1/20 Hello everyone, welcome to www.kireportscommunity.org #Nephsky #Medsky
Welcome to a #skytorial brought to you by @KIReports

2/20 Our author is Dr Saivani@ (@DrSaiVani1, @drsaivani.bsky.social)

Consultant Nephrologist, Kurnool Kidney Care, Kurnool, India. Today's topic is
Genetic testing (Next Generation Sequencing)—A Sherlock Holmes in discovering the truth of #Nephsky, #Medsky

#MedTwitter #nephtwitter @ISNkidneycare #XTwitter

3/20 What are the developments in genetic testing?

Genetic testing underwent a sea change from slow and laborious Sanger analysis to high-throughput simultaneous screening of multiple disease genes after the introduction of Next Generation Sequencing (NGS). This has led to the ease of genetic testing. @ISNkidneycare
#Nephsky #Medsky

4/20 Quick check!

What is the biggest advantage of NGS?

a) Simultaneous and high-throughput screening
b) Easily available and affordable
c) Single gene analysis
d) No need for DNA

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5/20 What are the different types of next-generation sequencing?
For more info please read
https://doi.org/10.1002/mgg3.82
#Nephsky #Medsky

Next Generation sequencing – Which test to order?	
Whole genome sequencing	**Entire genome is sequenced, both introns and exons are sequenced**
Whole Exome sequencing	Here only protein producing genes (Exons) are analyzed
Targeted gene approach Only target gene approached	a)DNA Capture technique b)Long Range locus rich PCR technique(LR based PCR screening)
Infographic by Dr Sai Vani @DrSaiVani1	

6/20 Did you know ADPKD accounts for approximately what proportion of patients with ESKD?

a) <1%
b) 2-3%
c) 5-10%
d) >20%

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Demographics - ADPKD
common monogenic genetic disorder
Point population prevalence – 3.96 per 10,000 people
Accounts for 5-10% of the ESRD population
4th leading cause of CKD
Infographic by Dr Saivani Y @DrSaiVani1

7/20 🫁

🧬

Why is genetic testing not first-line in typical ADPKD?

🧬

a) Low sensitivity
b) Lack of mutations
c) Imaging + Family history often suffices.
d) High false negatives . #Nephsky #Medsky

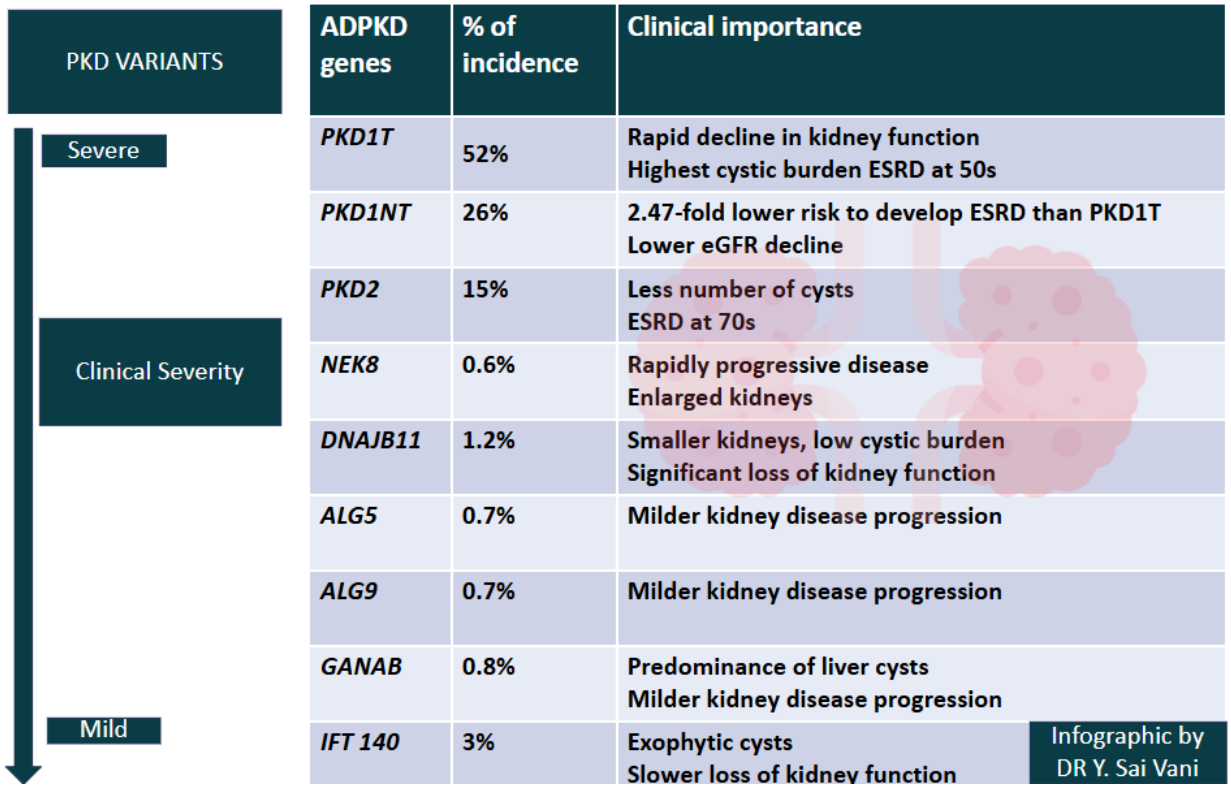
8/20 🫁 Do you know various genes involved in the ADPKD phenotype, with their incidence patterns and clinical severity? #Nephsky #Medsky

genetic architecture

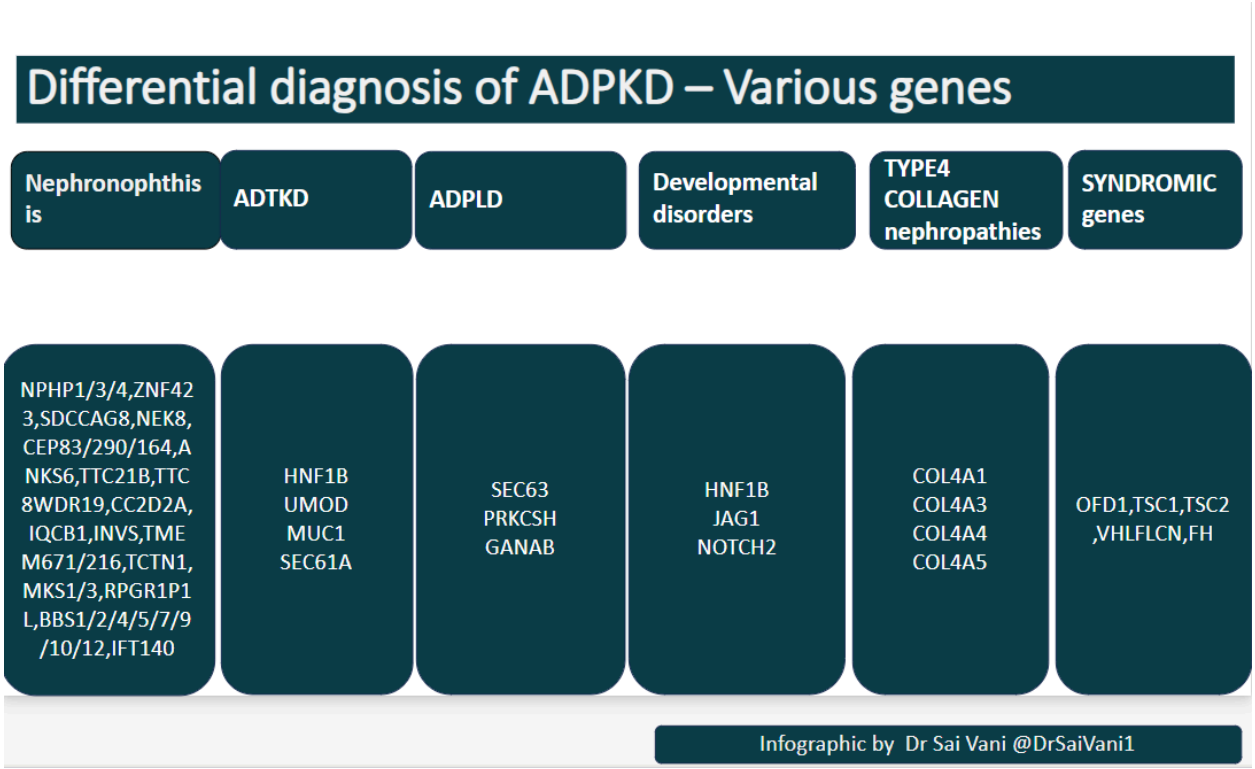
✔️ PKD1 (~85%)
✔️ PKD2 (~15%)
✔️ Minor genes (IFT140, GANAB, DNAJB11…)

👉 Think spectrum, not single-gene disease

For more information read
https://www.ajkd.org/action/showPdf?pii=S0272-6386%2825%2900933-3



9/20 Do you know various important genes for differential diagnosis of ADPKD presenting with the cystic kidney morphology? #Nephsky #Medsky



10/20 🧠 We will recap the importance of the common ADPKD genes, polycystin1 and polycystin2. #Nephsky #Medsky

PKD 1	PKD2
localized on chromosome 16p13.3	4q 21-22,
Contains 46 exons	15-exon single-copy gene
Difficult to quantify due to large gene	Can be quantified
Secretes polycystin1 – unknown function	Transient receptor potential channel protein
Both proteins regulate cellular functions like proliferation, apoptosis, and fluid secretion	
Infographic by Dr Saivani Y @DrSaiVani1	

11/20 🧠 Did you know about the importance of allelic heterogeneity (phenotypic heterogeneity) of the disease? #Nephsky #Medsky

Allelic heterogeneity drives phenotype

🔴 Truncating (PT) → severe, early
🟢 Non-truncating (NT) → milder, late

But exceptions exist: phenotype ≠ genotype always

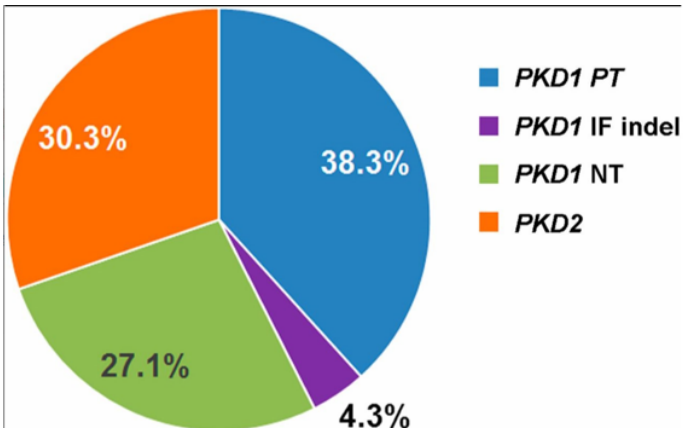


Figure from Refining genotype and phenotype correlation in ADPKD, Hwang et al., JASN, 2025

https://doi.org/10.1681/ASN.2015060648

12/20 Though a clinico-radiological hint is suffice for diagnosis, genetic testing helps in prognostication through PROPKD score #Nephsky #Medsky
https://doi.org/10.1681/asn.2015010016

PROPKD CALCULATOR

Variable	Points
Male	1
Hypertension before 35years of age	2
First urological event before 35years of age	2
Mutation	
PKD2 mutation	0
Non truncating *PKD1* mutation	2
Truncating *PKD1* mutation	4
PROPKD SCORE	SUM

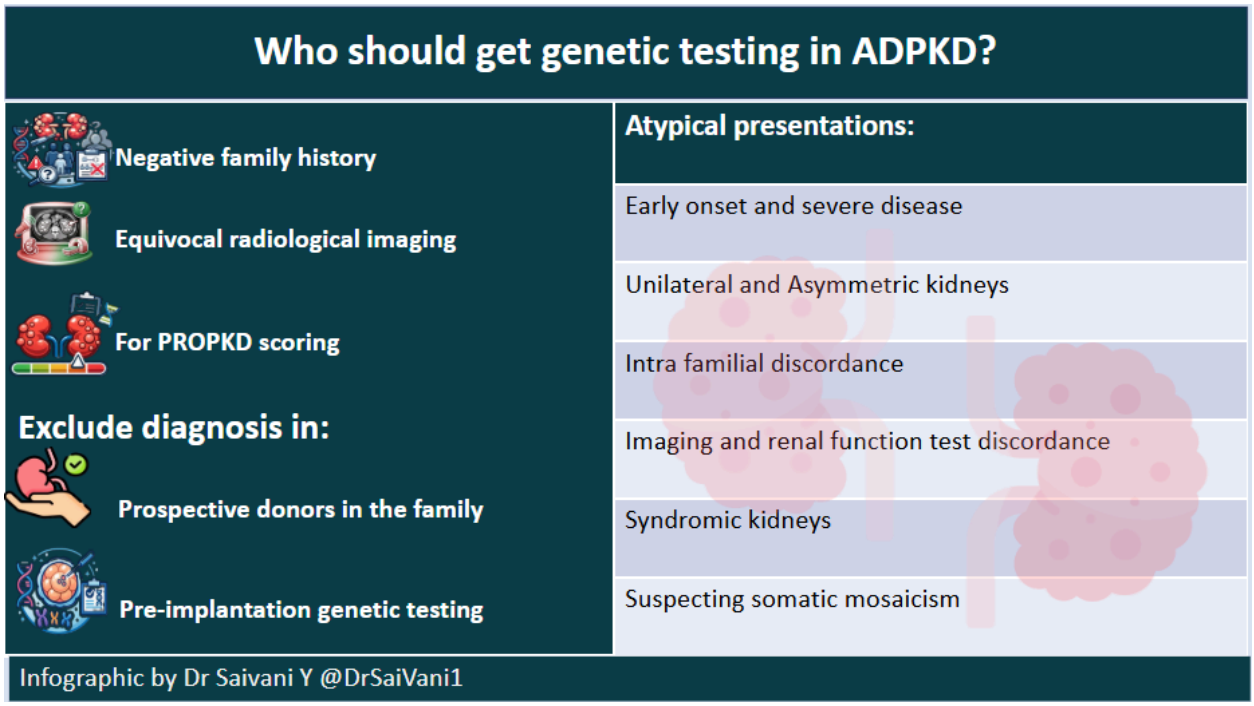
CLINICAL SCENARIO
A 32-year-old male with hypertension,hematuria,PKD1 truncating mutation

Male	1
Hypertension before 35	2
First urological event before 35	2
PKD1 truncating mutation	4
PROPKD SCORE	9

PROPKD SCORE	0	1	2	3	4	5	6	7	8	9
RISK OF PROGRESSION TO ESRD	LOW 70.6 median age for ESRD onset Eliminates evolution to ESRD before age 60				INTERMEDIATE 56.9 median age for ESRD onset			HIGH 49 median age for ESRD onset Risk of rapid progression and a 92% chance of reaching kidney failure before 60		

Infographic by Dr Sai Vani @DrSaiVani1

13/20 The various indications for genetic testing are clinically needed, and some are evolving. For more information, reach out this article #Nephsky #Medsky
https://doi.org/10.1016/j.kint.2024.06.031



14/20 Genetic testing is beneficial not only in diagnosis but also to exclude the diagnosis in ADPKD, especially.

1) To select the kidney donor within the family
2) Before preimplantation genetic testing

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15/20 Let us more learn more about prenatal genetic testing

This sophisticated method involves performing intracytoplasmic sperm injection (ICSI) on the ovum on day three at the blastocyst stage, during which 5-10 trophectoderm cells are taken and sent for genetic testing to identify ADPKD-negative embryos. #Nephsky #Medsky
In prenatal genetic testing, noninvasive and invasive methods exist.

Prenatal genetic testing

Non-invasive	Invasive
Less accurate	Accurate
Performed at 6-7 weeks	Chorionic villous sampling performed at 11-14 weeks Amniocentesis performed at 15-16 weeks
No risk of pregnancy loss	0.5% loss of pregnancy

Infographic by Dr Sai Vani @DrSaiVani1

16/20 Why is PKD1 genetic analysis complex? #Nephsky #Medsky
PKD-1 analysis = Technical nightmare
Contains two parts (1 - 33 and 33 - 46 exons)
GC-rich
6 pseudogenes are present.
False positives + mapping errors

17/20 What are pseudogenes? Why is this task more difficult?
Pseudogenes are nonfunctional genes that resemble functional genes, cannot produce proteins, and lack regulatory elements. High sequence similarity to functional genes makes it difficult to identify the variants, and often located near areas of segmental duplication makes them more difficult to analyze.
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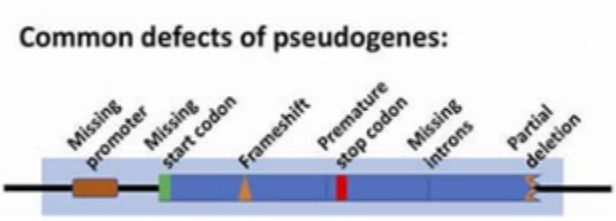

18/20 @ISNkidneycare Let's go for a quiz. #Nephsky #Medsky
Which method of NGS is outstanding in accuracy?
A) Whole exome sequencing
B) Whole genome sequencing
C) DNA capture-based targeted screening
D) LR-based PCR-targeted screening

19/20 @ISNkidneycare The answer is D
The long-range locus-rich PCR technique is excellent in accuracy but laborious. The following table illustrates the differences between various techniques.#Nephsky #Medsky
For more information read following article
https://doi.org/10.1080/14737159.2017.1358088

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Character	**LR based PCR screening**	**DNA Capture based screening**	**Whole genome screening**
Target gene selection	At time of PCR	At time of capture	After sequencing
Capture efficiency at rich Guanosine cytosine(GC)	High	low	low
False positive rate	low	high	high
Allelic drop out bias	high	low	low
Off target capturing	Minimal	yes	yes
Ambiguous mapping	low	high	high
Labor intensity	High	Low	Low
Computational intensity	low	high	highest
Cost of research	High	low	highest
Sequencing of promoter and introns	If included in primer region	If included in probe design	yes
Infographic by Dr Saivani@DrSaiVani1			

20/20
Huge thanks to all my mentors
Happy learning!
The link for the blog