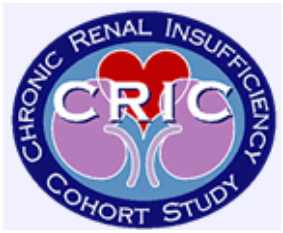
Driving Biological Problem:
Systems Biology of Diabetic Complications



Matthias Kretzler / Eva Feldman

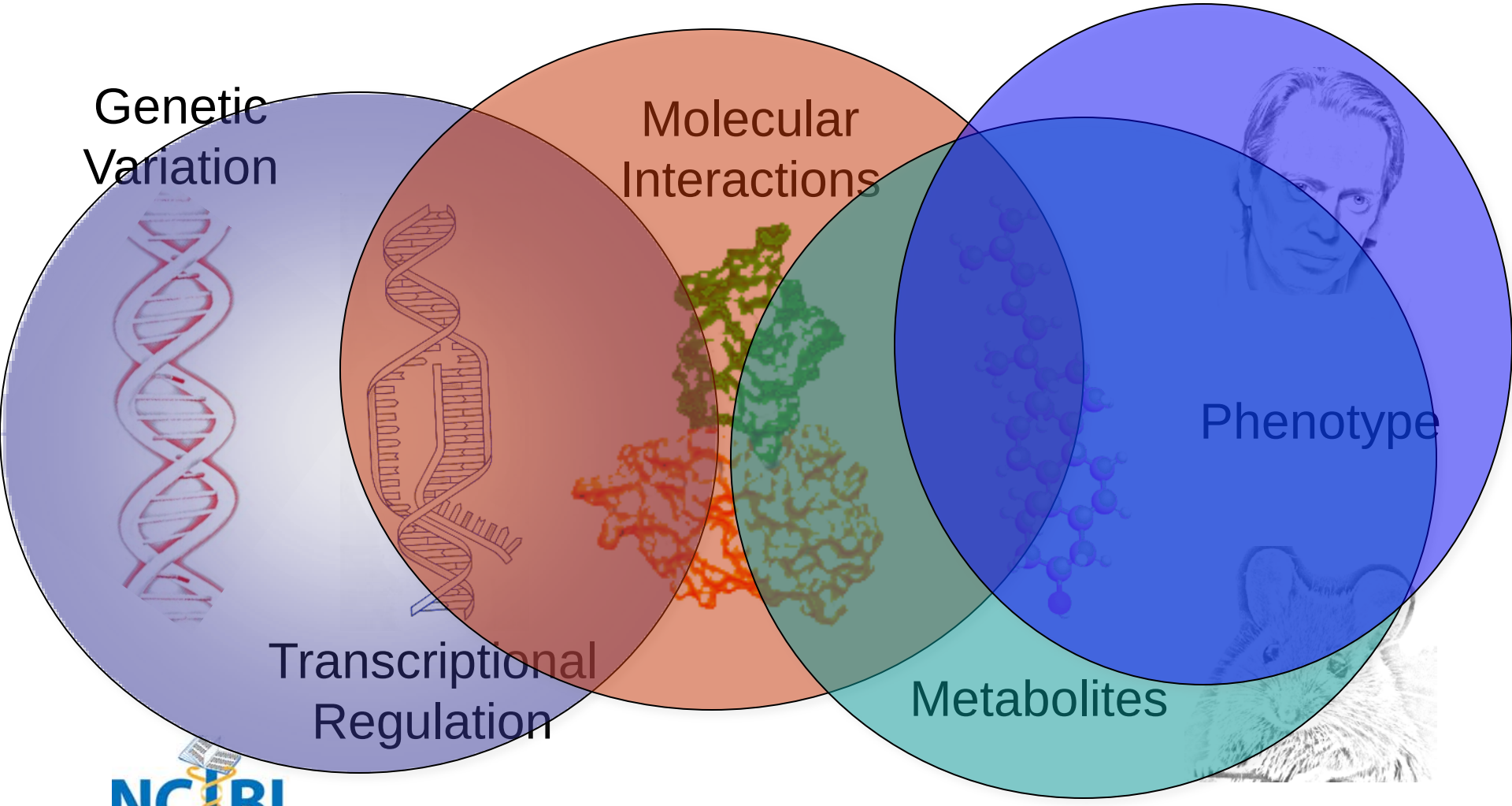
Internal Medicine / Neurology

Center for Computational Medicine and Biology

Multi-level analysis of a system disease: Our unique opportunity in diabetic endorgan damage

- Diabetes as a disease process affecting the patients on a molecular, cellular, tissue and organismal level
- Matched by multi-level research at Michigan:
  - Integration across tissues:
    - Shared response pattern to Diabetes
      - Diabetic nephropathy (DN)
      - Diabetic peripheral neuropathy (DPN)
  - Integration across species:
    - Shared responses between mouse and man
      - Human early and progressive DN and DPN biopsies
      - Murine STZ-, db/db-, Chip- DN/DPN mouse models
  - Integration along functional cascade:
    - High resolution clinical and structural phenotypes (structured follow-up >40 years)
    - GWAS, mRNA, uRNA, Proteome, Metabolites in tissue, plasma and urine
  - Integration of response to therapeutic interventions:
    - Shared response pattern to treatment intervention in human and murine systems
      - Renin-Angiotensin, TZD, …

Analytical Hierarchy

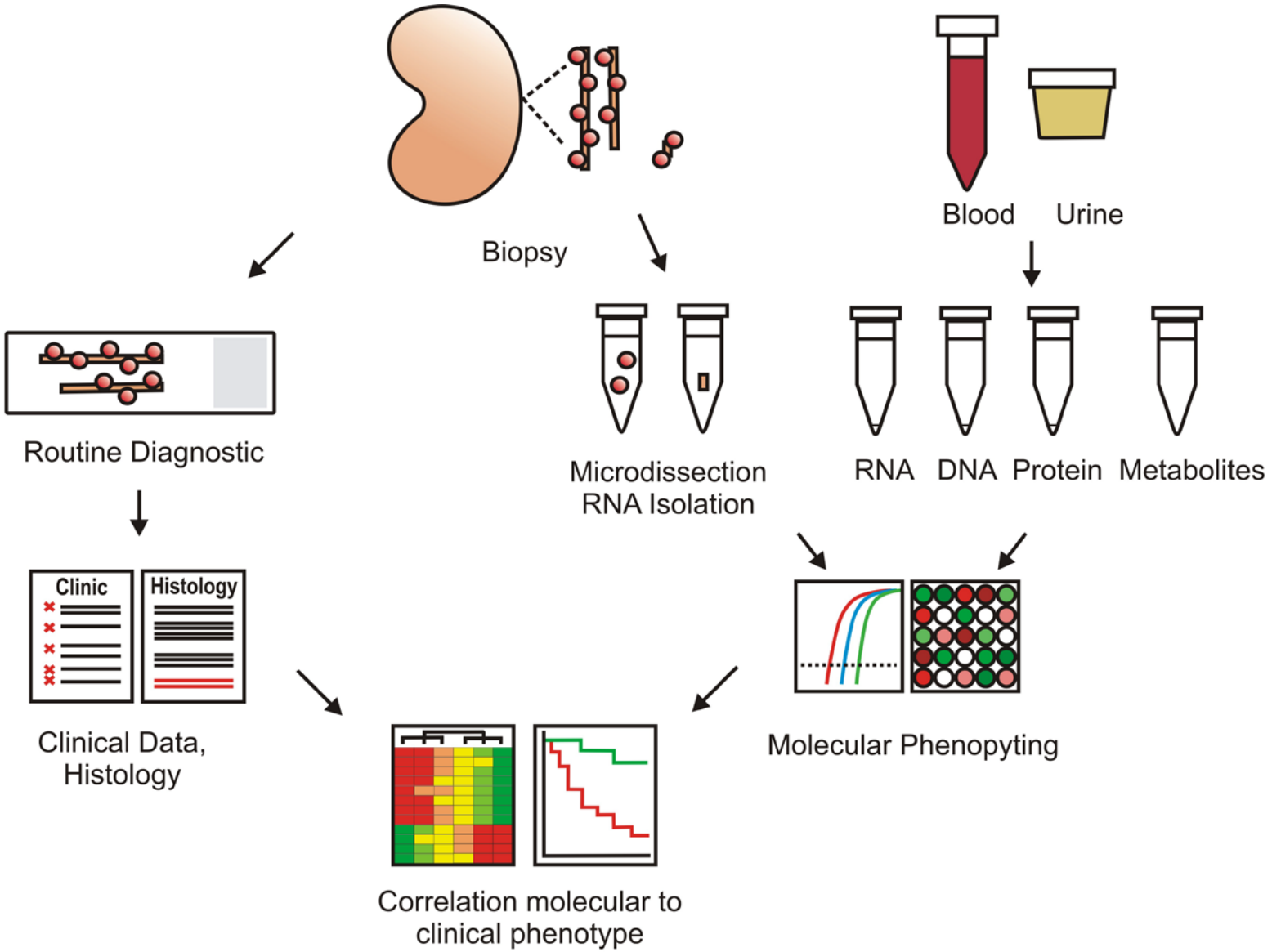


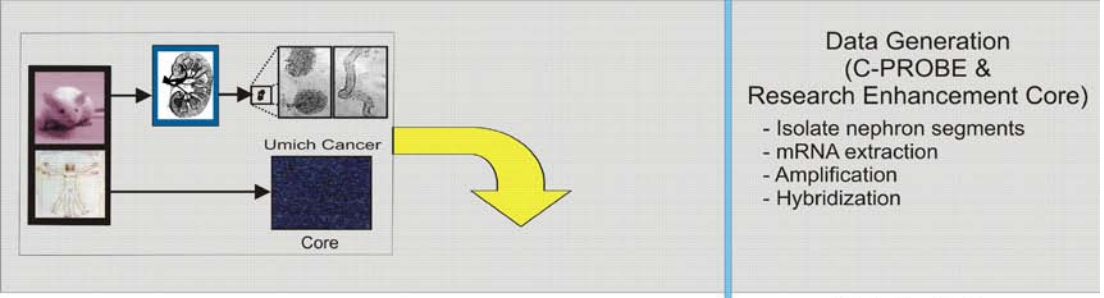
Gene-expression map of DN and DPN

- Human renal biopsy consortium
  - Micro-dissected renal biopsies
  - Linear amplification and hybridization to Affymetrix chips HG_U133
- Diabetic Nephropathy
  - Early DN (II-III) protocol biopsies (n=66)
  - Prog. DN (III-IV) indication biopsies (n=23)
- Reference samples (n=182)
  - Living related donor pretransplant-biopsies (LD, n=27)
  - Cadaver donor pretransplant-biopsies (CD, n=4)
  - Tumor nephrectomies (TN, n=5)
  - Thin membrane disease (TMD, n=5)
  - Minimal change disease (MCD, n=12)
  - Hypertensive Nephropathy (HN, n=20)
  - IgA-Nephropathy (IgA, n=27)
  - Lupus-Nephritis (SLE, n=32)
  - Membranous Nephropathy (MGN, n=17)
  - FSGS (FSGS, n=10)

- Human sural nerve biopsies
  - ranked by Myelin Fiber Density
  - Progressor group (n=18):
    - MFD $\geq$ 500 fibers/mm^2
  - Non-progressor group (n=18):
    - MFD $\leq$ 100 fibers/mm^2

Study layout: Diabetic Nephropathy





Data Generation
(C-PROBE &
Research Enhancement Core)

- Isolate nephron segments
- mRNA extraction
- Amplification
- Hybridization

Data Analysis
(Applied Systems
Biology Core)

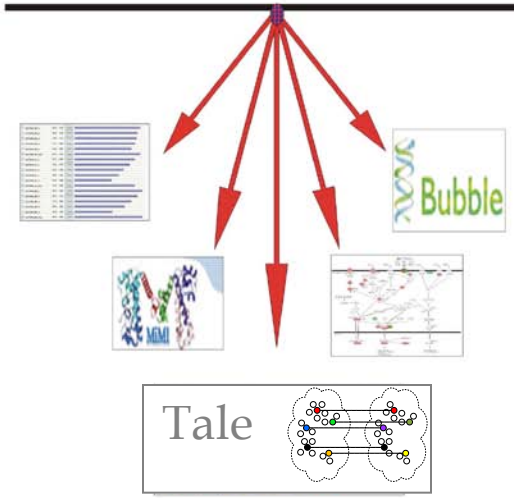
- Normalization of Affy Chips
- Upload into Expression Database

GenePattern

Parallel
documentation
&
communication
channel

Candidate selection

- Analysis in GenePattern pipeline
- Comparison to other murine datasets:
  same disease stage
- What elements are specific?



- Docs
- Log files
- Wiki
- Tutorials
- eMail archive
- Chat room

Integration into functional
context

*interactive data mining in the
SAKAI portal, using e.g:*

- GO:
  * categorization of regulated mRNAs
- MiMi:
  * molecular interaction analysis
- Genomatix Suite:
  * definition of transcriptional modules
  * prediction of gene regulation by
    promoter modeling
- Ingenuity:
  * integration into canonical pathways
- Bubble:
  * interactive construction of Bayesian
    Networks

U Mich Expression Database

↓

Data processing pipeline:
MIT / Broad
GenePattern pipeline

↓

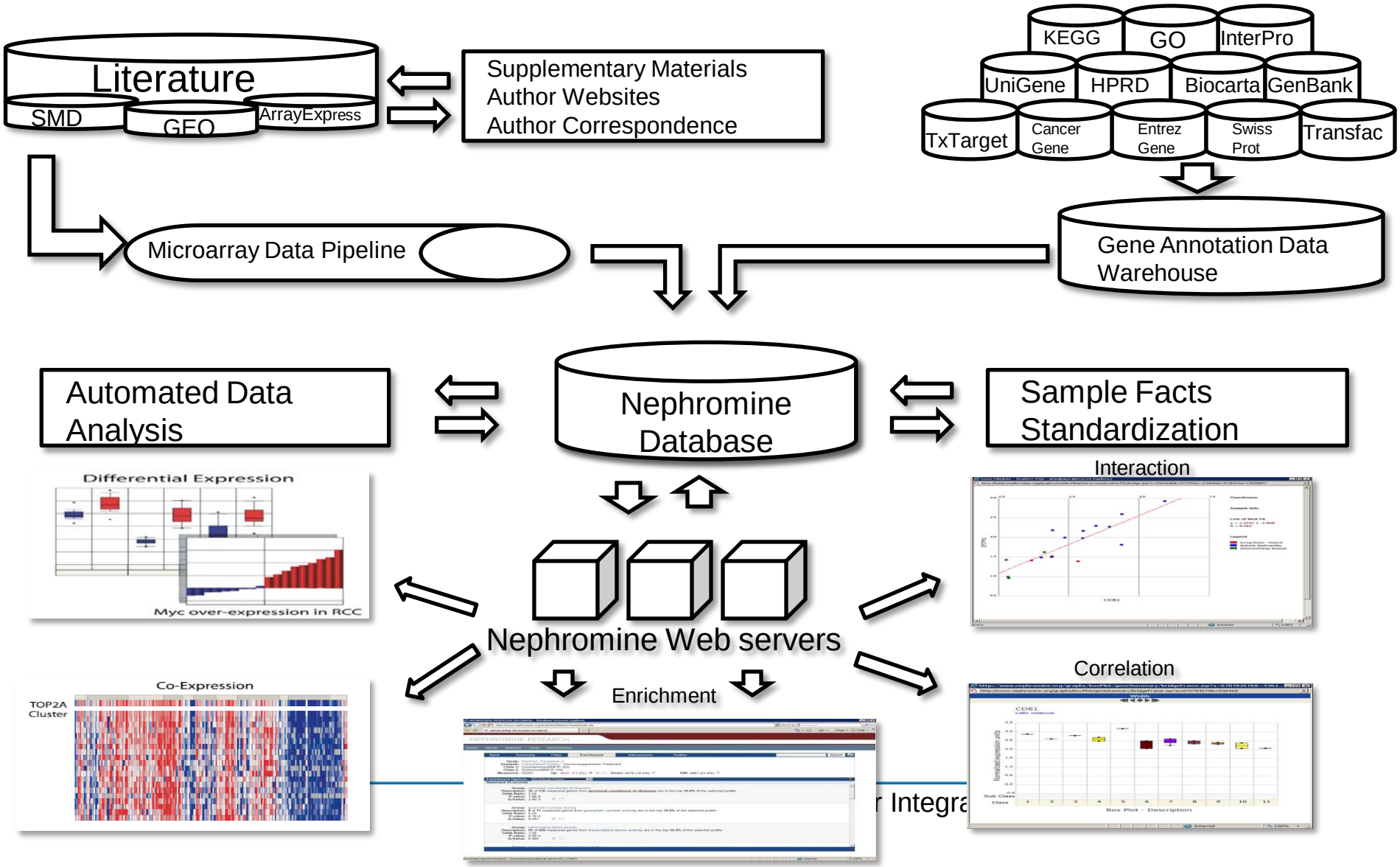
Data analysis pipeline:
NCIBI tool suite
in Sakai Portal based interface

↓

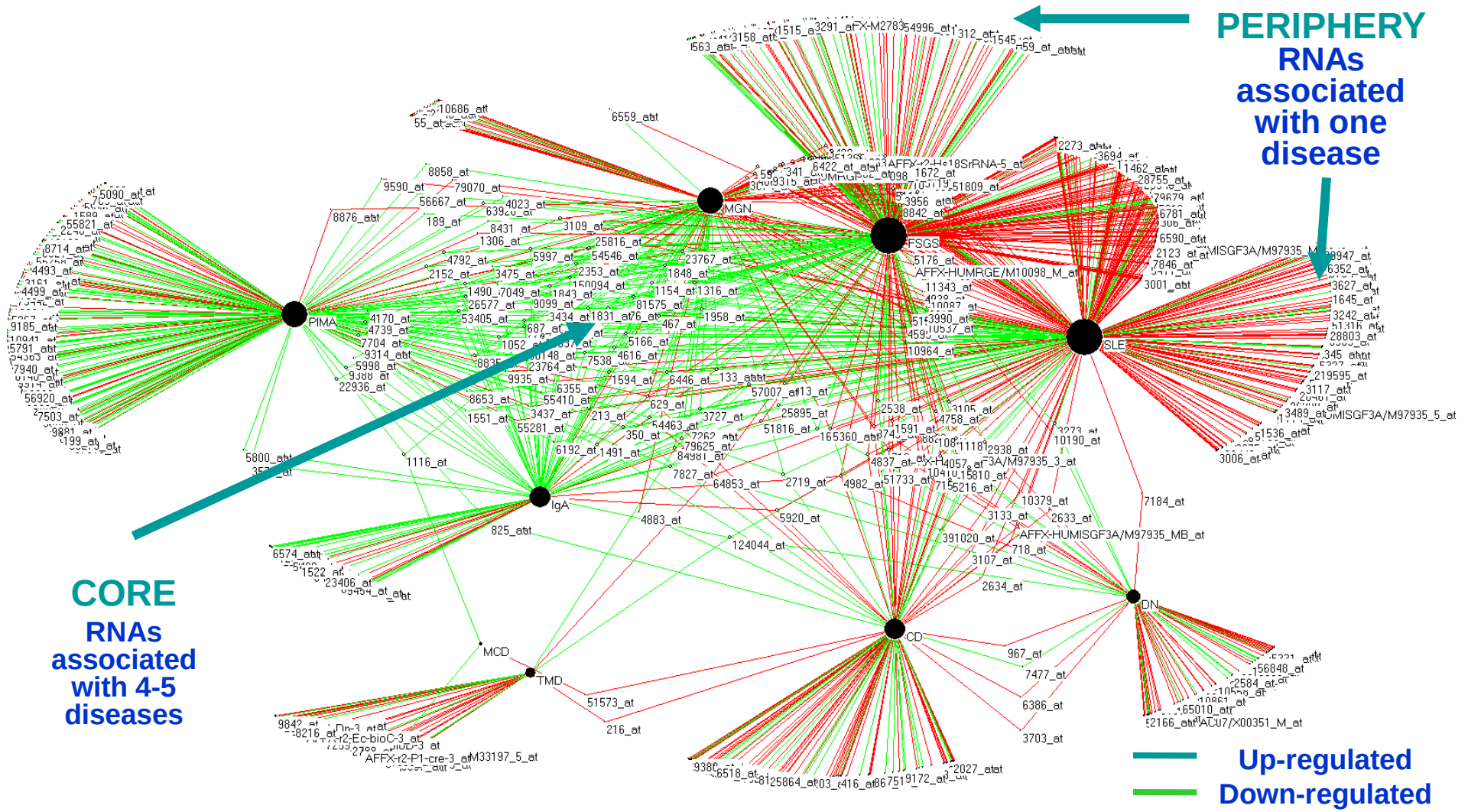
Serving the international community:
Applied Systems Biology Core in the
O'Brian Renal Center

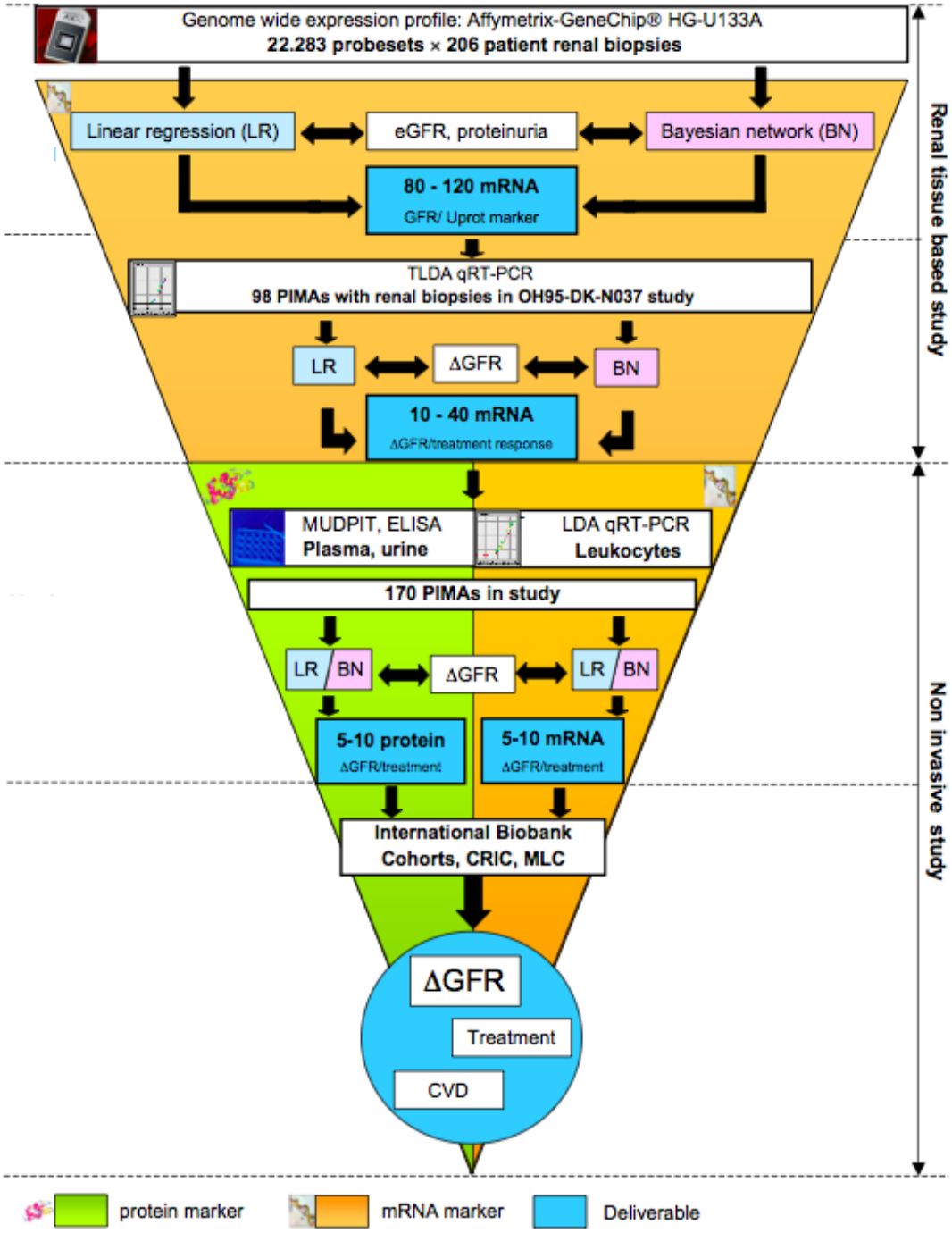
42 collaborative groups, 4 continents,
10 funded collaborative grants

www.nephromine.org: Domain specific systems biology search tool



Redefining disease categories:
Global molecular network view of renal disease





Molecular diagnosis of DN:

Strategy for tissue based and non-invasive marker definition and verification: Moving from tissue based mRNA markers to plasma and urine based protein and metabolites

Available cohorts for study:

1. NIDDK PIMA NIDDK cohort (n=180)
2. Chronic Renal Insufficiency Cohort (CRIC, n=3600)
3. International Renal Biobank Network (n=2800)

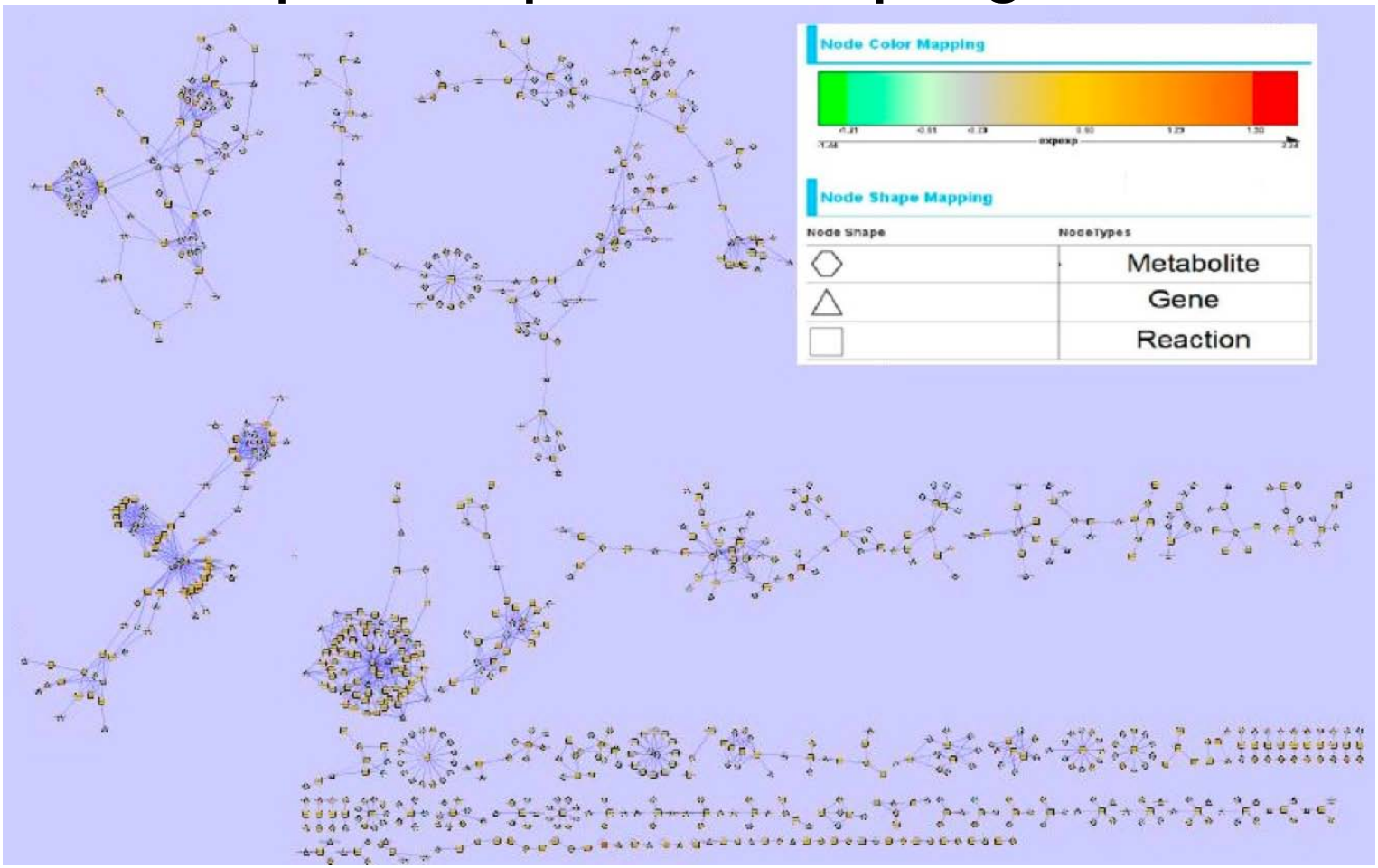
Metabolites as diagnostic marker of DN

Advantages compared to transcripts and proteins:

- Metabolites are readily obtainable in biofluids and are freely filtered into the urine.
- Metabolites have a well-defined precise nomenclature and role in biological processes.
- Metabolites are stable compounds which do not undergo further modifications.
- Metabolic pathways have the potential to provide a disease (stage) associated fingerprint.

Mapping of transcriptional data set in global metabolic networks

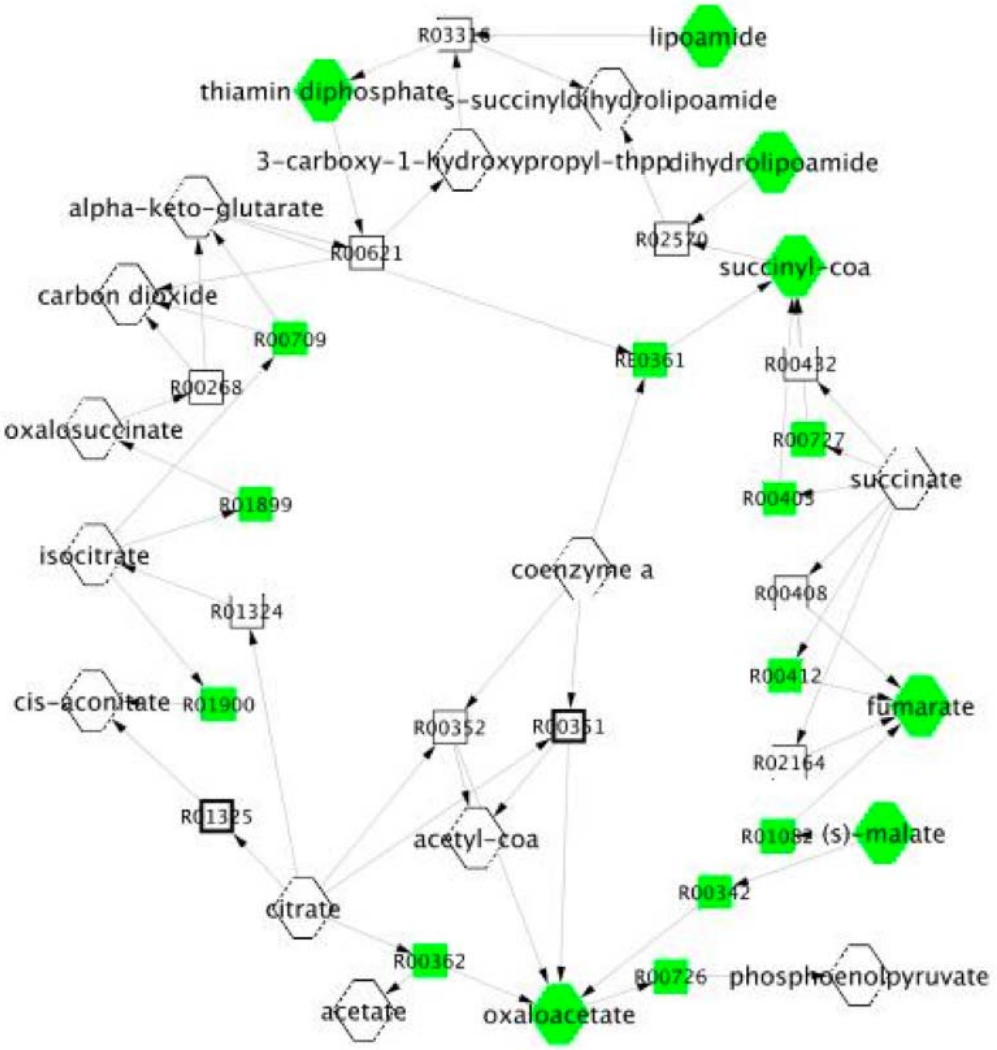
Mapping metabolite pathways from transcriptional profiles in progressive DN



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.

METSCAPE:

Mapping metabolite pathways from transcriptional profiles in 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

Multilevel data integration in DN: Integrating genetics with genomics

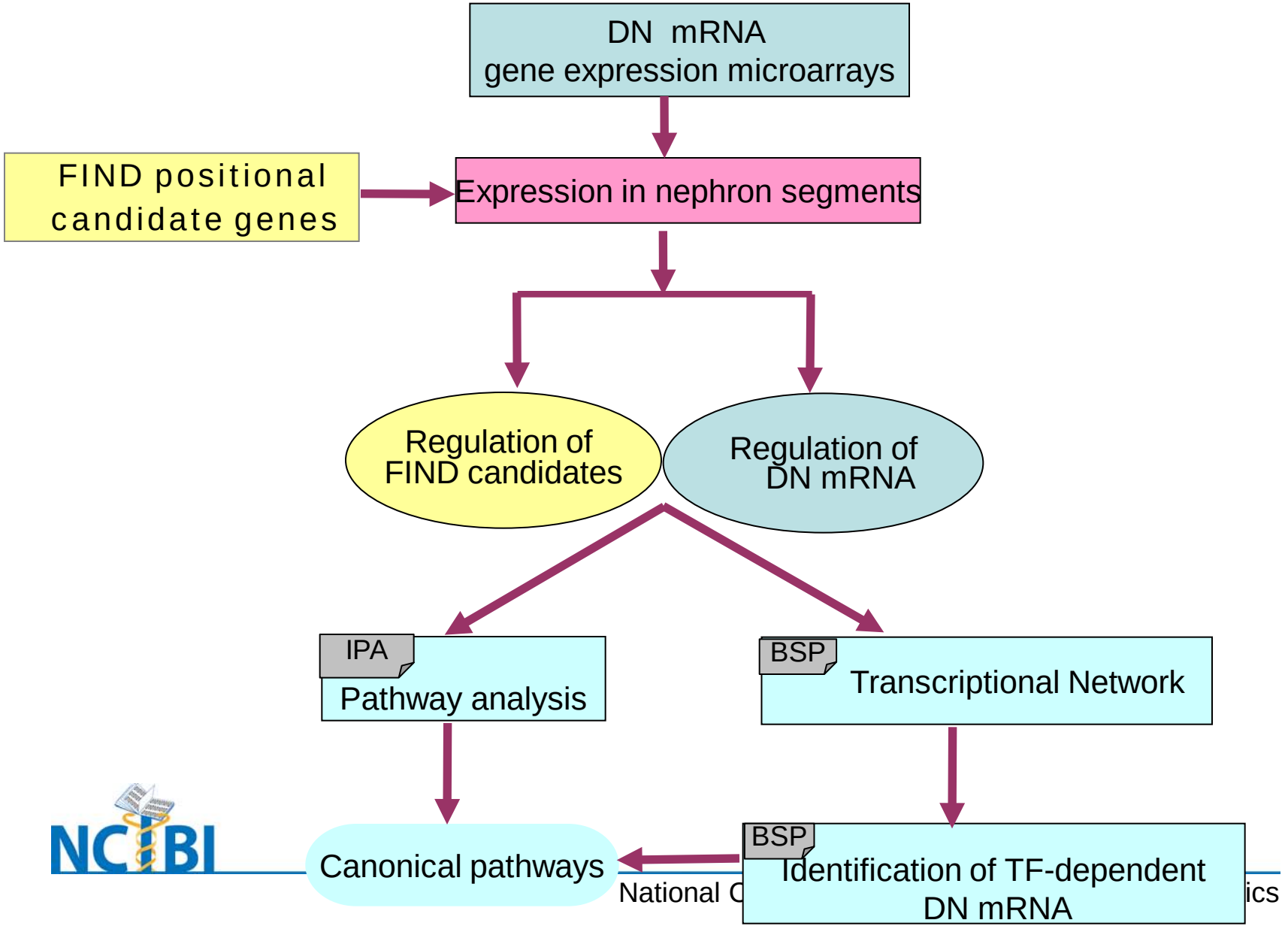
- Challenge:
  - Genome Wide Association Studies (GWAS) generate multiple putative functional variants:
    - Prioritize targets for further validation
    - Integrated targets into functional context of disease
- Strategy:
  - Integration with transcriptional analysis can define:
    - Functional status of a gene in disease
      - Differential mRNA expression
    - Functional status of gene environment:
      - Regulation of signalling or metabolic pathways
      - Regulation of transcriptional networks

Integrating GoKind and FIND with DN transcriptomic profiles

- GoKind has identified regions with strong DN associations
  - SNP's in non-coding sequence
  - Defining SNP function using in silico promoter modeling

    (Poster Y. Bai for proof of concept)
- FIND (Family Investigation of Nephropathy and Diabetes):
  - Coarse association scan for Albumin/Creatinine Ratio (ACR) and DN at population level
    - Mixed effects models adjusted for family structure
  - Positional candidates
  - Integration of positional candidate genes obtained in FIND consortium with DN gene expression profiles

Schema of Integration



FIND associated genes expressed in DN in nephron segments?

- 37 FIND-SNP associated genes

- Microalbuminuric DN (protocol biopsies)

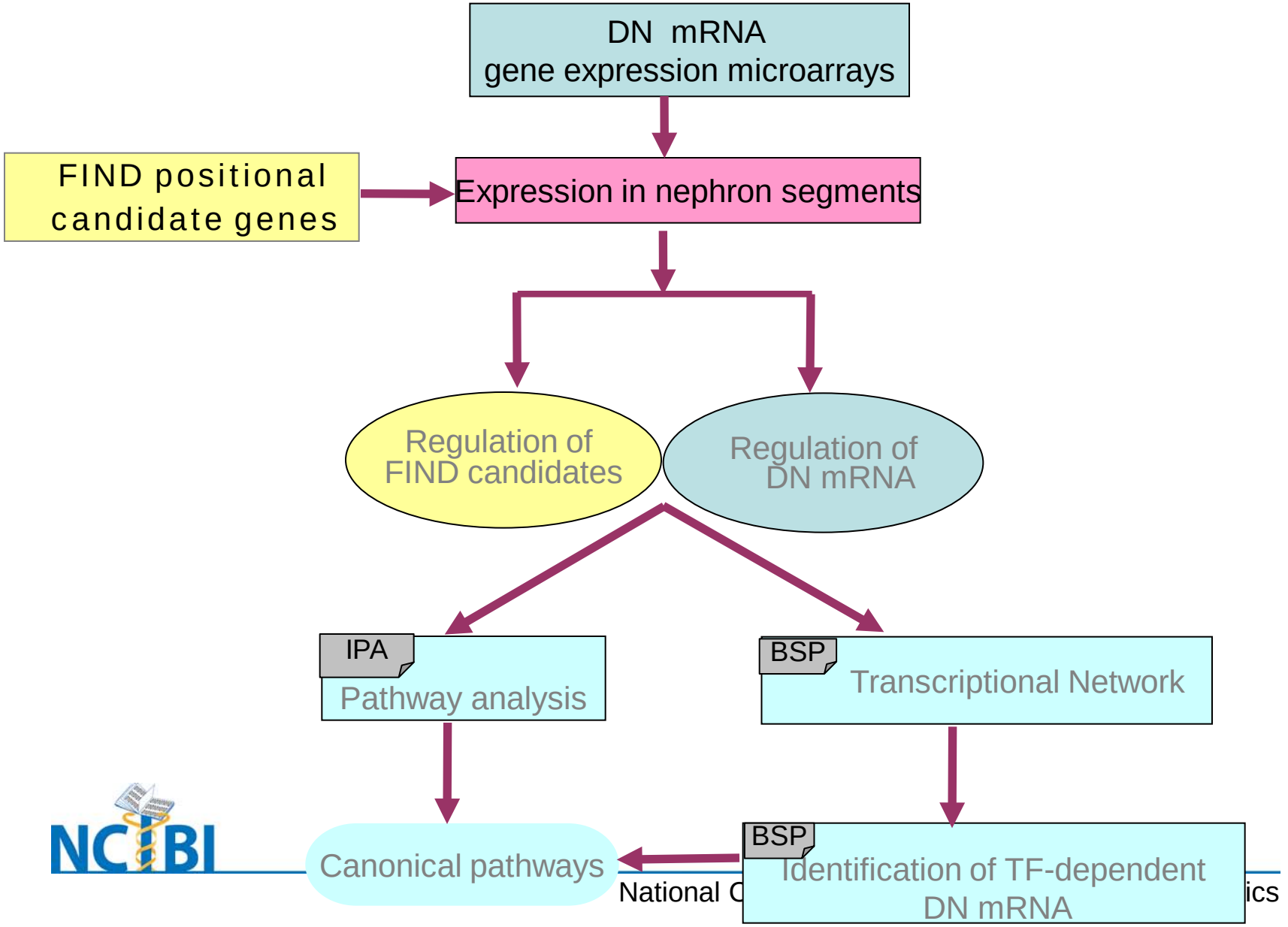
  Glomerular compartment (n=22):

  – **33 / 37**

  Tubulo-interstitial compartment (n=22):

  – **35 / 37**

Schema of Integration

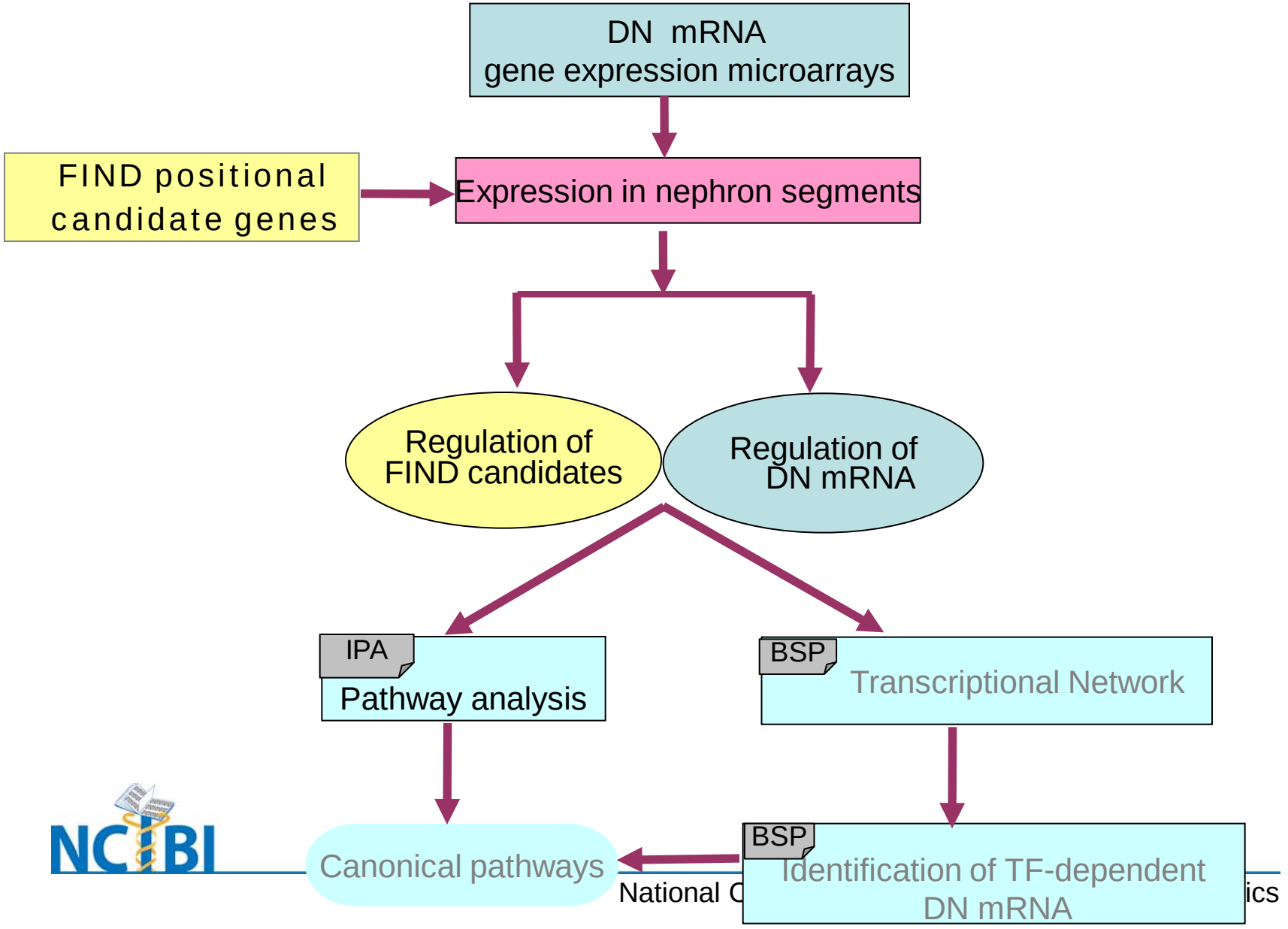


Candidate genes regulated in DN

Microalbuminuric DN versus non-diabetic living donor biopsies

- FIND candidates
  - Glomeruli:
    - » 20/ 33 (q<0.01)
  - Tubulo-interstitium:
    - » 11/ 35 (q<0.01)
  - Both compartments
    - » 9 / 33
- Is enriched compared to random gene set

Schema of Integration



Integration into functional context: Pathway mapping

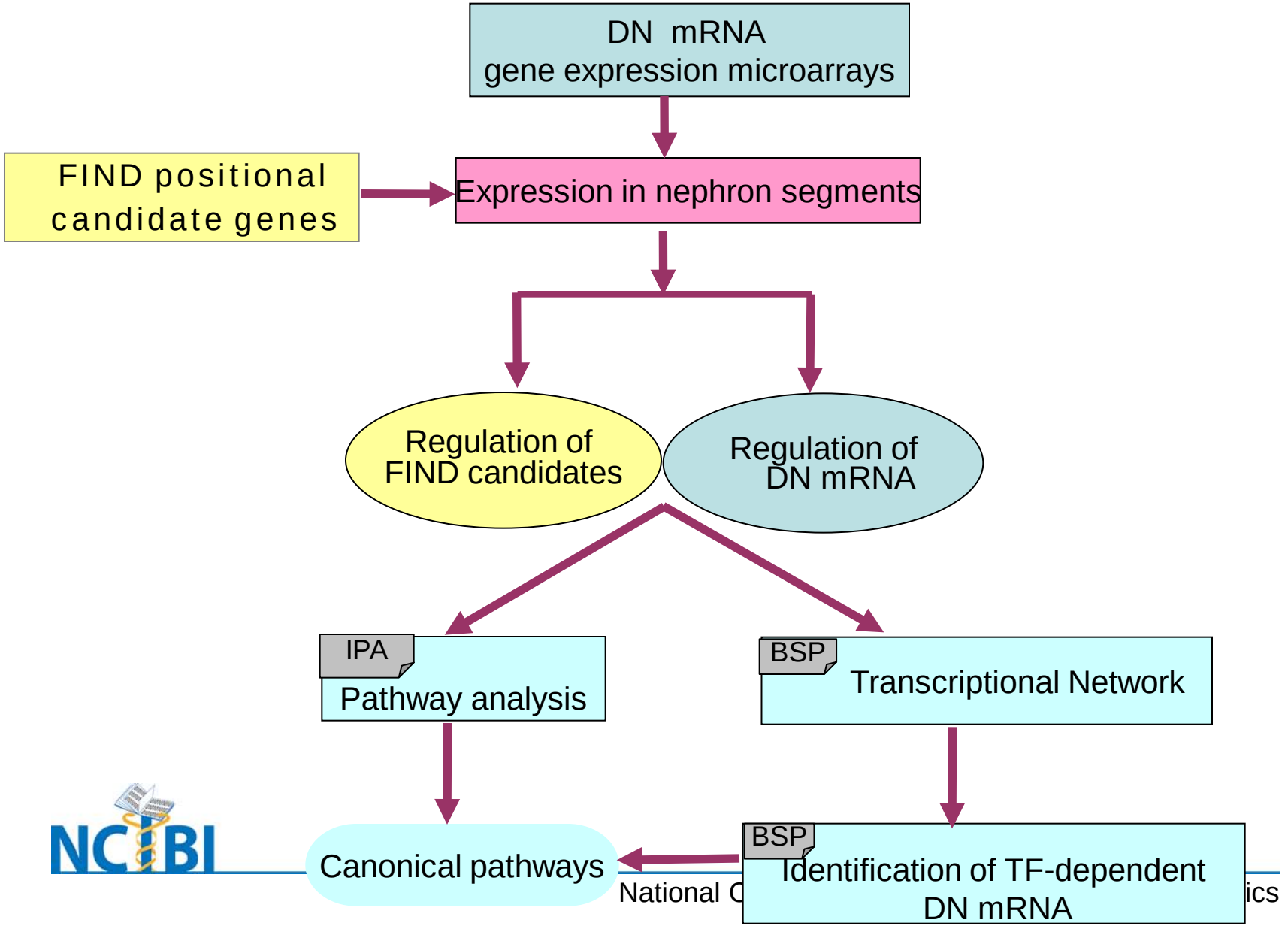
- Gene associated with highly regulated pathways in DN
  => Relevance of gene for disease process

- Strategy:
  - Genes passing expression filter steps were mapped into canonical pathways with regulation in the DN expression profiles
    - Genes associated with highest number of pathways
    - Genes associated with highly regulated pathways
  - Limitation:
    - Biased towards well-characterized molecules / pathways
  - Advantage:
    - Integration into functional context and prior knowledge

FIND associated Genes mapped with top 154 canonical pathways regulated in DN glomeruli

Gene Name	Number of pathways	Examples
PRKAR2B (PKA Regulatory Subunit IIb)	19/154	ERK/ MAPK Signaling, Insulin Receptor Signaling, ……
RXRA (retinoid X receptor, alpha)	13/154	PPAR±/RXR ± Activation, PPAR Signaling , ..
IFNAR2 (interferon (alpha, beta and omega) receptor 2)	2/154	Interferon Signaling
TEK (Tie2 / Angiopoetin Receptor)	1/154	IL-8 Signaling
FBXW7 (F-box and WD repeat domain containing 7)	1/154	Protein Ubiquitination Pathway
GMDS (GDP-mannose 4,6-dehydratase)	1/154	Fructose and Mannose Metabolism

Schema of Integration

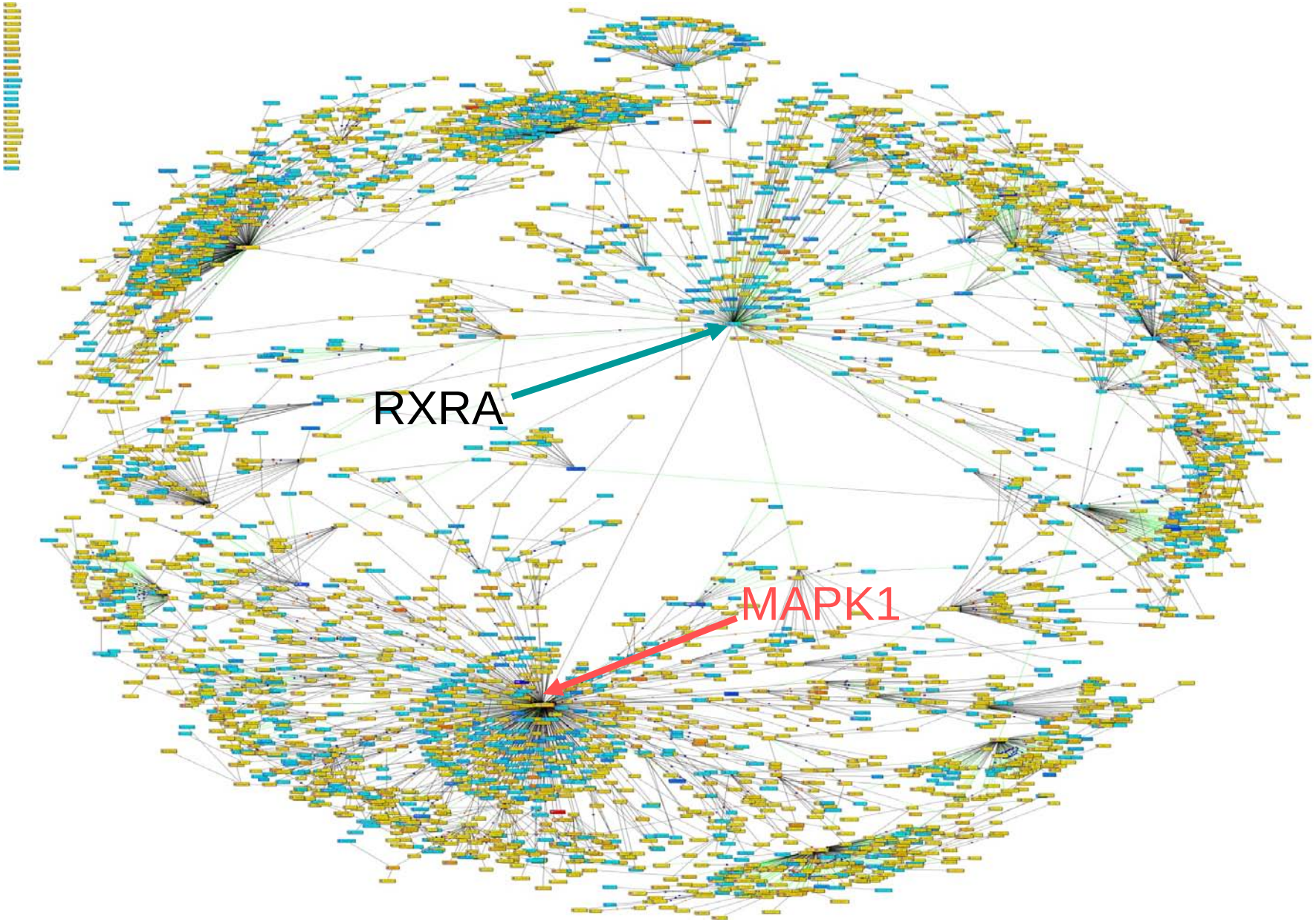


Integration into functional context: Transcriptional networks

- Transcription factors are promising targets
  - Potential to alter expression and influence transcriptional cascades

- Strategy:
  - Define functional characteristics of transcription factors in candidate gene set
  - Evaluate regulation of transcription factor dependent mRNAs in DN gene expression data sets

Differentially regulated transcripts (q<0.01)in early DN glomeruli



RXRA transcriptional network in DN

Nodes: DN regulated genes

- MESH Term
  (Type II Diabetes + Pubmed Co-cited)

Connections (Edges)

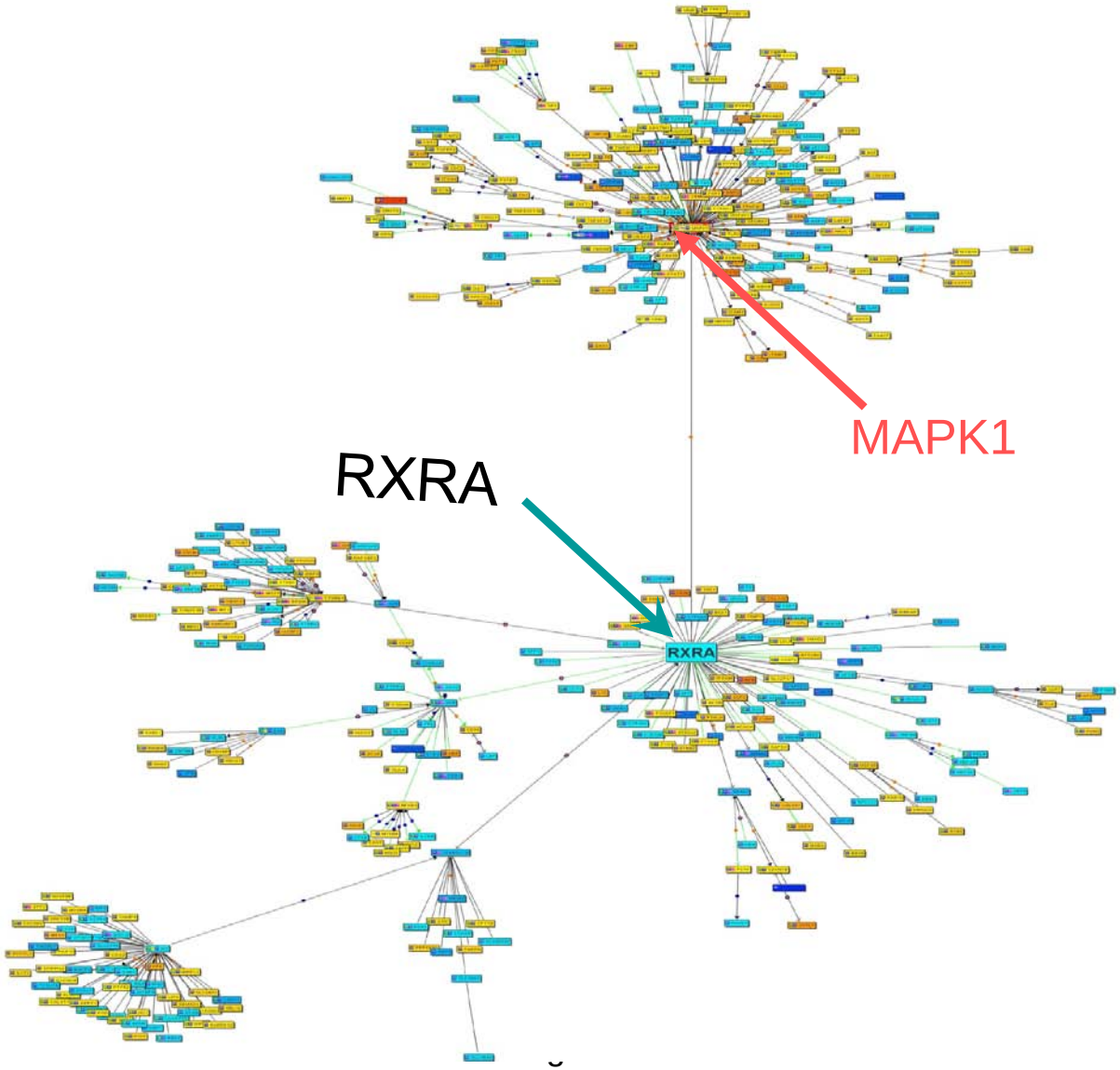
- Promoter binding site + Pubmed Co-cited

Red /Orange :

Up-regulated

Blue :

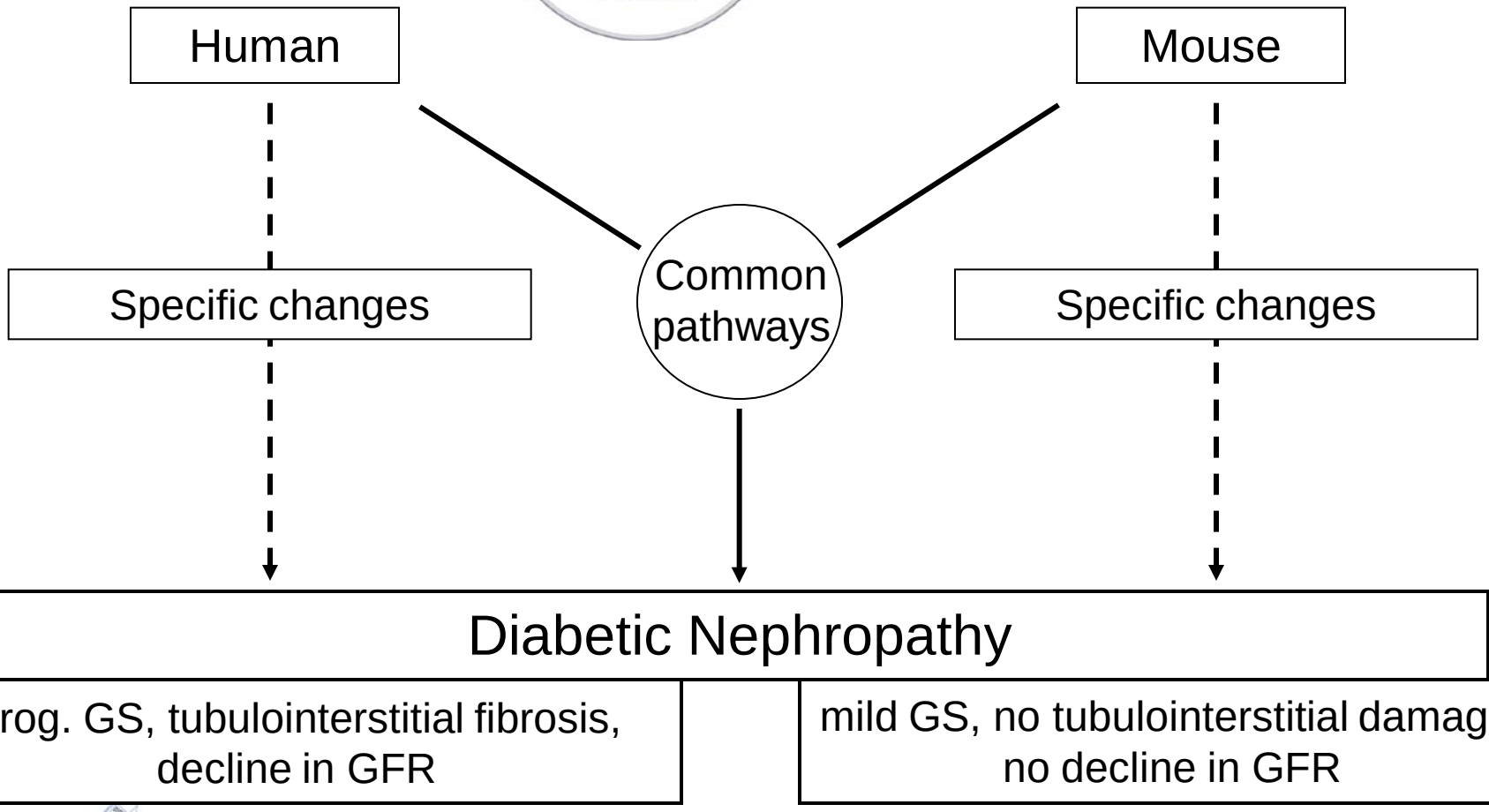
Down-regulated



In silico prediction of RXRA function in diabetic glomerulopathy:

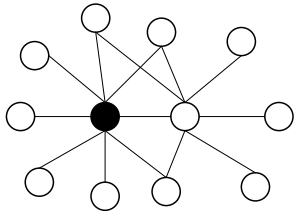
- Top functional categories of RXRA dependent genes with regulation in diabetic glomerulopathy:
  - Glucocorticoid Receptor Signaling
  - IL-8 Signaling
  - Apoptosis Signaling
  - BMP signaling pathway

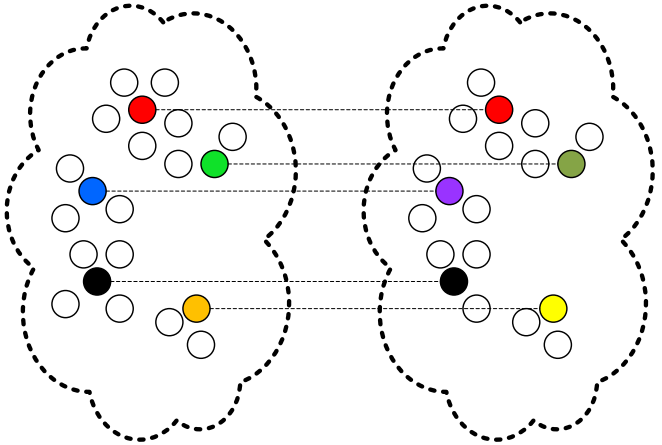
Human versus murine DN/DNP

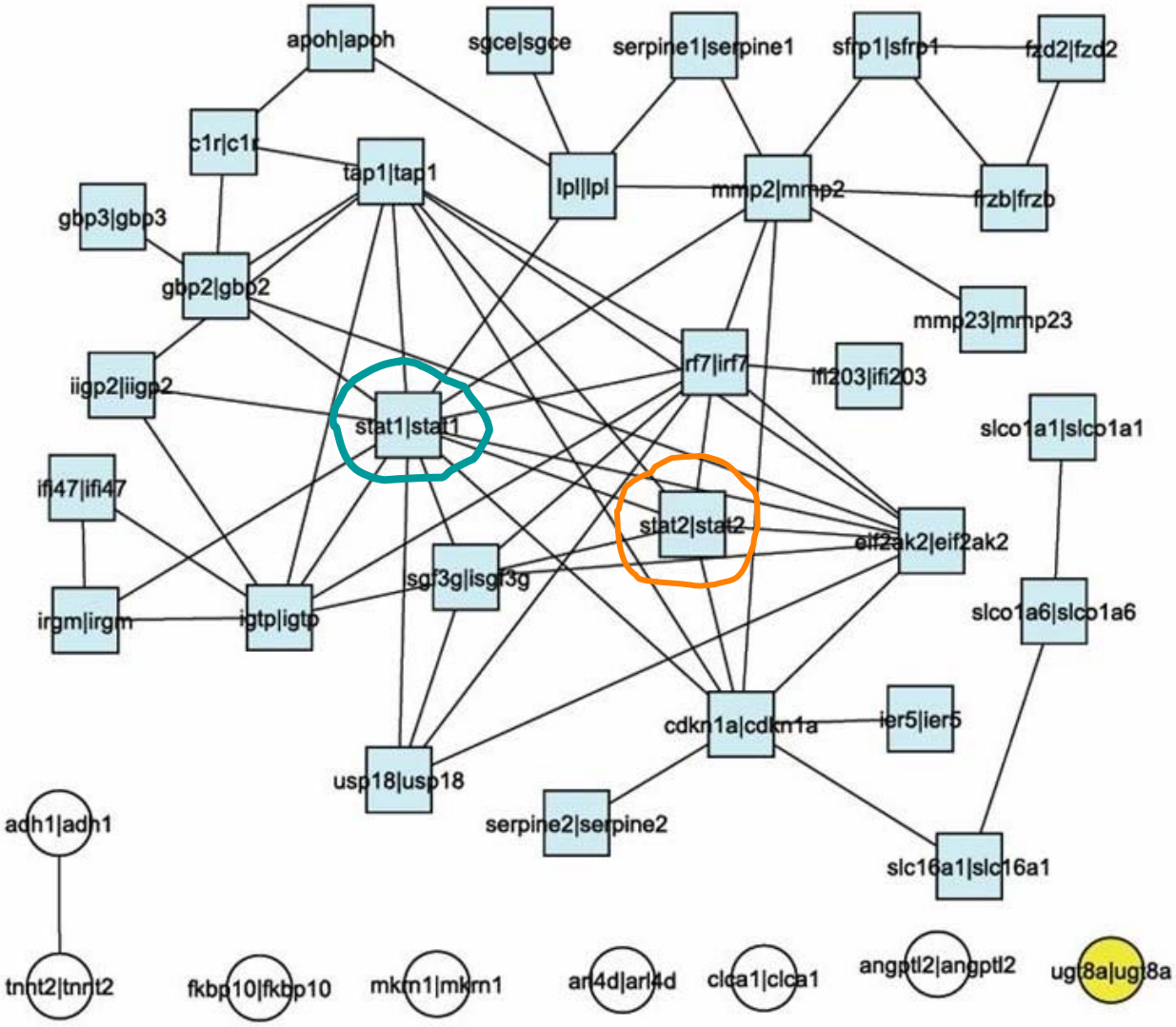


Defining shared transcriptional responses on a network level

- Compare complex regulatory networks for conserved network structures
  - Subopitmal graph matching tools required
- TALE Indexing Technique (Y.Tian and Y. Patel, 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







Mouse:
- 168 nodes, 510 edges

Human:
- 471 nodes, 3258 edges

- Transcriptional networks shared between murine and human DN (STZ and GIPR^{dn}-mice)
  - Networks integrating gene expression values with NLP (PubMed abstracts) and automated promoter analysis
    - Nodes indicate aligned gene pairs
    - Edges represent the conserved interactions in both models.

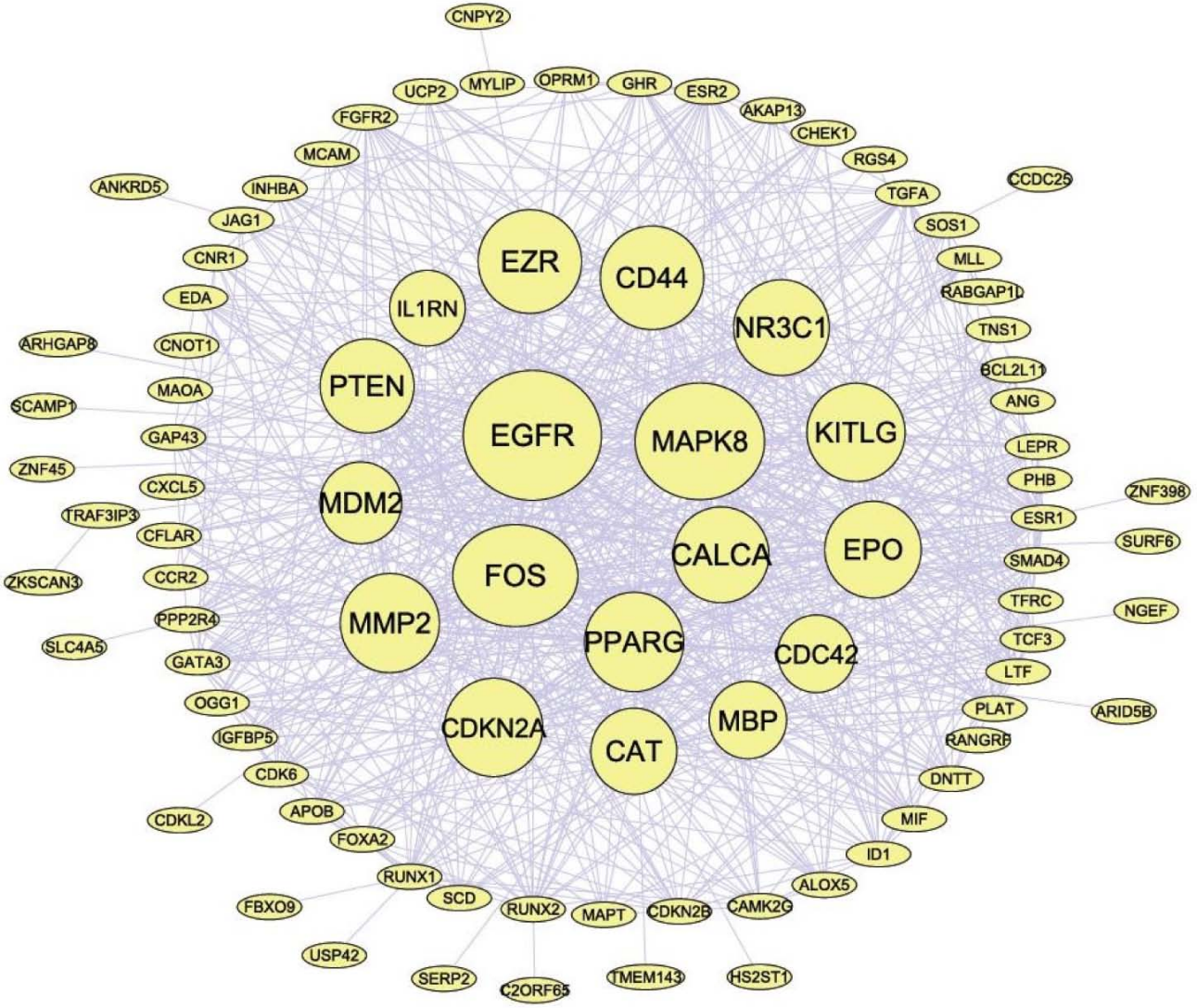
Defining cross organ conserved regulatory networks

- **Identification of significantly differentially expressed genes**
  4,680 (DPN) and 4,630 (DN)

- **Significantly enriched GO categories:**
  - 55 conserved, 13(DPN) and 77 (DN) tissue specific
- **Extraction of the transcriptional regulatory network:**
    - Differentially expressed genes as nodes are linked via NLP of PubMed abstracts at sentence level
  - DPN (nerve): 2,935 nodes and 26,000 edges
  - DN (kidney): 3,151 nodes and 28,000 edges

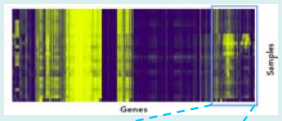
Network comparison DN - DPN

- Shared network:
  - 91 nodes as key hubs of cross-tissue conserved regulation.
  - Well-known diabetes-related genes:
  - PPAR-g, LEPR

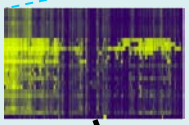


Global Informatics Strategy

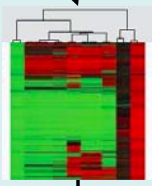
Affymetrix Microarray Platform (UMCCC)



Differentially Expressed Genes (DEGs) (NCIBI GenePattern Server)



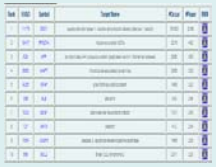
Clustering of DEGs (NCIBI Chinese Restaurant Clustering)



Functional Analysis

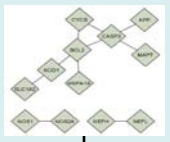
Functional enrichment
NCIBI ConceptGen

Literature mining
NCIBI SciMiner



Gene network
BiblioSphere, SciMiner, NCIBI SAGA/TALE

PPI Network
NCIBI MiMI, Cytoscape, SAGA/TALE



Key pathways/genes in DPN

Experimental Validation

RT-PCR, Western, Immunohistochemistry, Transfections

Validated markers of DPN

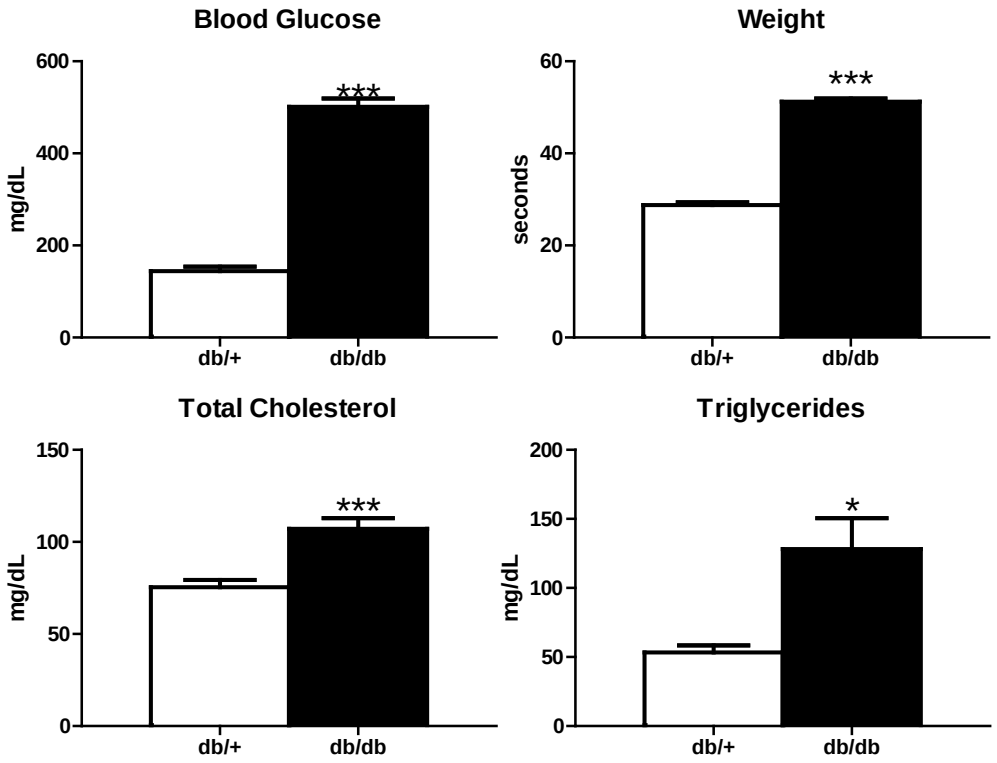
Future Directions

Validation of targets in Schwann cells & targeted metabolomics

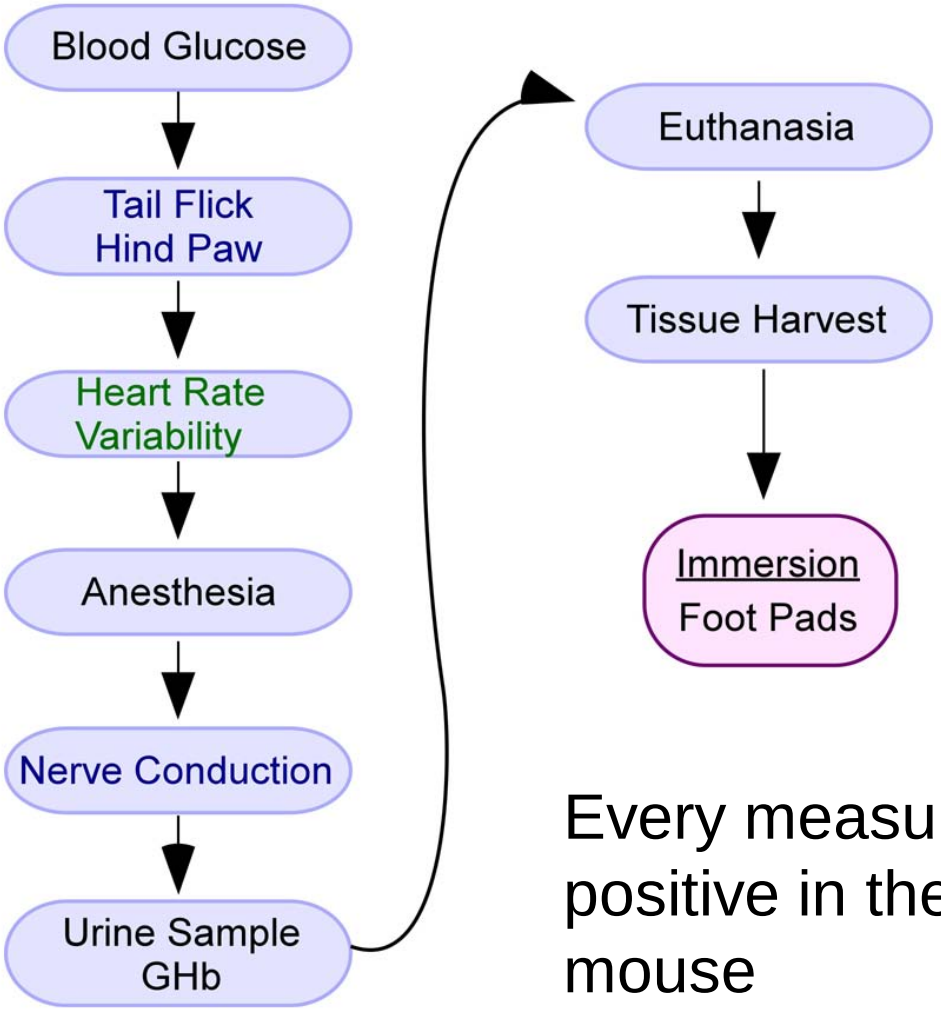
Novel Clinical Tools

Mouse Model of Diabetes

- The BKS-db/db mouse model of Type 2 diabetes
- Develops severe obesity, diabetes and dyslipidemia following 24 weeks of diabetes



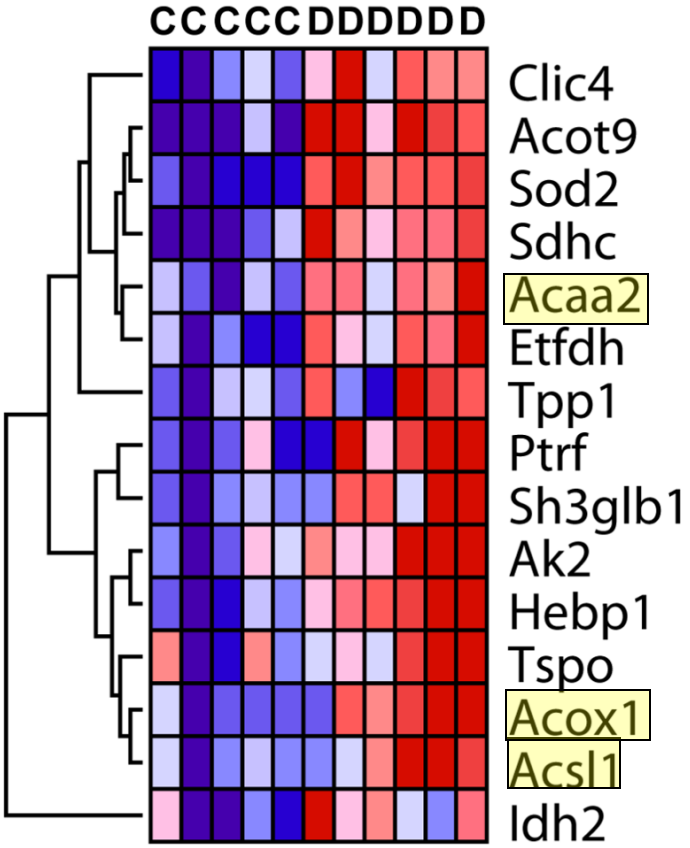
Neuropathy Phenotyping



Every measure of DPN is positive in the BKS-db/db mouse

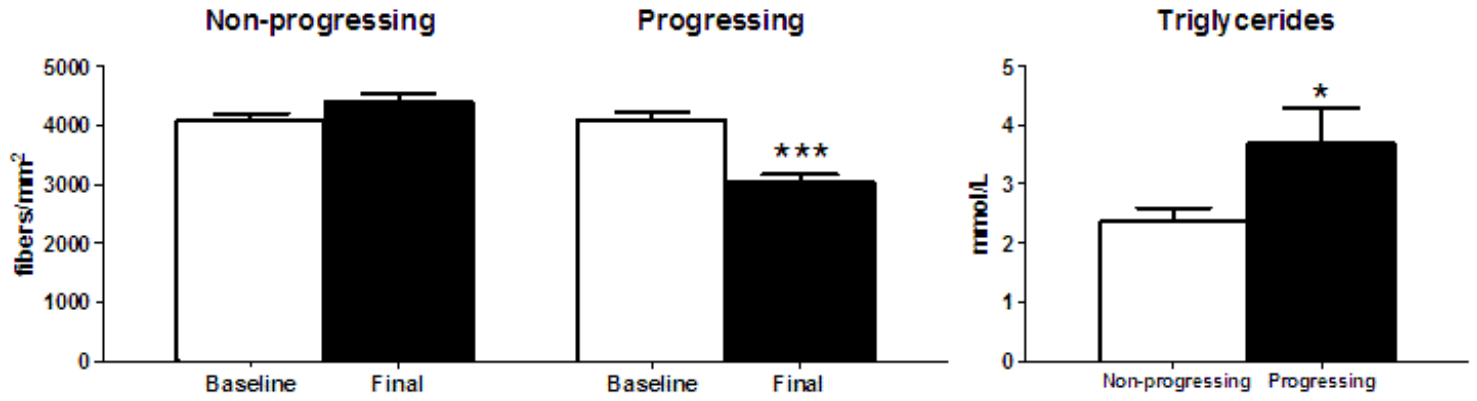
Gene Expression Profiling

- Affymetrix arrays are run on the peripheral nerve of the diabetic mice
- NCIBI Chinese Restaurant Clustering identifies a regulated cluster
- Enriched for mitochondria and lipid metabolism (yellow)
- **What is the significance of the lipid metabolism enrichment?**



Lipid Hypothesis of DPN

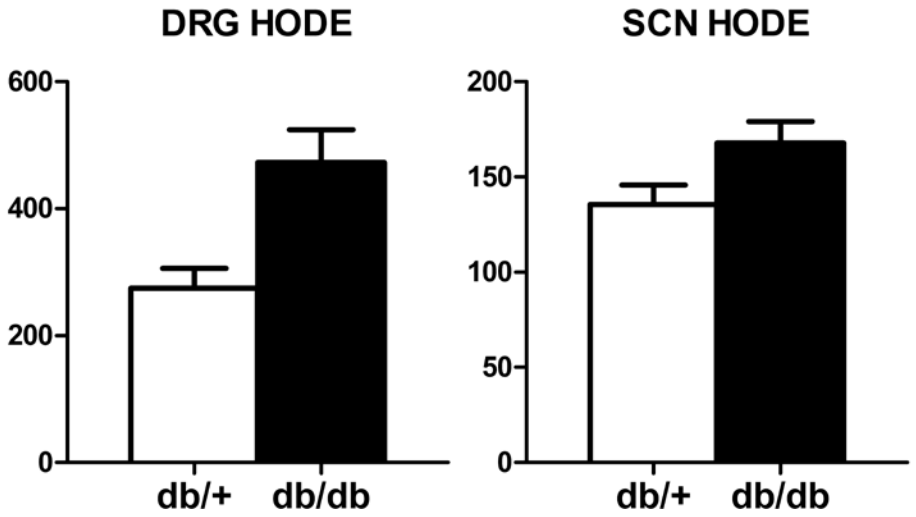
- Recent evidence indicates that changes in lipids accelerate DPN progression
- Our *post hoc* analysis of a DPN clinical trial supports this hypothesis



Elevated Triglycerides Correlate with Progression of Diabetic Neuropathy
Wiggin TD, Sullivan KA, Pop-Busui R, Amato A, Sima AA, Feldman EL
Diabetes, In Press

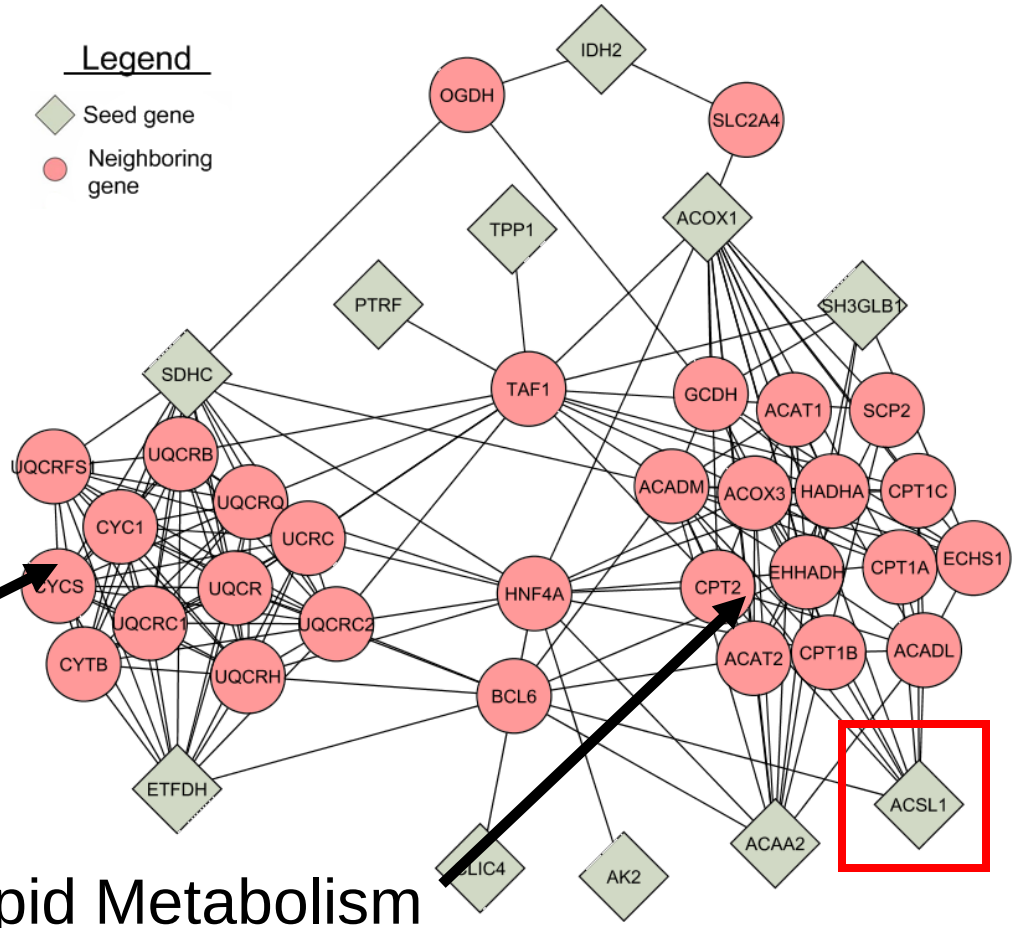
Oxidized Lipids Increased in Mouse Model of DPN

- The effect of dyslipidemia may be connected to the increased abundance of oxidized lipids in the peripheral nerve.



Molecular Interaction Network

NCIBI MiMI is used to construct a molecular interaction network for the genes in the regulated cluster



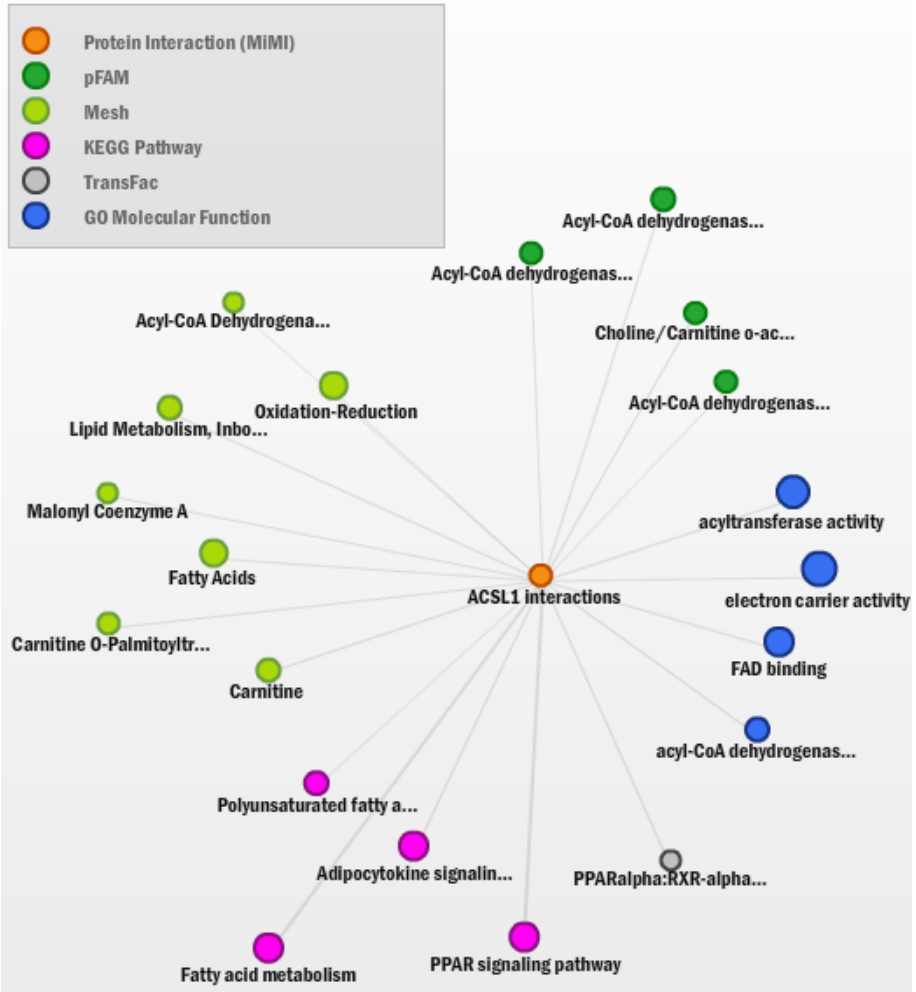
Cytochrome C

Lipid Metabolism

ConceptGen Network for Lipid Metabolism Gene

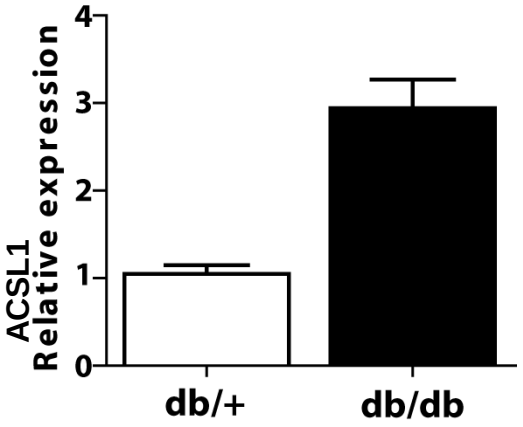
- NCIBI ConceptGen is used to construct a cloud of associated concepts around our lead hit, Acsl1

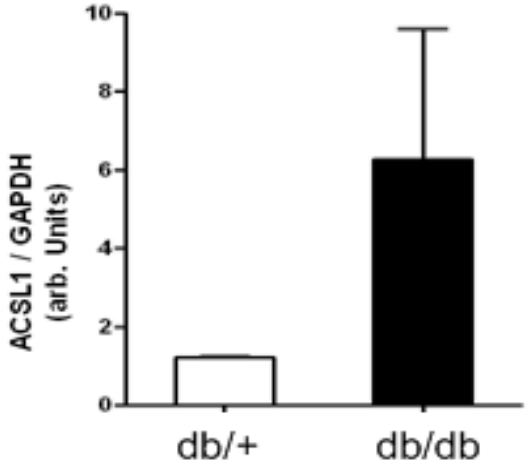
- PPAR signaling & Oxidative stress, highly relevant DPN pathways, are found

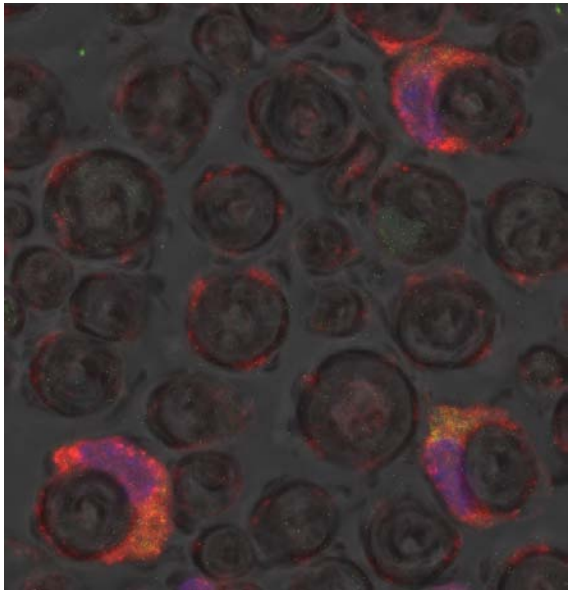


Acsl1 Focused Confirmation

- Acsl1 regulation is confirmed on the transcript and protein level
- Expression is localized to the Schwann cell cytoplasm

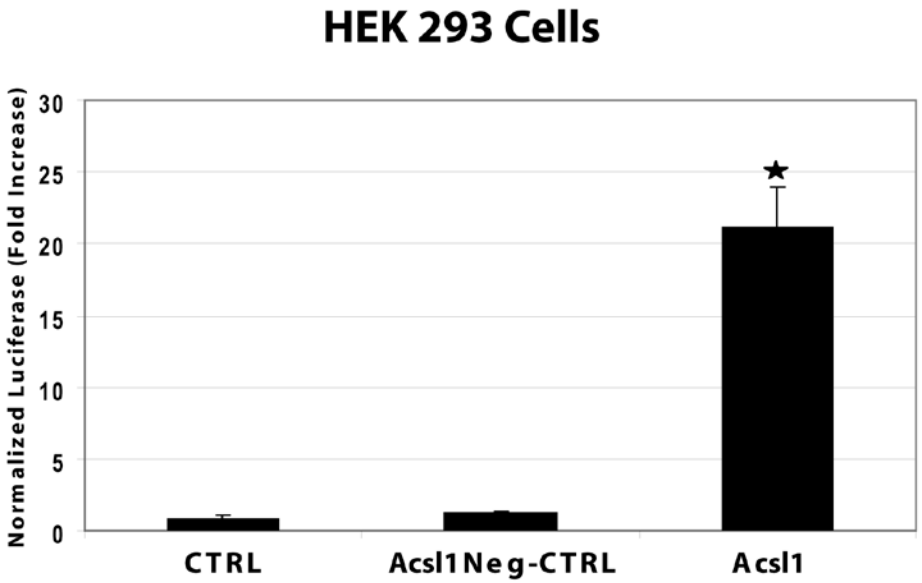






Promoter Functionality

- The Acsl1 *cis*-promoter was cloned into a luciferase reporter in HEK293 cells to confirm its ability to drive expression

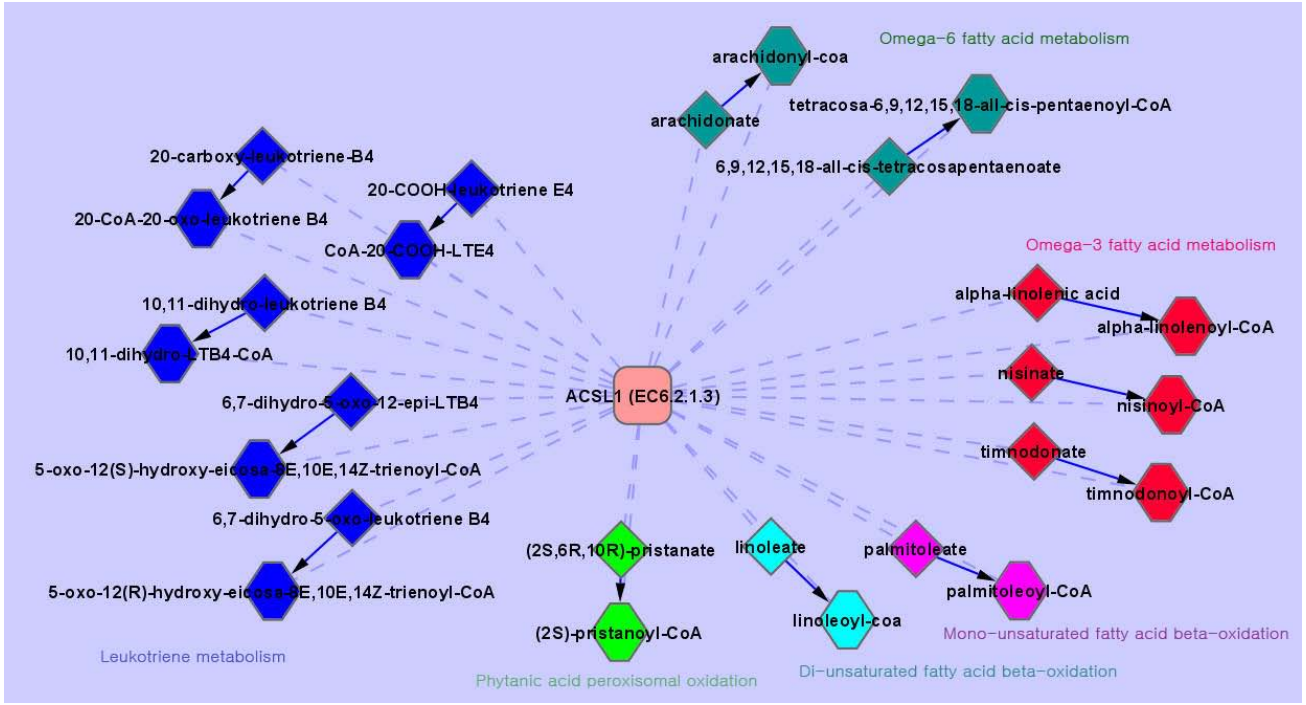


Conclusions

- An informatics approach identified lipid metabolism as relevant to DPN
- Oxidized lipids are greatly increased in the nerve of diabetic mice
- One specific gene, Acsl1, is prioritized by network analysis
- Acsl1 regulation correlates with increases in oxidized lipids
  - In a preliminary study, Acsl1 regulation precedes DPN development in human nerve

Future Directions

- We will use an informatics approach to identify metabolites that may be affected by Acsl1 regulation
- These metabolites may be useful as biomarkers of disease or as targets for therapy



Emerging strategy for multi-systems map of diabetic complications

Integrated analysis of transcriptional regulation:

- Identification of candidate molecular diagnostic markers
- Shared transcriptional regulatory programs of converging signaling pathway
  - Integrating transcriptional networks and clinical phenotypes across models, tissues and species with comparative genomics
  - Pathways shared between human and murine DN delivers tested animal models for experimental validation

- Next steps:
  - Integration along all steps of the regulatory continuum using data sets rapidly becoming available
    - GWAS, mRNA, uRNA, Proteome, Metabolites, Clinical and Structural Phenotypes
  - Evaluate the diagnostic power in prospective studies
  - Modulate identified pathways in functional studies

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NIDDK:

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R24 DK082841
R21 DK079441-01
+ intramural Bench to Bedside
U01 DK076139-01
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JDRF
Nephcure
ALR