Evaluating Living Donors for Genetic Kidney Disease

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DISCLOSURES

NONE
Objectives

• Appreciate that genetic causes of ESRD are underrecognized
• Realize that family history and ancestry increase risk of ESRD in living donors
• Review the benefits and challenges of genetic testing in living donor candidates
• Understand the role of genetic counselors and geneticists in evaluation for genetic disease
• Study case examples to illustrate the role and impact of genetic testing
• Recognize that testing living kidney donors for genetic disease, *if done responsibly*, can inform risk of future ESRD and improve decision making for physician and donor
Case history #1


- JF is admitted in March 2012 with anemia, low platelets and creatinine of 5, needs to start chronic dialysis.

- JF is evaluated at the University of Iowa, diagnosed with aHUS secondary to CFH (p.Leu1189Argfs*2).


- Age 39 (October 2022): JF is well on eculizumab (creatinine 1.1) (#Her sister lost 2 transplants within 3 yrs, had a cPRA 100%, and never transplanted again).
Making a (genetic) diagnosis

- **Why:** Necessary to recognize the problem, predict course, prognosticate and determine management
- **How:** Identify a pattern, select diagnostic tests, assemble a differential, establish a diagnosis
- **Patterns of renal disease:**
  - Cystic kidney disease: e.g., ADPKD
  - Renal developmental defects (CAKUT): – e.g., hypoplasia, dysplasia, renal agenesis, vesicoureteric reflux
  - Glomerular diseases: Proteinuria (esp. severe) or hematuria +/- RBC casts: e.g., Alport syndrome, Fabry disease, FSGS
  - Tubulointerstitial disease: Bland urine, minimal proteinuria: e.g., ADTKD
  - Disorders of tubular transport: Gitelman syndrome, Dent disease
Tools to make a renal diagnosis

- History and physical exam
- Kidney function testing
- Imaging studies
- Kidney biopsy
- Genetic testing

Cystinuria
Fabry disease
Polycystic disease
Gordon syndrome
Why make a genetic kidney diagnosis?

• A genetic diagnosis is a diagnosis; may not need genetic testing.
• Sequencing maybe more specific, cost-effective, simpler diagnostic test
  – Advanced CKD or ESRD – biopsy findings unhelpful
• Risk of post transplant recurrence of disease
  – aHUS from CFH or CFI variants – high rate of recurrence;
    DGKε and MCP variants have a low risk of recurrence
  – Genetic forms of FSGS - low rates of recurrence (except NPHS1)
  – primary hyperoxaluria – high recurrence rate with kidney transplant alone
• Allows screening of at-risk living donor candidates
  – **first** make a genetic diagnosis in affected individual
  – Useful approach for 1° relatives of patients with CKD/ESRD
Types of genetic variants

1. Genetic change: **Single Nucleotide Variants (SNVs), small insertions and deletions**

3 billion base pairs per haploid genome

- Each of us have about 3,600,000 **SNVs**; 350,000 **indels**; 440 are in coding sequence; **3 LOF variants and 20 predicted deleterious variants**
- 4 million bp differences between two individuals: still makes us 99.94% identical

**Wild type vs variant**
- heterozygous vs homozygous
- heterozygous vs hemizygous

Karczewski et al., *Nature 2020, 581, 434-443*
Genetic change: **Copy Number Variants (CNVs)**

Duplications, deletions of large tracts of DNA

Many genes have risen as gene duplication events contributing to genetic diversity and evolution

- e.g., COL4A5 and A6
- CYP11B1 and CYP11B2
- CLCKA and CLCKB

But not - PKD1 and PKD2

Adjacent: PKD1 and TSC2
Challenges with genetic testing

• Lack of distinguishing phenotype thus defying classification
  – Incomplete phenotype, overlapping phenotype, phenocopy
• Clinicians' awareness of available tests and how to order
• Choice of tests – individualized vs panel of tests vs comprehensive testing
  – e.g. ADPKD: PKD1, PKD2, IFT140, GANAB; PKD phenocopy: HNF1B
  – e.g. FSGS – recessive, dominant: 40 + genes
    Also ‘non FSGS genes’ that cause FSGS (COL4 genes, LMX1B, TTC21B, CLCN5)
• Expense of test and who pays for it
• Interpreting test results
  – ACMG criteria: Pathogenic /likely pathogenic variants vs VUS vs benign/likely benign
  – Should be relevant to disease
• Risks of testing – psychological risk, insurance risk, overdiagnosis, false reassurance
• Need for genetic counseling – before/after
Risk of ESRD in living related donors

• 40% of living donors are biologically related to their recipients

\textbf{aHR for ESRD post donation in a 1\textsuperscript{st} degree relative: 1.7}

• Living donors between 1994-2016

<table>
<thead>
<tr>
<th>Relationship to Recipient</th>
<th>aHR</th>
</tr>
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<tbody>
<tr>
<td>Biological Parent (N = 14,540)</td>
<td>2.01</td>
</tr>
<tr>
<td>Biological Child (N = 20,174)</td>
<td>1.87</td>
</tr>
<tr>
<td>Full Sibling (N = 33,182)</td>
<td>1.6</td>
</tr>
<tr>
<td>Other Biological Relation (N = 10,215)</td>
<td>19.79</td>
</tr>
<tr>
<td>Non-Biological (N = 45,216)</td>
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Exome sequencing and diagnostic yield in ESRD

- 3315 patients with all categories of renal disease
  - 1128 patients in the AURORA cohort (ESRD cohort in a statin trial)
  - 2187 patients in the Columbia CKD cohort (28.3% with family history)
- Diagnostic variants in 307 patients (9.3%) in 66 monogenic disorders
  - 206 autosomal dominant; 42 recessive; 54 X-linked; 5 dual diagnosis
  - 6 genes account for 63% of diagnosis

Groopman et al., NEJM 2018, doi: 10.1056/NEJMoa1806891

Exome sequencing

In unselected population positivity rate ~10%
Diagnostic yield in kidney transplant candidates

Study 1

- 635 patients on transplant waitlist at the Charite, Berlin, Germany:
  - 119 of 635 patients (18.7%) had a known genetic cause of kidney disease (mostly ADPKD)
  - 340 of 635 patients (53.5%) had an undetermined cause of kidney disease
    - Of these 87 had ESRD prior to age of 40
    - Diagnostic variants in 16% of patients with undetermined diagnosis and < 40 yr

Overall, 20% of the waitlist were shown to have a genetic cause of kidney disease

Schrezenmeier et al., Genetics in Medicine (2021) 23:1219–1224
EVALUATING LIVING KIDNEY DONORS FOR GENETIC DISEASE

• Phenotype the recipient candidate
  – History/physical, urine studies, ultrasound/CT/MRI, renal biopsy, genetic testing

• Establish a diagnosis or a differential diagnosis in the recipient candidate
  – May need genetic testing – single gene or limited panel or comprehensive panel
  – Use appropriate screening test for living related donor
    • e.g ultrasound in donor if recipient has ADPKD.
      – But limited value in younger individuals
      – < 30 yr old (NPV ~90%)
      – 30-40 yr old (NPV ~ 98.2%)
  – Focused genetic testing of the living donor for familial variant

Do not test living donor with a comprehensive renal gene panel
Proband III-2 with hematuria, ESRD, lenticonus, normal audiogram.

Renal biopsy:
- Light: FSGS
- EM: GBM lamellations with segmental thinning
- IF: segmental mesangial and capillary loop IgM, C3

Diagnosis:
Consistent with Alport
Consistent with X-linked inheritance

- 36 yr old sibling III-1 wants to donate.
  - No hematuria, proteinuria
  - No hearing defects
  - No lenticonus
- Genetic test: splicing variant in intron 38 of COL4A5 (3657-9A>G) in III-2 confirms X-linked Alport

Genetic diagnosis in III-2: Alport – COL4A5 (X-linked)

Case 1: Hereditary nephritis (?Alport) with at-risk donor sibling

Donor III-1 negative and cleared to donate.

Thomas, C. P. et al., Am J Transplant. 2016, 10.1111/ajt.13970
Case 2. Cystic kidney disease with negative family history

**Background:**
Proband II-2: large cystic kidneys (> 18 cms) with CKD5
52 yr old dad – recurrent nephrolithiasis, no cysts on CT.
50 yr old mom – Hemolytic anemia, no cysts by ultrasound
26 yr old sister wants to donate. Ultrasound: no cysts

Genetic testing of II-2: p.Glu2771Lys in PKD1
Previously reported pathogenic variant in *PKD1*
Genetic diagnosis: ADPKD-PKD1

- 26 yr old sibling II-1 wants to donate
- Genetic counseling for II-1 prior to testing
- Genetic testing of II-2: pathogenic variant in *PKD1*.

Donor II-1 negative and proceeds to donation
Case 3. **Just IgA nephropathy?**

62 y/o old female (II-3) presents to the transplant center with CKD5 from IgAN

No family history of kidney disease.

She needs a kidney transplant

40 yr old daughter (III-3) wants to donate – but she has microscopic hematuria
Case 3. Just IgA nephropathy?

Genetic testing of II-3 (transplant candidate): p.Gly121Ser in COL4A3
Likely pathogenic variant
Is this contributing to CKD in proband?

- a. 40 yr old daughter III-3 wants to donate
- b. Genetic counseling for III-3 prior to testing
- c. Genetic testing of III-3: likely pathogenic variant in COL4A3

**Significance for donor:** Thin Basement Membrane Disease or **Autosomal dominant Alport disease**

Donor II-1 positive for familial variant; advised against donation

*Thomas C.P et al., Transplant International 2021 34:2696*
What genetic test to choose?

- Consider focused genetic testing when the diagnosis is clear or the differential diagnosis is limited
  - Fabry Disease: GLA gene; Cystinosis: CTNS (Sanger)
  - Limited panel-based testing: e.g., PKD panel, kidney stone panel (NGS)

- Consider broad based screening if differential diagnosis is broad
  - (whole) Exome/Genome sequencing (NGS)
  - Broad or comprehensive panel (NGS)
    - e.g., KidneySeq™ (Univ of Iowa), Renasight™ (Natera), KidneyCode™ (Invitae)

We are talking about the affected individual not the asymptomatic donor
1. Accurate variant interpretation is essential for clinical action.
2. American College of Medical Genetics has developed guidelines.

- Complex process
  - 28 evidence codes by which to score a variant
  - 20 rules for combining codes

- Goal – reach one of five conclusions by which to predict variant effect
  - Pathogenic (P)
  - Likely Pathogenic (LP)
  - Variant of Uncertain Significance (VUS)
  - Likely Benign (LB)
  - Benign (B)

- Expert disease-specific knowledge is essential to properly interpret ACMG rules.
- Additional testing/analysis can change conclusion (e.g., segregation, functional study).

*Richards et al.*, *Genetics in Medicine* 2015, 17: 405
Interpreting a test report

• ACMG classification
• Is it relevant to the phenotype?
  – Pathogenic or likely pathogenic variants may not be relevant
  – Some VUSes may be relevant (the evidence is just inconclusive)
    • Consult with geneticist
    • Additional phenotyping
    • For recessive variants: test parents to see if *cis or trans* (phase)
    • Segregation analysis
    • Functional studies
• Does the genetic variant conform to expected mechanism of disease?
• Dealing with uncertainty
Role of genetic counselor/geneticist

- Assist with counseling before and after genetic testing
- Help interpret genetic test report
- Aid in establishing variant’s relevance in patient with disease
- Determine testing strategy for asymptomatic healthy donor
- Consider counseling even with non-genetic predictive testing
  – e.g. ultrasound/MRI for at risk donors with family history of PKD
Sequential genetic testing of living related donors for inherited renal disease to promote informed choice and enhance safety of living donation.

Transplant candidates (TC) with known/suspected genetic disease
n=24 (mean age: 50.5)

- Tubulo-interstitial: 16.7%
- Cystic: 29.2%
- CAKUT: 16.7%
- Glomerular: 37.5%

Genetic Testing Methods
- Next-Gen Seq: 22 of 24
- Probe extension - MALDI-TOF: 1 of 24
- Chromosomal microarray: 1 of 24
- MLPA: 1 of 24

Diagnosis confirmed (50%)

- PKD1 n=6
- COL4A3 n=2
- COL4A5 n=1
- MUC1 n=1
- HNF1B n=1
- SDCCAG8 n=1

Solve rate

- Inconclusive n=2
- Negative n=10

Focused variant testing of
17 related LDs

- Cystic: 100%
- Glomerular: 33%
- CAKUT: 25%
- Tubulointerstitial: 25%

CONCLUSION: The inclusion of genetic testing clarified the diagnosis in recipient candidates, helped exclude disease in LDs, and improved their safety and informed decision making in LDs.
Living Donor (LD) Candidate

Family History +ve (see Table 1)
- At least one family member with known or suspected genetic kidney disease, or
- At least one family member with unknown cause of kidney disease, especially early onset

Counsel donor and recipient

Familial disease known
- Disease focused or comprehensive genetic testing (recipient candidate)
  - Diagnosis confirmed
    - Test for familial variant in LD
      - POSITIVE: Counsel against donation
      - NEGATIVE: Permit donation
  - Testing -ve
    - Counsel LD about residual uncertainty
    - Individualize decision about donation

Familial disease unknown
- Comprehensive genetic testing (recipient candidate)
  - Diagnosis confirmed
    - Test for familial variant in LD
      - POSITIVE: Counsel against donation
      - NEGATIVE: Permit donation
    - Individualize decision about donation
  - Testing -ve
    - Counsel LD about residual uncertainty
Testing starts with the transplant candidate

Genetic Testing of Transplant Candidate

- Phenotype transplant candidate
- Counsel transplant candidate
- Choose appropriate gene panel

Test results positive
- P/LP
  - Gene matches phenotype
    - Yes or uncertain*
      - Conform to disease mechanism?
        - Zygosity matches inheritance pattern
        - Variant matches molecular mechanism
      - No
        - Lack of evidence for genetic disease
          - No
            - No
          - Yes
            - Consult geneticist
              - Consider additional studies

- VUS
  - Gene matches phenotype
    - No
      - Lack of evidence for genetic disease
        - No
          - No
        - Yes
          - Is additional testing required*? (e.g., CMA)

Test results negative
- P/LP variant
  - Genetic diagnosis confirmed
    - Proceed to focused donor testing
- VUS
  - Consult geneticist
    - Consider additional studies
    - Consider testing donor for VUS

* Consult geneticist if necessary

C.P. Thomas et al., Am J Transplantation, 2023 (in press)
Conclusion

• Genetic disease accounts for 20% of the waitlisted transplant population (in Europe)
• Living donors have an increased risk of ESRD, which is greater with a positive FH
• Testing living donors must follow an assessment of the transplant candidate’s cause of ESRD and should use an appropriate test validated to exclude familial disease
• Genetic counseling and/or geneticist consultation may be required for interpretation of identified variants in affected candidate
• Exercise caution when using broad based gene panels in asymptomatic living donor candidates
• Sequential genetic testing of living-related donors for inherited renal disease promotes informed choice and may enhance the safety of living donation
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Questions?
Session Survey

Christie P. Thomas, MD | April 19th 2:00 PM-2:45 PM