Update for MMPC 9-08

# AMDCC Update

- History
- Current structure and projects
- Models
- Phenotying protocols
  - Aperio
  - Neuropathy
  - ?Retinopathy
  - ?Ranking
- Pilot Projects…and a bit of data
- Interaction with MMPCs

History of AMDCC

- First funded in 2001.

- 5 year consortia of 8 large groups devoted to phenotyping and identifying the best models of diabetic nephropathy, vasculopathy, cardiomyopathy, neuropathy. Uropathy and retinopathy added later as single investigator units.

- Accomplishments
    - Definition and pathologic description of each complication
    - Adoption of common phenotyping standards
    - Strain analysis and comparisons. Issues with B6 esp. for nephropathy
    - Screening of multiple models; many for several complications
    - Impact of diet analyzed (somewhat)

- Problems
    - Difficulties in sharing of models for cross-phenotyping due to quarantine and other institutional issues
    - No "perfect" models emerged for any complication
    - Little novel hypothesis-driven or generating work
    - Problems disseminating our findings, despite robust data upload capabilities

Current AMDCC/MMPC structure (2006 onward)

D **Coordinating and Bioinformatics Unit (CBU)**
(http://grants.nih.gov/grants/guide/rfa-files/RFA-DK-05-012.html)

1. Receive phenotyping data from the MGHC, MMPCs and PB sites
2. Facilitate meta-analysis of data
3. Manage joint AMDCC-MMPC website
4. Coordinate semi-annual meetings of AMDCC and MMPC

External Advisory Board (EAB)

NIH Program Staff
(NIDDK, NHLBI & NINDS)

Phenotyping data uploaded to website By MGHC & MMPCs

Organ-specific phenotyping data generated by PB sites uploaded to website

C **Mouse Metabolic Phenotyping Centers (MMPCs)**
(http://grants.nih.gov/grants/guide/rfa-files/RFA-DK-05-008.html)

1. Models made by the MGHC will be fully phenotyped by the MMPCs across all relevant complications and for a full duration of diabetes
2. All phenotyping data will be sent to Coordinating and Bioinformatics Unit (CBU)

Open communication to ensure the rapid exchange of ideas and information

A **Pathobiology (PB) Sites**
(http://grants.nih.gov/grants/guide/rfa-files/RFA-DK-05-011.html)

1. Individual PIs (PB sites) submit applications for peer-review proposing the construction of up to 2 new mouse models of diabetic complications
2. Selected PIs comprise the Executive Steering Committee (ESC) and work together to prioritize the proposed models
3. ESC submits their recommendations to the EAB and NIH Program staff for approval
4. It is anticipated that ~10-20 models will be made

Completed strains shipped to dedicated MMPC Diabetic Complications Phenotyping Core(s)

Ideas for model generation sent to MGHC

Completed strains shipped back to PB sites for hypothesis-driven study of basic mechanism(s)

B **Mouse Generation & Husbandry Core (MGHC)**

1. Generate strains prioritized by the ESC and approved by the EAB and NIH
2. Breed enough animals to distribute to the PB sites for mechanistic studies and to the MMPCs for general phenotyping across relevant complications
3. Provide appropriate animal husbandry (e.g. western diet, STZ, etc.)
4. Provide basic phenotyping of animals (e.g. glycemia, urine collection, etc.)

Indicates the movement of information

Indicates the movement of mice

Current AMDCC investigators/projects

Abel, E. Dale – Modeling Diabetic Cardiomyopathy and Microangiopathy (H, V)

Bottinger, Erwin – Role and Mechanisms of Epithelial Injury in Diabetic Nephropathy (K)

Brosius, Frank – Recapitulating transcriptional pathways of human diabetic nephropathy in mice (K)

Coffman, Thomas – Angiogenic Signals in Diabetic Complications (K, V)

Daneshgari, Firouz – Diabetic Uropathy Pathobiology Site (Bladder)

Davis, Richard – Atherosclerosis and other complications in the hyperlipidemic BKS diabetic mice (V, others)

Feldman, Eva – Mitochondrial SOD as a Target for Diabetic Neuropathy (N)

Goldberg, Ira – Creating Glucose Responsive Cardiovascular Complications (H, V)

Harris, Raymond – Generating Mouse Mutants with Diabetic Nephropathy (K)

Levi, Moshe – Novel Models of Diabetic Nephropathy (K)

McIndoe, Richard – Coordinating and Bioinformatics Center Director

Maeda, Nobuyo – Dyslipidemia, Lipoic Acid and Diabetic Vascular Complications in Humanized Mice (V)

Sharma, Kumar – Adiponectin and Nox 4 in Diabetic Kidney Disease (K)

Smithies, Oliver – Bradykinin, Nitric Oxide and Mitochondrial DNA Damage in Diabetic Complications (K, others)

Current AMDCC:
Restricted and evolving goals
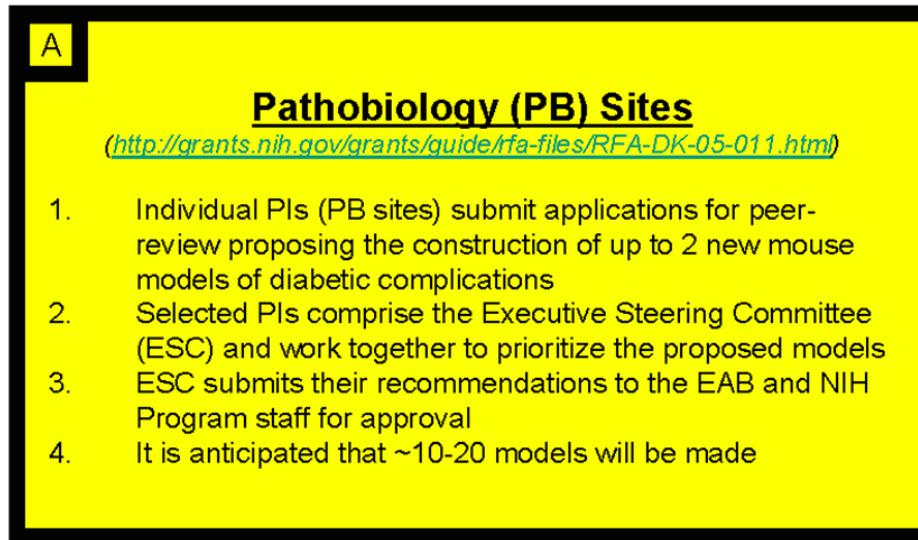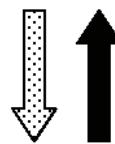
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Model Generation and Husbandry Core
(JAX)

Current AMDCC Models at JAX

Import, re-derive, cryopreserve, GQC, and general husbandry of the Akita mutation on several genetic backgrounds (FVB, 129/Sv and DBA)
    Stock No. 6867 - FVB.B6-*Ins2Akita*/MlnJ - from Dr. Mary Loeken
    Stock No. 7562 - D2.B6-*Ins2Akita*/MatbJ - from Dr. Matthew Breyer
    Stock No. 7688 - 129S6.B6-*Ins2Akita*/CofJ – from Dr. Thomas Coffman

Phenotype FVB.BKS(D)-*Leprdb, FVB.B6-Ins2Akita*, and D2.B6-Ins2Akita mice

Import the B6.Bdkrb1/2 and B6.*Bdkrb2* nullizygous stock to create a compound mutants with B6-Ins2 Akita

Develop and produce bradykinin 1 null, conditional bradykinin 2 targeted mutation on a C57BL/6J background

Import the congenic 129.*eNOS* "knockout" stock to be mated to the B6.eNOS "knockout" stock maintained at TJL to create a compound mutant

Speed congenic production and distribution of D2.Cg-*Nr1h4tm1Gonz*/J (D2.*FXR*-/-) mice

Import 129. JAK2 Stop/flox knockin mice to be placed on 129SvEv background.

Import podocyte-specific (nphs2) Cre mice on B6 and 129/Sv backgrounds

Current AMDCC Models at JAX

Develop, produce and distribute C57BL/6J mice containing a knock-in allele of lipoic acid synthetase

Produce a speed congenic with multiple mutations: nerve-specific SOD2 mutant on a BKS background (BKS.Cg- *Leprdb Sod2tm1Shs* Tg(Nes-cre)1Kln/J) for distribution

Develop, produce and distribute FVB/NJ mice carrying the HAHNox4pTRE transgene.

Import, re-derive, develop, cryopreserve, GQC, and general husbandry of a compound smooth muscle specific SOD2 mutant (*Sod2tm1Shs* SM22-creER [a.k.a. Tagln-Cre]) on C57BL/6J background

Develop, produce, and distribute a conditional *eNOS* on a congenic129 and DBA/2J background

Develop and produce tetracycline inducible C57BL/6J mice carrying 2 transgenic constructs [TRE/hAR (human aldose reductase) and H2-Kd/rtTA]. Mate selected founder line to Stock No.2207 – B6.129S7-*Ldlrtm1Her*/J

Import, re-derive, develop, cryopreserve, GQC, and general husbandry of a compound mutant B6.Cg-Ins2Akita *Gt(ROSA)26Sortm1(Ntn1)Abel* Tg(cre/Esr1)5Amc, on C57BL/6J

AMDCC approach to complications
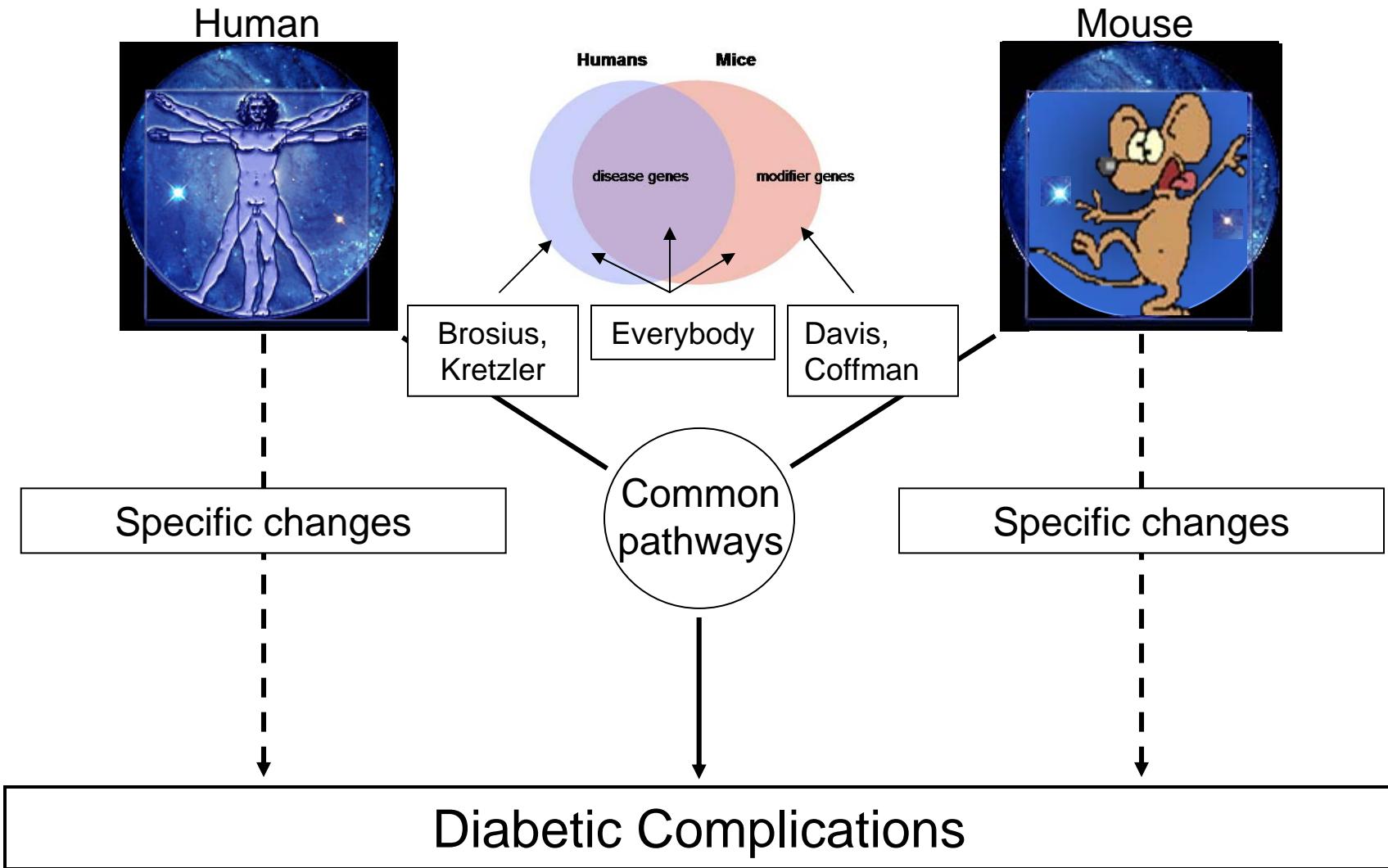
RAS/Kinin
1. B1R/B2R – Smithies
2. Ren tg – Coffman
3. Ace2 - Coffman

Lipids
1. ACS – Abel
2. LAS – Maeda
3. FXR – Levi
4. 5-LO - Davis
5. CD36 – Susztak/Bottinger
6. ApoE – Breslow
7. Adiponectin – Sharma

Glucose Uptake/Metab
1. hAR – Goldberg
2. Ove27 - Epstein
3. GluT – Brosius
4. *AGE - AM Schmidt*

ROS
1. SOD2 – Daneshgari, Feldman, Abel
2. eNOS – Harris, Breyer, Smithies
3. LiAS/Vit C – Maeda
4. Nox4 – Sharma
5. hAR – Breslow, Goldberg, Brownlee
6. CIRKO/Mitochondrial – Abel
7. Mpv17 – Bottinger

Endothelial Dysfxn Angiogenesis
1. Netrin – Abel
2. eNOS/prostacyclin – Harris
3. VEGF – Coffman
4. Hifs – Coffman

Cytokines/Signaling
1. VEGF – Coffman
2. Jak/STAT – Brosius
3. TGFB – Bottinger
4. CTGF - Maeda
5. Dcn – Sharma
6. *PKC - King*

Others
1. Leptin
2. Osteopontin – Susztak

Inflammation
1. NOD
2. *NFkB – (man) Kretzler*

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AMDCC Protocols

Diabetes Induction

Low-Dose Streptozotocin Induction Protocol (mouse)

Low-dose STZ + high-fat diet (mouse)

Dissection

Animal SPT Catheter Implantation

Isolation of rodent eyes for analysis of diabetic retinopathy

Removal and Preservation of the Urinary Bladder

Histology

En face preparation and quantification of aortic surface area covered by atherosclerotic lesions

Mouse dissection and preparation of heart (aortic sinus) and brachiocephalic artery for lesion quantitation by sectioning

Phenotype Assay

[Aortic Banding in Mice](#)

[Atherosclerotic Morphometry in Pigs](#)

[Cardiac AMPK Assay](#)

[CATALASE](#)

[Conscious Cystometry Bladder Function Testing](#)

[Creatinine Clearance by HPLC](#)

[Creatinine Companion Protocol Assay (Exocell)](#)

[Determination of Glomerular Filtration Rate in Conscious Mice using FITC-inulin](#)

[Echocardiography: Mouse](#)

[Evaluation of Mitochondrial Function](#)

[Frequently sampled intravenous glucose tolerance test](#)

[Glucose "Clamp" for measurement of whole-body insulin stimulated glucose disposal](#)

[Glucose uptake in isolated cardiomyocytes.](#)

[Intraperitoneal Glucose Tolerance Testing (IPGTT)](#)

[Isoproterenol](#)

[Lipid Profiles in Mice](#)

[Lipoprotein separation by FPLC](#)

[Measurement of Left Ventricular Hemodynamic Parameters in Intact Mice](#)

[Measurement of Left Ventricular Performance in Langendorff Perfused Mouse Hearts](#)

[Mesangial Index Quantification](#)

[Morphometry: Mouse](#)

Phenotype Assay (con't)

Murine Microalbuminuria ELISA (Albuwell M kit)

NADH Oxidase Activity

Nerve Conduction Velocity Tests

Neuropathy Phentoyping Protocols

Phenotypic Characterization of the Working Heart

Plantar Analgesia Test for Hind Paw/ Tail

Plasma Insulin (LINCO ELISA)

Podocyte Count and Density Analysis

Quantification of atherosclerosis at the aortic sinus

Serum, Blood and Body Weight Measurements in Pigs

Tail Flick

Thiobarbituric acid reactive substances

TRAP

Ultra Sensitive Rat Insulin ELISA Kit

Ultracentrifugal separation of HDL alone and calculation of non-HDL

Ultracentrifugal separation of VLDL, LDL and HDL

Western Analysis used in Oxidative Stress Protocols

Technique

Determination of Podocyte Number and Density in Rodent Glomeruli

Vascular perfusion of mice

WT-1 Staining Protocol for Podocytes

AMDCC Definition of Neuropathy

- Evidence of clinical loss of sensory function
  - Tail flick and hind paw withdrawal latencies (TF and HP)
  - = "Behavior measure" (Neurodiab)

- Electrophysiological evidence of nerve impairment
  - Sensory and motor distal latencies and nerve conduction velocity (NCV)
  - = "Electrophysiology measure" (Neurodiab)

- Anatomical evidence of nerve fiber loss
 - Intraepidermal fiber density (IEFD
  - = "Anatomy measure" (Neurodiab)
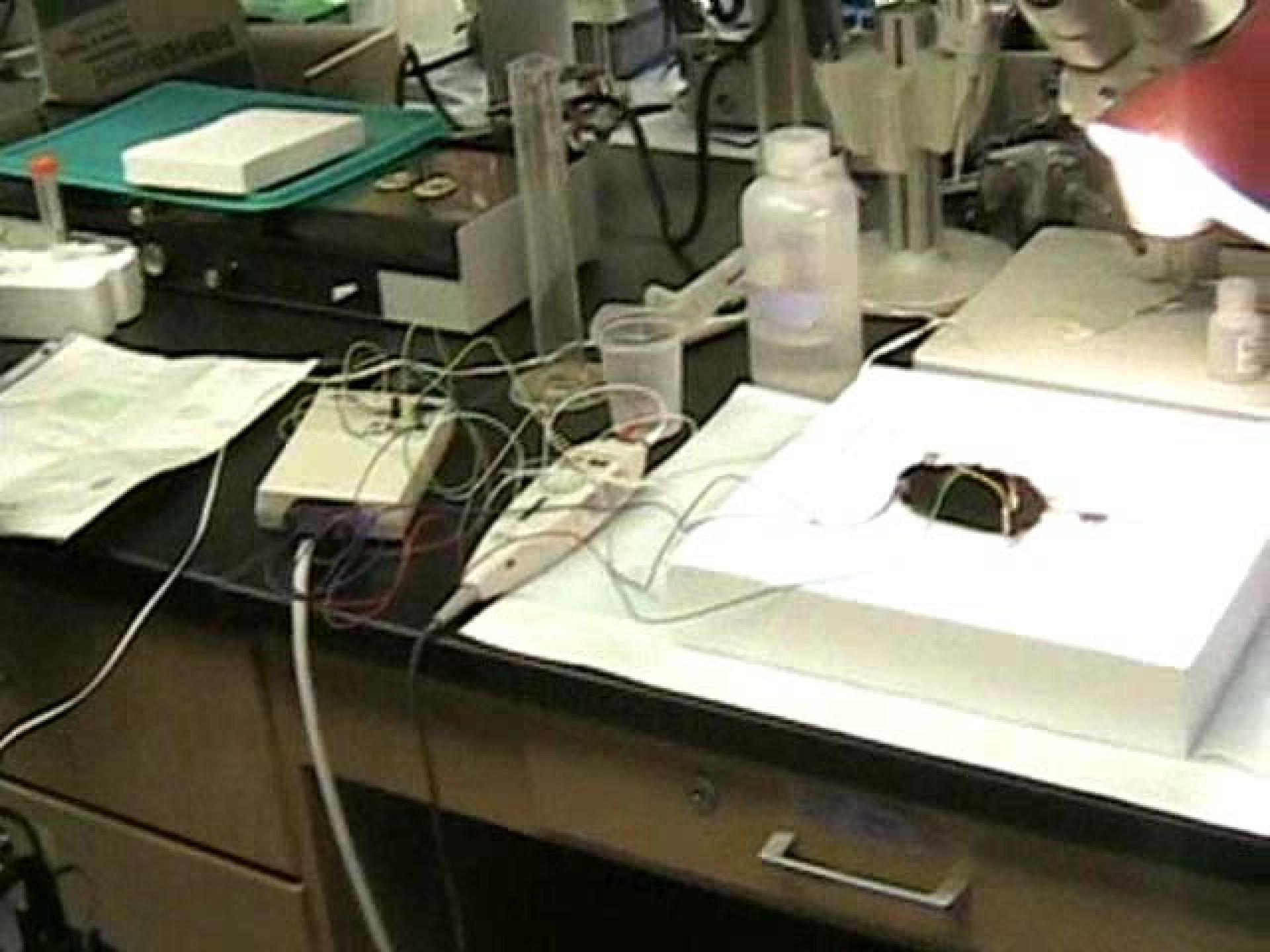
C57BLKS Mouse Has Diabetic Neuropathy
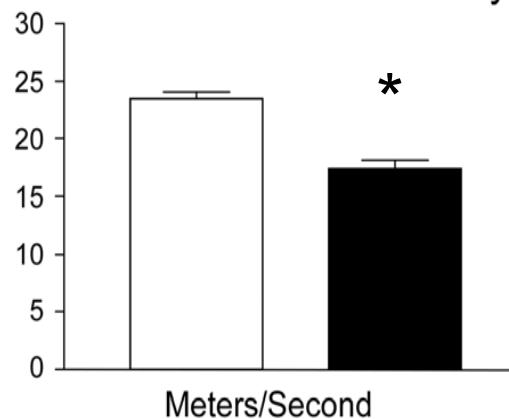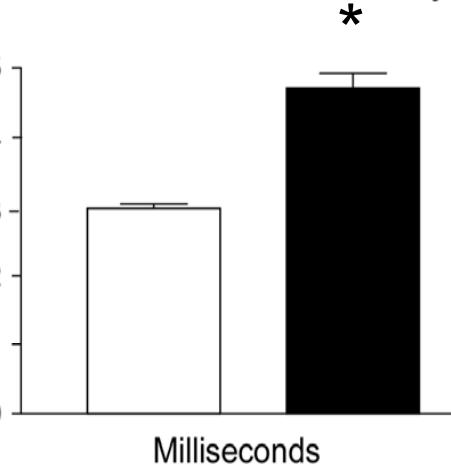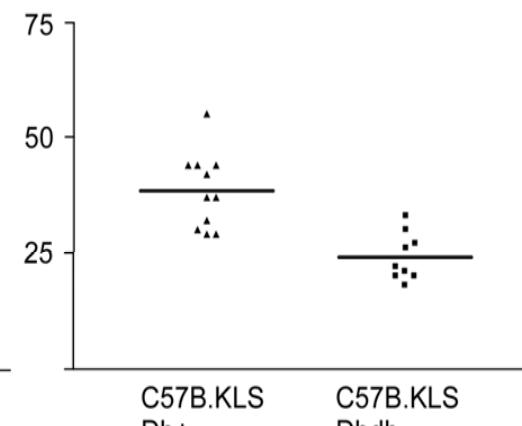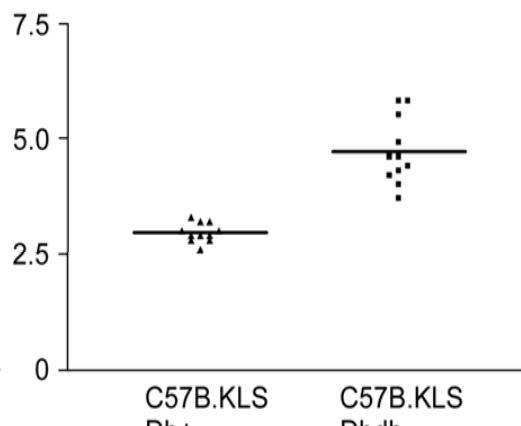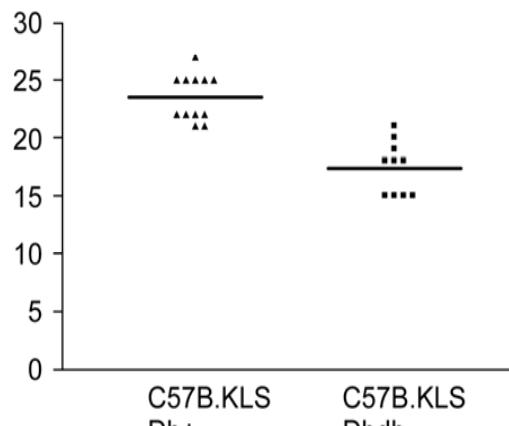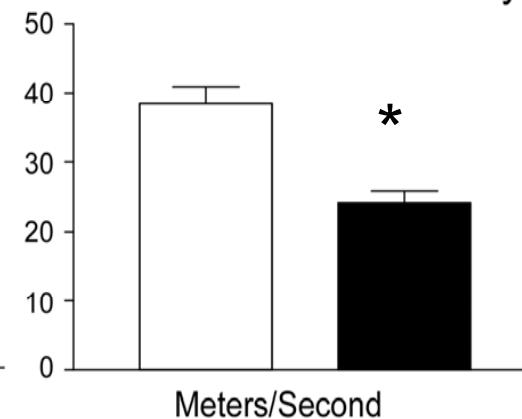
* P< 0.001

IENF in KLS db/db Mice with Early Diabetic Neuropathy
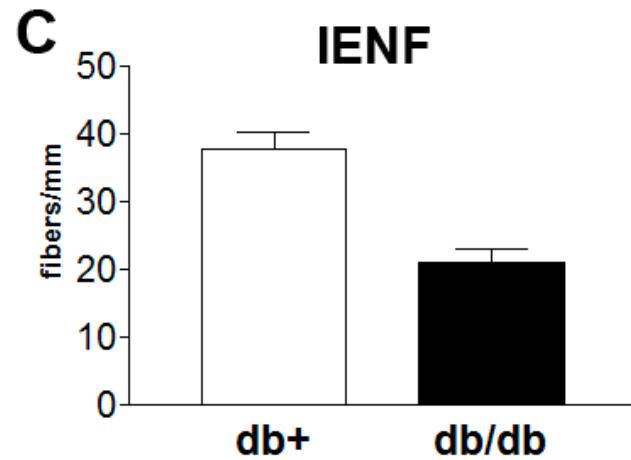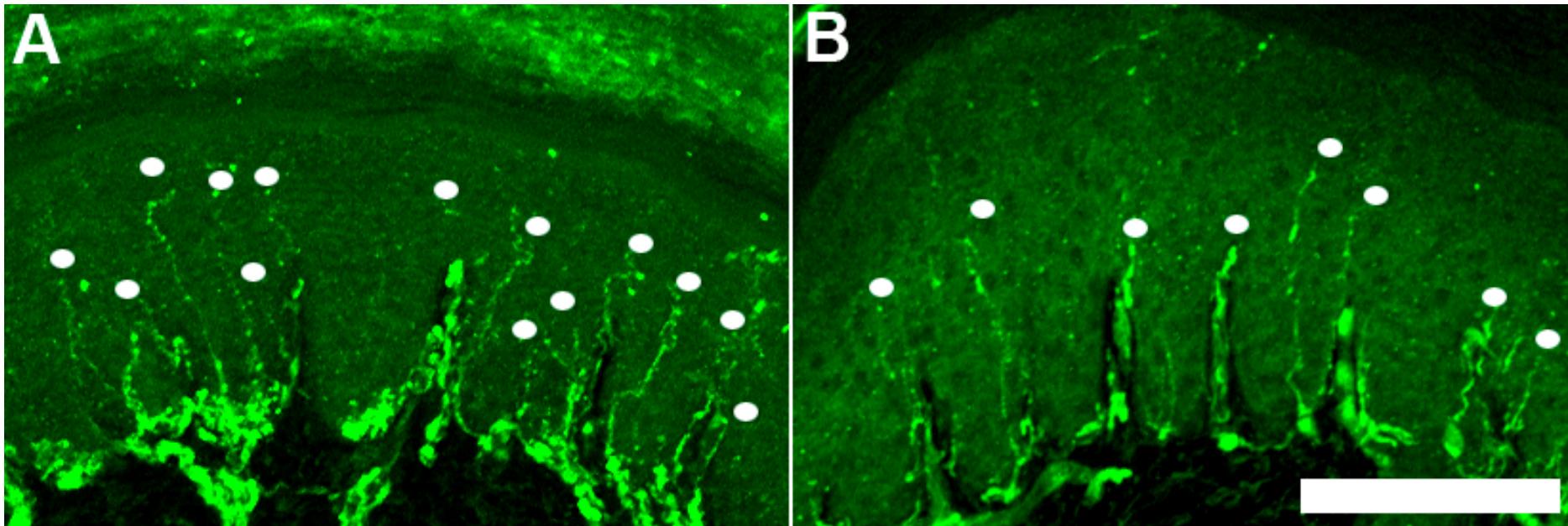
Sullivan et al, in press, Neurobiol Dis

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Diabetic Nephropathy
Classic Models

	Humans	Rodents
• Proteinuria	++	+
• Decline in GFR	++	+/-
• CKD	++	-
• Mesangial expansion	++	+
• Nodular GS	++	-
• GBM thickening	++	+
• Arteriolar hyaline	++	+/-
• Interstitial fibrosis	++	-
• Tubular atrophy	++	-

Proposition for "Best Murine Models" Assessment

- **Clinical:**

Uprot	2
Decreased GFR	2

- **Morphological lesions:**

Nod sclerosis, GBM	2
Art hyaline	2
Int fibrosis	2

Ideal model   **10**

From Agnes Fogo

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EAC questions re: "mid-course corrections."

- **Should future AMDCC efforts be more heavily weighted toward gene discovery or validation of human genetic findings?**

- **How can the information on the genetic architecture of complex traits and the discovery of genetic variants for types 1 and 2 diabetes be incorporated into AMDCC plans and milestones?**

- **Should efforts directed at mapping QTLs associated with diabetic complications be expanded under the AMDCC?**
  - **As a corollary, is a mouse FIND/GoKIND project feasible (are mouse SNP maps sufficiently developed to permit WGA?) and scientifically valuable?**

- **Should additional efforts be made to "tap" this resource (Kretzler) as a way to identify candidate genes for AMDCC study?**

- **Should new efforts be made by the consortium (and JAX) to address Gene x Environment interactions?**

- **Is the value of outbred mouse lines being fully utilized for the study of diabetic complications?**

Murine-Human Transcriptomic Comparisons in Diabetic Nephropathy and Neuropathy

- Harness the capabilities of genome wide analyses for an integrated view of regulatory networks activated in diabetic endorgan damage:

    - Specific Aim 1:
        - Define transcriptional networks activated in humans and in various AMDCC models of murine diabetic complications using transcriptional pathway mapping and promoter modeling tools.
    - Specific Aim 2:
        - Compare transcriptional networks between humans and mice with diabetic complications and define key pathways to be altered in murine models.
    - Specific Aim 3:
        - Utilize a web-based search tool for effective mining of the data sets generated in Aims 1 and 2 by the diabetes complication research community.
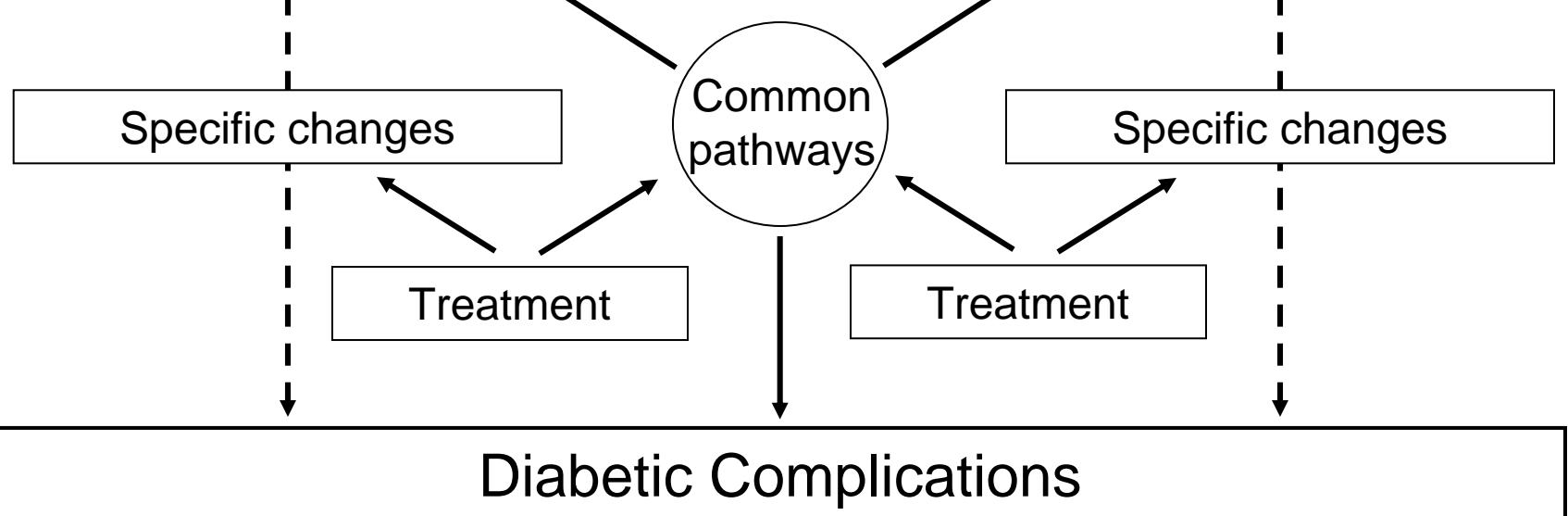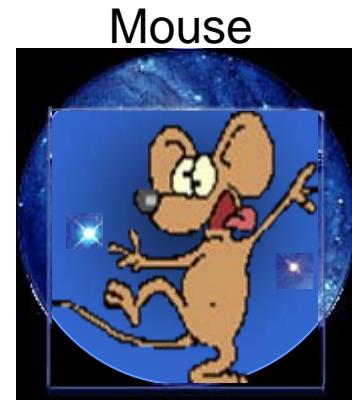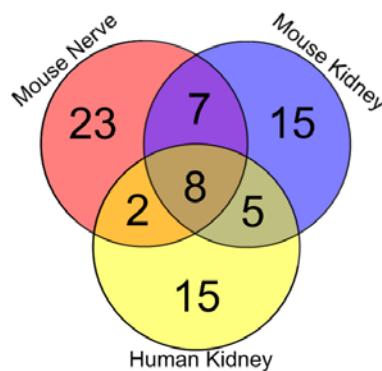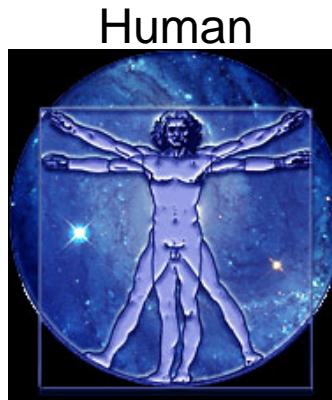
Jak/Stat pathway in human DN
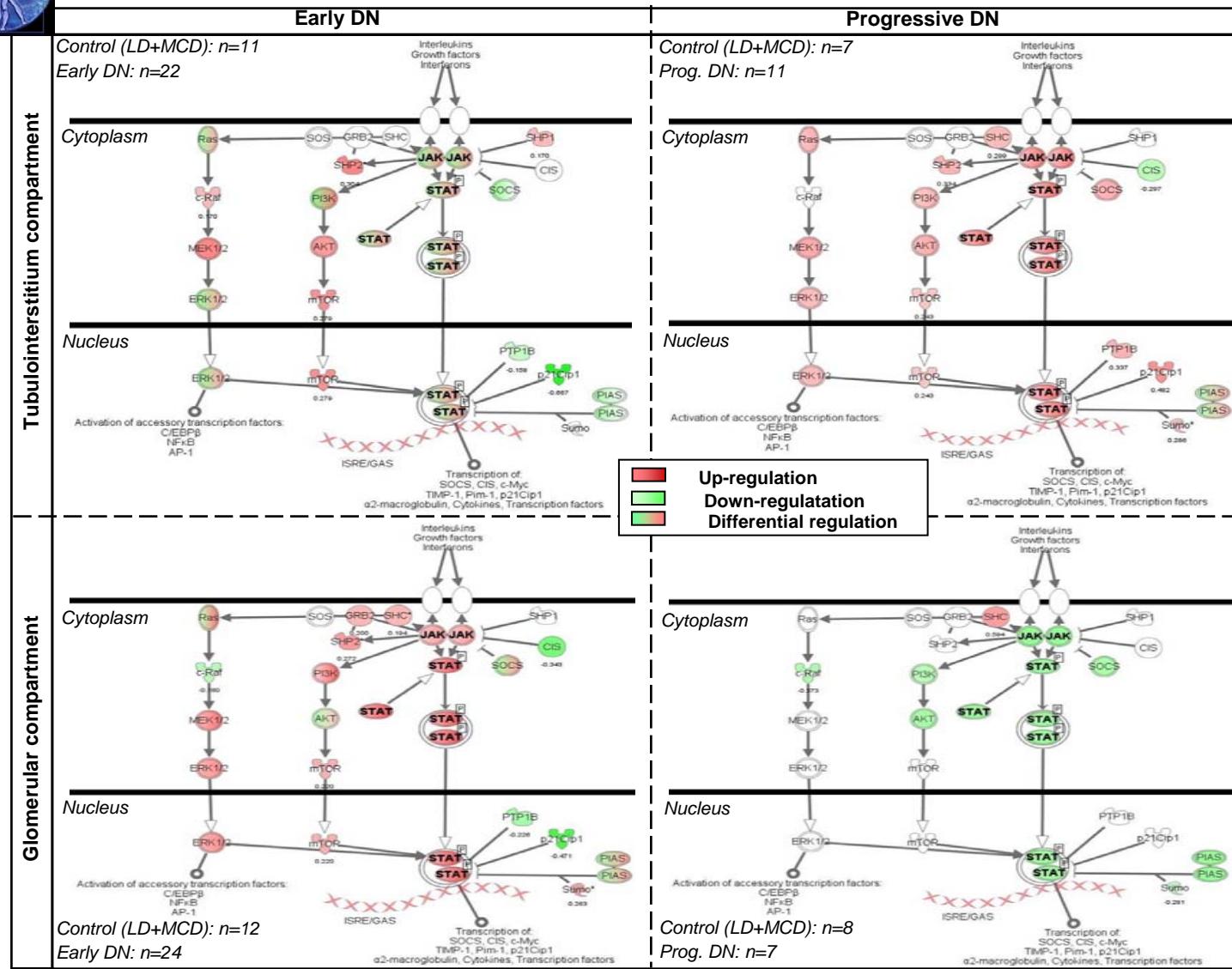
Structural Overview:
www.nephromine.org
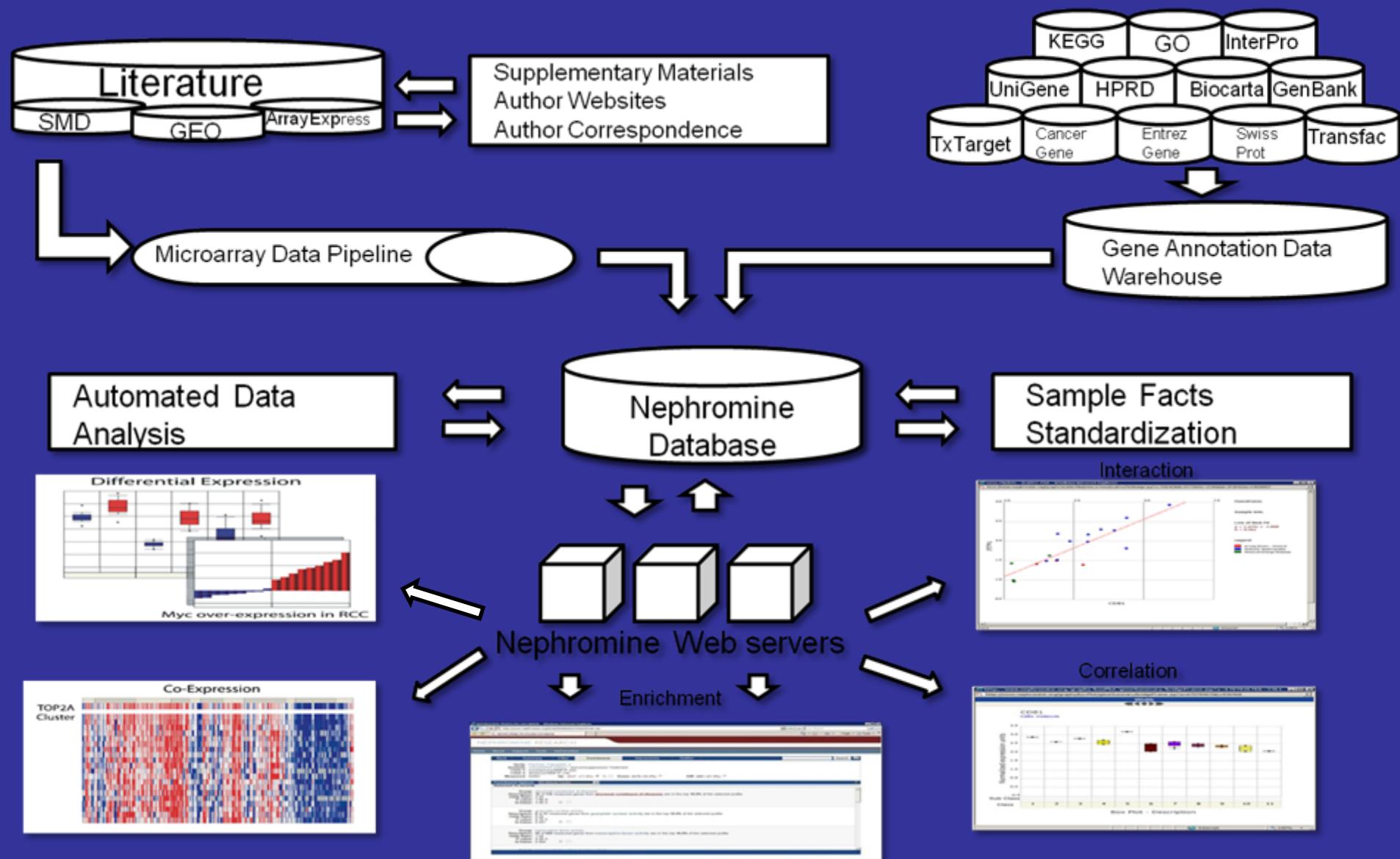
Current AMDCC/MMPC structure (2006 onward)
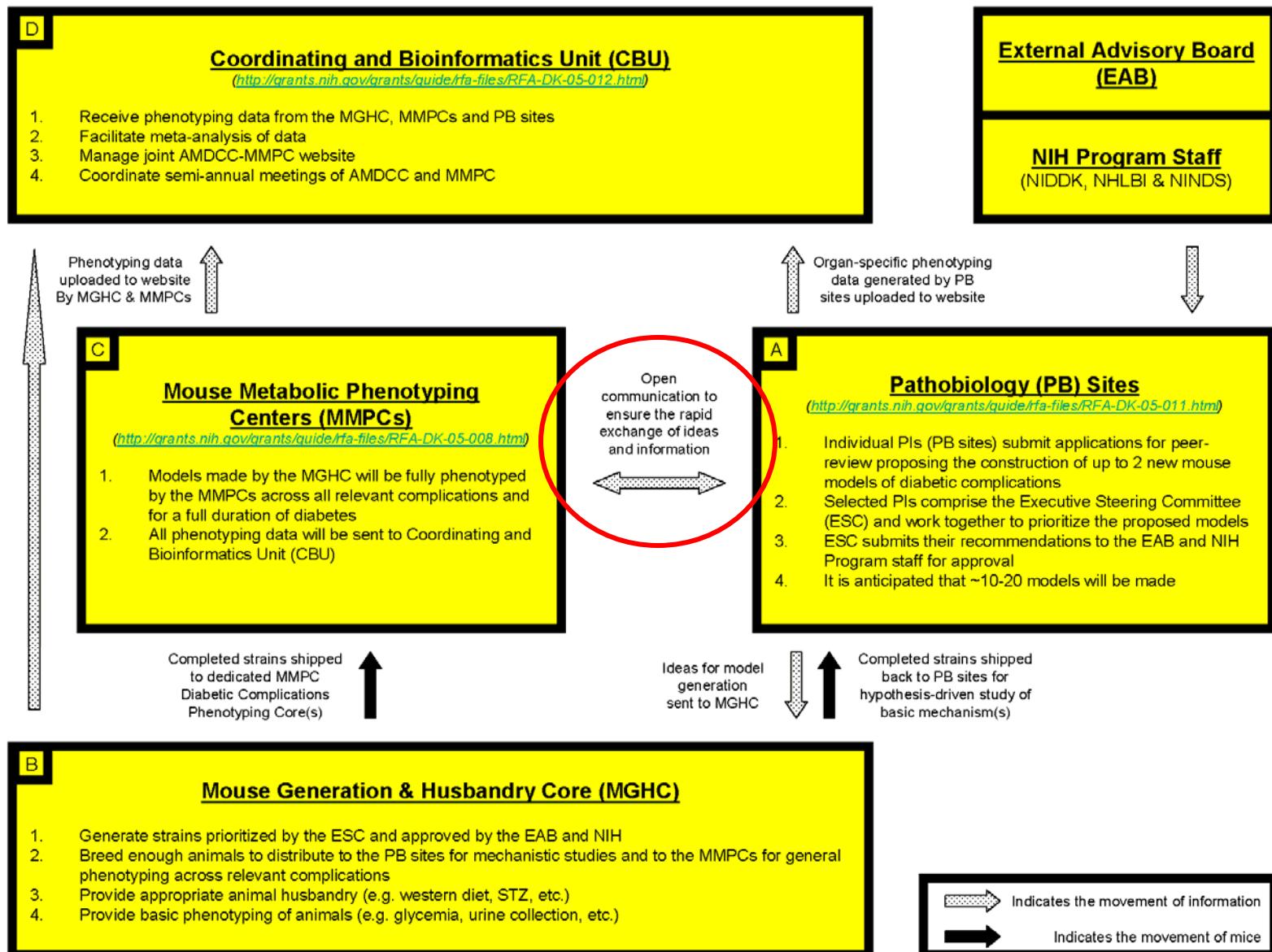
How can the AMDCC and MMPC best interact?

- Codify phenotyping methods and standards (and pricing?).
- Identify models that should be phenotyped for multiple complications and coordinate this phenotyping
- Fill in gaps in phenotyping (we can do neuropathy)
- Address effects of aging, diet and other environmental factors in a coordinated manner.
- Cross-validate and rank (??) models.
- Produce reviews and position papers
- ??