Evaluating Living Donors for Genetic Kidney Disease

Christie P Thomas, MD

Medical Director, Kidney Transplant Program
Professor of Internal Medicine, Pediatrics, Obstetrics and Gynecology
Vice-Chair, Faculty Advancement, Department of Internal Medicine
University of Iowa Carver College of Medicine

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, an 18 yr old female, European ancestry, history of *ITP* donates kidney to her 25 yr old sister[#] with HUS in 2001.
- Age 28: JF develops proteinuria late in pregnancy – delivers healthy baby Feb 2012.
- 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 30: JF, treated with eculizumab, receives a living *unrelated* kidney transplant, Feb 2014.
- Age 39 (October 2022): JF is well on eculizumab (creatinine 1.1)

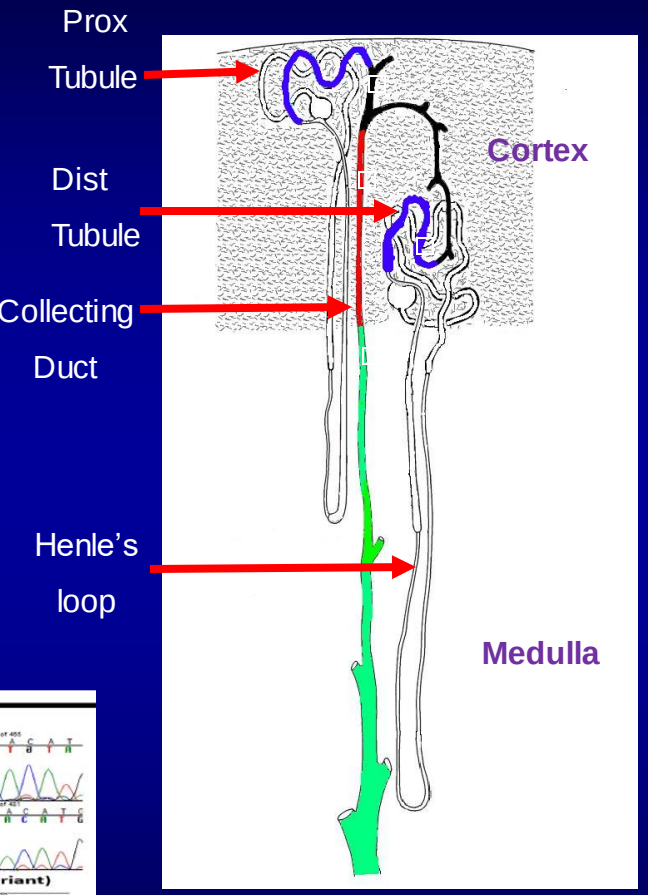
([#]Her sister lost 2 transplants within 3 yrs, had a cPRA 100%, and never transplanted again).

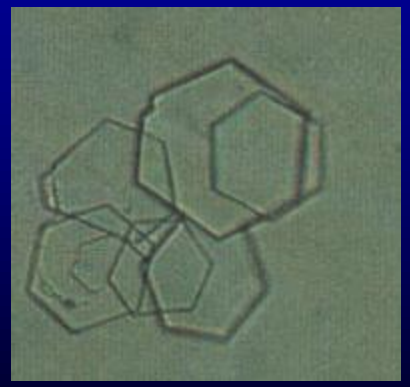
Making a (genetic) diagnosis

- 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

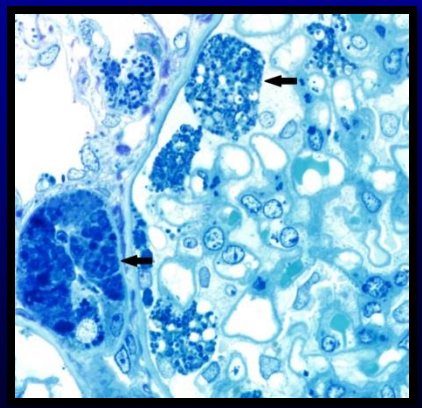
Tools to make a renal diagnosis

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

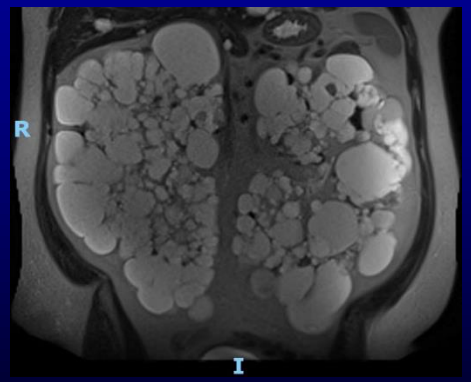




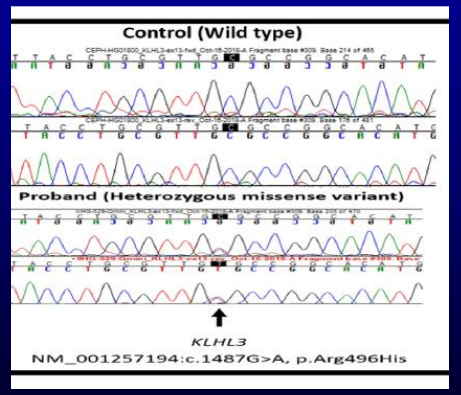
Cystinuria



Fabry disease



Polycystic disease



Gordon syndrome

Why make a genetic kidney diagnosis?

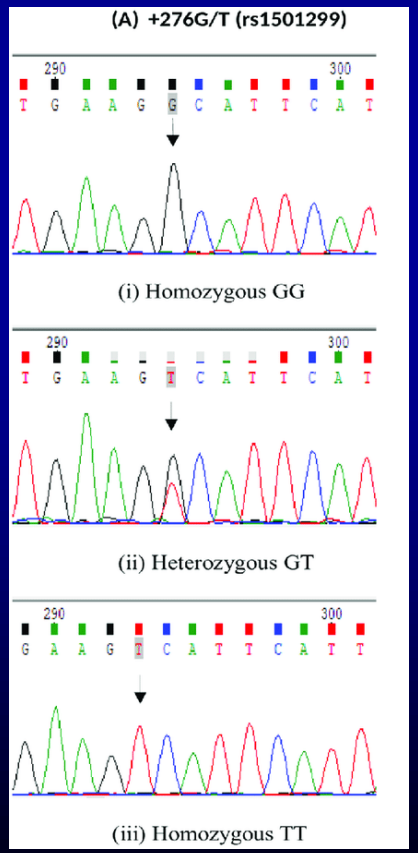
- A genetic diagnosis is a diagnosis; may not need genetic testing.
- Sequencing maybe more specific, cost-effective, simpler diagnostic test
  - Advanced CKD or ESRD – biopsy findings unhelpful
- Risk of post transplant recurrence of disease
  - aHUS from CFH or CFI variants – high rate of recurrence;
    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^{o} 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

Small insertions and deletions=indels



- 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

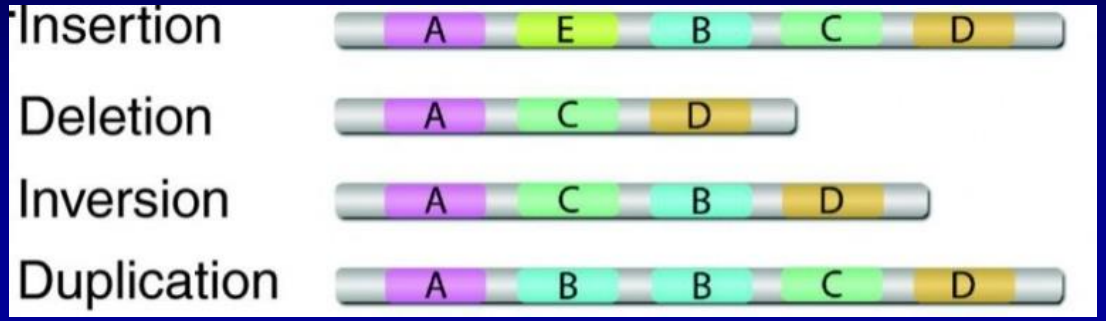
heterozygous vs homozygous

heterozygous vs hemizygous

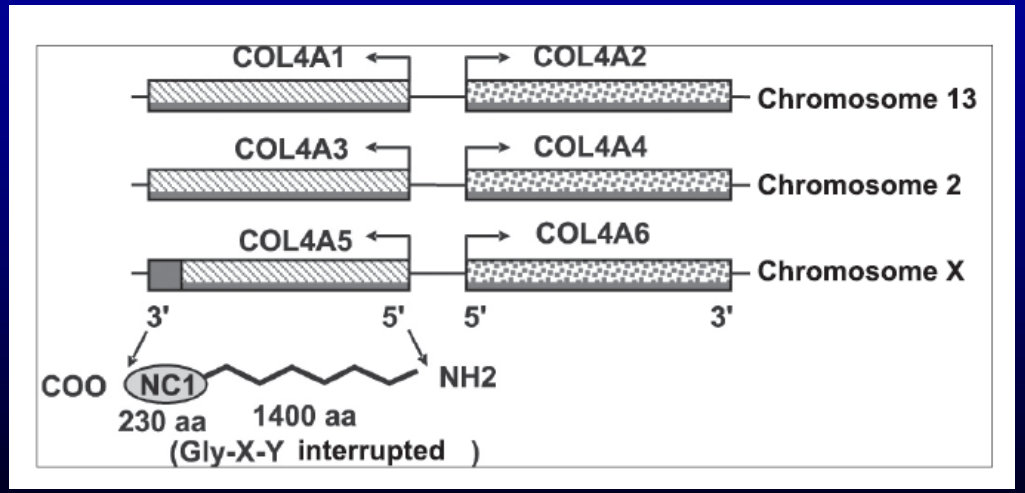
Karczewski et al., Nature 2020, 581, 434-443

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

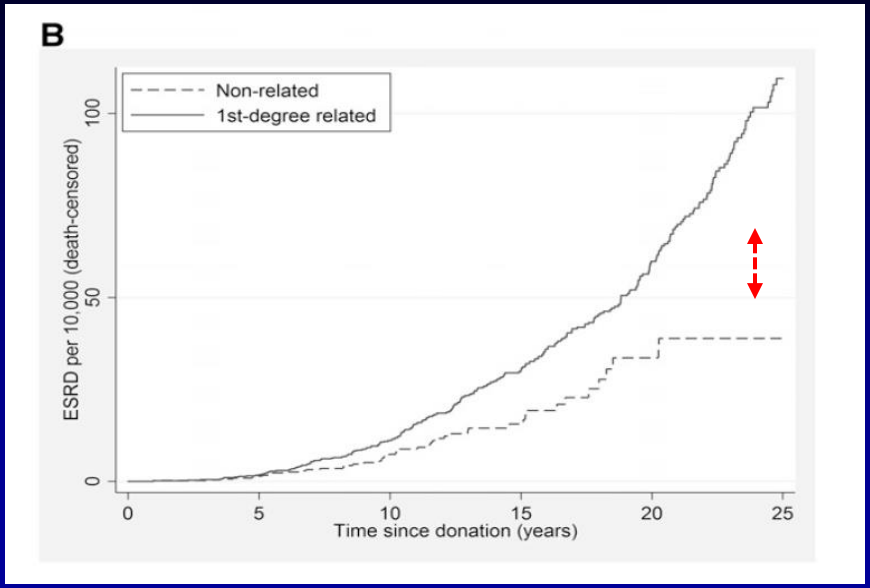
But not - PKD1 and PKD2
Adjacent: PKD1 and TSC2

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

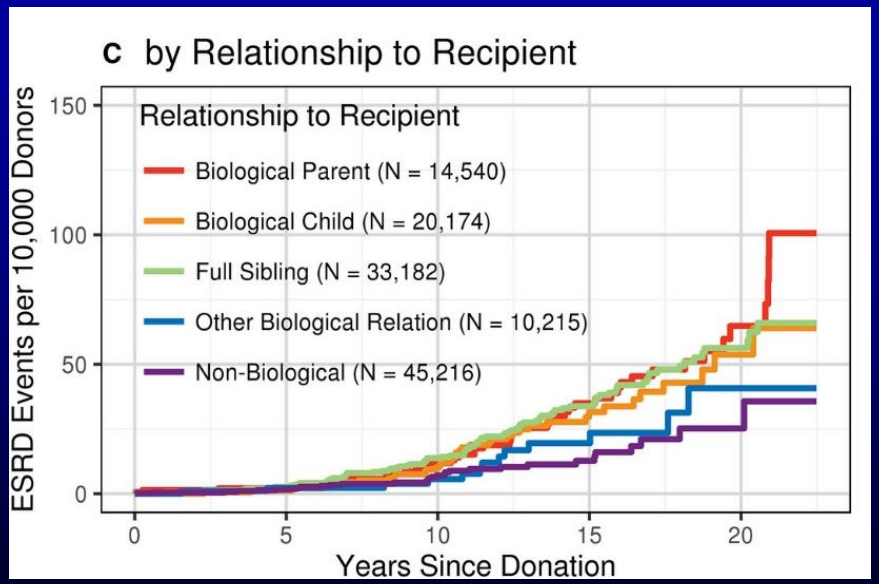
    Also 'non FSGS genes' that cause FSGS (COL4 genes, LMX1B, TTC21B, CLCN5)
- Expense of test and who pays for it
- Interpreting test results
  - ACMG criteria: Pathogenic /likely pathogenic variants vs VUS vs benign/likely benign
  - Should be relevant to disease
- Risks of testing – psychological risk, 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

aHR for ESRD post donation in a 1st degree relative: 1.7



- Living donors between 1994-2016

Relationship	aHR
Parent vs other	2.01
Sibling vs other	1.87
Child vs other	1.6
Identical twin vs other	19.79

Massie, A.B. et al., JASN 2017;28:2749-2755
Wainwright, JL., AJT 2018; 18: 1129-1139

Exome sequencing and diagnostic yield in ESRD

- 3315 patient 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

Table 2. Diagnostic Yield and Heterogeneity of Genetic Diagnoses across Clinical Diagnostic Categories.

Clinical Diagnosis	Sequencing Performed	Diagnostic Variants Present	Diagnostic Yield	Distinct Monogenic Disorders Detected	Singleton Genetic Diagnoses
	number of patients		*percent*	*number*	
Congenital or cystic renal disease	531	127	23.9	27	20
Glomerulopathy	1411	101	7.2	23	14
Diabetic nephropathy	370	6	1.6	3	2
Hypertensive nephropathy	319	8	2.5	6	4
Tubulointerstitial disease	244	11	4.5	10	9
Other	159	6	3.8	4	2
Nephropathy of unknown origin	281	48	17.1	28	17
Total	3315	307	9.3	66*	39*

* A total of 27 genetic diagnoses were found multiple times, 21 of which were found among patients in different clinical diagnostic subgroups.

Exome sequencing

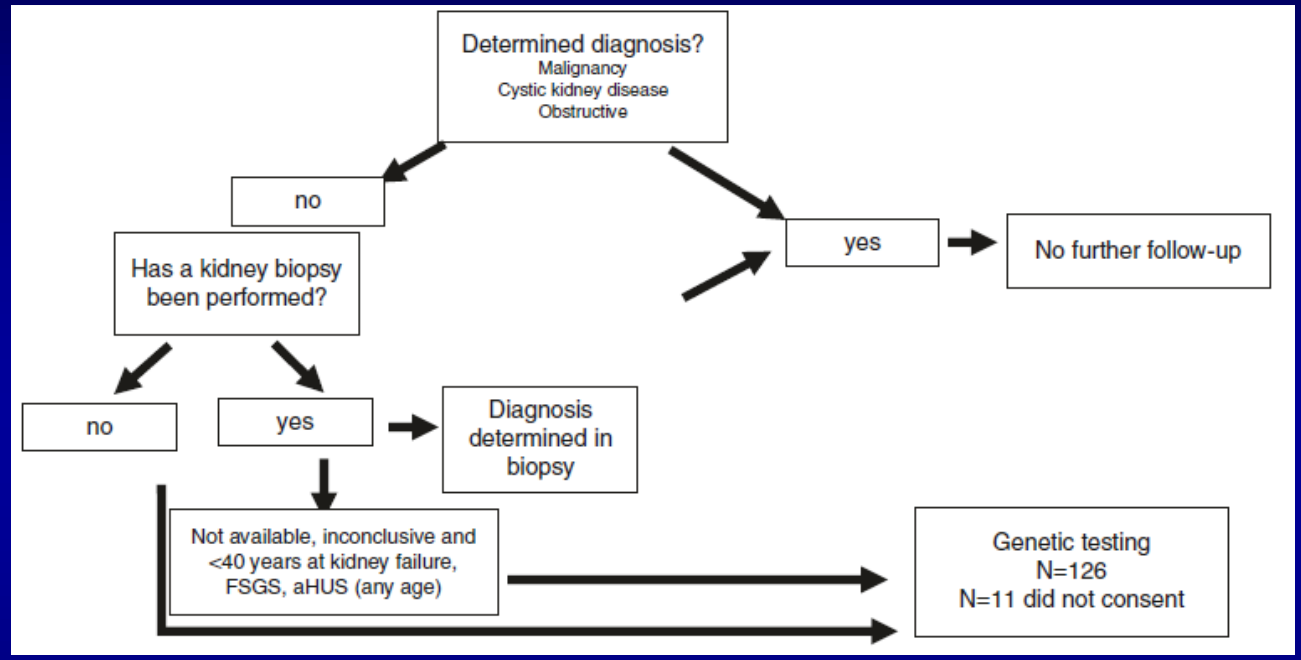
In unselected population positivity rate ~10%

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

Diagnostic yield in kidney transplant candidates- I

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

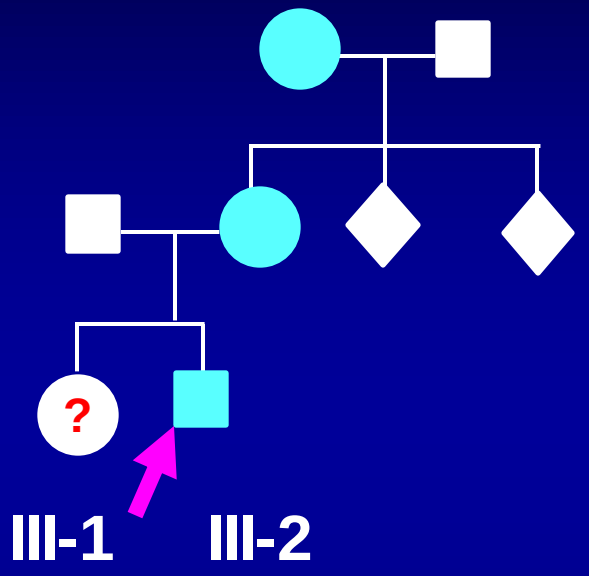
EVALUATING LIVING KIDNEY DONORS FOR GENETIC DISEASE

- 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

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

Proband III-2 with hematuria, ESRD, lenticonus, normal audiogram.



Donor III-1 negative and cleared to donate.

Renal biopsy:

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

Diagnosis:

Consistent with Alport

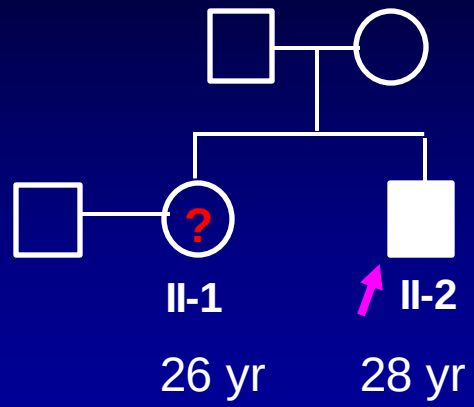
Consistent with X-linked inheritance

- 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)

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

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

Donor II-1 negative and proceeds to donation

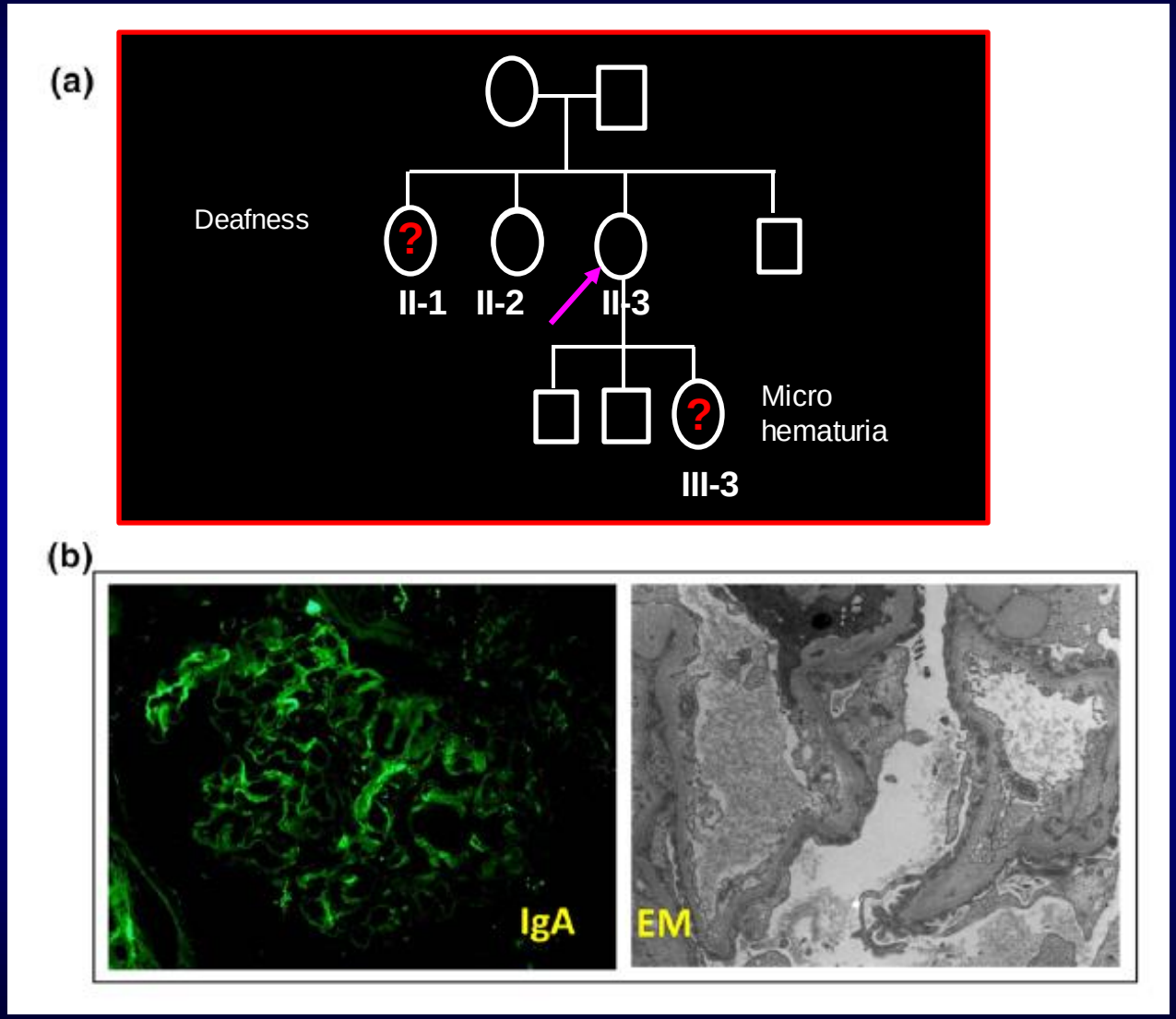
Case 3. Just IgA nephropathy?

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

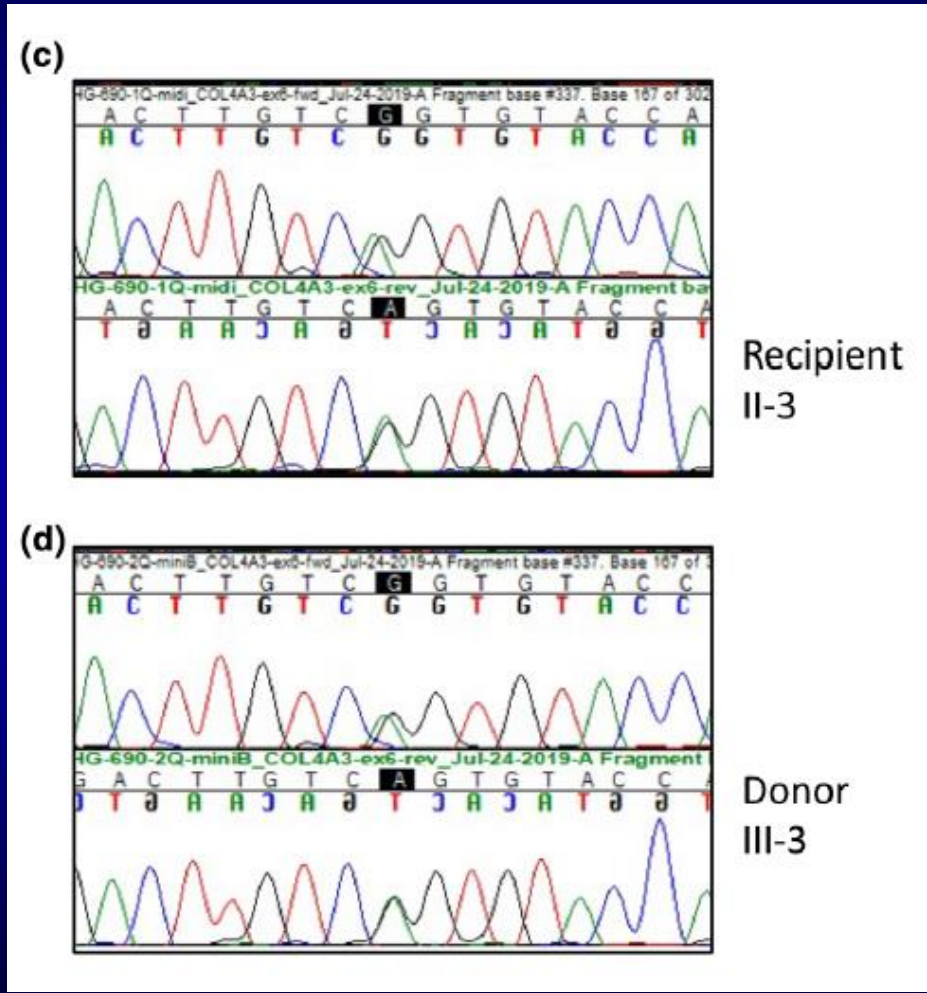


Case 3. Just IgA nephropathy?

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

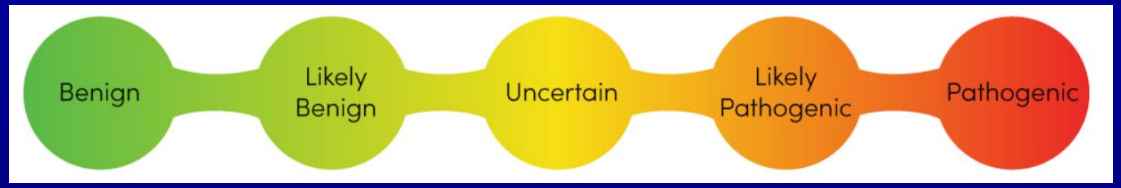
What genetic test to chose?

- 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

Data Analysis and Variant Identification: Variant Annotation

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

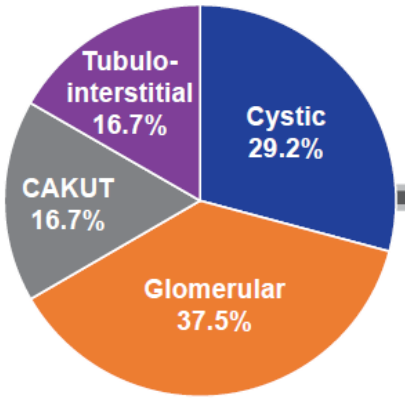
- ACMG classification
- Is it relevant to the phenotype?
  - Pathogenic or likely pathogenic variants may not be relevant
  - Some VUSes may be relevant (the evidence is just inconclusive)
    - Consult with geneticist
    - Additional phenotyping
    - For recessive variants: test parents to see if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

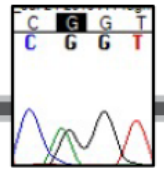
Role of genetic counselor/geneticist

- 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)





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%)

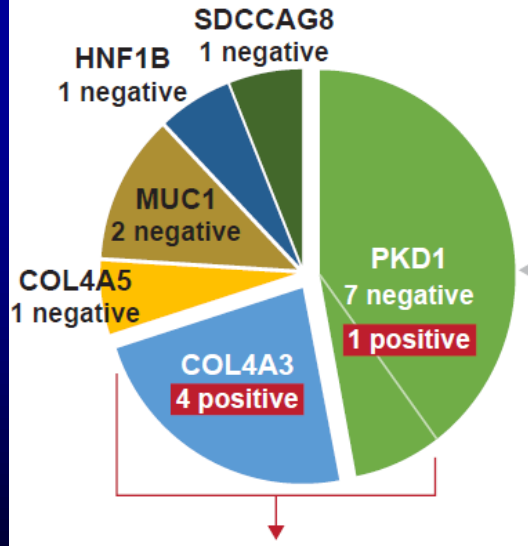
PKD1 n=6 | COL4A3 n=2 | COL4A5 n=1 | MUC1 n=1 | HNF1B n=1 | SDCCAG8 n=1

Inconclusive n=2

Negative n=10

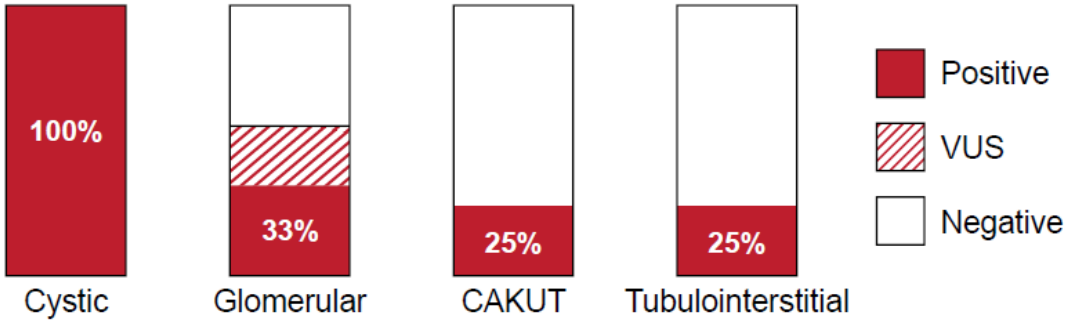
Solve rate

Related asymptomatic living donor (LD)



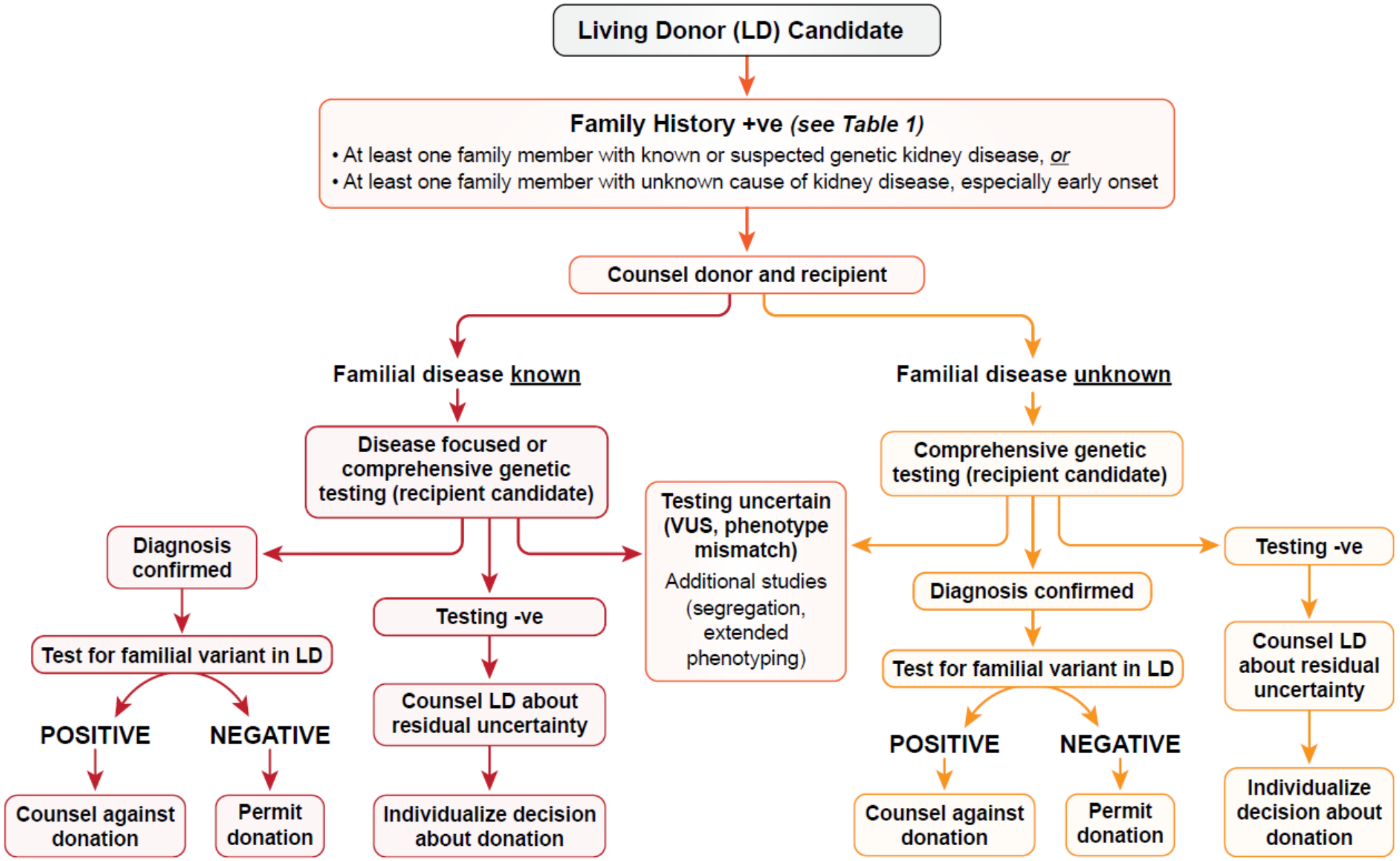
Positive LDs counseled against donation

Focused variant testing of 17 related LDs



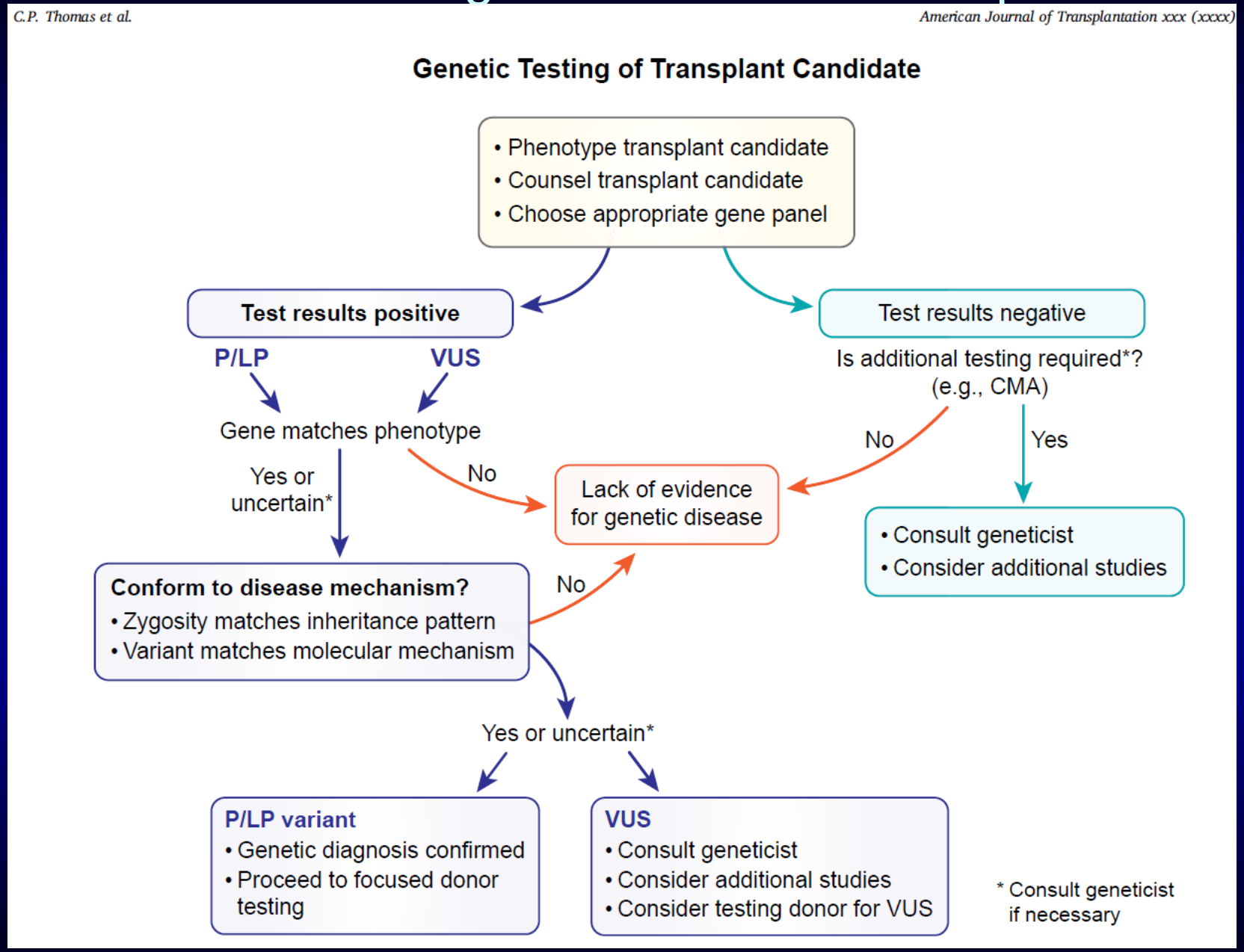
CONCLUSION: The inclusion of genetic testing clarified the diagnosis in recipient candidates, helped exclude disease in LDs, and improved their safety and informed decision making in LDs.

Thomas C.P et al., Transplant International 2021 34: 2696



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

Testing starts with the transplant candidate



Thomas C.P 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

Thanks to

Mycah Kimble, Colleen Campbell, Adela Mansilla, Richard Smith, Iowa Institute of Human Genetics (iihg@uiowa.edu)

Margaret Freese, CGC
Genetic Counselor, Renal Genetics Clinic

Angela Korsun, Gwen McNatt and Alan Reed
University of Iowa Organ Transplant Center

Questions?

Session Survey

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