Defining Feature: excessive glomerular protein leak into the urine

".....when the bubbles settle on the surface of the urine, it indicates disease of the kidneys and the complaint will be protracted..."

Hippocrates – 400BC
Relapse

Remission
Despite 2500 years of clinical description...

many molecules and mechanisms remain a mystery...
Immune dysfunction

Genetic

Reactive

Immune activation
-innate/acquired

Epigenetic Influences

Genetic background
Characteristics of childhood NS

- heterogeneous:
  - isolated kidney disease
  - component of multi-system disorder
  - hereditary:- improper glomerular development and subsequent molecular malfunction
  - idiopathic
  - acquired - secondary damage
  - incongruent drug responses: steroid sensitive, steroid resistant, CNI, MMF

⇒ malfunction of the glomerular filtration barrier — complex macromolecular sieve and primary ultra-filter for plasma by the kidney
3D Glomerulus
1. Endothelium

2. Glomerular basement membrane

3. Podocytes
GFB malfunction: podocyte key culprit
GFB in disease

Normal

Nephrotic syndrome
“Podocentric” view – podocyte is the key culprit

- how dependent is protein filtration simply on the integrity of the slit diaphragms
- role of other GFB components
Clinical clues for a genetic basis

- monogenic diseases – AR/AD inheritance
- identification of causative genes by standard genetic tools
- to date: primarily expressed in podocytes or GBM
- positional cloning followed by mutation screening
- type of gene and/or mutation can indicate function
- *in vitro* experimental strategies and animal models: how does the mutation cause disease?
Scientific clues for a genetic basis

- glomerular cell biology: stereotypic response to injurious stimuli
- knockout models: mice, xenopus, zebrafish, \textit{C. Elegans}, drosophila, chick
- monogenic: single podocyte gene defects
- genetics of complex disease: rare variant hypothesis
- podocytes main target of injury
- \textit{but}, contribution of GBM and endothelium (+/- mesangial cells) may be underestimated
Molecular overview of the slit-diaphragm and podocyte cell–matrix interactions.

## Candidate genes (n=25)

### Direct linked
- **WT1**
- **NPHS1**
- **NPHS2**
- **ACTN4**
- **TRPC6**
- **PLCE1**
- **MYH9**
- **CDAP2**
- **INF2**
- **APOL1**
- **Arhgap24**
- **Cubulin**
- **MYOE1**
- **GLEPP1**
- **LAMB2**
- **LMX1B**
- **COL4A3/4A4/4A5**
- **COQ2**
- **COQ6**
- **PDSS2**
- **SMARCAL1**

### Indirect link - mouse models:
- **NEPH 1,2,3**
- **FAT 1,2**
- **PAX2**
- **PAR4**
- **NOTCH**
- **aPKC**
- **IL4**
- **Rac**
- **Podocalyxin**
- **S-laminin**
- **Mpv17**
- **others**
Other Genetic Influences

- epigenetic effects
- modifier genes: genetic pathways and protein complexes – other genes abnormalities may modulate disease
- functional redundancy
Clinical clues – genetic NS

- correct description of phenotype
- isolated kidney disease or syndromic
- onset < 2 years: >90% chance of genetic mutation
- onset < 5 years: 50% chance of genetic mutation
- treatment resistance
- family history
- pattern of inheritance: autosomal recessive, autosomal dominant, (sporadic)
- ethnic origin
- renal histology
Phenotype

Isolated kidney disease:
- Finnish type (CNF)
- diffuse mesangial sclerosis (DMS)
- focal segmental glomerulosclerosis (FSGS)
- minimal change (MN)

Syndromes:
- Denys Drash, Frasier, Galloway-Mowat,
- Nail Patella, Pierson, Lowe, Alports, Leigh,
- mitochondrial cytopathies, AMRF syndrome,
- Schimke
AGE OF ONSET
INHERITANCE
CLINICAL PHENOTYPE
(DRUG RESPONSE)
RENAL HISTOLOGY

Birth to 2 YEARS

Congenital
> 3/12

CNF
DMS
FSGS
Syndromes

Single gene mutations in
>90%

> 2 – 18 YEARS

FSGS
DMS
Minimal change Syndromes

>3/12-2years

DMS
FSGS
Minimal change Syndromes

Single gene mutations in
>50%

Single gene mutations less likely unless family history, predisposition alleles
Genes to consider

Age of onset
+
Inheritance & Phenotype
+/-
Renal Histology

**CNF**
NPHS1 (NPHS2)

**DMS**
PLCE1
WT1
CD2AP

**FSGS** (childhood)
NPHS2
WT1
PLCE1
CD2AP
NPHS1
MYOE1
COQ6
TRPC6
GLEPP1

**FSGS** (later onset)
TRPC6
ACTN4
CD2AP
MYH9/APOL1
NPHS2
WT1
INF2
Arhgap24
Arhgap24

**Collapsing FSGS** (non-syndromic)
CD2AP
COQ2
PDSS2
Glepp1

**Syndromes**
*WT1*
COQ2 and 6
PDSS2
SMARCAL1
SCARB2
PMM2
Lamb2
LMBX1

**FSGS + GBM**
Abnormality
Lamb2
LMBX1
COL4A3/COL4A4
Inheritance pattern

- Autosomal dominant
  - ACTN4
  - TRPC6 *
  - INF2*
  - LIMX1B*

- Autosomal recessive
  - NPHS1 *
  - NPHS2*
  - PLEC1*
  - LAMB2*
  - Arhgap24
  - APOL1/MYH9 * - African descent
  - GLEPP1

- many families have “private” gene mutations
Hereditary FSGS: AD

New Zealand

No Fhx renal disease/FSGS

FSGS, transplant

FSGS, transplant x2

FSGS, transplant

FSGS, CKD3

?
Which genes to test?

- Analyse phenotype
- CNS: NPHS1, NPSH2, WT1, LAMB2
- Later: NPHS1, NPSH2, WT1, LAMB2, PLCE1, APOL1/MYH9, TRCP6, INF2
- Use correct genetic tools
**NPHS1 mutations**

- congenital nephrotic syndrome: Finnish and FSGS
- Finns: two mutations: Fin major (nt121delCT), Fin minor (R1109X) - truncated, unstable proteins
- non-Finns: mutations distributed throughout the gene:
  - approx 60%: missense or non-frameshift deletions and insertions; approx 40%: nonsense, frameshift, splicing and promotor mutations
- steroid/drug resistant
- ? Predisposition to later onset NS
NPHS2 mutations

- 45 - 55% of familial FSGS
- additional role in sporadic FSGS – 8 to 20%
- present instead of NPHS1 mutations in ~ 5% of CNF
- di-genic inheritance of NPHS1 and NPHS2 mutations results in tri-allelic hit and congenital FSGS
- some mutations predictive of clinical course – have ethnic bias
- heterozygous R229Q variant – predisposition allele: later onset FSGS – also steroid resistance, microalbuminuria
WT1 and nephrotic syndrome

“Isolated” DMS/FSGS – rare: if detected likely be on DDS/FS spectrum

Denys Drash syndrome (DDS)

- congenital or early onset DMS
- intersex, Wilms’ tumour
- heterozygous missense mutations: exon 8/9

Frasier syndrome (FS)

- FSGS >5 years: consider chromosomes
- Intersex, FSGS, gonadoblastoma
- may be hereditary
- specific intron 9 mutations which disrupt normal splicing
- beware FSGS and delayed puberty
**PLCE1 Mutations**

- DMS/FSGS in first two years of life
- non-penetrance
- 15 - 28% incidence idiopathic DMS
- not a major cause of familial and later onset FSGS – screening of 69 families (231 individuals) completely negative
- may respond to steroids/ciclosporin – cannot be predicted by genotype
GBM nephrotic Genes

**LAMB2**
- laminin β-2: Pierson syndrome - ocular defects, congenital DMS with truncating mutations; FSGS with missense and non-truncating

**LMBX1**
- encodes transcription factor regulating collagen III and imp. for podocyte development: Nail patella syndrome and FSGS

**COL4A3/COL4A4**
- Alports and FSGS
- possible association with heterozygous *NPHS2 R229Q* variant as well as *MYH9*
- association with linkage to 11q24
Very Rare AR Syndromes

- **Schimke immuno-osseous dysplasia**
  - spondyloepiphysseal dysplasia, T cell immunodeficiency, FSGS
  - *SMARCAL1*: actin dependent chromatin remodelling

- **AMRF**
  - action myoclonus renal failure syndrome (FSGS) >15 years
  - FSGS
  - *SCARB2/LIMP2* lysosomal scavenger protein

- **Mitochondrial cytopathies**
  - metabolic
  - *COQ2* deficiency and Leigh Syndrome
  - collapsing FSGS

- **Carbohydrate-deficient glycoprotein syndromes**
  - *PMM2* (phosphomannomutase-2) gene mutations
  - FSGS
Idiopathic steroid sensitive NS

- rarely familial
- associated with polymorphisms in $ACE$, $VEGF$, $HLA$, $NPHS1$, $MDR1$, $MIF$
- exclusion of linkage to $NPHS2$ - distinct gene loci likely (homozygosity mapping: locus on chr 2p12-p13.2 (Ruf et al, 2003))
- familial and sporadic SSNS in Israeli + Bedouin populations: no linkage to chromosomal loci and no association with mutations in 80 podocyte genes (Landau et al, 2007)
- susceptibility genes: rare variant hypothesis
Genetic variants influencing human traits

- Mendelian diseases are caused by extremely rare variants with very large effect size (~1,000-fold)

- Complex genetic disease: genetic variants with individually small effect size (typically < 2 fold)
Exome/Genome Sequencing

- rare variants at low frequency in the general population could be primary drivers of nephrotic disease
- protein coding regions $\approx 1\%$ of the human genome but harbor 85% of mutations with large effects on disease-related traits
Aim of Genetic Studies

- To understand molecular mechanisms underlying SSNS – design of targeted treatment
- Genetic testing to identify “at risk” population
- More accurate diagnosis/prognosis
- Choosing appropriate treatments
- Gene/stem cell therapy
Gene Detection

- **Linkage**
  - single-gene ‘Mendelian’ disorders
  - inheritance patterns related to several 100’s to 1000’s genomic markers

- **Candidate gene approach**
  - small sample sizes
  - variants assayed limited to a to a select phenotype

- **GWAS**
  - susceptibility loci for complex genetic disease

- **Targeted next generation sequencing**
  - whole exome/whole genome
Research Approaches

Molecular Analysis

- next generation sequencing
- population genetics – likely to be complex inheritance
- detection of potential gene mutation
- examine effect on protein function *in silico*
- functional studies *in vitro/in vivo* – transgenics
- biology of relevant cell type in health and disease

Immunological Analysis

- role of innate/acquired immunity
- complement
- immune-related genes/mechanisms
Exome Sequencing

- **Test Population:** childhood NS

- **Approaches**

  **Targeted Capture:**
  - entire podocyte “genome” $\approx 7000$ genes
  - custom designed exon sequence capture hybridization
    - 385K array (NimbleGen) to capture exons of 4000 selected podocyte genes

  **Whole Exome:**
  - entire protein coding region
  - next generation sequencing - rare disease initiative:
    - RaDaR
    - https://bridgestudy.medschl.cam.ac.uk/index.shtml
Clinical Applications of Genetic Analysis

- Aetiology of disease – targeted therapy

- Bio-banking - RaDaR/NIHR Bioresource (national rare disease initiative)

- Diagnostics – genotype-phenotype to improve diagnosis and accurate prognosis

- Pre-transplant: genetic mutation confers minimal risk of disease recurrence

- Therapeutics - tailor made medicine in the clinic
Thank you and Questions