Genetics - what clinicians need to know

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• Kidney Foundation of Canada, US and Aus Alport Foundations
Discovery phase for inherited renal disease

Haemochromatosis in 1980s- rare
• 1º, dietary factors, Bantu cooking pots
• Sometimes familial tendency, HLA association
Inherited renal disease

- Many renal diseases are inherited even they only occur in one family member
- Many apparently sporadic diseases are genetic
Inherited kidney disease

• 50% renal failure in children, 20% in adults
  – One in 100 – Thin membrane nephropathy, structural anomalies
  – One in 500 – polycystic kidney disease
  – One in 5,000 - Alport syndrome, FSGS
  – One in 100,000 – cystinosis, von Hippel Lindau disease

• Underdiagnosed
Inherited renal disease

• All types of inherited renal disease can also present for the first time in adults

  • AR polycystic kidney disease
  • Nephronophthisis
  • Bardet-Biedl syndrome
  • Alagille syndrome
  • MELAS syndrome
  • Kearns-Sayre syndrome

  • LCAT deficiency
  • Cystinosis
  • Oxalosis
  • Alport syndrome
  • Fabry disease
Inherited renal disease

- Structural renal disease – single kidney, reflux, duplex system
- Cystic disease
- IgA disease
- SLE

- Complement abnormalities
1. Genetic testing – why test for mutations?
2. Inherited renal disease
   – Alport syndrome
   – C3 nephropathies
3. Identifying new genes – Scalp-Ear Nipple syndrome, IgA disease etc
4. New genetic treatments
5. What the renal community can do
   – MBS Item numbers for genetic testing
   – Registries and mutation databases
1. Genetic testing
Massively parallel sequencing

- DNA variants in exome, splice site and nearby introns
- Detects more mutations
- Confirms mutations
- More mutations = Better genotype-phenotype correlations
- Previously unsuspected double and triple mutations in the same gene, or different genes

- Gene discovery
- Modifying genes eg podocin mutations in Thin membrane nephropathy
Massively parallel sequencing
Why test for mutations

• Diagnosis – only need to test one family member, ‘cascade’ testing in other members
• Genotype-phenotype correlations and predicting clinical outcome
• Further mutations, modifying mutations
• Treatments will be tailored to mutation type
  – Missense mutations - 40%
  – Nonsense mutations (direct and downstream) – 40%
Children’s Hospital Westmead

5 years national podocin nephrin testing

Clinical Exome
Cost $1000-1500: Tested >50 renal patients - 50% gene diagnosis, 150-200 x cover
Launch Mid March, NATA accredited

www.kidney-research.org/genetest

3 Panels AUSCAMV4

Panel 1 aHUS/Complement 9 genes
Panel 2 Nephrotic 34 genes
Panel 3 Cystic 1A ADPKD etc 12 genes
Cystic 1B NPHP RP 39 genes
Cystic 1D ARPKD 14 genes
Cystic 1E BBS 14 genes
Cystinosis/Hyperpoxaluria
AUSCAM, Tubular CAKUT coming
2. Inherited renal diseases
   - Alport syndrome
   - C3 nephropathy
Alport syndrome

- 1:5000, so 4,500 people in Australia
- X-linked - 85%, AR - 15%
- Haematuria from age of 6, juvenile onset – renal failure before 30 years, hearing loss, eye changes
- 95% females have haematuria, 25% have renal impairment by 60 years
Expert Guidelines for the Management of Alport Syndrome and Thin Basement Membrane Nephropathy

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1: Definitions

• Alport syndrome - patients with the characteristic clinical features, a lamellated GBM with an abnormal collagen IV composition, and where a COL4A5 mutation (XL AS) or two COL4A3 or COL4A4 mutations (in trans) are identified or expected.

• TBMN – individuals with persistent isolated glomerular haematuria with a thinned GBM due to a heterozygous COL4A3 or COL4A4 (but not COL4A5) mutation.
2: Diagnosis of Alport syndrome -

- The diagnosis is highly likely if there is glomerular haematuria and a family history of Alport syndrome and no other cause for hematuria
- Or there is hearing loss, lenticiconus or retinopathy
- Or the GBM lacks the collagen IV α5 chain

- The diagnosis is confirmed with the demonstration of a lamellated GBM, or a *COL4A5* or two *COL4A3* or *COL4A4* mutations
Cornea in Alport syndrome
Lens in Alport syndrome
Central retinopathy
Maculopathy
Retinal temporal thinning
Macular hole
Giant macular hole
Peripheral retinopathy
# Ocular features

<table>
<thead>
<tr>
<th></th>
<th>Diagnostic</th>
<th>‘Severe’ mutations</th>
<th>Early onset renal failure</th>
<th>Distinguish female with AR from XL</th>
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<tr>
<td>PPD</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
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<td>Lenticous</td>
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<td>YES</td>
<td>YES</td>
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<td>Peripheral retinopathy</td>
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</tr>
</tbody>
</table>
3: Mode of inheritance

- The mode of inheritance is determined most accurately with the demonstration of a pathogenic mutation in the \textit{COL4A5}, or two mutations \textit{in trans} in the \textit{COL4A3} or \textit{COL4A4} gene
X-linked and AR disease

• The risks are very different for XL and AR disease
• Male with X-linked disease will have normal sons
• Female with X-linked disease will have half her sons affected and half her daughters affected

• AR – only one generation affected, suspect in a female with renal failure, lenticonus or retinopathy, consanguinity
Overall there are twice as many affected females as affected males

In each generation there are three times as many affected females as males
4. Genotype-phenotype correlations

- Mutations in *COL4A5* gene
- Early onset renal failure (before the age of 30 years) with these mutations (50%)
  - Large rearrangements, deletions and insertions
  - Nonsense mutations
  - Late onset renal failure
- Many missense mutations (40%) – late onset renal failure
- Genotype-phenotype correlation less clear for AR AS but probably the same
5: Family screening

- All affected members of a family should be identified. This includes all affected females, and most mothers of affected boys with XL AS
- Genetic testing is preferable
Worry more about genetic counselling for females
7: Management – males

- ACE inhibitors – even before the onset of proteinuria
- Especially where early onset renal failure is likely based on mutation characteristics and age in other family members
9. XL AS in females

- ‘Carriers’ versus ‘affected’; 15% risk of end-stage renal failure by the age of 60 years
- Most (85%) mothers of affected boys will be affected
- Monitor all females and treat with ACE inhibitors from onset of microalbuminuria
- Discourage females from renal transplantation
- If female persists with renal donation, recommend ACE inhibitors from the time of surgery
- 15% of mothers of an affected son are not affected and can be renal donors
12. AR AS – treatment

- All individuals with AR AS should be treated with ACE inhibitors from the time of diagnosis
- Managed by a nephrologist
What to do for a boy with suspected X-linked Alport syndrome?

- Test hearing and retinal photographs
- Test his mother for haematuria and her eyes for peripheral retinopathy
- Renal biopsy
- Genetic testing
- Examine his mother, and his siblings
- Start affected male and possibly family members on ACE inhibitors
Drusen in C3 nephropathy

• Mesangiocapillary glomerulonephritis type II ('dense deposit disease')
• All 7 of our patients with CFH mutations – both heterozygous and compound heterozygous
• 50% of patients have retinal atrophy by the age of 30 years
• Commonly complicated by haemorrhage, exudates and oedema
• Same process as age-related macular degeneration
Drusen – Dense deposit disease

- Haematuria, renal failure
- Mutations in Complement factor H gene
- C3 nephritic factor, low C3
- Face and shoulder girdle lipodystrophy
- Soft white deposits at macula
- Renal and retinal deposits have identical composition
- All patients by the age of 20
Dense deposit disease
Dense deposit disease
Dense deposit disease

- Retinal complications are common after years
- Vision is impaired
- Needs regular ophthalmological review
3. Gene discovery
Gene discovery

- Family (2 people) with a new disease
- One person with new AR disease

- Check for mutations in known genes
- Collected DNA from more family members
- Based on linkage
- Affected and unaffected family members are equally useful
Scalp-Ear-Nipple syndrome (MIM 181270) – Trevillian and May

- Ectodermal dysplasia
- Rare, autosomal dominant
- Scalp defect, prominent ears, rudimentary breasts and nipples
- Renal impairment
- ? Mutation in transcription factor
Renal abnormalities

- Renal cysts, renal failure
- Interstitial fibrosis
- Thinned GBM
LOD scores distribution across chromosomes
Missense mutations in \( KCTD1 \)

- c. 89C>A, p.Ala30Glu
- C.92C>T, p.Pro31Leu

- Transcriptional repressor of TFAP2 especially TFAP2\( \alpha \), but also TFAP2\( \beta \) and \( \gamma \) via BTB domain (Ding 2009)
- Expressed in breast, kidney, brain and ovary
Branchio-Oculo-Facial syndrome (OMIM 113620)

- **TFAP2A mutations** (Milunsky, Lin 2008)
- Autosomal dominant, *de novo* in 50%
- Branchial and periauricular skin defects
- Microphthalmia, anophthalmia, coloboma, hypertelorism, cleft lip, cleft palate, pits, prominent ears, hearing loss
- Associated with renal aplasia and cysts
Scalp- Ear-Nipple syndrome

- Fine hair, dystrophic nails, thinned GBM
- Thinned iris, cornea, retinal nerve fibre layer
- Thinned or absent skull posteriorly

- TFAP2 controls expression of keratin, collagen I $\alpha_1$ chain, collagen IV $\alpha_3$ and $\alpha_4$, laminin $\alpha_5$, $\beta_1$
- Explains skull defect, thinned membrane and astigmatism
IgA glomerulonephritis
4. New genetic treatments
Treatments for different types of mutations

• Missense mutations (where one amino acid is replaced by another)
  – Build up in ER and cause ER stress
  – ER stress in podocytes causes proteinuria
  – Overcome with PBA, a chemical chaperon

• Nonsense mutations (direct and downstream (those very complicated mutations); end in stop codon)
  – mRNA is destroyed by nonsense-mediated decay
  – Can be overcome with inhibitors eg cycloheximide
Gene editing

• Gene editing of patient fibroblasts
• Convert these to iPS/podocytes for injection
CRISPR technique

- Target and correct mutation
  1. Design ss DNA oligonucleotides
  2. Anneal ss oligos to generate ds oligo
  3. Dilute to working concentration
  4. Clone ds oligo into CRISPR nuclease vector
  5. Transform E coli cells and select for expression
  6. Analyse transformants for insert by sequencing
  7. Prepare purified plasmid DNA and transfec patient fibroblasts
Gene editing

- Laborious, separate process for each family
- Efficacy of correction
- Risks of transfected cells
Stem cells

PBA-treated mRNA change compared with untreated normal

iPS podocyte

<table>
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<th>Gene</th>
<th>COL4A3</th>
<th>COL4A4</th>
<th>COL4A5</th>
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<th>BAD</th>
<th>ATF6</th>
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Future treatments

• Cocktail of treatments – as for diabetes

• Directed against underlying mutation
• Directed against proteinuria and hypertension
• Stop interstitial fibrosis – eg miRNA
5. What can the renal community do?
The future

• Exome sequencing – Westmead, St V’s (Sydney), Melbourne genomic hub
• International trials
• Expert renal genetics clinics in each state
What can we do?

• Annual meetings on inherited renal disease – 3rd in 2015
• Mutation databases – LOVD and NCBI
• Orphan disease registries
• Patient support groups

• Funding for genetic testing – federal item number
• Mutation testing

• Guidelines for disease management - CARI
• Patient information sheets
• New genes – gene discovery (one person with AR disease)
• Common HREC applications

• KHA, ANZSN endorsement of these activities
Iris coloboma

- Structural abnormalities of the kidney – agenesis, reflux, cysts
- Often unrelated to kidney disease
Coloboma with unilateral kidney
Renal- (disc) coloboma syndrome

- 5% patients with reflux
- PAX2 mutations
- Interferes with vascular supply of the optic disc and urinary tract
- Varies from pit to ‘morning glory’ anomaly
- Usually asymmetrical
- No iris abnormality
- Visual defect varies from mild to severe
- No treatment
‘Morning glory’ anomaly
Retinal atrophy in MELAS syndrome

- One in 5,000
- A3243G mutation in mitochondrial DNA coding for tRNA(Leu)
- Myopathy, Encephalopathy, Lactic Acidosis, Stroke like syndrome, hearing loss, diabetes
- FSGS that progresses to renal failure by middle age
- 50% have retinal atrophy
Retinal atrophy on OCT in MELAS syndrome
Tuberous sclerosis

- One in 10,000
- AD, mutations in TSC1 and more often TSC2 genes
- TSC2 contiguous with PKD1 and patients with large deletions have TSC and large renal cysts
- 60% have multiple kidney angiomyolipoma
- 90% patients have a retinal hamartoma that eventually calcifies
- Useful diagnostically
- Vision remains normal
HANAC

- Rare
- Haematuria, tortuous vessels, muscle cramps
- Mutations affect von Hippel Lindau binding site on collagen IV α1 chain
Fabry disease

- One in 50,000, X-linked mutations in the GLA gene encoding α-galactosidase A
- Angiokeratoma, cardiomyopathy, neuropathy, renal failure
- Corneal verticillata and tortuous corneal and conjunctival vessels
- Must be distinguished from hypertension, HANAC

- K Nicholls
Von Hippel Lindau syndrome

- One in 36,000, AD mutations in VHL gene, 20% are *de novo*
- Plus somatic mutation
- Upregulation of VEGF
- 100% penetrance by 60 yrs
- Renal cysts, sometimes renal cancer
- 50% retinal haemangioblastoma
- Should be treated with laser to prevent retinal complications
Cystinosis

- One in 100,000
- 5% of children with renal failure
- AR, mutations in cystinosin gene
- Renal failure typically before 20 years
- Gritty eyes, with crystals in cornea, iris and retina

- Tsilou, 2006
Gitelman’s syndrome

- AR, mutations in SCL12A3
- Loss of function of sodium-chloride co-transporter
- Similar to features with thiazide diuretics
- Sclerochoroidal calcification