Mechanisms of hypertension and diabetic nephropathy

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The economic burden of kidney disease

- Hypertension and Diabetes are the leading cause of end stage renal disease. 66% of new cases.
- Number of patients with chronic renal disease is exploding currently estimated at 30 million or 13% of the population.
- Currently, approximately 500,000 Americans have been diagnosed with kidney failure. The number of Americans with this advanced stage of the disease is expected to grow to 785,000 by 2020.
- The annual cost of treating kidney disease is $42 billion, 25% of Medicare spending.

- There is a critical need for development of new therapies to slow or reverse the progression of chronic kidney disease.

http://www.asn-online.org/facts_and_statistics/kd-health-threat
Impaired autoregulation and 20-HETE, MMP2, TGF-β Interaction in the Pathogenesis of Renal Disease

Hypertension

↓ Autoregulation

↓ Myogenic tone

↓ 20-HETE

↓ Myoepithelial transformation

↓ TGF-β

↓ P ge

↓ MMP2, TGF-β

glomerulus

↓ P alb

Interstitial Fibrosis Tubulonecrosis

Proteinuria

Glomerulosclerosis

RENAL FAILURE
TGF Responses in Dahl S and R Rats

- Dahl R (n=13, 5 rats)
- Dahl S (n=15, 5 rats)
Effect of 20-HETE on TGF

Diameter of Afferent Arteriole (µm)

20-HETE in tubule (10 µm)
Effect of 20-HETE on Myogenic Response

Diameter of Afferent Arteriole (µm)

Pressure (mmHg)

20-HETE
HET-0016
Pgc during the Development of Hypertension in Dahl S rats

- SS
- 4A+

![Graph showing Pgc (mm Hg) with comparison between SS and 4A+ groups at different conditions: LS, HS, HS+HET0016.](#)
MMP-2 and TGF-β1 levels in Dahl S rats fed either a low salt or high salt diet

Hypertension
XL784 reversibility study in Dahl S rats

MAP (mmHg)

100 140 180 220

Vehicle (12)  Lisinopril + Losartan (12)  XL784 (12)  Lisinopril + Losartan + XL784 (12)

Protein excretion (mg/day)

0 50 100 150 200 250 300

Control 5 6 7 8 9 10

Weeks on High Salt Diet

*
Effect of Hypertension on the Expression of TGF-β in Dahl S Rats

**TGF-β1**

- Control
- TGF-β Ab

**TGF-β2**

- Control
- TGF-β Ab
Effect of TGF-β Ab on Proteinuria and Glomerular Injury in Dahl S Rats fed a High Salt Diet for 3 Weeks.

<table>
<thead>
<tr>
<th></th>
<th>Females (n=10)</th>
<th>Males (n=10)</th>
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</thead>
<tbody>
<tr>
<td>Low Salt</td>
<td>80 g/day</td>
<td>60 μg/min</td>
</tr>
<tr>
<td>High Salt</td>
<td>80 g/day</td>
<td>80 μg/min</td>
</tr>
<tr>
<td>TGF-β Ab</td>
<td>40 g/day</td>
<td>40 μg/min</td>
</tr>
</tbody>
</table>
Figure 1 B

6% BSA

FITC-Dx250

H2O

4% BSA

FITC-Dx250

4% BSA

H2O

H2O

H2O

H2O

4% BSA

FITC-Dx250
Labeling and Isolation of Glomeruli and Imaging

FITC-Dextran 250 kD
SD glomerulus albumin and dextran labeled
Figure 3A

![Graph showing % Fluorescence over Time (seconds). Lines represent different concentrations: 6-5% (12, 59), 6-4% (16, 108), 6-3% (17, 98), 6-2% (26, 100).]
Figure 5B

The graph shows the percentage of fluorescence over time (seconds) for two conditions: Vehicle (6, 37) represented by black circles and TGF-β1 (10ng/ml) represented by white circles. The data points are connected by lines and error bars indicate the variability. The x-axis represents time in seconds ranging from 0 to 50, and the y-axis represents the percentage of fluorescence ranging from 110% to 60%.
Hypertensive model

A

B

% Fluorescence

Time (seconds)

% Fluorescence

Time (seconds)

Expected Δ

Expected Δ
Effect of Hypertension on Glomerular Capillaries in Dahl S Rats

Control

High salt

High salt + anti TGFβ therapy
2 photon verification of TGF b effect

Fitc dextran
Low mw dextran
Albumin blue
Dahl S high salt 20X
Evidence Linking P4504A to salt sensitive Hypertension in Dahl S Rats

• The formation of 20-HETE is decreased in the kidney in Dahl S rats compared to other normotensive and hypertensive strains of rats.

• 20-HETE production in OM is reduced in Dahl S rats which contributes to elevated loop Cl- reabsorption in TALH. 20-HETE production is decreased in glomerulus but vascular production does not seem to lower than other strains.

• Chronic treatment with Fibrates increases the renal formation of 20-HETE, improves pressure natriuresis, and reduces blood pressure in Dahl S rats.

• The CYP4A region on chromosome 5 cosegregates with hypertension in a Dahl S X Lewis F2 cross.

• CYP4A genes are located on rat chromosome 5. Do they contribute to Hypertension in Dahl S rats?
Consomic rats are single chromosome substitutions

MCW: NHLBI program for Genomic applications - PGA

SSBN1
SSBN4
SSBN8
SSBN13
SSBN20
SSBN16

Note: the strain sequenced—is this BN
Blood pressure targets captured in the SS.BN Consomic strains

Telemetry Confirmation of renoprotective phenotype in SS.5^{BN} rats

MAP (mmHg)

Days on High Salt Diet

Protein excretion (mg/day)

‡ P<0.05 vs. other strains
CYP4A expression in the kidney of SS and SS.5^{BN} rats

Renal cortex

Renal outer medulla
Chronic administration of HET0016 reverses the phenotype of SS.5^BN rats

14 Days on High Salt Diet
Sequencing of CYP4A3 cDNA

Ref Seq
NM 175760 SD ccagcaatc... gtaaatatat ....attcagtttt... gataccctacacc
SS1 ccagcaatc... gtcagttataat ...attcagtttt... gatgccttacacc
SS2 ccagcaatc... gtcagttataat ...attcagtttt... gatgccttacacc
SS3 ccagcaatc... gtcagttataat ...attcagtttt... gatgccttacacc
SSBN51 ccagcaattt... gtcatataatat ...attcatgtttt... gatacccctacacc
SSBN52 ccagcaattt... gtcatataatat ...attcatgtttt... gatacccctacacc
SSBN53 ccagcaattt... gtcatataatat ...attcatgtttt... gatacccctacacc
Creation of GFP transgenic rats using Sleeping Beauty transposon

Add3, Dusp5 in FHH, 4A1 in SS rats
MAP and Proteinuria- SB Trasposon- CYP4A Transgenic rats vs Dahl S rats

8% NaCl diet

MAP (mmHg)

Proteinuria (mg/day)

Time (days)

Dahl S (n=7)
Line A (n=9)

* vs Dahl SSJr, p<0.05
Zinc finger nuclease KO technology (MCW, Guertz and Jacob)

\[ Fok1 \text{ nuclease domain} \]

\[ \text{Zinc finger motifs} \]

\[ \text{Agouti Coat Color Phenotype} \]

\[ \text{KO Add3, Dusp5 in FHH.1BN congenic, 4A2 in SS.BN5, MMP, TGFb in SS} \]

PNAS, 2006 103(44): p16370-16375
The FHH rat

The FHH rat is a genetic model of hypertension-induced renal disease

- Proteinuria- QTL chr 1
- Focal glomerulosclerosis
- Systolic hypertension (late)
- Pulmonary Hypertension
- Bleeding disorder
- Coat Color

*Kidney Int Suppl* 45: S2-S5, 1994
Substitution of Chromosome 1 from the BN rat restores the autoregulation of RBF in FHH rats

Pgc is elevated in FHH rats

MMP-2 and TGF-β1 levels are increased in the kidneys of FHH rats at 21 weeks.
Dilutional Palb measurements in SD and FHH rats

% Fluorescence

Time (seconds)

- ▲ FHH (8,82)
- ○ SD (12,206)
Infusion of FITC albumin in FHH and FHH.1\textsuperscript{BN} congeneric rats
Unbiased Discovery Based Approach:
Consomic rats are single chromosome substitutions.

MCW: NHLBI program for Genomic applications – PGA 2001

SSN1
SSN4
SSN8
SSN13
SSN16

SS diseased
BN normal
Albuminuria in FHH treated with LNAME

Transfer of Chrm 1 restores autoregulation of RBF in the FHH rat

Genetic map illustrating the introgressed regions in FHH.1^{BN} AR+ congenic strains

Left: indicates the location of genetic markers used to genotype the animals on chromosome 1 in FHH rats. The closed and open filled bars refer to Fawn hooded-hypertensive (FHH) and Brown Norway (BN) genomes, respectively.

Right: indicates known candidate genes in the 2.6 Mb region of interest. AR= Auto regulation; + present; - present.

- Similar to ribosomal protein L8
- Soluble aminopeptidase P1
- Max interactor 1
- Adducin 3 (gamma)
- Survival motor neuron domain containing 1
- LOC499376
- Dual Specificity Phosphatase 5 (DUSP 5)
- Structural maintenance of chromosome 3
- RNA binding motif protein 20
- Programmed cell death 4
- Soc-2 (Suppressor of clear) homolog

<table>
<thead>
<tr>
<th>Strains</th>
<th>FHH</th>
<th>BN</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
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<tbody>
<tr>
<td>A. Control</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>B. AR+</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>C. AR+ (original)</td>
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<tr>
<td>D. AR-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. AR+ (new)</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Region (Mb) | 6.1 | 4.6 | 2  | 2.6 |
Genes       | 19  | 17  | 6  | 11  |
Autoregulation | -  |  +  | -  | +   |

*Left*: indicates the location of genetic markers used to genotype the animals on chromosome 1 in FHH rats.
The closed and open filled bars refer to Fawn hooded-hypertensive (FHH) and Brown Norway (BN) genomes, respectively.

*Right*: indicates known candidate genes in the 2.6 Mb region of interest. AR= Auto regulation; + present; - present.
FHH and FHH.1\textsuperscript{BN} congenic rats

![Graph depicting the effect of RPP (mmHg) on % of control (100 mmHg) for FHH and FHH.1\textsuperscript{BN} congenic rats.](image)

![Bar chart showing RBF Autoregulatory Index for FHH and FHH.1\textsuperscript{BN} congenic rats.](image)
Restoration of myogenic response in AR+ FHH.1BN congenic strain E

![Image showing a micrograph with perfusion pressure at 60 mmHg and 140 mmHg.]

**Graph:**
- Y-axis: Afferent arteriole diameter (μm)
- X-axis: Perfusion pressure (mmHg)
- Data points:
  - FHH (4)
  - FHH Ca$^{+2}$ free (4)
  - AR+ (4)
  - AR+ Ca$^{+2}$ free (4)
Restoration of RBF autoregulation mitigates proteinuria and renal injury

![Images showing proteinuria and renal injury](image)

![Graph showing protein excretion](image)

Glomerular injury score:
- FHH: 3
- AR+: 1

Protein excretion (mg/day) vs. Age (weeks):
- FHH (10)
- AR+ (6)

* indicates statistical significance.
Decision tree for the prioritization of candidate genes for transgenic studies.

1. Positional candidates
   - Quantitative PCR
     - Differentially Expressed
       - No
         - Comparative Seq. Analysis
           - No
             - Stop
           - Yes
             - Sequence Variant in Regulatory Element
               - Yes
                 - Stop
               - No
                 - Alter Promoter activity
                   - No
                     - Stop
                   - Yes
                     - Sequence Variant in Conserved Region
                       - Yes
                         - Candidate for Transgenics
                       - No
                         - Stop
      - cDNA Sequence
        - Stop codon, splice variant or Point mutation altering protein structure
          - Yes
            - Candidate for Transgenics
          - No
            - Stop
SNP analysis in coding region of Add3 gene: 37 SNPs

<table>
<thead>
<tr>
<th>Exon/Intron mRNA Position of SNP</th>
<th>Exon2 147</th>
<th>Exon2 229</th>
<th>Exon13 1847</th>
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<tr>
<td>ENSRNOT00000017600(BN)</td>
<td>C/Threonine</td>
<td>C/Aspartic Acid</td>
<td>A/Lysine</td>
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<tr>
<td>NC-0015100.2(SD)</td>
<td>C/Threonine</td>
<td>G/Serine</td>
<td>A/Lysine</td>
</tr>
<tr>
<td>NM-031552(SD)</td>
<td>G/Serine</td>
<td>T/Aspartic Acid</td>
<td>C/Aspartic Acid</td>
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<tr>
<td></td>
<td></td>
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<tr>
<td>BN gDNA</td>
<td>C</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>ACI gDNA</td>
<td>C</td>
<td>T</td>
<td>A</td>
</tr>
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<td>FHH gDNA</td>
<td>G</td>
<td>T</td>
<td>C</td>
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<tr>
<td>SHR gDNA</td>
<td>G</td>
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<tr>
<td>FHHRV mRNA</td>
<td>G</td>
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<td>C</td>
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<tr>
<td>FHHCV mRNA</td>
<td>G</td>
<td>T</td>
<td>C</td>
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<tr>
<td>FHM8RV mRNA</td>
<td>C</td>
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<tr>
<td>FHM8CV mRNA</td>
<td>C</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>SHR mRNA</td>
<td>G</td>
<td>T</td>
<td>A</td>
</tr>
</tbody>
</table>

19 SNPs in Dusp5 gene
FHH and FHH.1\textsuperscript{BN} congenic rats

Autoregulation of RBF: - + - + +
Proteinuria: + - + ?

Rab-38 - responsible for the re-uptake of filtered protein in the proximal tubules

“Candidate region”
Rab38 Sequencing

FHH.BNRab38
FHH

Rab 38 regulates reuptake and processing of filtered albumin
Reasons for lack of progress in preventing diabetic nephropathy

• **Problem:**
  No rodent model of diabetic nephropathy exhibits progressive renal disease and lesions resembling those seen in man. Cell models not particularly informative.

• **Solution:** We combined the genome of the FHH rat that develops renal disease but not diabetes with that of the GK rat that develops type II diabetes but not renal disease.
Glucose Intolerance in T2DN Rats

Glycemia (mg/dL)

Time of IPGTT (minutes)

- 12 months
- 9 months
- 6 months
- 3 months

BN
Progressive Proteinuria in T2DN Rats

- Male BN
- Male T2DNmimic

* denotes different from age-matched BN
# denotes different from previous age in group-matched
Diabetes model

A

% Fluorescence vs. Time (seconds)

- STZ (8,135)
- SD (12,206)

B

% Fluorescence vs. Time (seconds)

- T2DN 6 months (4,69)
- SD (12,206)

Expected Δ
Glomerular Basement Membrane in 12 month T2DN Rats
Effect of tight diabetic control on diabetic nephropathy
MMP-2 and TGF-β1 levels in T2DN rats

MMP2 levels (pg/mg of protein)

<table>
<thead>
<tr>
<th></th>
<th>3 month T2DN (4)</th>
<th>6 month T2DN (4)</th>
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<tbody>
<tr>
<td>0</td>
<td>50</td>
<td></td>
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<tr>
<td>50</td>
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</tr>
<tr>
<td>100</td>
<td>150</td>
<td></td>
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<tr>
<td>150</td>
<td>200</td>
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</table>

TGF-β levels (fg/mg of protein)

<table>
<thead>
<tr>
<th></th>
<th>3 month T2DN (4)</th>
<th>6 month T2DN (4)</th>
</tr>
</thead>
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<tr>
<td>0</td>
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<tr>
<td>600</td>
<td>800</td>
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</table>

Type-2 diabetes
Effects of XL784 on proteinuria in T2DN rats

- Vehicle (12)
- Lisinopril (10)
- XL784, 50 mg/kg (10)
- Lisinopril + XL784, 50 mg/kg (11)
- XL784, 150 mg/kg (10)

Protein excretion (mg/day)

Time (months)

Control 1 2 3 4
Effect of an ACE Inhibitor and MMP on diabetic nephropathy

Vehicle

Lisinopril

XL784
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