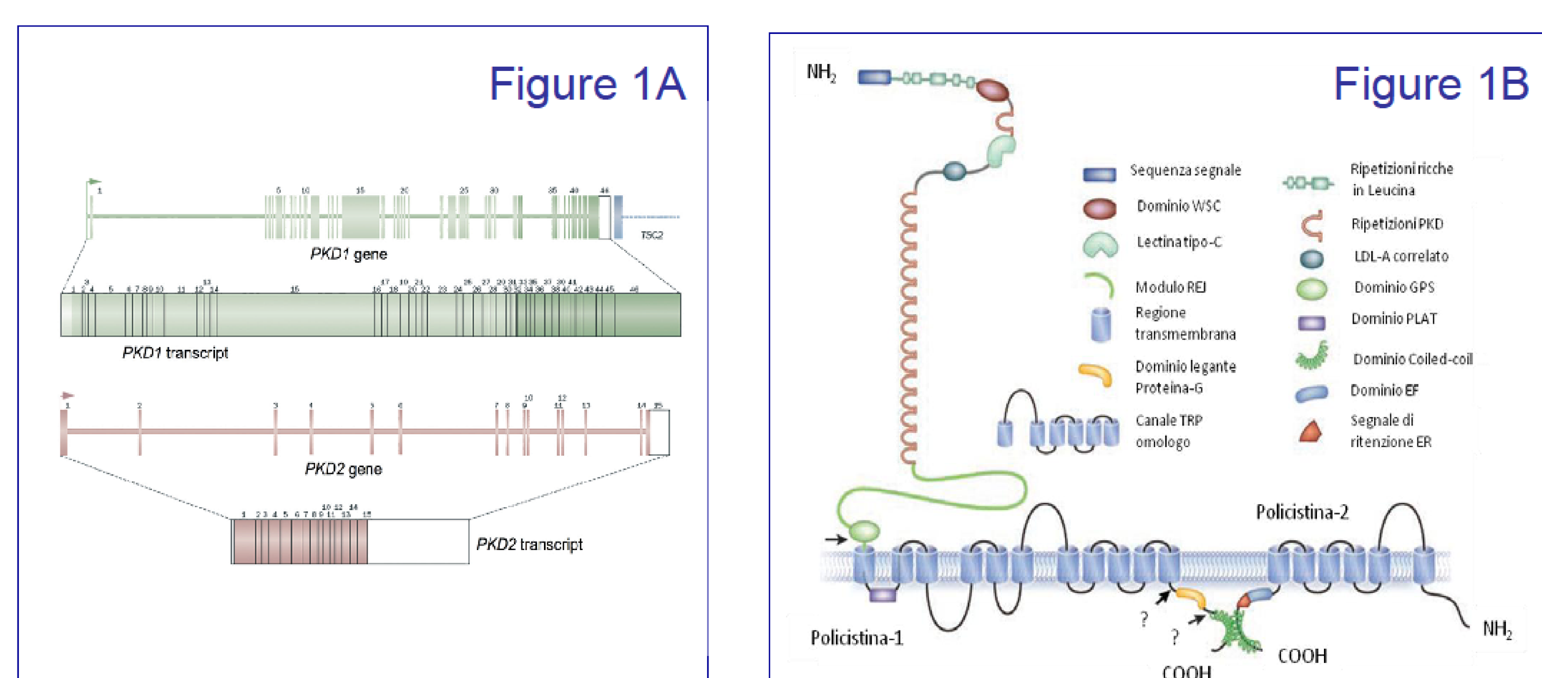
PKD1 AND *PKD2* MUTATION ANALYSIS IN 90 UNRELATED ITALIAN PEDIGREES WITH AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE (ADPKD): SAGER SEQUENCING *vs* NEXT GENERATION SEQUENCING (NGS).

Sterpeta Diella[1,2], Matteo Accetturo[2], Giovanni Stallone[1], Silvana Chicca[3], Paola Pontrelli[2], Barbara Infante[1], Elena Ranieri[1], Giuseppe Grandaliano[1], Loreto Gesualdo[2], Maddalena Gigante[1]

[1]University of Foggia, Dept. of Medical and Surgical Sciences, Foggia, ITALY; [2]University of Bari "Aldo Moro", Dept. of Emergency and Organ Transplantation, Bari, ITALY; [3]"Sandro Pertini" Hospital, Nephrology and Dialysis, Roma, ITALY

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD), is characterized by the development of renal cysts leading to end-stage renal failure. ADPKD is typically diagnosed by imaging but the diagnosis may be uncertain, especially in young individuals (<30 years) and in patients with a negative family history. ADPKD is caused by mutations in *PKD1* or *PKD2* genes encoding for policistin-1 and -2, respectively (Figure 1A, B)



The mutational analysis of ADPKD is complicated by extensive allelic heterogeneity and by the presence of six highly homologous sequences of *PKD1* exons 1–33. Here, we report our comprehensive mutation analysis of PKD1 and PKD2 genes in 137 Italian ADPKD patients from 90 unrelated pedigree using both Sanger Sequencing and Next Generation Sequencing (NGS).

METHODS

PKD1 and *PKD2* genes were analyzed using Sanger sequencing, Multiplex Ligation-dependent Probe Amplification (MLPA) and NGS by multiplexing indexed paired-end libraries from long range PCR using Illumina Nextera XT kit and Illumina MiSeq instrument (Figure 2)

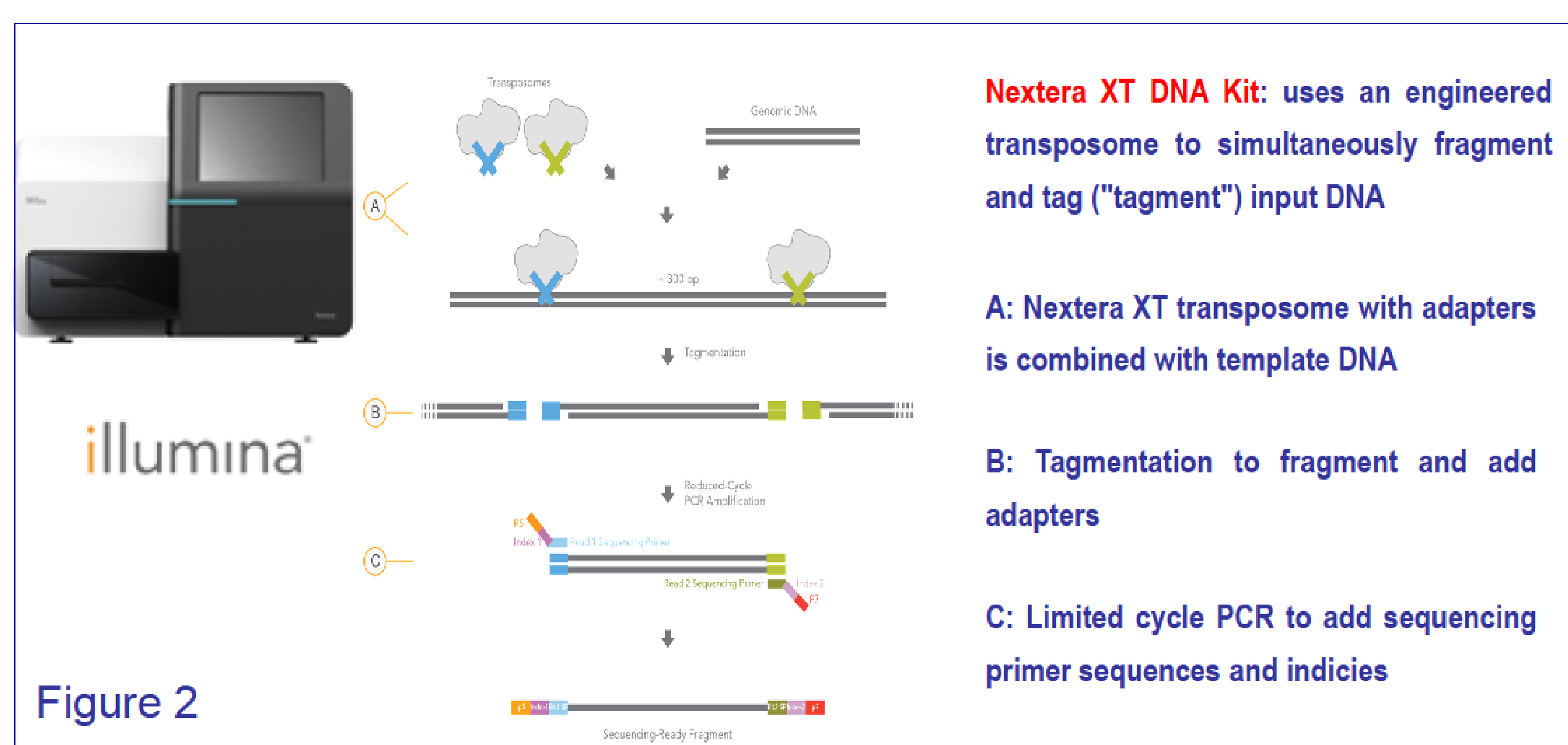


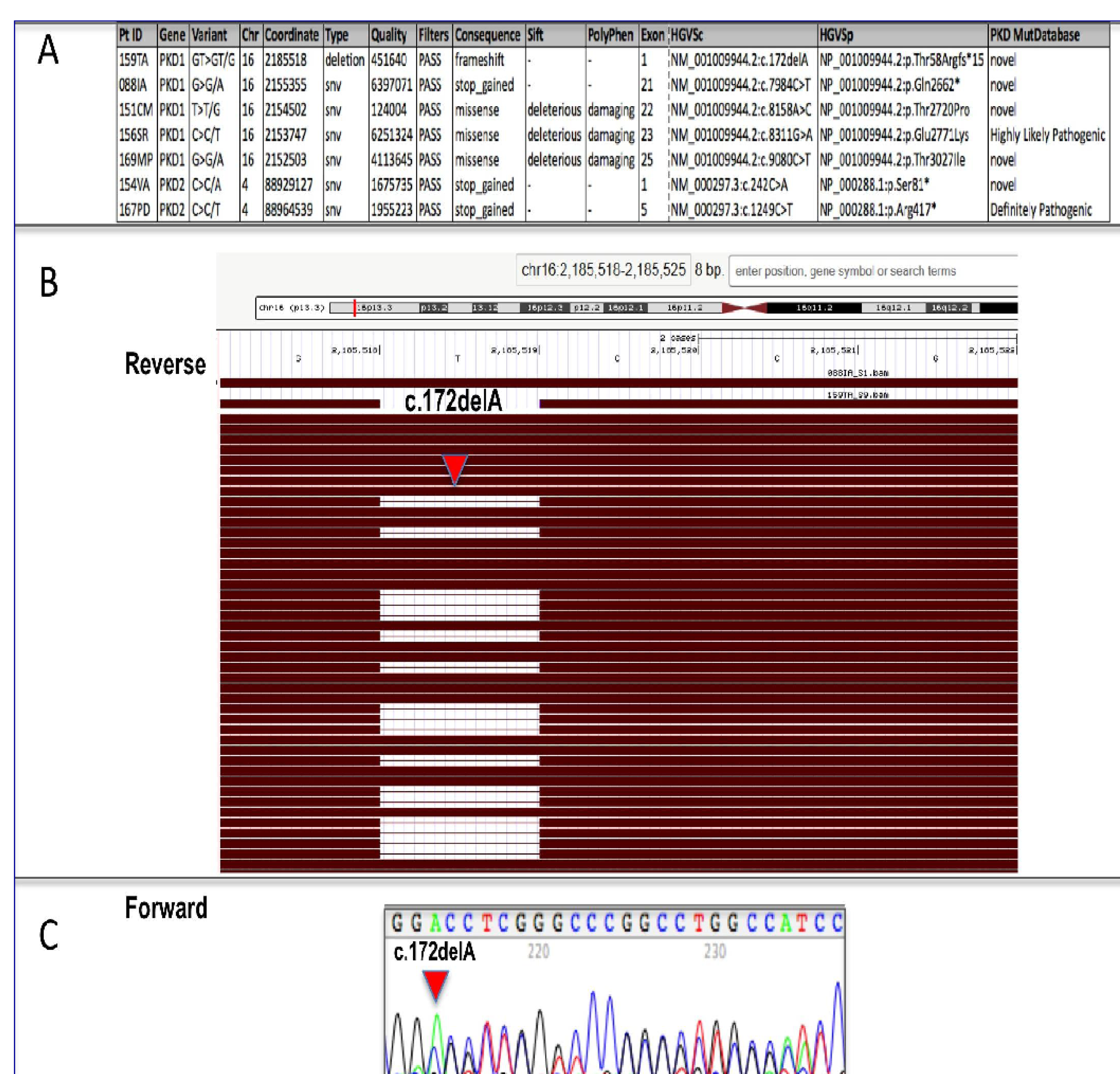
Figure 2

RESULTS

We found 70 (48 novel) definitively (40) and highly/likely pathogenic (30) mutations and 6 novel indeterminate and likely neutral mutations. We achieved an overall detection rate of 90%. Nine out of 78 positive pedigrees resulted *PKD2* carries (12%), 69/78 were PKD1 carriers (88%) and 12/90 (13%) resulted mutation negative. 12 *de novo PKD1* mutations were identified in sporadic patients. Both parents of these probands have been analyzed for detected mutations demonstrating that all 12 mutations occurred *de novo*. We found the largest number of *de novo* mutations reported in a single study (15%).

RESULTS

Ten out of 90 pedigrees (3 previously analyzed by Sanger sequencing - 1 mutation-positive and 2 mutation-negative - and 7 not previously genotyped) were analyzed by NGS using Illumina Nextera XT and Illumina MiSeq: we confirm the presence of a previously identified nonsense mutation, the lacking of clear pathogenic mutations in the other two previously analyzed patients, the presence of pathogenic or probably pathogenic mutation in 6/7 novel patients and the absence of clear pathogenic mutations in one pedigree (Figure 3A). All NGS results were confirming by Sanger sequencing, achieving sensitivity and specificity of 100% for this small number of samples (Figure 3B and C).



Pt ID	Gene	Variant	Chr	Coordinate	Type	Quality	Filters	Consequence	Sift	PolyPhen	Exon	HGVSc	HGVSp	PKD MutDatabase
159TA	PKD1	GT>GT/G	16	2185518	deletion	451640	PASS	frameshift	-	-	1	NM_001009944.2:c.172delA	NP_001009944.2:p.Thr58Argfs*15	novel
088IA	PKD1	G>G/A	16	2155355	snv	6397071	PASS	stop_gained	-	-	21	NM_001009944.2:c.7984C>T	NP_001009944.2:p.Gln2662*	novel
151CM	PKD1	T>T/G	16	2154502	snv	124004	PASS	missense	deleterious	damaging	22	NM_001009944.2:c.8158A>C	NP_001009944.2:p.Thr2720Pro	novel
156SR	PKD1	C>C/T	16	2153747	snv	6251324	PASS	missense	deleterious	damaging	23	NM_001009944.2:c.8311G>A	NP_001009944.2:p.Glu2771Lys	Highly Likely Pathogenic
169MP	PKD1	G>G/A	16	2152503	snv	4113645	PASS	missense	deleterious	damaging	25	NM_001009944.2:c.9080C>T	NP_001009944.2:p.Thr3027Ile	novel
154VA	PKD2	C>C/A	4	88929127	snv	1675735	PASS	stop_gained	-	-	1	NM_000297.3:c.242C>A	NP_000288.1:p.Ser81*	novel
167PD	PKD2	C>C/T	4	88964539	snv	1955223	PASS	stop_gained	-	-	5	NM_000297.3:c.1249C>T	NP_000288.1:p.Arg417*	Definitely Pathogenic

Figure 3.
A. Pathogenic Mutations identifed by NGS.
B. Representative example of visual inspection of NGS aligment showing c.172delA (p.Thr58Argfs*15) mutation.
C. Sanger sequencing confirmation showing frameshift.

CONCLUSIONS

In summary, we performed a comprehensive mutation screening of *PKD1* and *PKD2* genes in 90 unrelated Italian families. Our study represents a significant advance in the molecular diagnosis of ADPKD Italian patients because it (i) analyzes the largest Italian cohort; (ii) reports the largest number of *de novo* mutations in a single study, demonstrating that the prevalence of *PKD1 de novo* mutations may have been underestimated and emphasizing the importance of molecular screening in patients without family history; (iii) provides for the first time a new NGS method for Italian patients with a detection rate comparable to Sanger sequencing but with significantly lower costs and faster diagnostic times.

52 ERA
53--FP
C) Renal development and cystic diseases.
Sterpeta Diella
DOI: 10.3252/pso.eu.52era.2015
ePosters supported by F. Hoffmann- La Roche Ltd.

PosterSessionOnline.com