Present & Future Role of Molecular Genetic Diagnostics in ADPKD

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KDIGO Controversies Conference on Autosomal Dominant Polycystic Kidney Disease (ADPKD)

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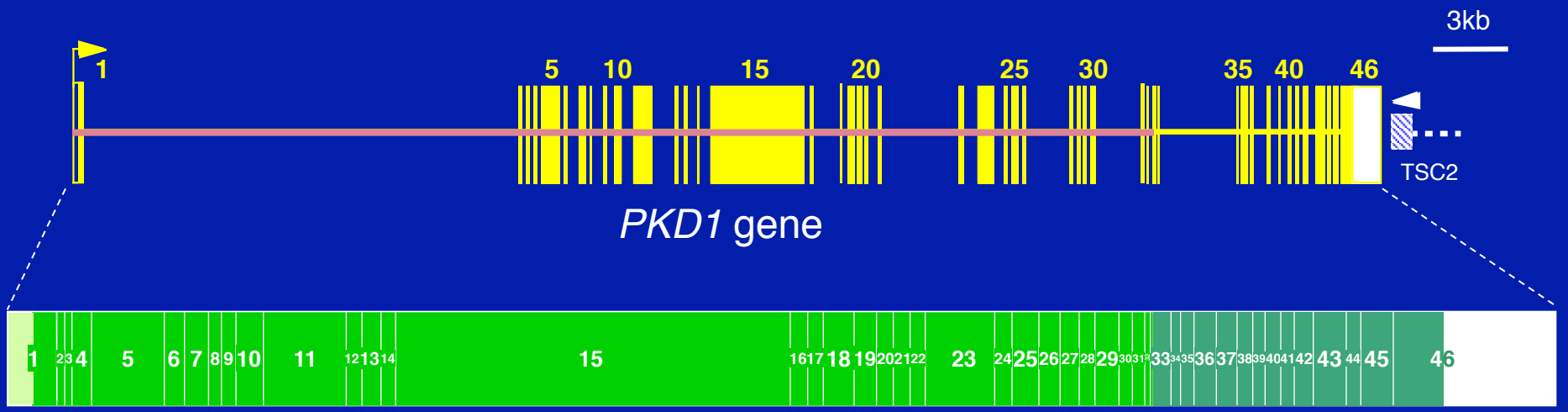
Disclosure of Interests

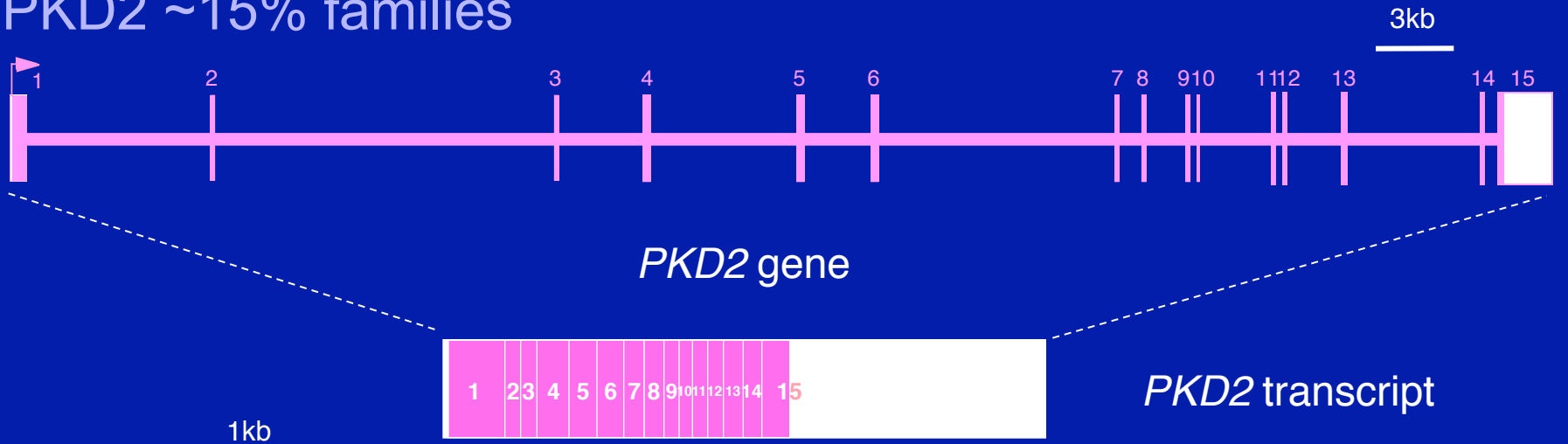
- Otsuka Pharmaceuticals: Research grant

Mutations to *PKD1* or *PKD2* cause ADPKD



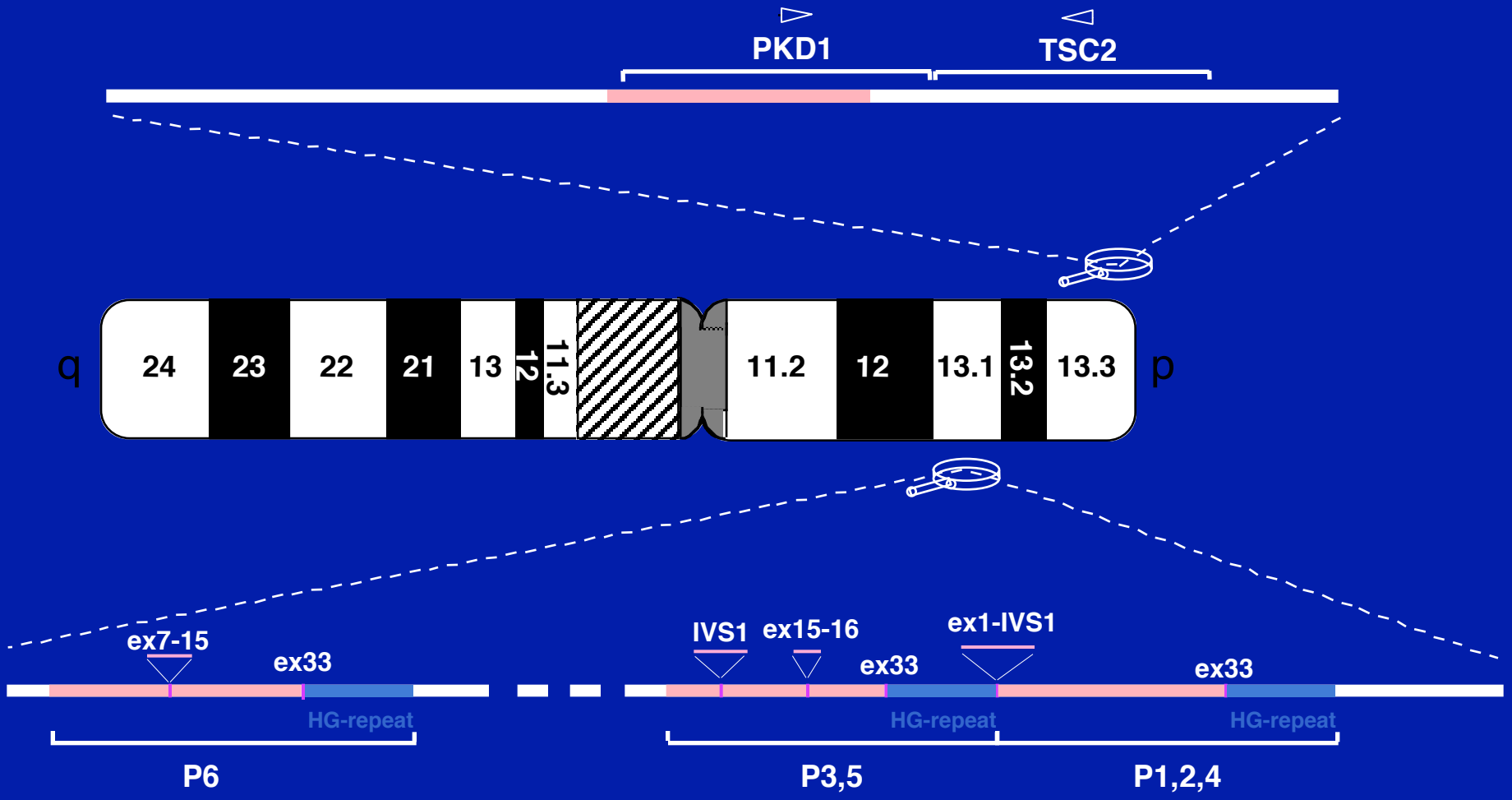
PKD1 ~85% families

PKD2 ~15% families



Hughes et al Nat Genet (1995); International Consortium, Cell (1995); Mochizuki et al Science (1996)

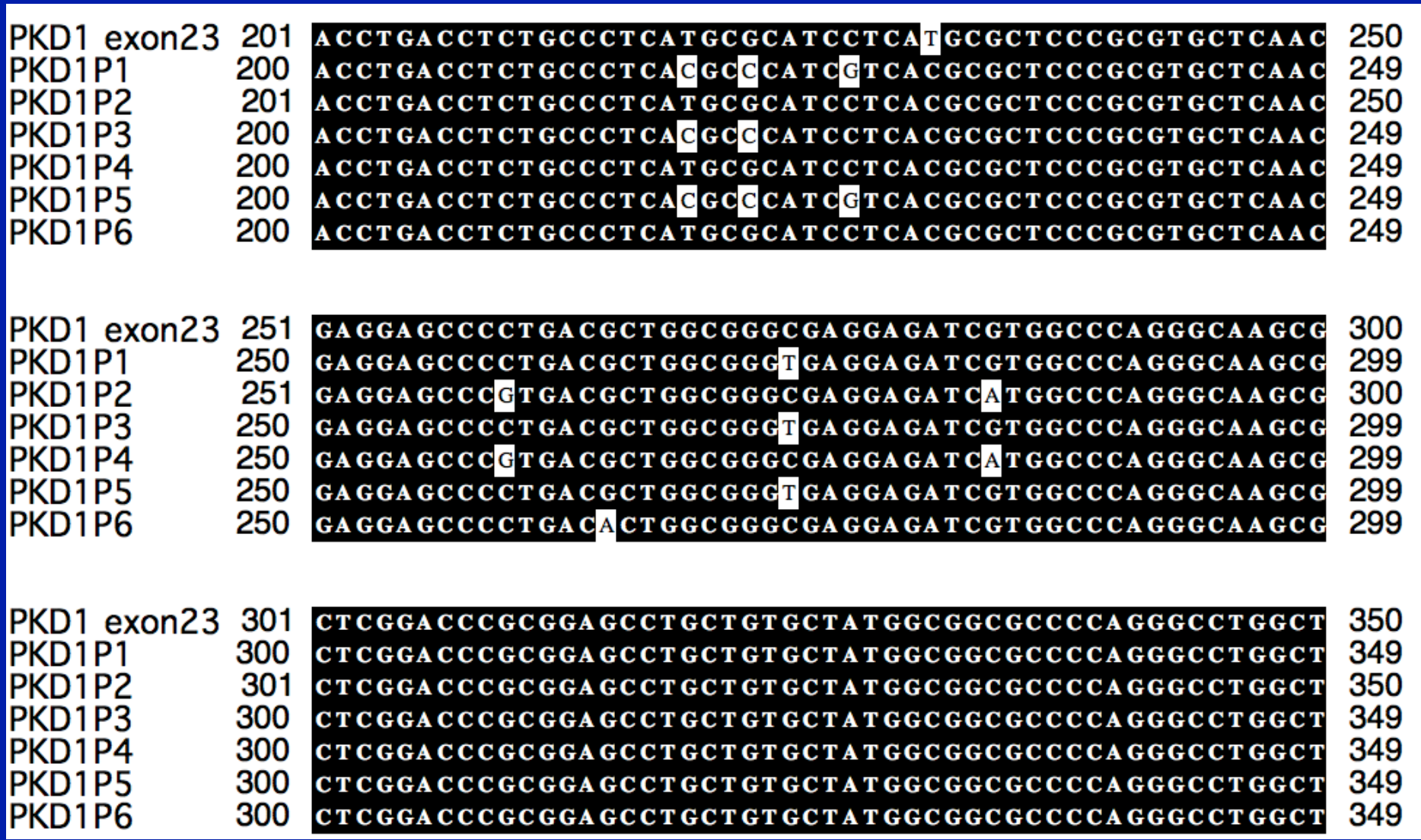
The *PKD1* region is duplicated in 16p13.1



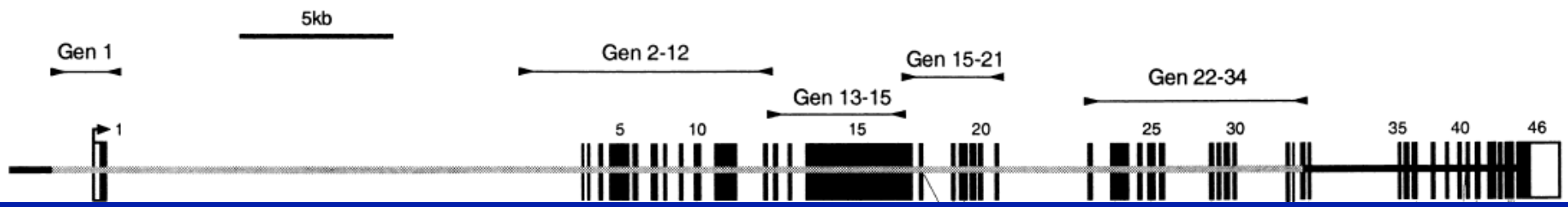
6 *PKD1*-like pseudogenes; up to 99% identity to *PKD1*

European Consortium, Cell 1994

Sequence similarity between *PKD1* and the six pseudogenes

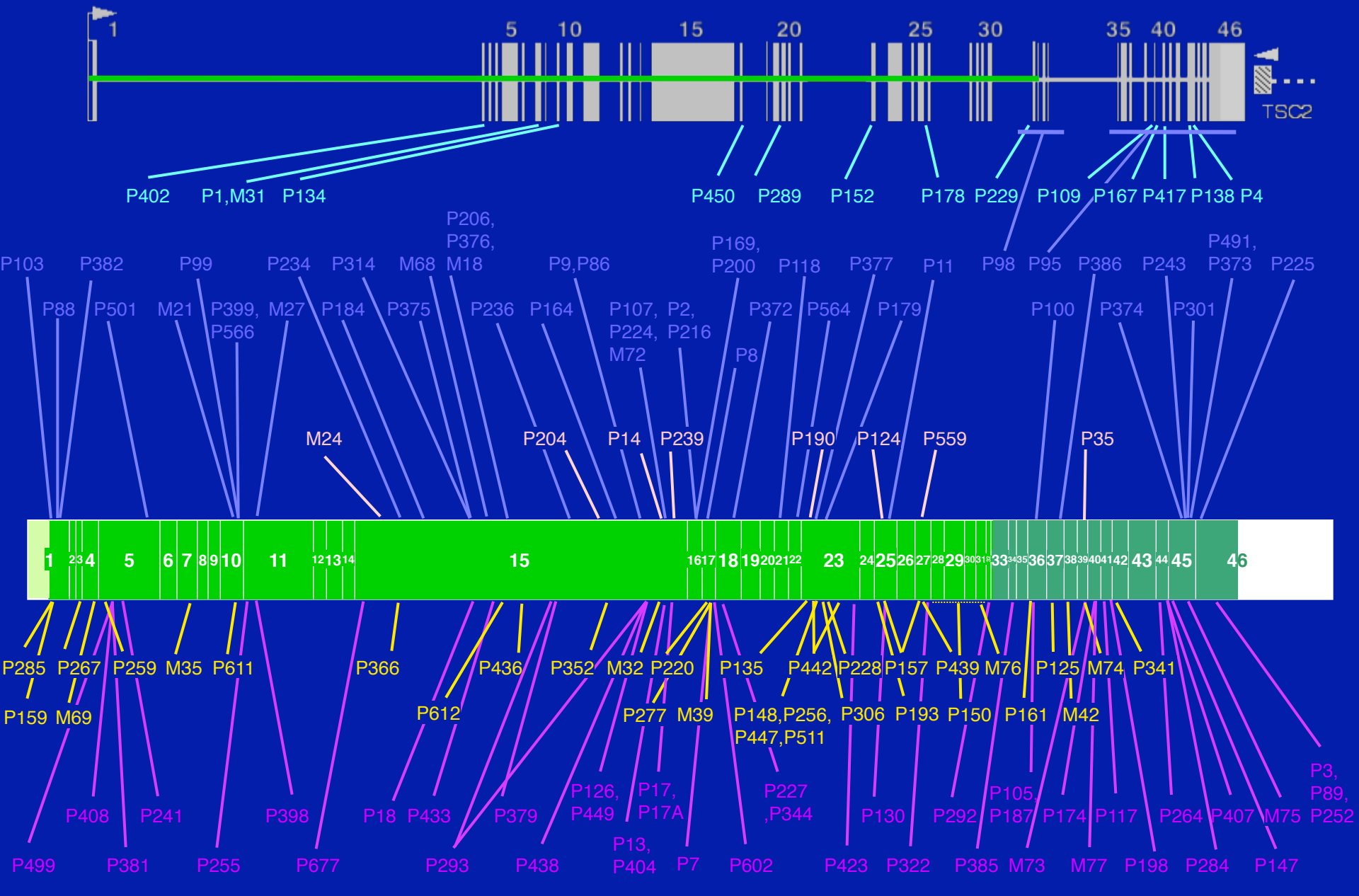


Sanger screening protocol for ADPKD

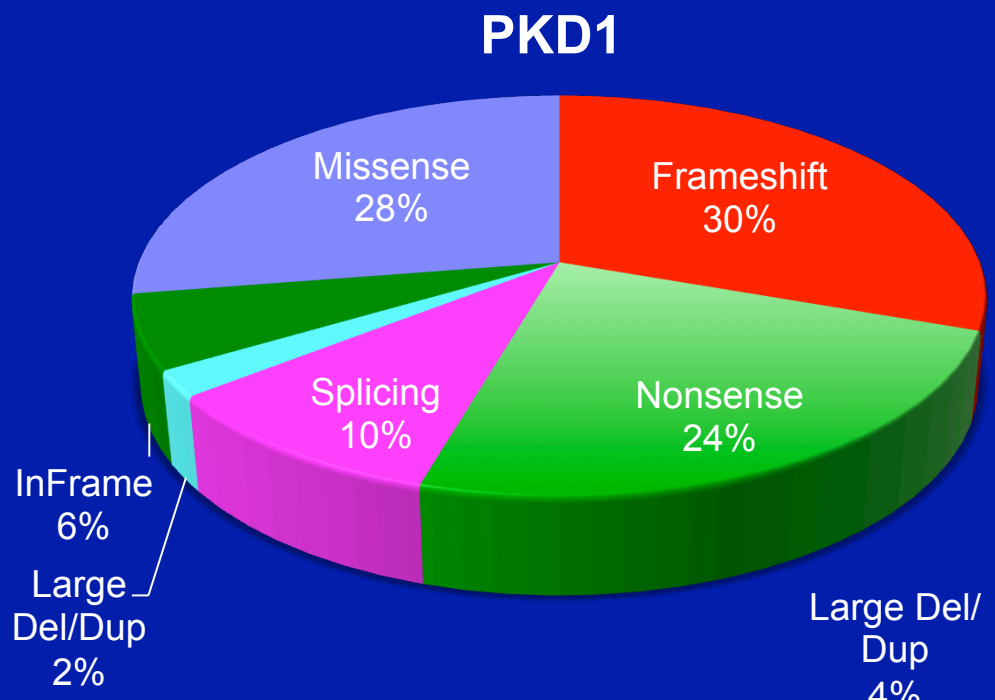


- 5 locus specific amplicons cover the duplicated part of *PKD1*
  - *PKD1* exons and flanking intronic regions: 46 amplicons
  - PKD2 exons and flanking intronic regions: 17 amplicons
    - Total 63 amplicons

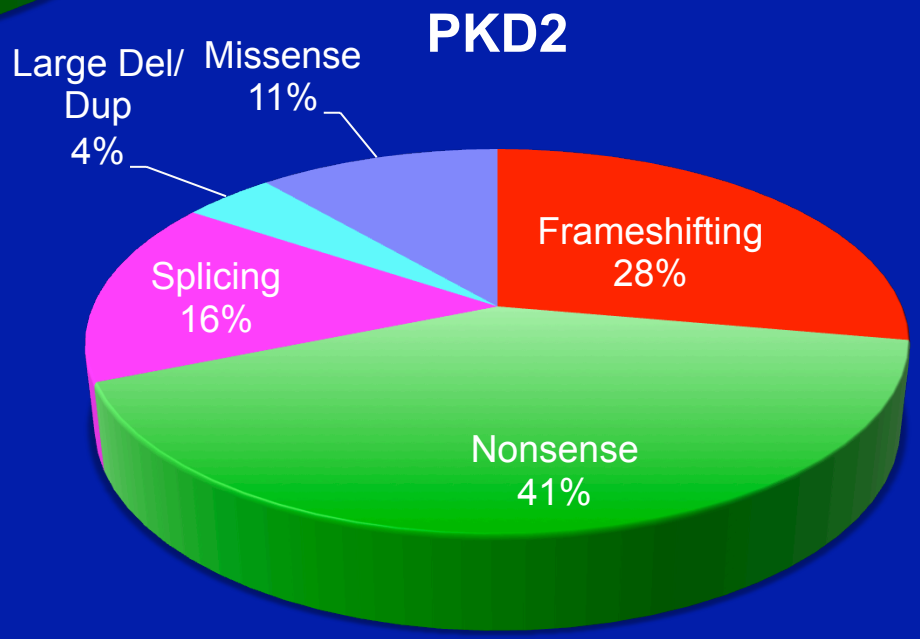
High level of allelic heterogeneity in PKD1



Mutation Types in ADPKD: HALT PKD population



No mutation identified in ~8% families



Non-definite mutations in 34% *PKD1* and 11% *PKD2*

Update to ADPKD mutation database (PKDB): Version 2.95 http://pkdb.mayo.edu

- Total 2544 variants
  - PKD1 = 2293 PKD2 = 251
- 1425 Likely Pathogenic mutations (2020 families)
  - PKD1 = 1250 (1677 families)
  - PKD2 = 175 (343 families)
- 189 Indeterminate change
  - PKD1 = 181; PKD2 = 16
- 867 Neutral Polymorphisms
  - PKD1 = 911 (94%); PKD2 = 58 (6%)

Diagnostics of ADPKD usually performed by renal imaging

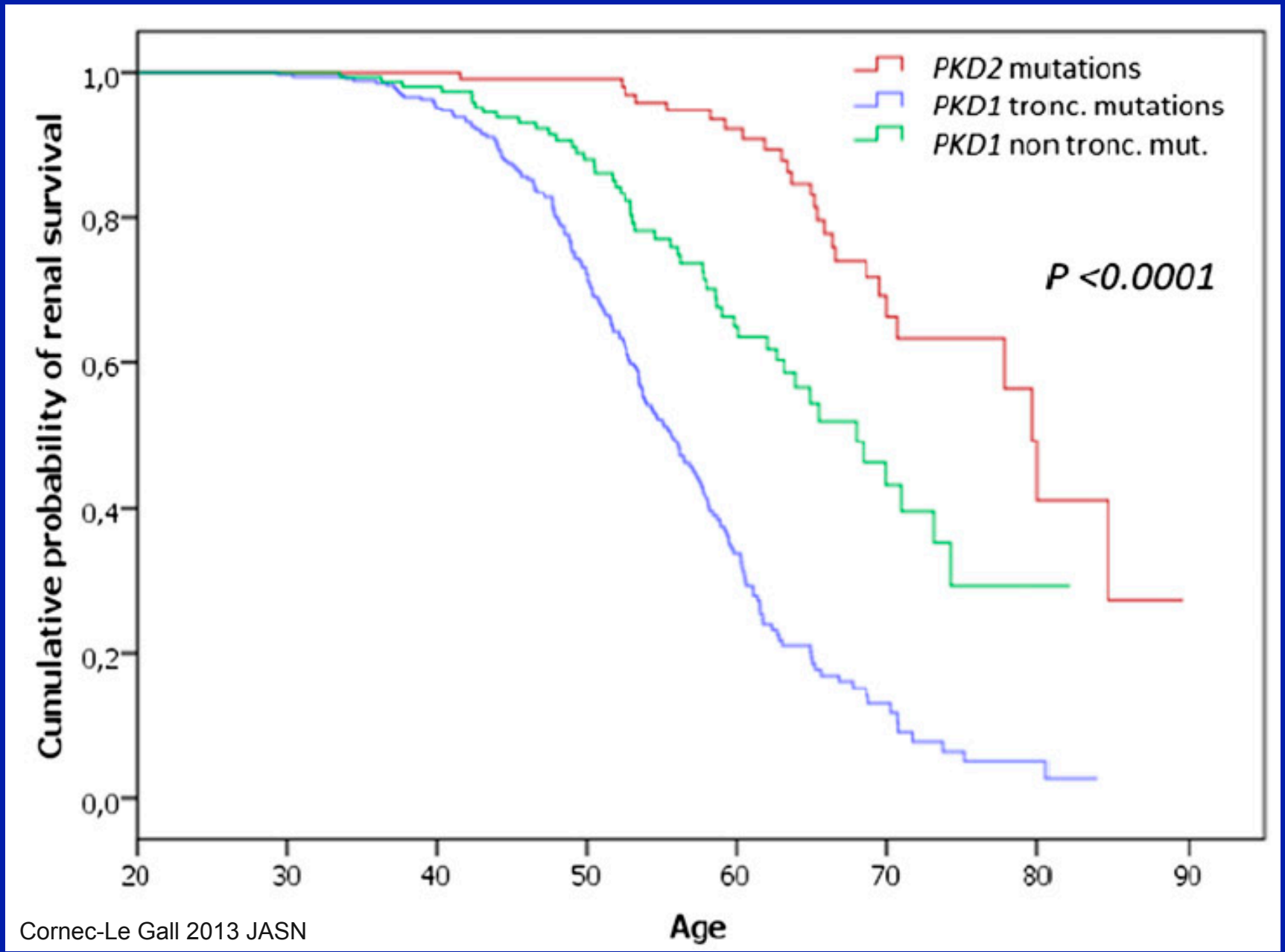
- Imaging methods can usually accurately diagnose ADPKD in adults

- Imaging diagnostics less reliable in younger adults
  - Especially in families with less severe disease
    - *PKD2* and hypomorphic *PKD1* mutations

Gene-based diagnostics for ADPKD

- Genetic testing may be helpful when imaging results are equivocal and firm diagnosis required
  - Living related donors
    - Confirming negative diagnosis in young potential donor when imaging results may be unreliable
    - Clarifying the diagnosis in a potential donor when 1 or 2 cysts detected by imaging
- Individuals with a negative family history and/or an unusual disease presentation: to clarify the diagnosis
  - Early onset ADPKD
  - Mild PKD
  - Atypical radiological presentation
- Once therapies available: testing of young patients to obtain a firm diagnosis before starting treatment

Knowing the gene and the mutation are of prognostic value in ADPKD



Cornec-Le Gall 2013 JASN

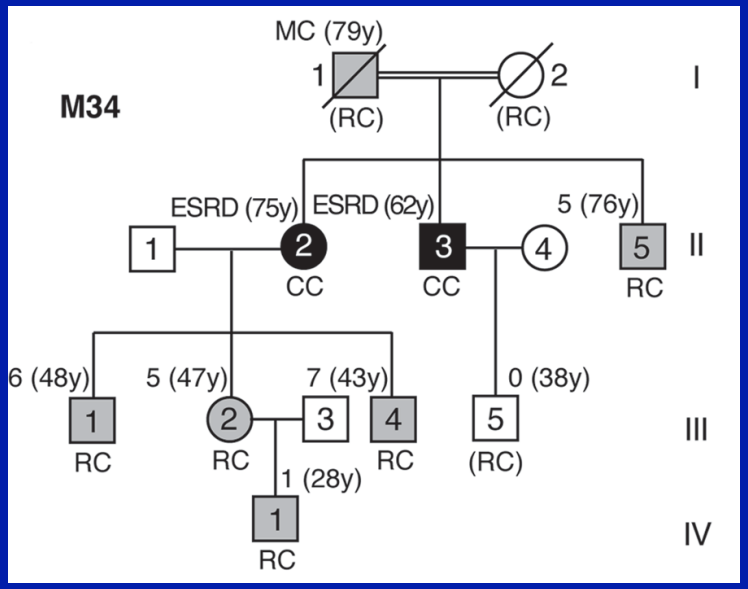
Gene-based prognostics in ADPKD

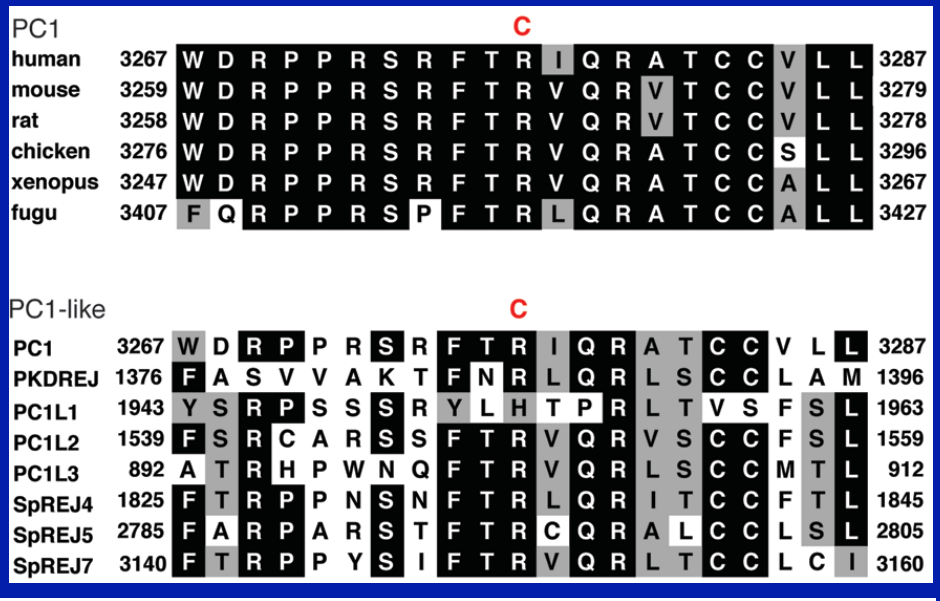
- Gene and mutation data can provide information about the severity of disease
  - Truncating *PKD1* mutations associated with more severe disease
  - PKD2 mutations associated with milder disease
  - Hypomorphic *PKD1* mutations associated with milder disease
- Prognostic information can help patient management
  - Planning for ESRD
  - Provide reassurance for those with predicted less severe course
  - Select patients for clinical trials
  - Select patients treatments

Resolve complex ADPKD cases

- Negative family history
  - Determine if disease is ADPKD
    - Especially with mild disease
  - *De novo* mutation
    - Reduced risk in sibs
    - Mosaicism – can complicate risk prediction
- Early onset cases
  - Some due to combinations of ADPKD alleles
    - Of value for avoiding further early onset cases in a family
- Marked intrafamilial variation
  - Allelic/genic combinations
  - Mosaicism

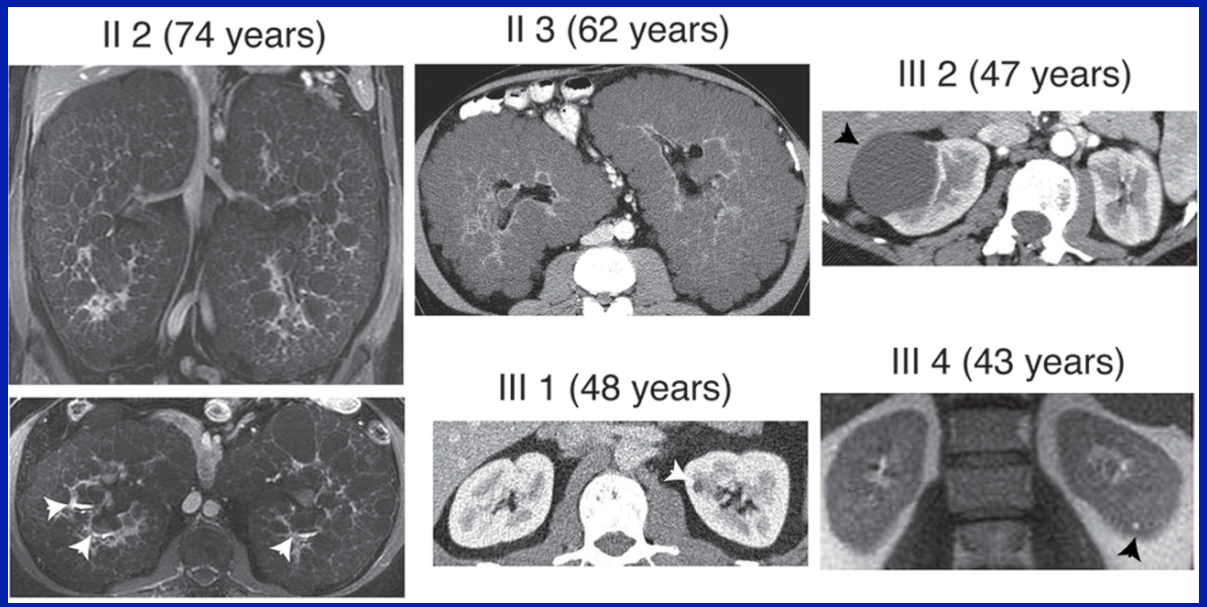
Hypomorphic *PKD1* allele in homozygosity: extreme intrafamilial phenotypic variability





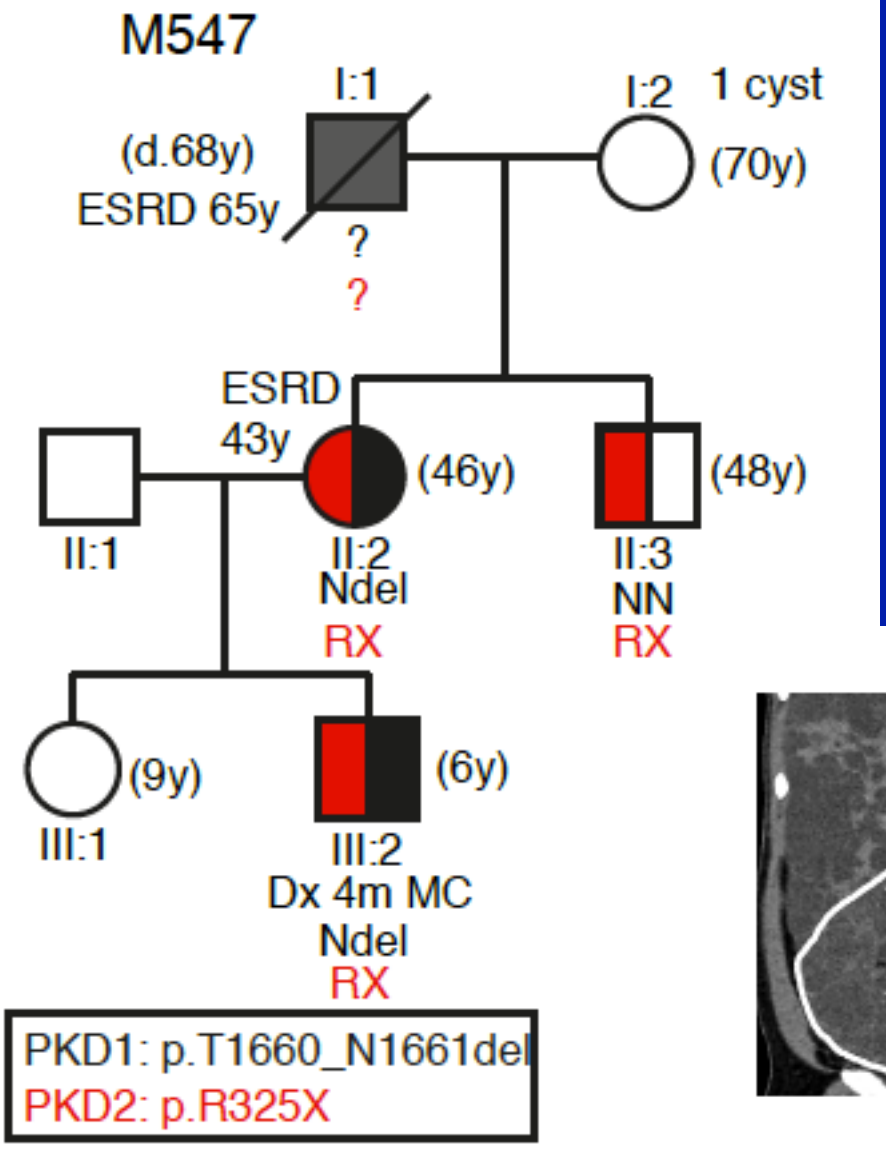
Consanguineous US family of French origin homozygous or heterozygous for R3277C

Unusual, reniform kidneys with multiple small cysts in homozygotes

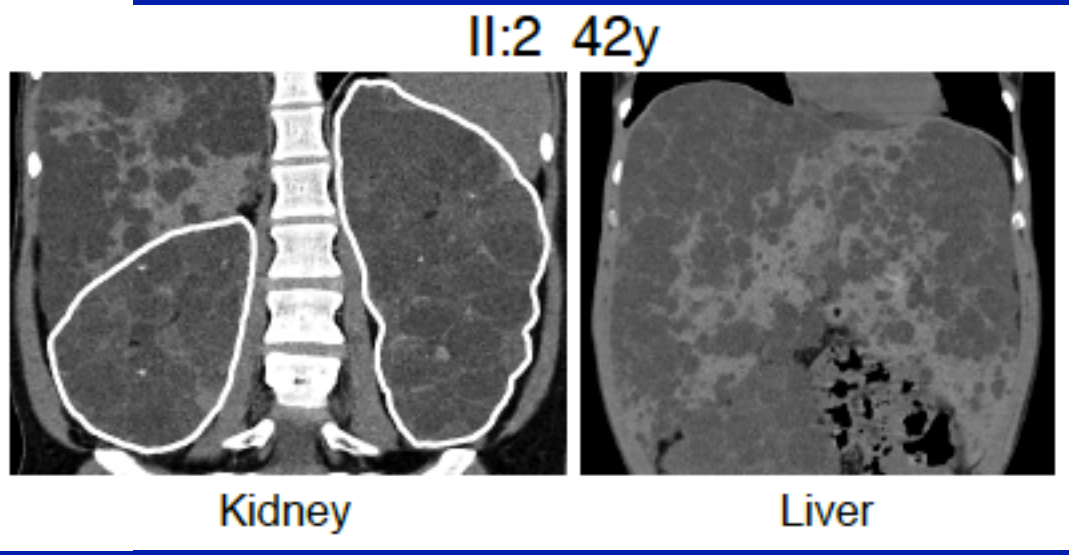


Rossetti et al 2009 KI

Family with *PKD1* and *PKD2* mutation: intrafamilial phenotypic variability



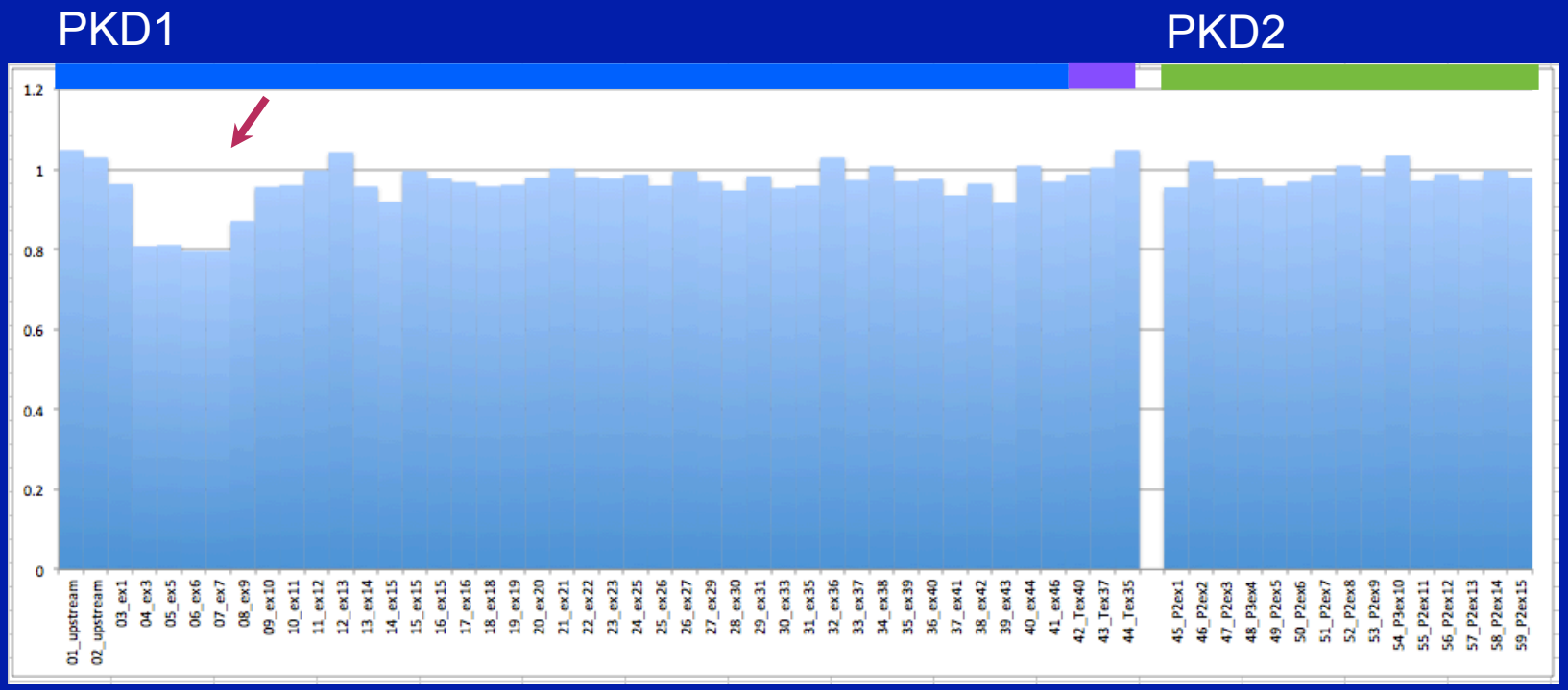
Patients with both mutations have more severe disease



Gainullin in preparation

MLPA assay for *PKD1* and *PKD2*

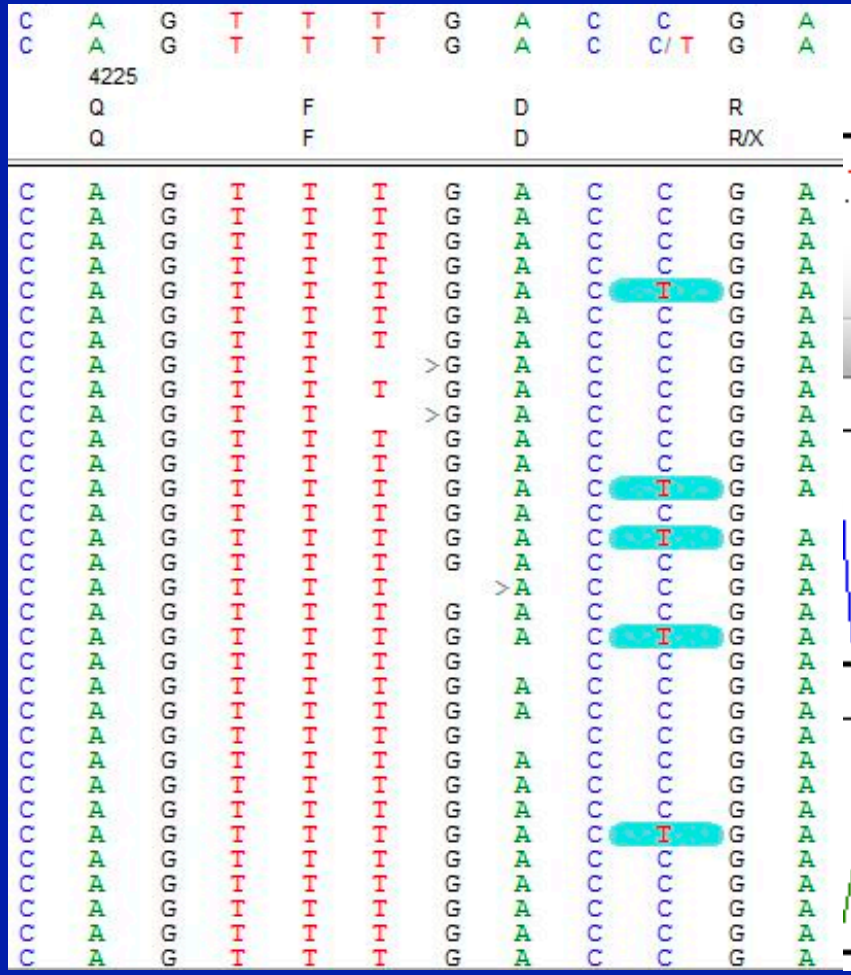
- 3-4% of mutations are large rearrangements
- PKD1 deletion of exons 3-9, 40% mosaic

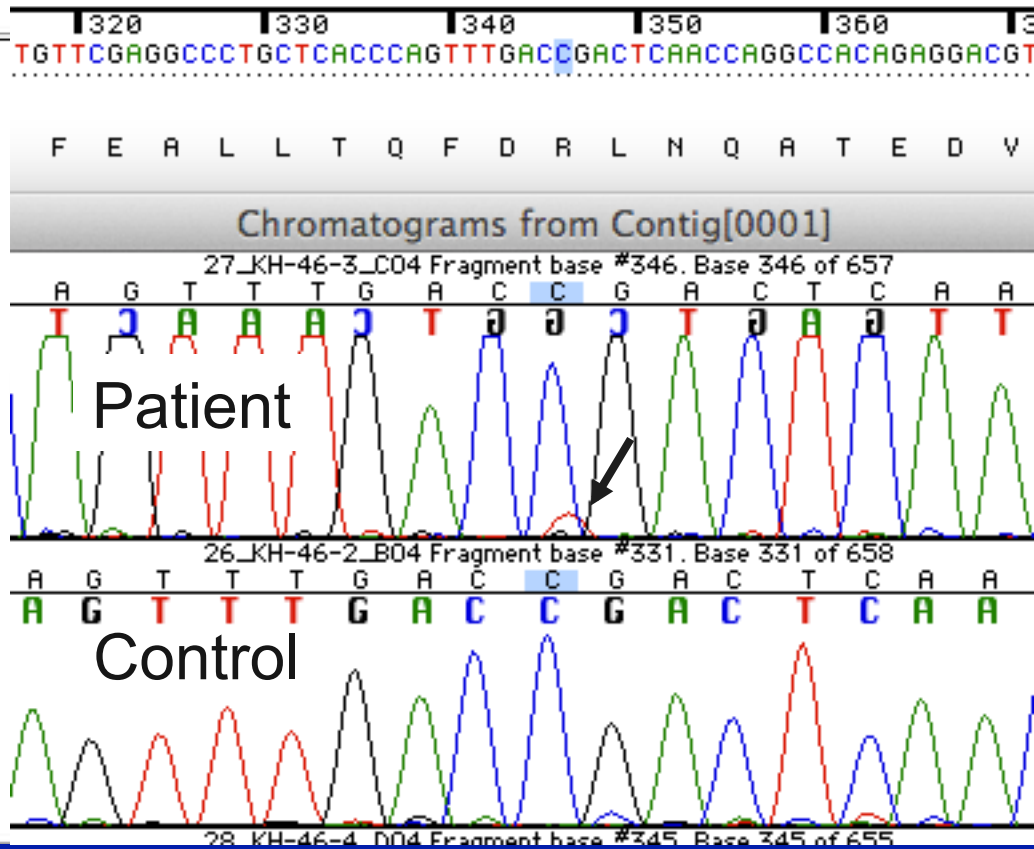


Screening for mosaics employing next generation sequencing

ADPKD patient with mild disease (S.Cr. 1.9 at 77y) and a negative family history was mutation negative by Sanger sequencing

NGS analysis identified the nonsense mutation *PKD1*: p.R4228X at a low level



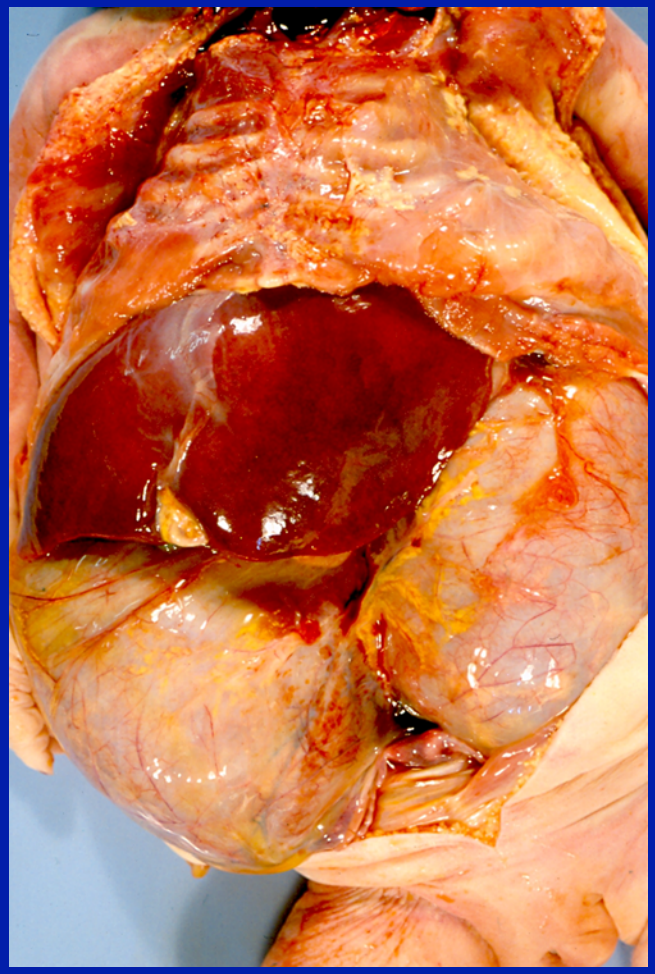


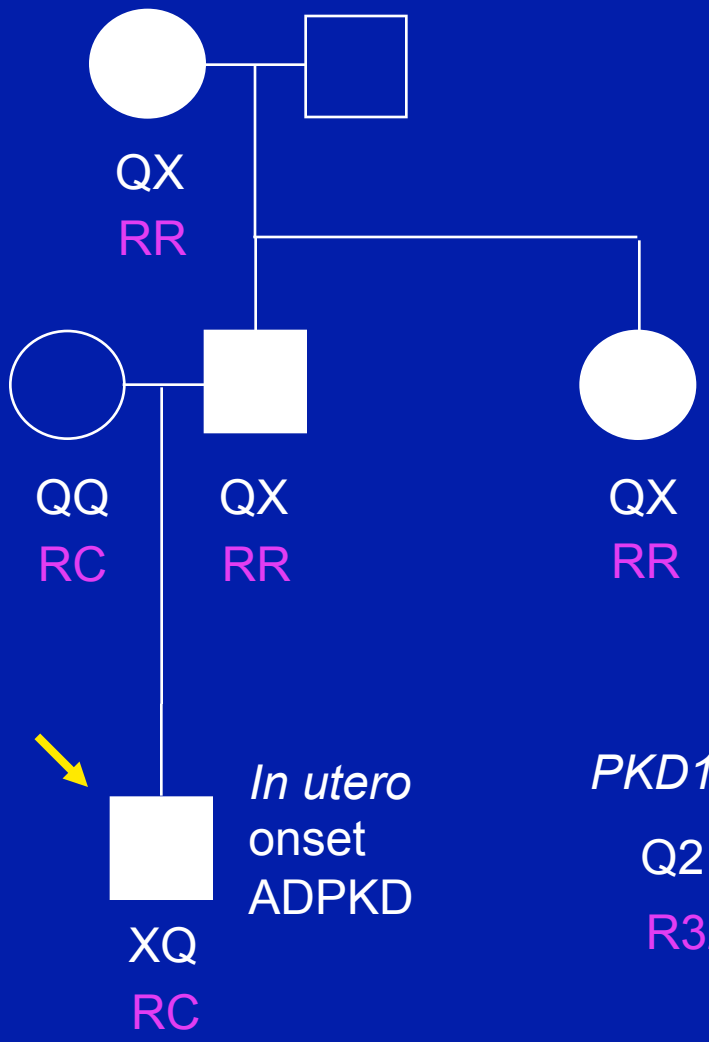
In utero onset ADPKD

- Rarely (<1%) ADPKD presents *in utero* with enlarged and echogenic kidneys in a family with otherwise typical ADPKD
- These cases can be confused with ARPKD
- Increased risk of recurrence in sibs
  - Suggests simple genetic mechanism

Zerres 1993 JMG

Early-onset disease associated with co-inheritance of a truncating and hypomorphic *PKD1* allele





PKD1 Variants

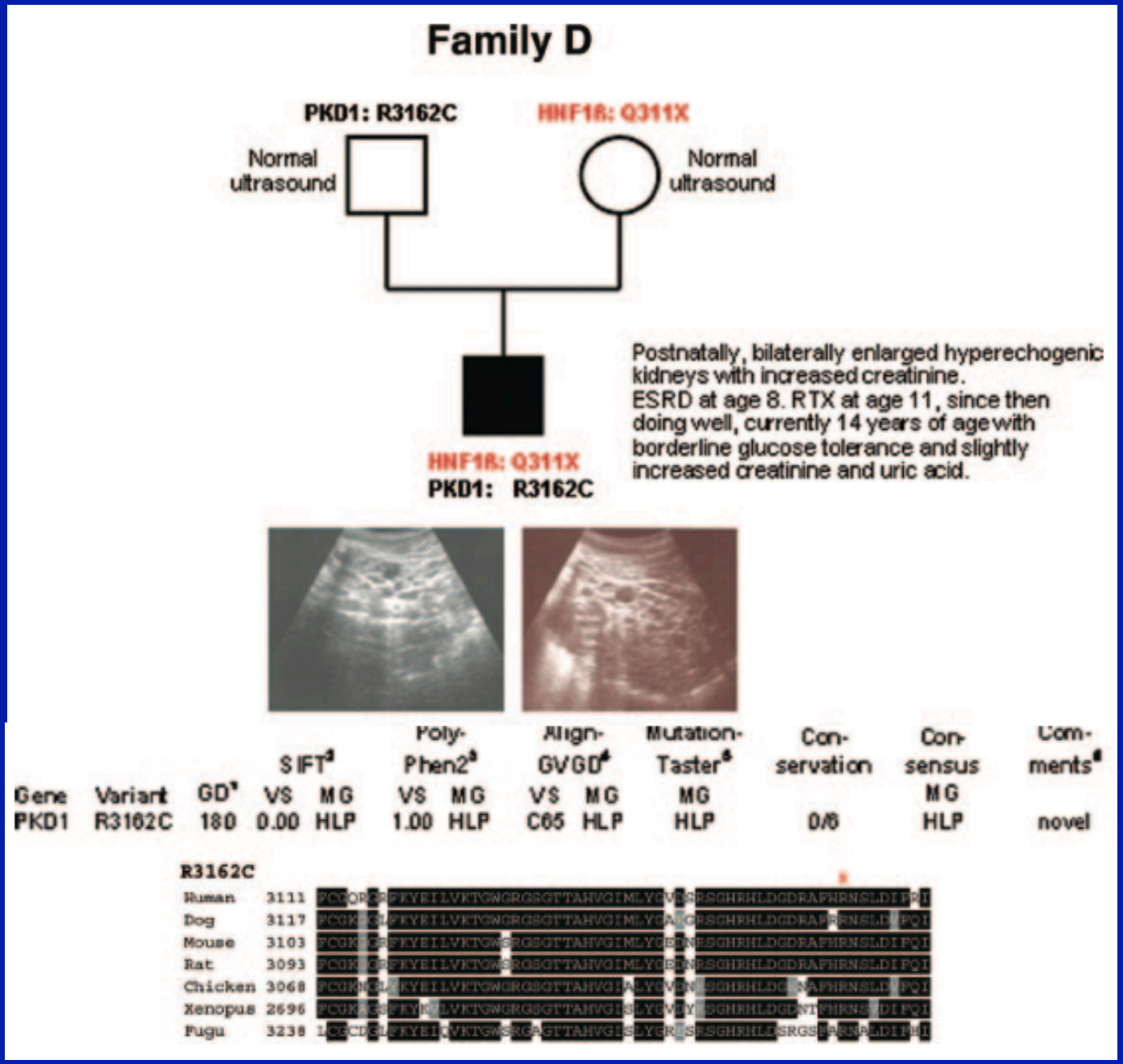
Q2158X

R3277C

Rossetti et al 2009 KI

Other explanations for early onset ADPKD

- Not all EO cases due to co-inheritance of ADPKD alleles
- Co-inheritance of mutations at other loci may cause EO PKD
  - *HNF1B*
  - *PKHD1*
- Analysis of candidate panel or whole exome screen may be appropriate in unresolved EO cases



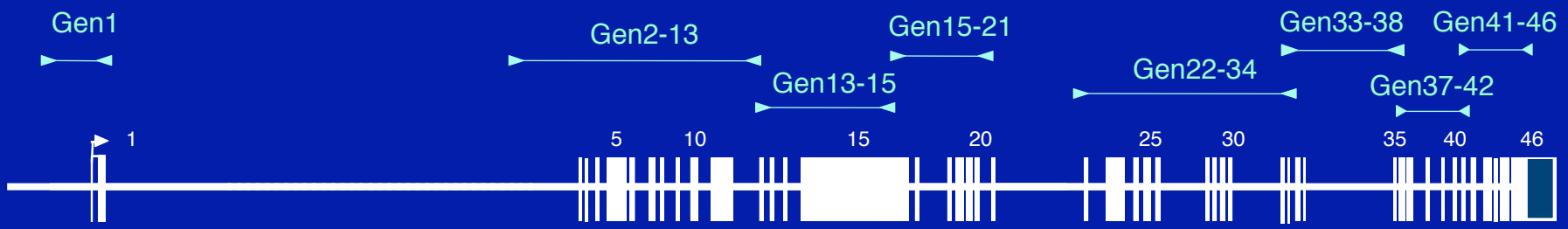
Example of combination of *PKD1* and *HNF1B* allele causing EO PKD

Bergmann 2011 JASN

Gene-based diagnostics in ADPKD is complex

- Genetic and extreme allelic heterogeneity
  - Completely screen *PKD1* and *PKD2* required
- Segmental duplication of *PKD1*
  - Locus specific enrichment required
  - Exon capture methods unreliable
- Many variants of uncertain significance
- Many *PKD1* non-truncating changes hypomorphic
  - Identification of hypomorphic alleles difficult
- Genetic test is expensive and not always informative
- Reports often uninformative and difficult to understand
  - Clinical testing only available through one vendor in US:
  - Athena Diagnostics - ~$5000 with MLPA testing
  - Recent Supreme Court ruling may open US market

Next-generation sequencing allows rapid analysis of multiple patient samples



- *PKD1* and *PKD2* amplified as 14 long-range products
  - exon capture unreliable for *PKD1* because of genomic duplication
- Potential for higher throughput and reduced cost
- Mutation detection rate likely to be comparable
- Introns, UTRs and promoters could also be screened

Rossetti 2012 JASN

Molecular Diagnosis of Autosomal Dominant Polycystic Kidney Disease Using Next-Generation Sequencing

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Table 4 NGS Analytic Sensitivity and Specificity (Variants Detection)

	Sanger sequencing		
NGS	Variant alleles (positive)	Reference alleles (negative)	Total
Variant alleles (positive)	248	0	248
Reference alleles (negative)	2	1825	1827
Total	250	1825	2075

Table 6 Comparison of Reagents, Sequencing Costs, and Time of Labor for Sanger Sequencing and NGS

Method	Purpose	Quantity	Cost ($)			Labor time (days)
			Per sample	Per run	Per subject	
Sanger sequencing ($N = 25$)	LR-PCR *(PKD1)*	250	2.40	600.00	24.00	5
	Standard PCR *(PKD2)*	400	1.50	600.00	24.00	4
	Purification	200	2.40	480.00	19.20	1
	Sequencing primers	3050	0.10	305.00	12.20	NA
	Sanger sequencing	1600	3.00	4800.00	192.00	5
	Data analysis	NA	NA	NA	NA	4
	Total			6785.00	271.40	19
NGS ($N = 25$)	LR-PCR (*PKD1* and *PKD2*)	250	1.45	362.50	14.50	2
	LR-PCR product quantification	250	0.12	30.00	1.20	0.5
	DNA fragmentation	25	6.50	162.50	6.50	0.5
	Library preparation	25	20.00	500.00	20.00	3
	Library quality assessment	25	0.20	5.00	0.20	0.25
	NGS sequencing (MiSeq)	1	990.00	990.00	39.60	1
	Data analysis	NA	NA	NA	NA	1
	Total			2050.00	82.00	8.25

Mutation-based diagnostics in ADPKD is likely to be more widely employed

- Mutation identified in 90% cases
  - Definite (truncating) mutations in ~65% families
- Bioinformatic scoring of non-definite mutations increasingly reliable
  - Recurrent mutations ~50% in recent studies
  - Mutation database of value
    - Identify pathogenic mutations
    - Highlight hypomorphic changes
- Of diagnostic and prognostic value
- Mutation type may in the future influence treatment options
  - Similar to cystic fibrosis
- Cost of test needs to decrease
- Reliability and interpretation of results needs to improve