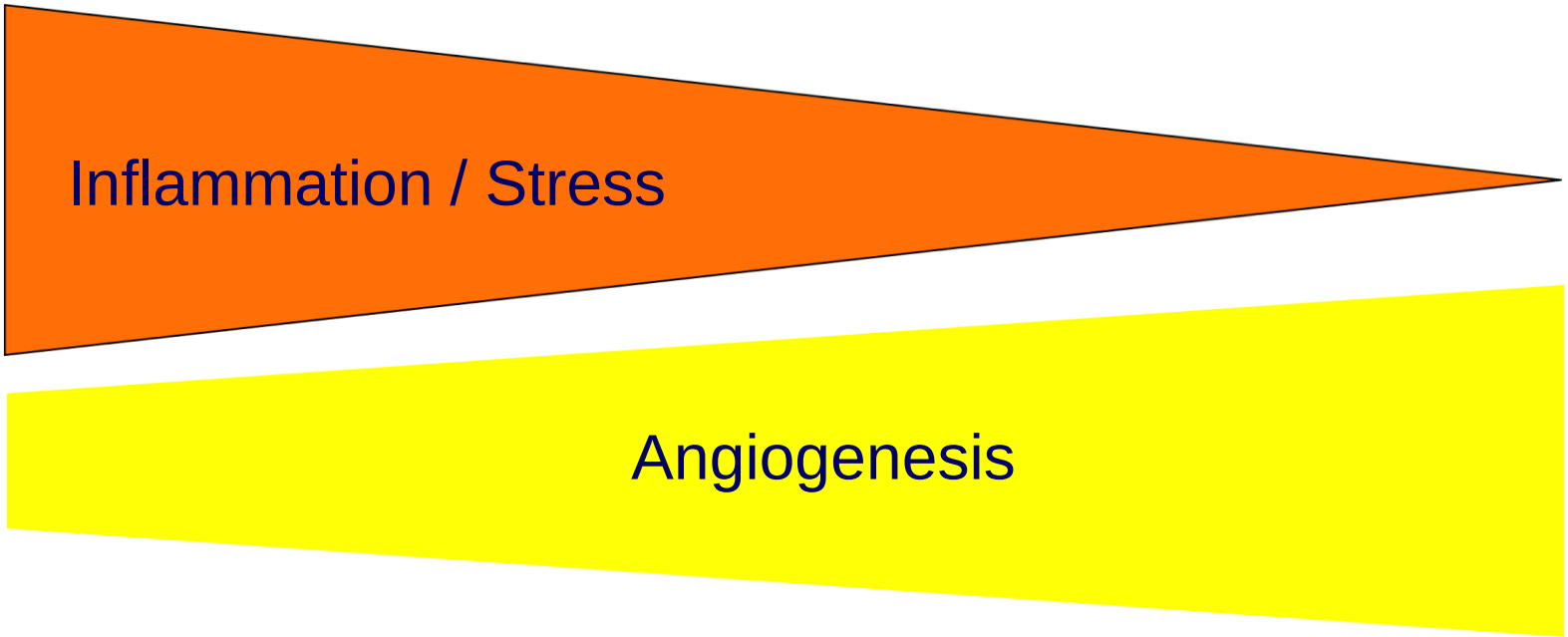


shared interactions
human interactions
murine interactions

Functional networks enriched

The top Biological processes enriched in the shared network .(Zscore>4.0)

BKS db/db eNOS -/-	BKS db/db	STZ DBA/2
Hum-eNOS-/-	**Hum-Db/db**	**Hum-STZ**
response to external stimulus	blood vessel development	blood vessel development
positive regulation of MAP kinase activity	vasculature development	vasculature development
cytokine-mediated signaling pathway	transmembrane receptor protein tyrosine kinase signaling pathway	enzyme linked receptor protein signaling pathway
response to wounding	angiogenesis	transmembrane receptor protein tyrosine kinase signaling pathway
response to stress	positive regulation of cellular metabolic process	angiogenesis
inflammatory response	cell surface receptor linked signal transduction	
transmembrane receptor protein tyrosine kinase signaling pathway	inflammatory response	
regulation of MAP kinase activity		



Current State

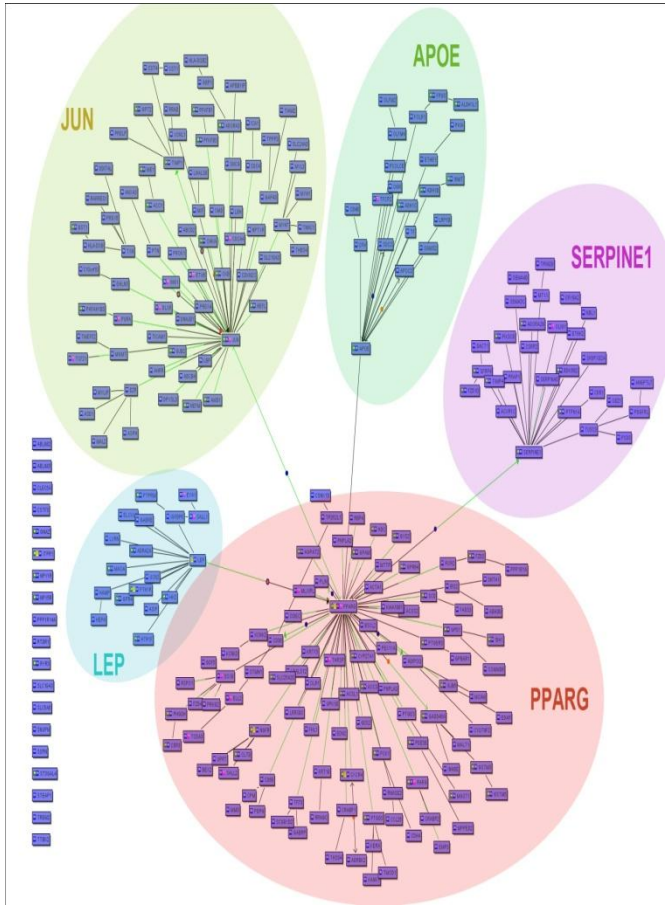
- Neuropathy
- Cardiomyopathy
- Atherosclerosis
- Retinopathy
- Nephropathy

Current State: Neuropathy

- Transcriptomics:
  - Human: Functional enrichment analyses identified "lipid metabolism" and "inflammation" over-represented in the DEGs in humans with progressive DPN vs. nonprogressors.
  - Mouse: ~4,000 DEGs in diabetic mouse sciatic nerve, which are enriched with 'lipid metabolism', 'oxidative stress', 'mitochondrion', 'response to stress', 'apoptosis' and 'axonogenesis' functions.
  - Regulatory network analysis suggests increased lipid metabolism may lead to elevated mitochondrial oxidative stress thus contributing to DN progression.

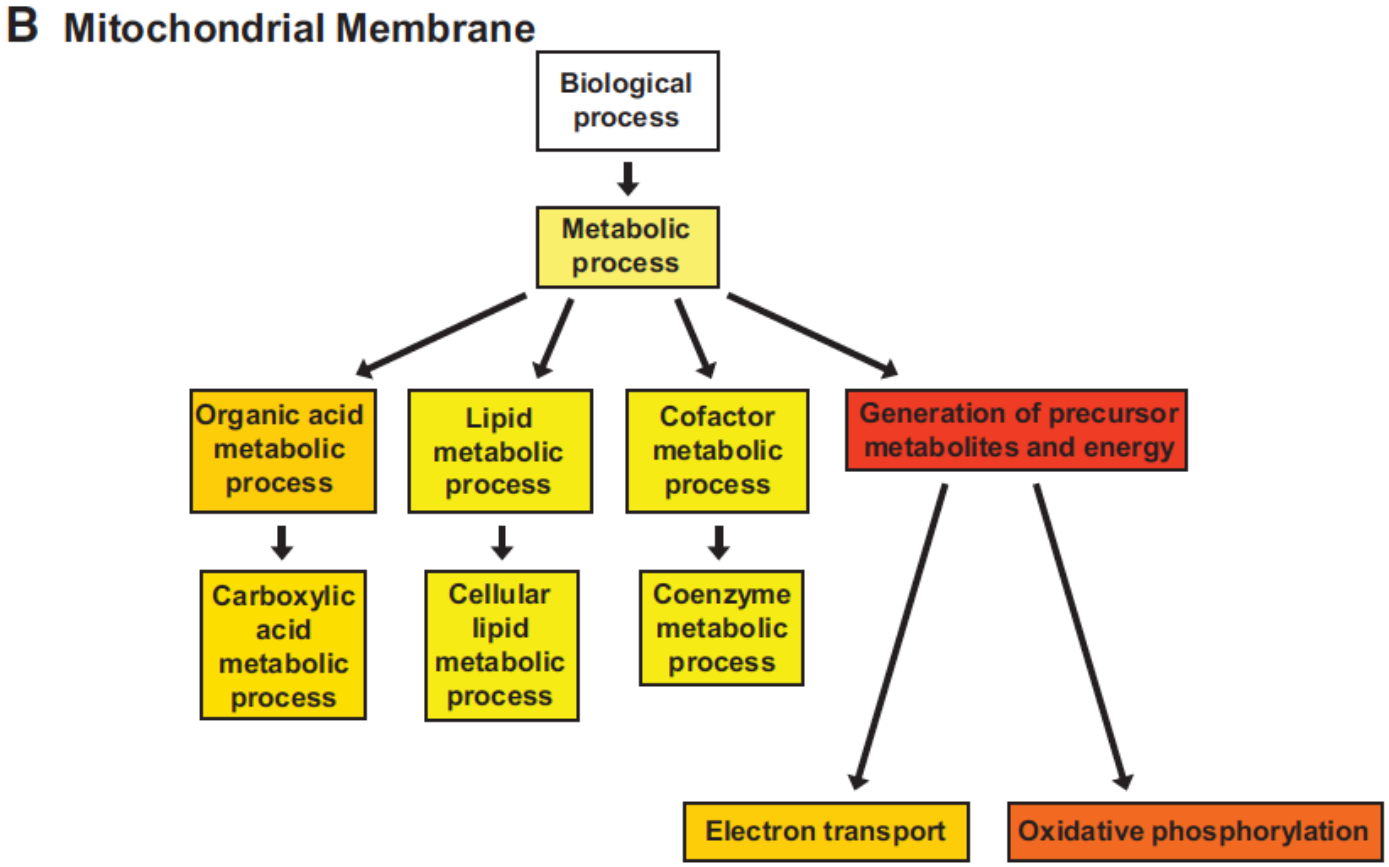
Progressive diabetic neuropathy

Genomatix BiblioSphere PathwayEdition
– Gene co-citation network (PubMed abstract) at sentence level



- **PPARG** (peroxisome proliferator-activated receptor gamma) – regulates glucose and lipid metabolism. Agonists of PPARG ameliorates DN and nephropathy in animal models
- **APOE** (apolipoprotein E) – regulates normal catabolism of triglycerides and cholesterol. Polymorphism is linked to diabetic nephropathy
- **LEP** (leptin) – controls appetites with LEPR. Mutations associated with type 2 diabetes
- **JUN** (c-Jun oncogene) – controls cell cycle and death
- **SERPINE1** (serpin peptidase inhibitor E Type 1) – regulates fibrinolysis. Elevated plasma level is associated with higher incidences of diabetes.

Current State: cardiomyopathy Mitochondrial proteomics



Bugger et al., Diabetes *58:*1986–1997, 2009

Current State: cardiomyopathy

•“Proteomics approaches highlighted the unique susceptibility of cardiac mitochondria to injury relative to other mitochondrial populations … [and] highlighted differences in the pathophysiology of mitochondrial dysfunction between type 1 and type 2 diabetes.” Dale Abel

•“the fact that proteomic changes do not reflect or predict actual metabolic flux rates in these tissues emphasizes the importance of using a systems biology approach including metabolite measurements (metabolomics) in combination with comparative
•proteomics to better inform the complex interaction of
•transcriptional and protein changes in the adaptation of
•mitochondria to diabetes.” Bugger et al., Diabetes *58:*1986–1997, 2009

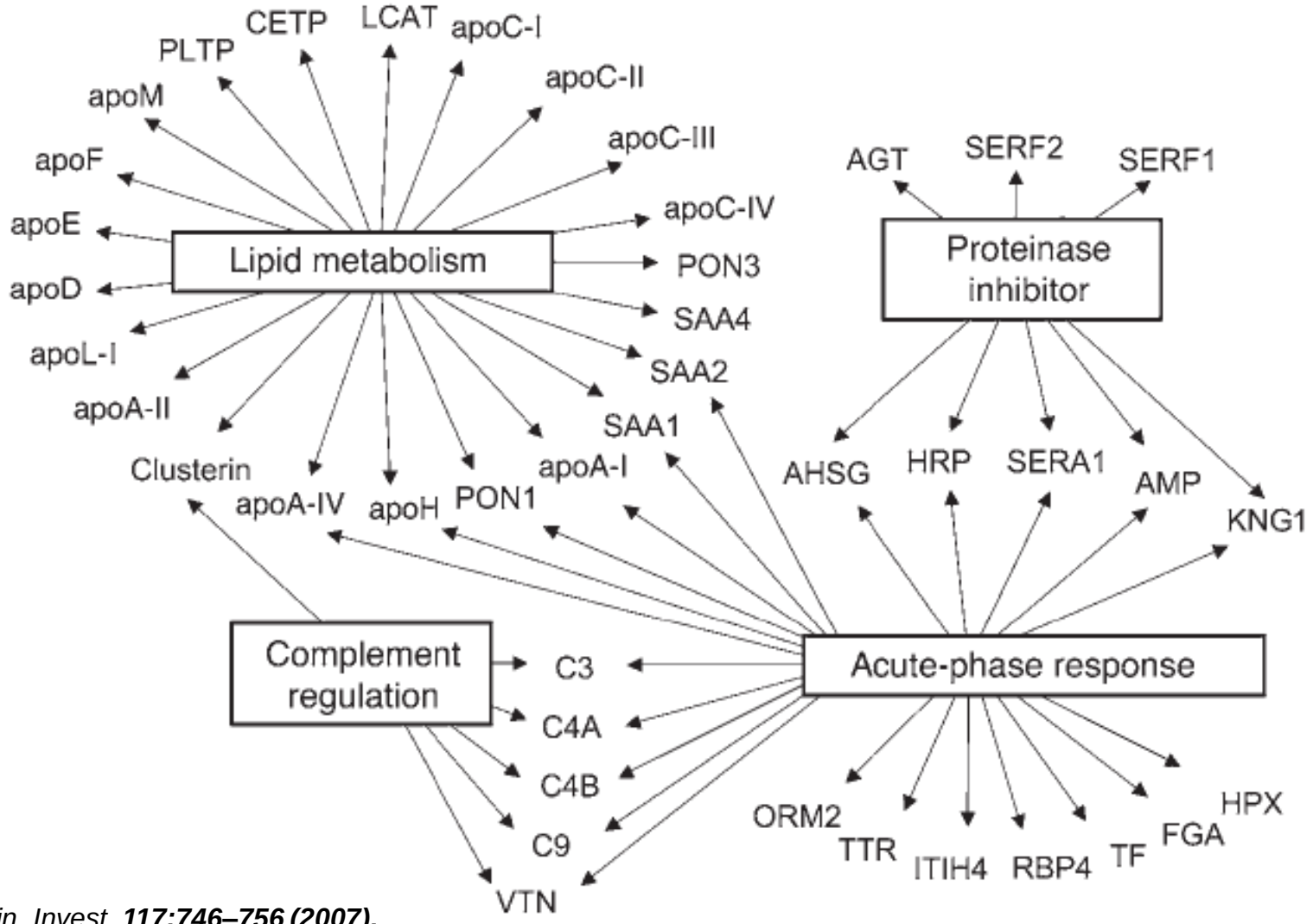
Current State: Athero diabetes

not done as far as I know. Jay Heinecke also has done some of this, but on macrophages without diabetes. Ira Goldberg

The basic answer is there is almost nothing on networks and diabetic atherosclerosis. I am attaching a recent review of network analysis of atherosclerosis (independent of diabetes). Richard Davis

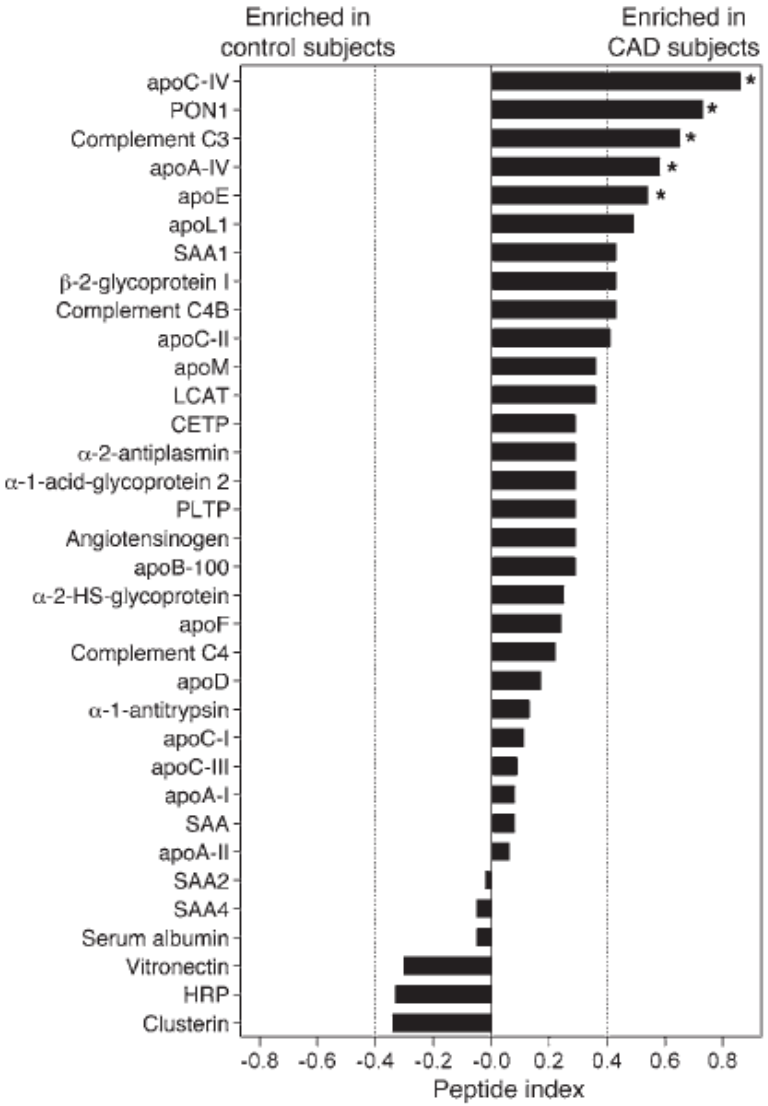
Doing a systems bio approach to changes in macrophages that are in plaques undergoing progression or regression. Our collaborators are at the Inst for Systems Biol, Seattle. We are planning to add an arm in which we make mice diabetic with STZ and test the effects of hyperglycemia on macs in progressing and regressing plaques, again using systems biol approaches. Basically, we use laser capture to isolate macs from plaques, then get the RNA and do microarrays. The systems folks then take the data and torture it with their algorithms." Ed Fisher

Current State: Athero HDL proteomics



J. Clin. Invest. ***117:746–756 (2007).***

Current State: Athero HDL proteomics



J. Clin. Invest. ***117:746–756 (2007).***

Current State: Retinopathy: Retinal vessel transcriptomics

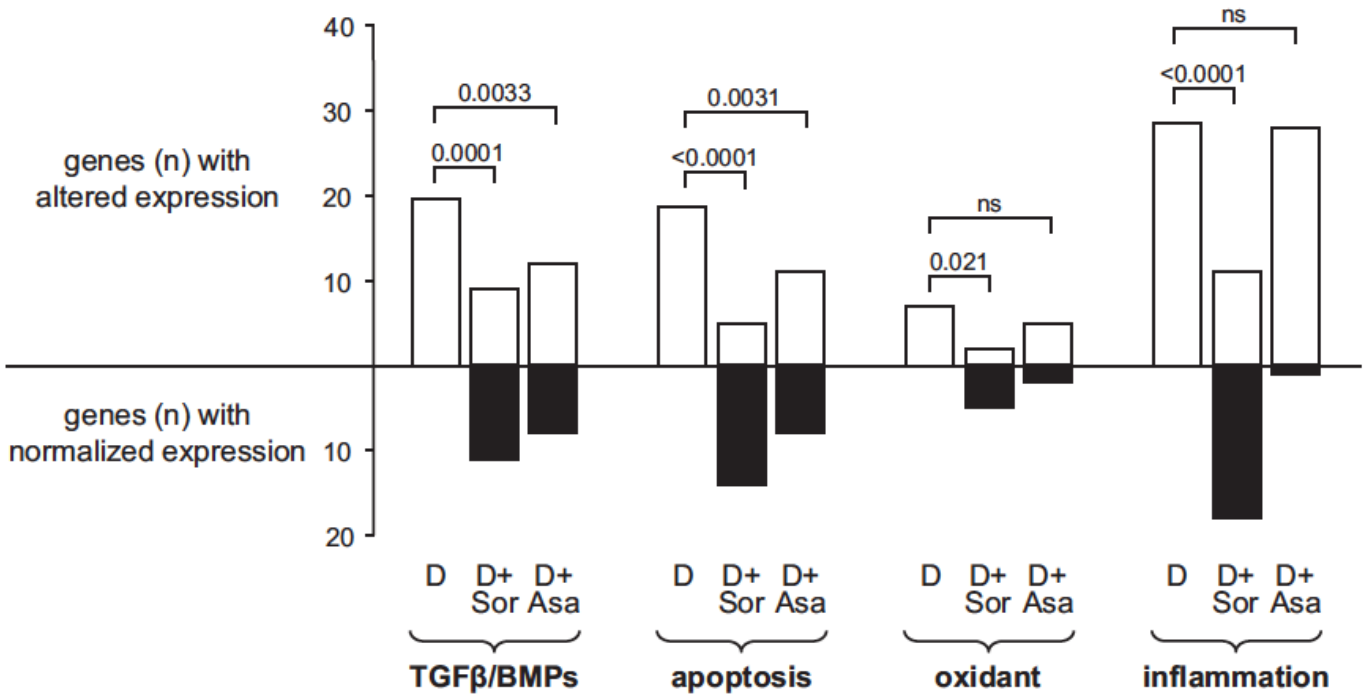
TABLE 2
Functional clustering of the known genes differentially expressed in retinal vessels in diabetes

Functional category and known function*	Upregulated	Downregulated
TGF-β/BMPs pathway		
20 genes		
TGF-β activation	16	—
BMP activation	3	1
Oxidative stress		
7 genes		
Pro-oxidant	3	—
Antioxidant	2	1
Pro-/antioxidant	1	—
Inflammation and response to injury		
29 genes		
Inflammatory mediators	5	—
Acute-phase response proteins	9	—
IFN-γ pathway	8	—
Antigen presentation	3	1
Anti-inflammatory	3	—
Matrix and vascular remodeling		
35 genes		
Matrix	4	1
Cell adhesion	7	1
Actin organization	3	2
Vascular remodeling	17	—
Cell cycle		
7 genes		
Cell cycle progression	1	2
Cell cycle arrest	1	1
Proliferation inhibition	2	—
Apoptosis		
19 genes		
Proapoptotic p53 pathway	5	2
Proapoptotic	7	1
Antiapoptotic	2	2
Other		
39 genes		
Metabolism	8	1
Miscellaneous†	6	2
Function unknown‡	15	7

*The 127 known genes differentially expressed in diabetes were assigned to functional categories based on review of information from NCBI (National Center for Biotechnology Information) Entrez Gene integrated with data from published literature. Individual genes are listed in all pertinent categories based on their known functions. The complete list of the genes included in each functional category is presented in supplemental Tables 2–8. †Includes genes related to functions that cannot be assigned to any of the major categories; ‡as stated in bibliographical sources.

***Diabetes* 58:1659–1667, 2009**

Current State: Retinopathy: Retinal vessel transcriptomics



Diabetes **58:1659–1667, 2009**

Current State: Retinopathy: Retinal proteomics in response to ARB

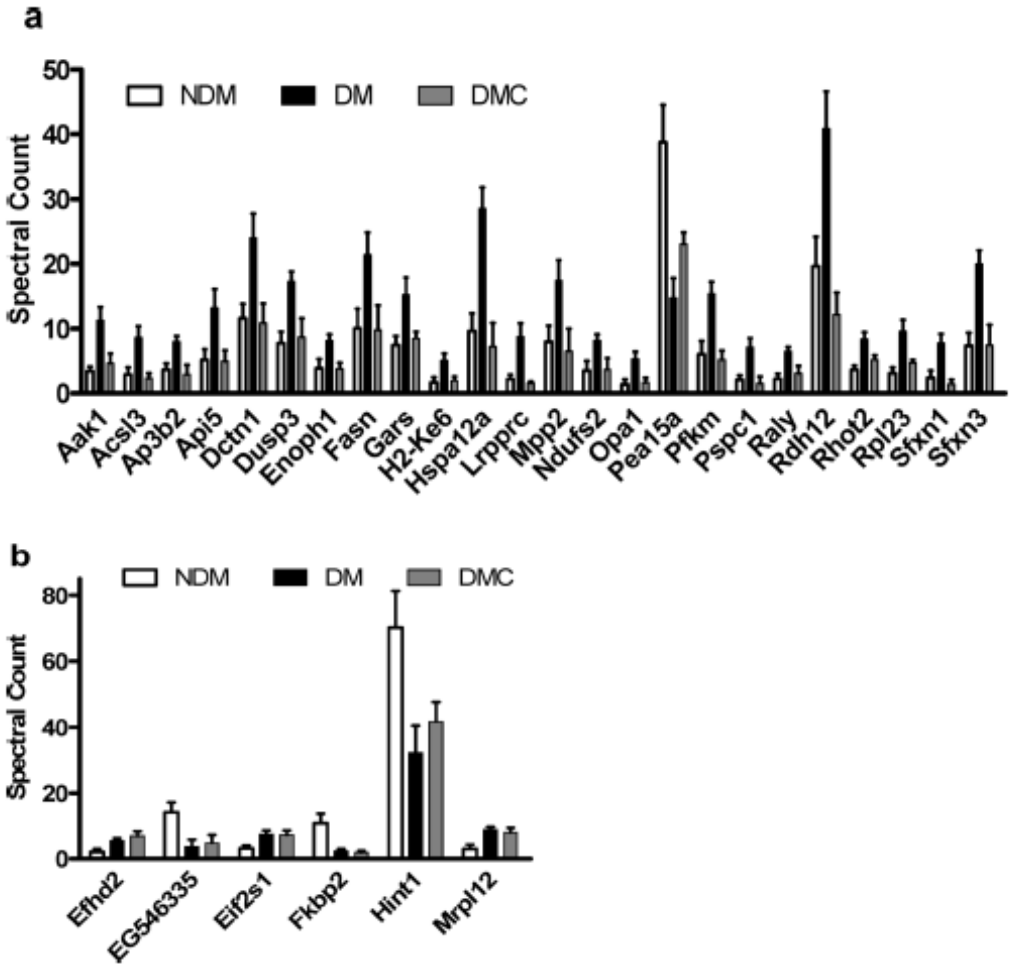
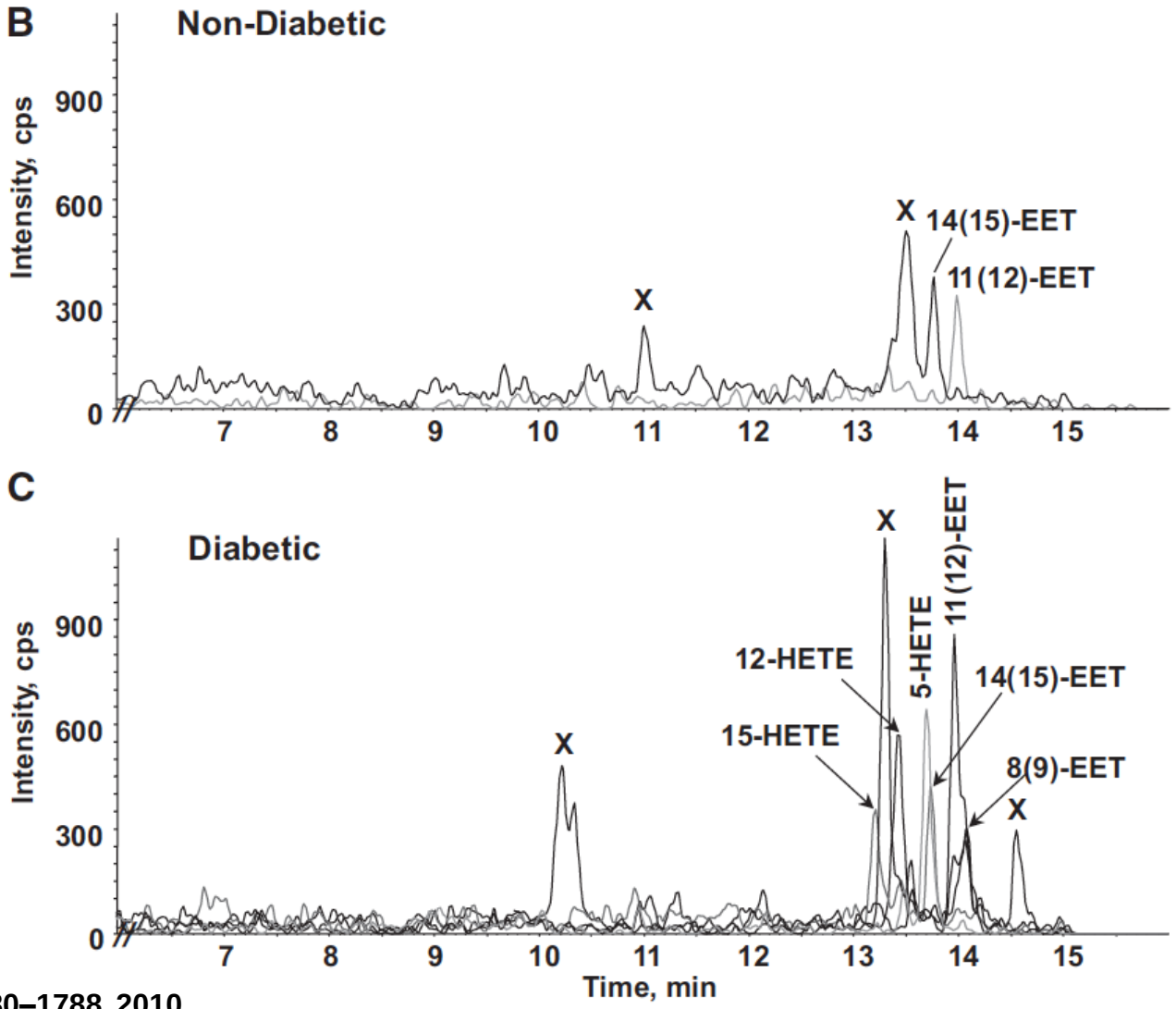


Figure 4. Effect of candesartan on proteins in diabetic mouse retina. Diabetic mice (DM), nondiabetic mice (NDM), and diabetic mice treated with candesartan (DMC). Twenty-four proteins were normalized by the treatment of candesartan (a) ($P < 0.05$, NDM vs DM and DM vs DMC) and 6 proteins were not reversed by the treatment of candesartan (b) ($P < 0.05$, NDM vs DM and NDM vs DMC). Error bars represent standard error of the mean (S.E.M). $n = 5$ mice.

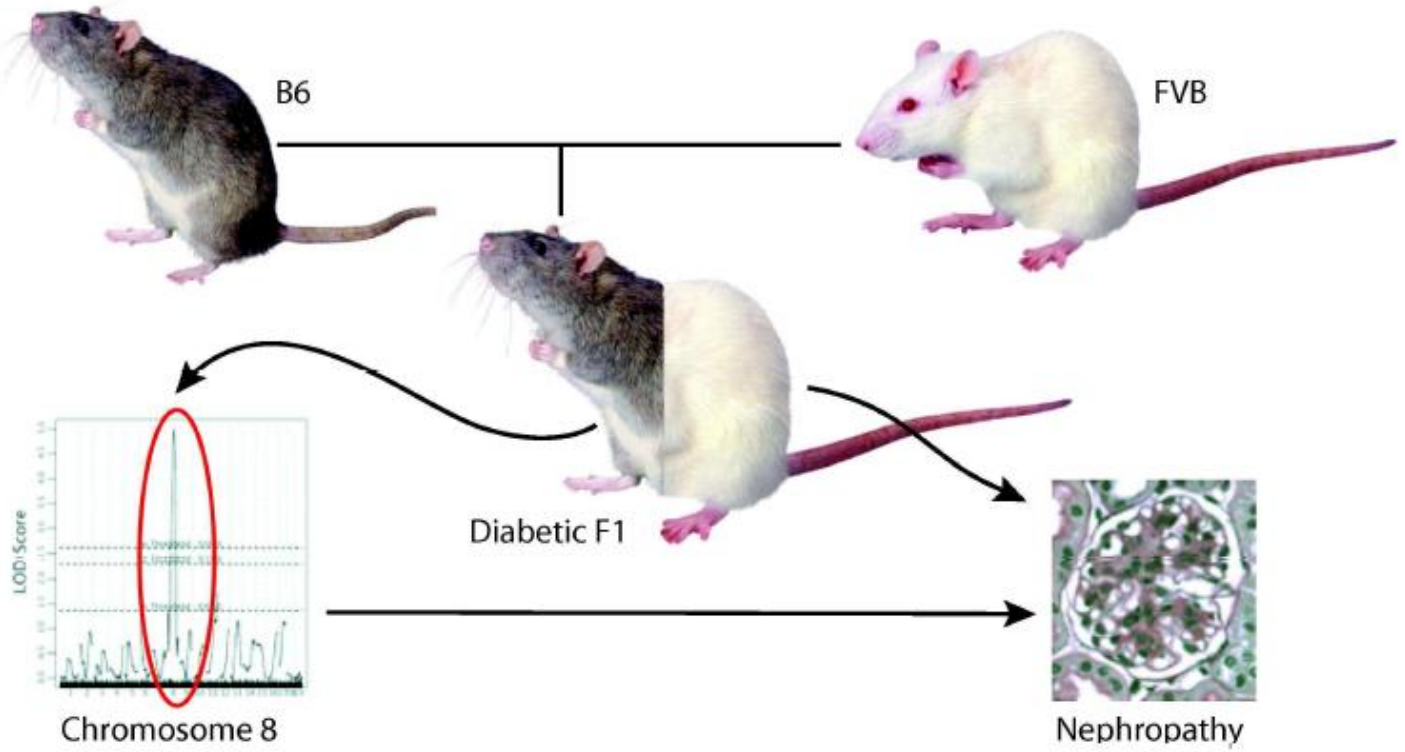
Journal of Proteome Research ***2009, 8, 5541–5549***

Current State: Retinopathy
vitreous lipidomics correlates with progression



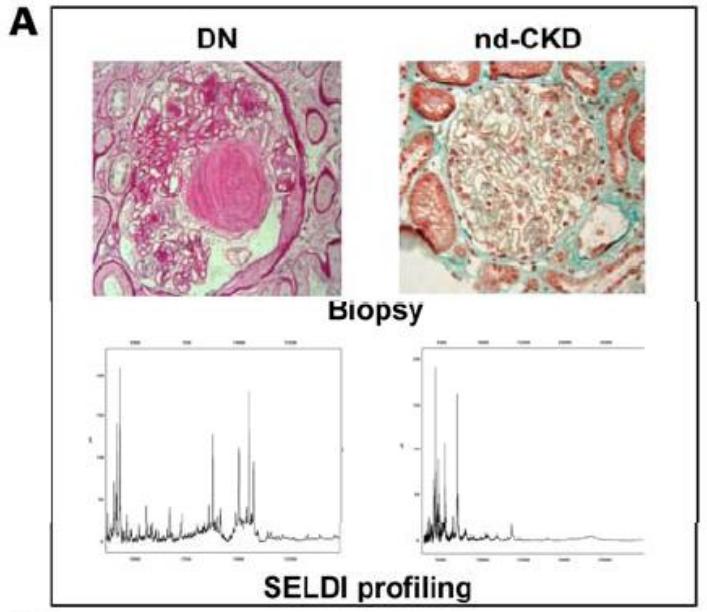
Diabetes 59:1780–1788, 2010

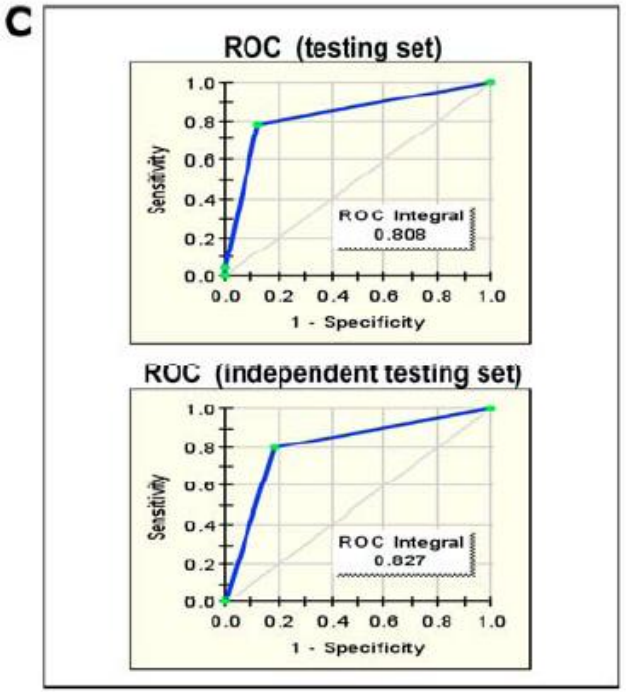
Current State: Nephropathy
GWA mapping and GWE
in Mouse strain F1s to identify DN locus



Chua, et al. Kidney Int. 2010 Jun 2

Current State: Nephropathy
Urine proteome as biomarker for progression





Diabetes Care. 2010 Jul 29. [Epub ahead of print]

Themes

1. Much opportunity for systems approaches to diabetic CV complications

2. GWAS, transcriptomics can be used to predict changes in function. New functional associations can also be predicted. These need to be verified as not all are borne out and the directionality of effect can be opposite.

3. Targeted or focused proteomics, metabolomics and lipidomics are likely to give the most direct indication of underlying functional alterations and are most likely to be the best biomarkers in urine and plasma (but mRNAs…).

1. Human-model organism systems biology comparisons are fruitful.

Questions to answer for report

1. What is the current status of the area in terms of basic, translational and clinical research with regards to all diabetic complications?
    - Has there been any therapeutic success coming from the system biology approach ?
        - The issue becomes understanding the end points for success of treatment. Could be most useful as means to generate biomarkers for surrogates of disease end points.

2. What is the potential impact of these studies on the field of complications research? Is there sufficient and meaningful significance to the idea/approach to move the field of complications research forward?
    - Identifying potential biomarkers, understanding major pathways affected.
    - Identify targets for intervention

3. Are there technical or scientific barriers preventing progress in the area? **How would one perform cross-complications analysis?**
    a. Longitudinal studies will be needed for biomarker /system biology analysis to develop markers that not only predict disease but also predict therapeutic outcome. Combined clinical/translational approaches to tissue specific complications.
    b. With large studies should try to capture as many complication specific tissues as you can—both models and human.
    c. Animal models would be useful for those complications that lend themselves to isolation of tissues (e.g. bladder or nerves).
        1. One issue with mice is one never knows how disease progression is affected by genetic manipulations given in the human not all individuals progress to nephropathy.

        2. Should use multiple mouse strains longitudinal design given one end point (ACR) may change based on disease progression and may not be useful as a disease progression indicator depending on the time being evaluated.

    d. Using tissues for biomarker analysis/system biology analysis will require specific annotation for complication specific phenotyping in order to correlate with disease etiology.
        Biopsy of tissues in the clinical trials would be important to advance the field. Consider autopsy specimens of trial patients.

4. Are there clear objectives that can be outlined for this area which, if achieved, will enhance our understanding and treatment of complications? Are there existing human samples or resources with DNA analysis, omics, histology and pathology that could be utilized to help achieve these goals?
    1. Existing human resourced: DCCT- EDIC, GoKIND, FIND for example. Some have serial collections and are continuing collection.
    2. Potentially doing a time course study with mouse model of specific complications collection tissues/samples during disease progression followed by Omic type of studies to look for predictive markers. Will need human tissue to validate mouse observations.

5. Can this area identify new circulating blood and urine biomarkers? Where would you put resources?

    Attention should be to metabolomics given the functional nature of the data. Also, proteomics since well worked out.

6. Can existing NIH resources be leveraged?

    Increase the bioinformatics infrastructure to help with cross-platform, cross-species analysis.

7. Can this area of research assist our understanding of all diabetic complication? yes.