An efficient and comprehensive strategy for genetic diagnostics of polycystic kidney disease (ADPKD/ARPKD)

BIOSCIENTIA HUMAN GENETICS

Tobias Eisenberger[1], Christian Decker[1], Milan Hiersche[1], Ruben C. Hamann[1], Lars Pape[2], Henry Fehrenbach[3], Eva Decker[1], Steffen Neuber[1], Valeska Frank[1], Hanno J. Bolz[1], Burkhard Toenshoff[4], Christoph Mache[5], Kay Latta[6], Carsten Bergmann[1,7]

[1]Bioscientia Center for Human Genetics, Ingelheim, Germany; [2]Clinic for Pediatric Kidney, Liver and Metabolic Diseases, MHH Hannover, Germany; [3]Department of Pediatrics, Children's Hospital Memmingen, Germany; [4]Department of Pediatric Nephrology, University Heidelberg Medical Center, Germany; [5]Department of Pediatrics; Medical University Graz, Austria; [6]Clementine Children's Hospital, Frankfurt, Germany; [7]Renal Division, Department of Medicine, University Freiburg Medical Center, Freiburg, Germany

Background

Renal cysts are clinically and genetically heterogeneous conditions. Autosomal dominant polycystic kidney disease (ADPKD) is the most frequent life-threatening genetic disease and mainly caused by mutations in *PKD1*. The presence of six *PKD1* pseudogenes and tremendous allelic heterogeneity make molecular genetic testing challenging requiring laborious locus-specific amplification. Up to 5% of ADPKD patients present with an early and severe phenotype. Affected families have a high recurrence risk for severe clinical courses, sometimes clinically indistinguishable from the recessive form ARPKD with mutations in *PKHD1*. Notably, both ADPKD genes can also be inherited in a recessive way and many mutations occur *de novo*, i.e. the family history is unremarkable. Further phenocopies are known and mutations in genes that typically cause ciliopathies with extrarenal features such as nephronophthisis, Joubert and Bardet-Biedl syndrome may sometimes mimic PKD especially in the prenatal setting and early childhood. Overall, increasing heterogeneity illustrates the need for a more comprehensive genetic testing approach targeting all genes that may have to be discussed for differential diagnosis.

Methods

We established and validated a sequence capture based NGS testing approach for all genes known for cystic and polycystic kidney disease including *PKD1* (in total, n = 40 genes). The complete genomic region of the *PKD1* gene was targeted by an optimized custom SeqCap EZ choice library (NimbleGen). In total, 55 positive control samples were included in the validation cohort and sequenced on an Illumina MiSeq or HiSeq1500 system. NGS data analysis was performed with an in-house bioinformatic pipeline as previously described[1,2]. Results from NGS were compared to results from preceded Sanger sequencing. Sophisticated bioinformatic read simulation was applied to increase analytical depth of the validation study. For exonic areas in the duplicated region in *PKD1* with impaired discriminative mapping of reads between master gene and pseudogene due to high sequence homology relaxed filter criteria of ≥ 8% of total reads at the position showing the alteration were applied. Copy number variation (CNV) analysis was performed using VarScan and results compared to previous results from MLPA analysis.

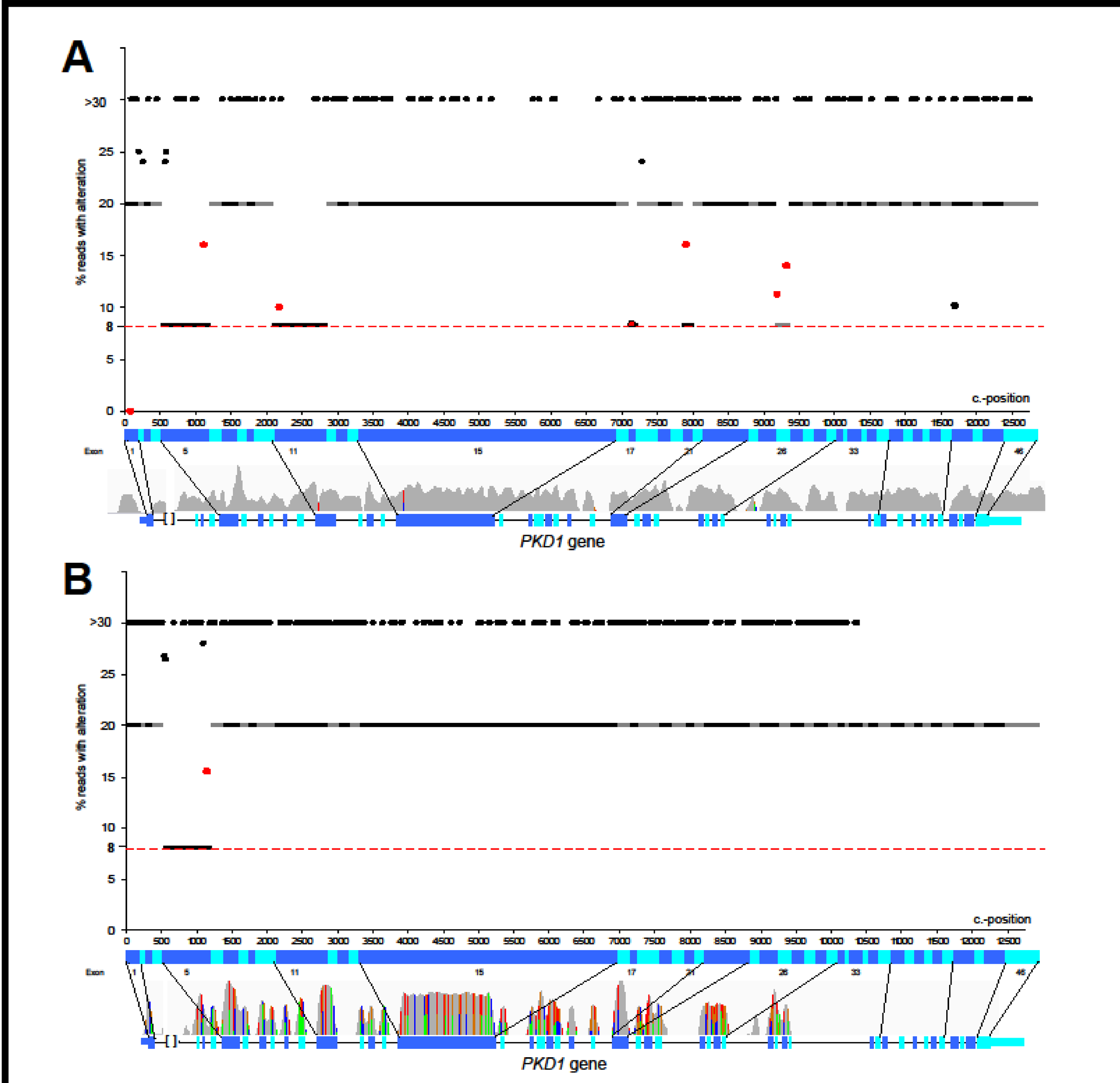

Figure 1. Detection level and distribution of *PKD1* variants in our cohort and by variant simulation
Percentage of alternative reads detected by our NGS approach for all *PKD1* variants (black dots) from our validation cohort (**A**) and from read simulation by Wgsim (**B**). Variants highlighted with red dots in the duplicated region required second-step analysis with a lower detection threshold (8% alternative reads, red dashed line) to be detected in these critical exons.

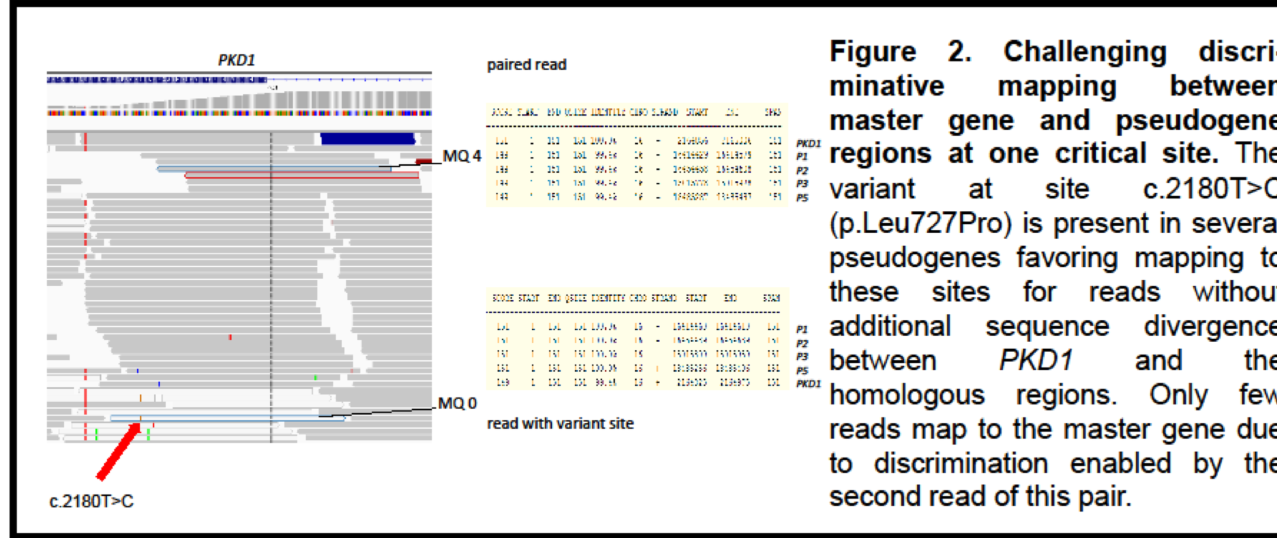

Figure 2. Challenging discriminative mapping between master gene and pseudogene regions at one critical site. The variant at site c.2180T>C (p.Leu727Pro) is present in several pseudogenes favoring mapping to these sites for reads without additional sequence divergence between *PKD1* and the homologous regions. Only few reads map to the master gene due to discrimination enabled by the second read of this pair.

Variant statistics	# Variants	# Detected	Detection rate
Sanger verified variants (SNVs, indels)	683	681	99.6%
Different variants	160	159	99.4%
Definitely and putatively pathogenic mutations	63	62	98.4%
Different mutations	58	58	100%
Novel mutations (different ones)	36	36	100%

Table 1. Validation statistics in *PKD1* mutation positive patient cohort

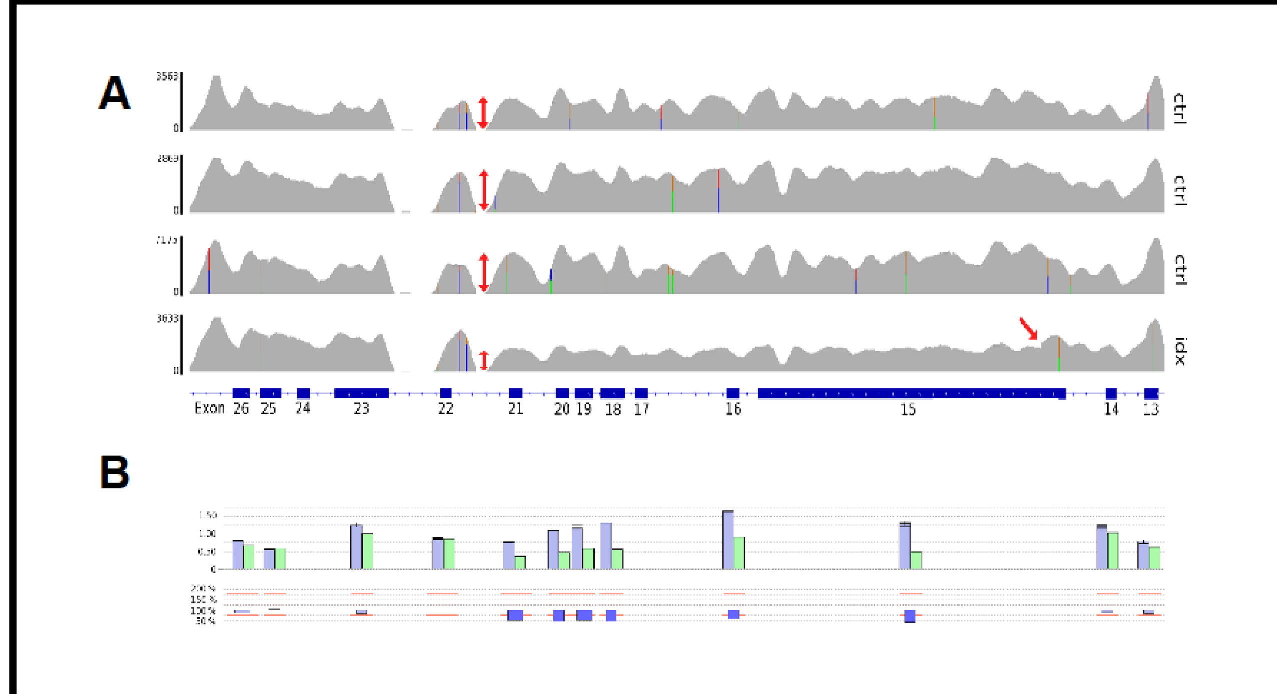

Figure 3. CNV detection of multiple exon deletion in *PKD1* for patient 46. A Coverage plots (IGV) of three control (ctrl) patients versus the index (idx) sample with a drop in coverage at *PKD1* exons 15-21 for the index (red double arrows). **B** Result from MLPA analysis displayed by MLPA module in JSI SeqPilot software.

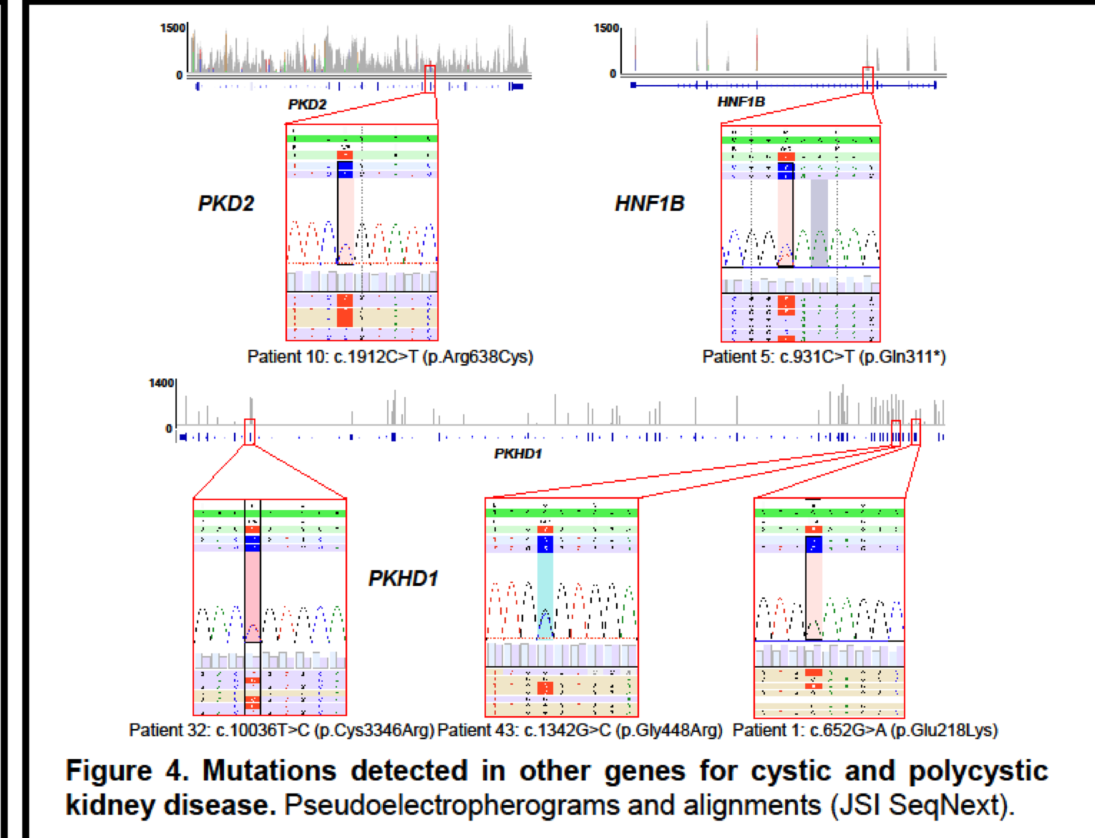

Figure 4. Mutations detected in other genes for cystic and polycystic kidney disease. Pseudoelectropherograms and alignments (JSI SeqNext).

Results

- Sufficient coverage of *PKD1* and all other genes known for cystic and polycystic kidneys was obtained.
- The approach is highly sensitive (99.7%) and specific (99.8%) (Table 1).
- We applied a mapping algorithm mainly with standard settings, filter and variant calling criteria that is sufficient for unambiguous detection of sequence variants in most of the *PKD1* regions (Fig. 1A).
- Variant simulation in duplicated *PKD1* region with high variant density underscored the sensitivity of our approach (Fig. 1B).
- Caution is required at sites where competitive mapping of read pairs between master gene and pseudogene regions impairs efficient read discrimination (Fig. 2).
- CNV analysis is feasible for *PKD1* and all other targeted genes (Fig. 3).
- Our approach enables the parallel analysis of all genes for cystic and polycystic kidney disease (Table 2, Fig. 4).

Patients with mutations in more than one gene

Pat.	*PKD1* mutation	Class.	Additional variant(s)	Class.	Comment
5	c.9484C>T (p.Arg3162Cys) (het)	LH	*HNF1B*: c.931C>T (p.Gln311*) (het)	DP	Patient with unremarkable family history and PKD phenotype carries causative *HNF1B* nonsense mutation *in trans* to a likely hypomorphic *PKD1* variant
3	c.7345_7356del (p.Thr2449_Gly2452del) (het)	PP	*NPHP1*: deletion exons 1-20 (het)	DP	*PKD1* revealed as major disease locus in a patient with suspected ARPKD and negative family history; *NPHP1* deletion might represent accidental carriership
43	c.12671_12674del (p.Thr4224Serfs*133) (het)	DP	*PKHD1*: c.1342G>C (p.Gly448Arg) (het); *MRE11A*: c.1516G>T (p.Glu506*) (het)	P; DP	Detection of pathogenic *PKHD1* mutation in a patient with suspected ARPKD (family history unclear at time of initial testing) may have been misleading
17	c.4697_4698insA (p.Ser1567Glufs*11) (het)	DP	*PKHD1*: c.3407A>G (p.Tyr1136Cys) (het)	PP	Detection of probably pathogenic *PKHD1* mutation in a patient with suspected ARPKD and negative family history may have been misleading

Table 2. Mutations and variants identified in other genes for cystic and polycystic kidney disease.
LH - likely hypomorphic; DP - definitely pathogenic; PP – probably pathogenic; P – pathogenic.

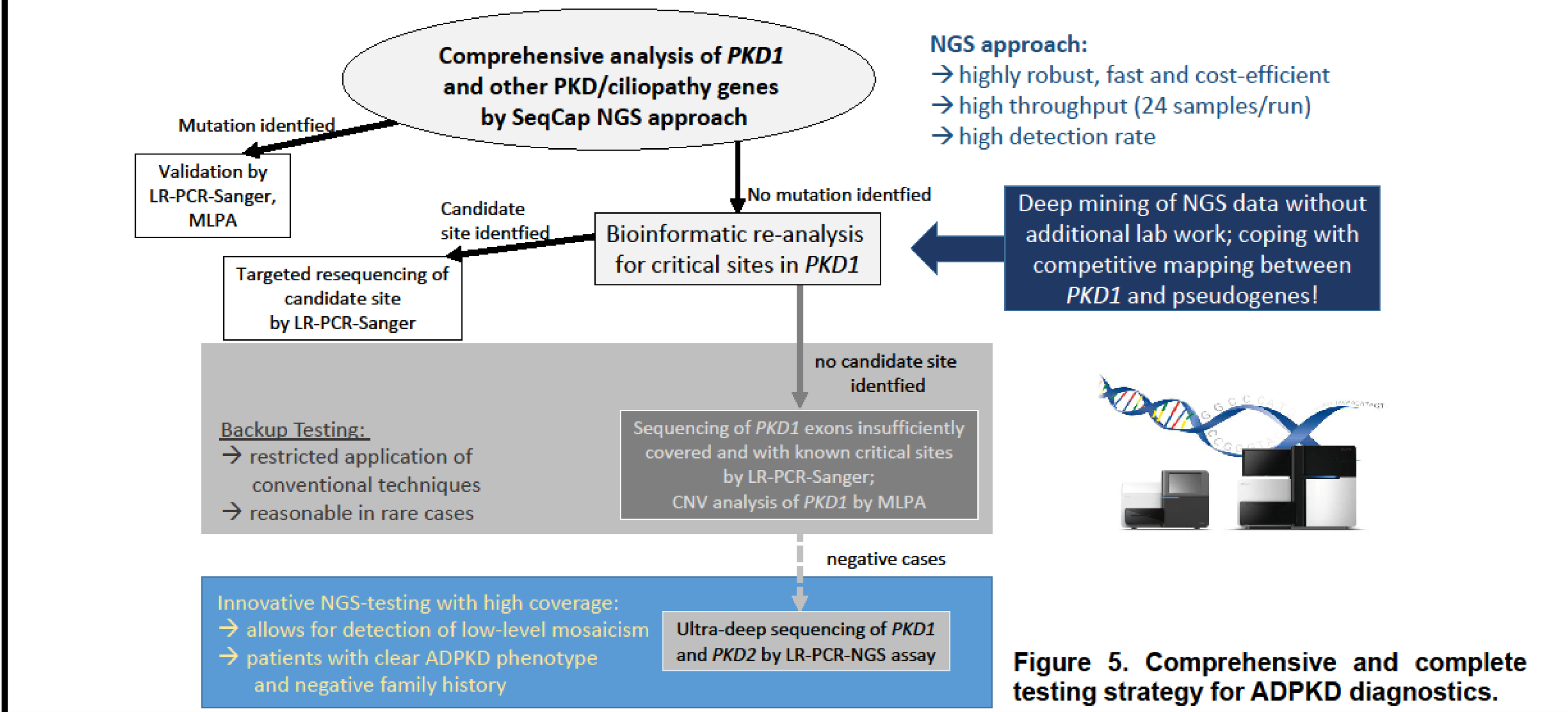

Figure 5. Comprehensive and complete testing strategy for ADPKD diagnostics.

Conclusions

- We established and validated a sequence capture based NGS testing approach for all genes known for cystic and polycystic kidney disease including *PKD1*, which is equally sensitive as established methods.
- An additional advantage over conventional Sanger sequencing is the detection of copy number variations (CNVs).
- Common NGS may have some limitations and pitfalls when applied in complex regions like *PKD1*.
- We propose a time- and cost-efficient diagnostic strategy for comprehensive molecular genetic testing of polycystic kidney disease (Fig. 5) which will be of particular value when therapeutic options for PKD emerge (Tolvaptan/Jinarc is already approved for ADPKD treatment in some countries and was recently recommended by the European Medicines Agency (EMA) for approval).

Reference
(1) Eisenberger T, Decker C, Hiersche M, Hamann RC, Decker E, Neuber S, Frank V, Bolz HJ, Fehrenbach H, Pape L, Toenshoff B, Mache C, Latta K, Bergmann C. An efficient and comprehensive strategy for genetic diagnostics of polycystic kidney disease (2015) PLoS One 3;10(2):e0116680, 2015.
(2) Eisenberger T, Neuhaus C, Khan AO, Decker C, Preising MN, et al. (2013) Increasing the yield in targeted next-generation sequencing by implicating CNV analysis, non-coding exons and the overall variant load: the example of retinal dystrophies. PLoS One 8: e78496.

contact: Carsten Bergmann
Zentrum für Humangenetik, Bioscientia
Konrad-Adenauer-Str. 17, 55218 Ingelheim
Tel.: 06132/781-476; carsten.bergmann@bioscientia.de

DOI: 10.3252/pso.eu.52era.2015
ePosters supported by F. Hoffmann- La Roche Ltd.
PosterSessionOnline.com