Fundació Puigvert

Diagnosis of autosomal dominant polycystic kidney disease using efficient *PKD1* and *PKD2* targeted next generation sequencing

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INTRODUCTION

Autosomal polycystic kidney disease (ADPKD) is caused by mutations in *PKD1* in 85% of cases and *PKD2* in the remaining 15%. Approximately 70% of genomic *PKD1* region (exons 1-33) is duplicated 6 times within 6 pseudogens. This, together with the high GC content, the absence of mutation hot spots and the high allelic heterogeneity, makes the molecular diagnostics of ADPKD challenging. To date, sequencing of *PKD1* repeated region requires long-range amplifications followed by nested PCRs, combined with Sanger sequencing of all 46 *PKD1* and 15 *PKD2* exons. If no mutations are identified, multiplex ligation-dependent probe amplification (MLPA) analysis is performed to identify potential insertions and deletions.

AIMS

- To validate NGS technology for genetic diagnostics of ADPKD in a validation cohort: 36 patients with known mutations in *PKD1* and *PKD2*
- To apply this approach in a discovery cohort including 12 ADPKD patients with unknown mutations

MATERIALS AND METHODS

Assay workflow

Library preparation (TruSeq Illumina) → **Capture probes** of *PKD1* and *PKD2* genomic region (Custom NimbleGen SeqCap EZ Choice Library) → **Sequencing:** - HiSeq 2000 Illumina (Validation cohort) - MiSeq Illumina (Discovery cohort) → **Bioinformatic analysis** In-house pipeline (1) → **Candidate pathogenic variants**

Pathogenicity evaluation

All the variants were validated by Sanger sequencing

- Mutations that could give rise to premature protein (truncating mutations) → **DEFINITELY PATHOGENIC**
- Missense and noncanonical splicing variants → SCORING SYSTEM → - Conservation in orthologs - *In silico* predictors: SIFT, Polyphen - Allele frequency: dbSNP, 1000 genomes - ADPKD mutation database → **- HIGHLY LIKELY PATHOGENIC - LIKELY PATHOGENIC - INDETERMINATE VARIANTS - NEUTRAL VARIANTS**

RESULTS

Panel design

- The capture probes designed covered all the coding regions
- Capture probes could not be designed in 19% of intronic and intergenic sequences

Depth of coverage

	Mean (x)	≥100x (%)	≥50x (%)	≥20x (%)
VALIDATION COHORT				
PKD1	331	85	92	96
PKD2	481	97	99	99
DISCOVERY COHORT				
PKD1	81	52	65	87
PKD2	174	68	93	99

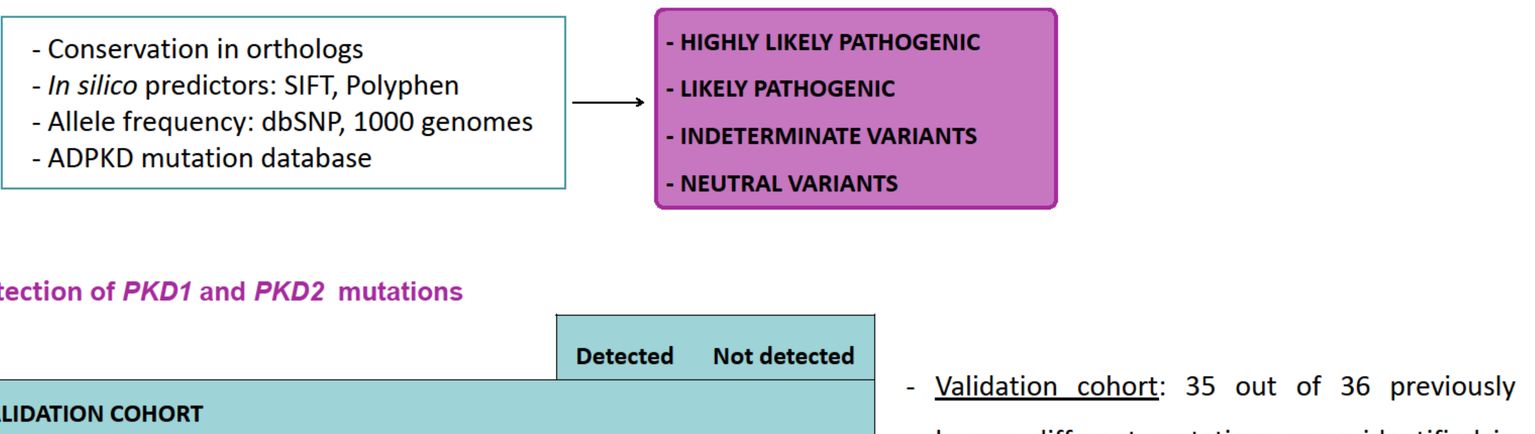
Detection of *PKD1* and *PKD2* mutations

	Detected	Not detected
VALIDATION COHORT		
Nº of mutations in *PKD1* duplicated region	24	1
Nº of mutations in *PKD1* no duplicated region	6	0
Nº of mutations in *PKD2*	5	0
Total (%)	**35 (97)**	**1 (3)**
DISCOVERY COHORT		
Nº of mutations in *PKD1* duplicated region	8	1
Nº of mutations in *PKD1* no duplicated region	2	1
Nº of mutations in *PKD2*	1	0
Total (%)	**11(85)**	**2 (15)**

- Validation cohort: 35 out of 36 previously known different mutations were identified in their correct heterozygous state.
- Discovery cohort: 11 out of 12 mutations were detected in 12 patients (1 patient carried a mutation in *PKD1* and another in *PKD2*).
- Not detected mutations: the 3 mutations not detected by our NGS approach were located in poorly covered regions: exon 1 , 23 and 44 of *PKD1*

Detection of large deletions

Two previously known large deletions were correctly identified (yellow peaks). The normalized relative copy number of an exon in a sample (SVD-ZRPKM values) for the 36 patients of the validation cohort is shown. (A) Patient 03-106-P6 *PKD1* deletion (g.2154344-2186386). (B) Patient 11-571-P2: *PKD2/ABCG2* deletion (g.88952828-89050618)



CONCLUSIONS

- In-solution capture coupled to NGS is an efficient approach to detect all types of ADPKD pathogenic mutations, including structural variants.
- Our approach is cost- and time- effective, and meets the sensitivity and specificity criteria required for genetic diagnostics of ADPKD.

References

(1) Trujillano D et al 2013 *J Med Genet* 50(7):455-62

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51ERA-EDTA 38--MP

D) Genetic diseases and molecular genetics.

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DOI: 10.3252/pso.eu.51era.2014

Poster presented at: 51 ERA-EDTA CONGRESS AMSTERDAM 2014

PosterSessionOnline.com