ALBERT-LUDWIGS-
UNIVERSITÄT FREIBURG

BIOSCIENTIA
HUMAN GENETICS

Preimplantation Genetic Diagnosis (PGD) and Childhood Diagnostic Evaluation

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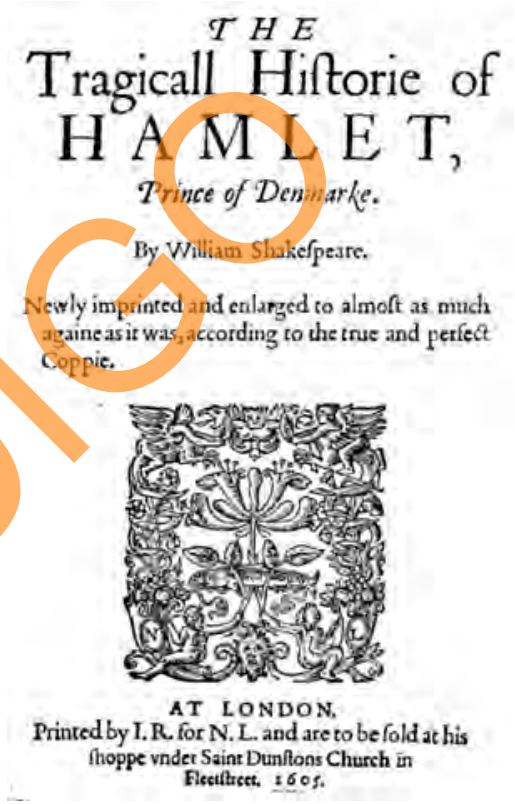
Disclosure of Interests

Employee of Bioscientia, Institute for Medical Diagnostics, Center for Human Genetics

“Nature cannot choose his origin”

http://shakespeare.mit.edu/



THE
Tragicall Hiſtorie of
HAMLET,
Prince of Denmarke.

By William Shakeſpeare.

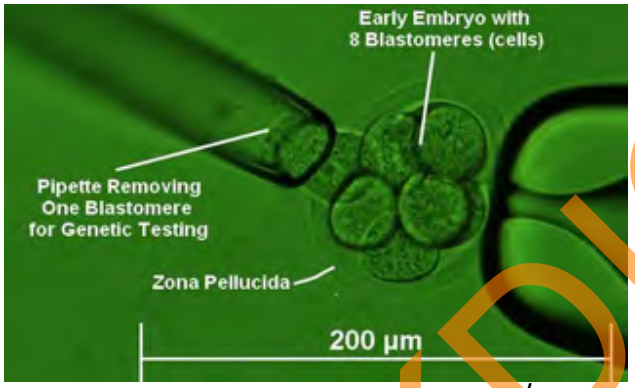
Newly imprinted and enlarged to almoſt as much againe as it was, according to the true and perfect Coppie.

AT LONDON,
Printed by I. R. for N. L. and are to be ſold at his ſhoppe vnder Saint Dunſtons Church in Fleetſtreet. 1605.

Who we are is predetermined and beyond our control

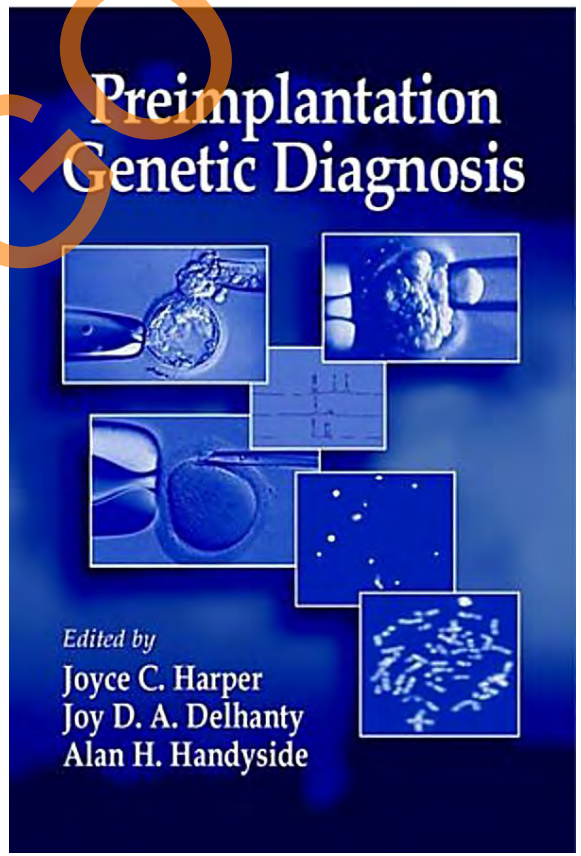
Preimplantation genetic diagnosis (PGD)

Procedure used to test early human embryos for serious inherited genetic conditions or chromosomal abnormalities



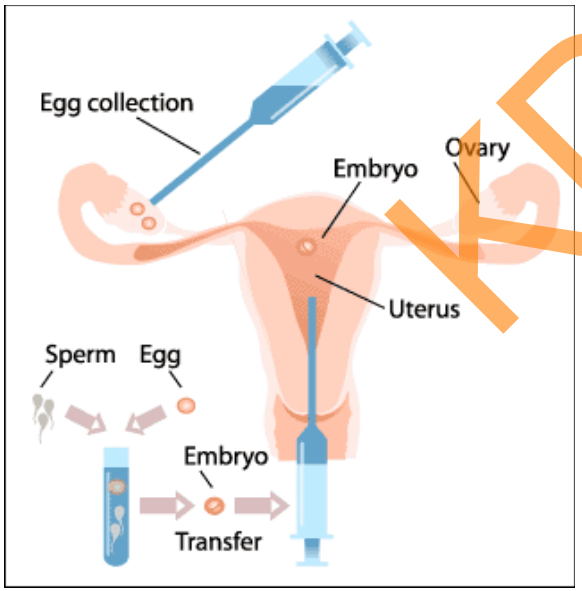
http://medicine.missouri.edu/fertility/

http://www.cpmc.org

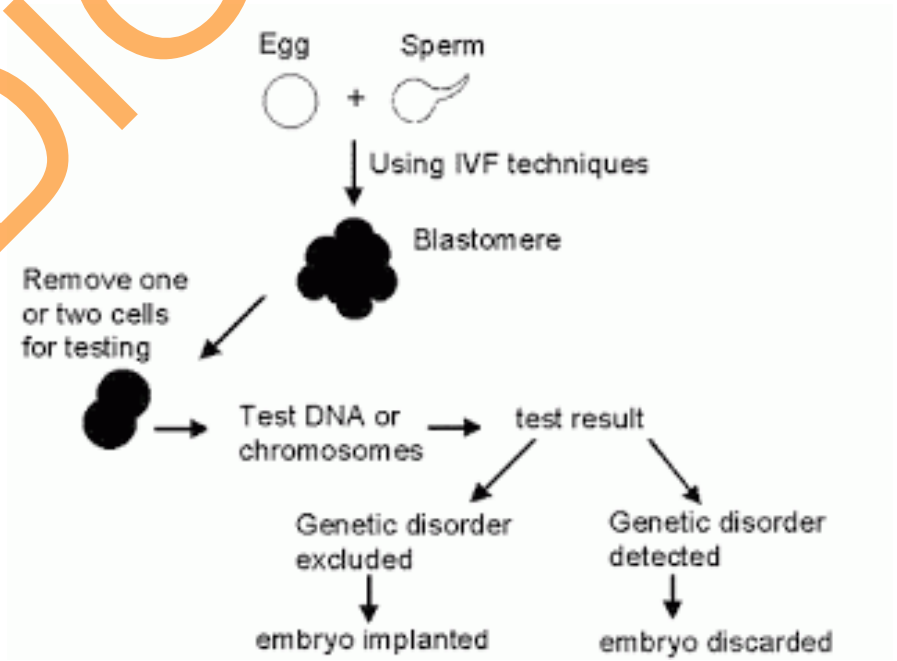


Preimplantation genetic diagnosis (PGD)

1. Creation of an embryo by *in vitro* fertilisation (IVF)
2. Removal of 1-2 cells from the embryo
3. Genetic testing of these cells for specific genetic condition
4. Subsequent transfer of unaffected embryos to woman‘s uterus

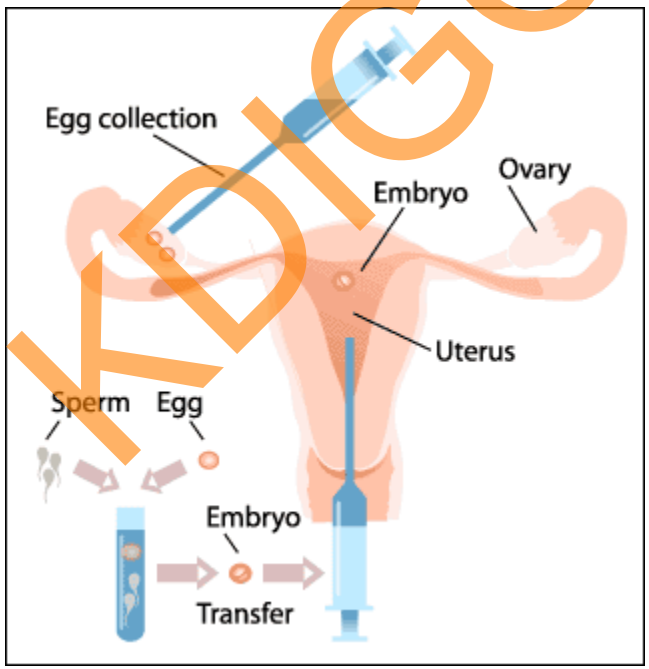


http://www.ivf.net.in/ivf&icsi/



Preimplantation genetic diagnosis (PGD)

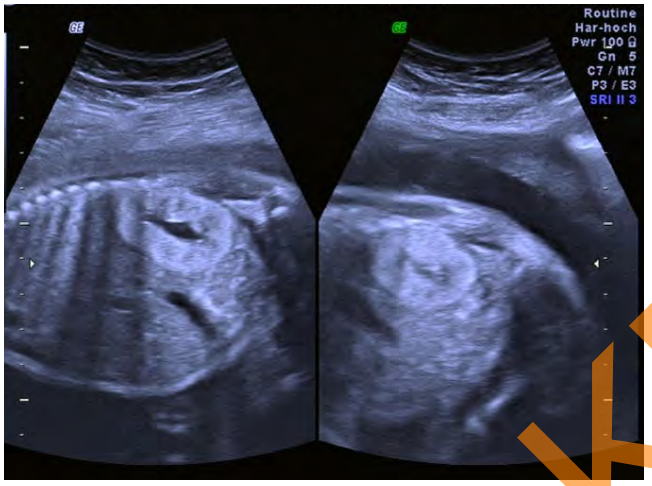
Although fertile couples have to use assisted reproductive technology, PGD is a psychologically and ethically preferable option for many patients.



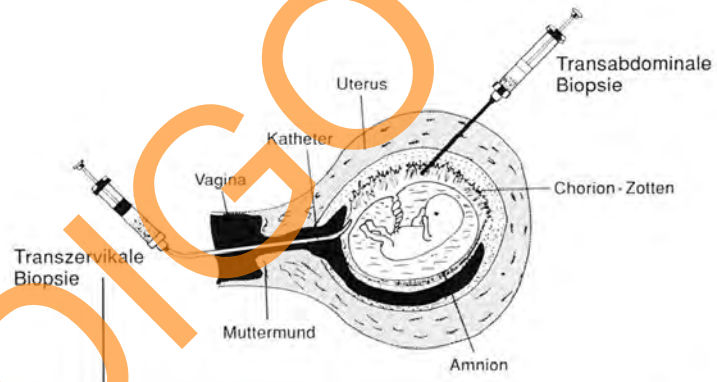
http://www.ivf.net.in/ivf&icsi/

Other options for prenatal diagnosis

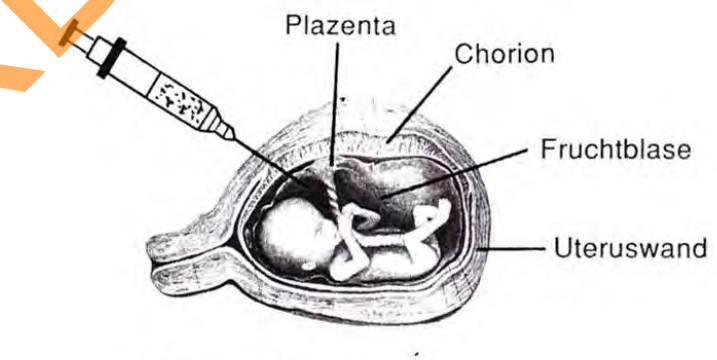
Non-invasive and invasive techniques



Prenatal ultrasound
Often only late detection of findings, rather unreliable



CVS
(11-12th gw)

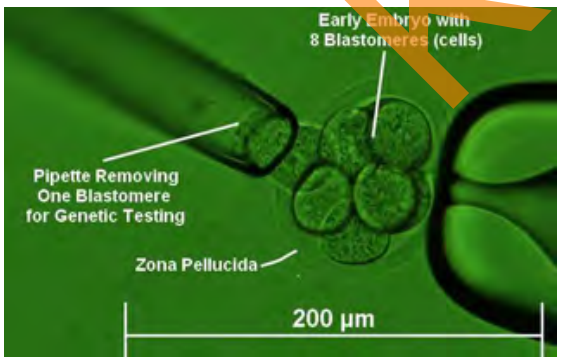


AC
(15-17th gw)

Small risk for miscarriage

What is the current status of PGD in developed countries?

- First PGD performed in 1990 (Handyside et al., *Nature* 1990)
- Multiple approaches validated with improved sensitivity and accuracy of PGD
- Indications for PGD have expanded from childhood onset recessive genetic disorders to include adult-onset disorders, cancer predisposition alleles, and blood and HLA typing etc.
- Laws and regulations on PGD differ significantly



http://medicine.missouri.edu/fertility/

http://www.cpmc.org

PGD discussion German Parliament

Foto: Deutscher Bundestag/Lichtblick/Achim Melde

Gesetz
zur Regelung der Präimplantationsdiagnostik
(Präimplantationsdiagnostikgesetz – PräimpG)

Vom 21. November 2011

Der B … d tech-
sen:

Das … er Prä-
1990 (BG … Maßnah-
zes vom … ssionen
worden … assenen
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… esregie-
… Zustim-

1. Nach … für die
… implan-
… tationsdiagnostik durchgeführt werden darf, einschließlich der Qualifikation der dort tätigen Ärzte und der Dauer der Zulassung,

2. zur Einrichtung, Zusammensetzung, Verfahrensweise und Finanzierung der Ethikkommissionen für Präimplantationsdiagnostik,

3. zur Einrichtung und Ausgestaltung der Zentralstelle, der die Dokumentation von im Rahmen der Präimplantationsdiagnostik durchgeführten Maßnahmen obliegt,

§ 3a
Präimplantationsdiagnostik; Verordnungsermächtigung

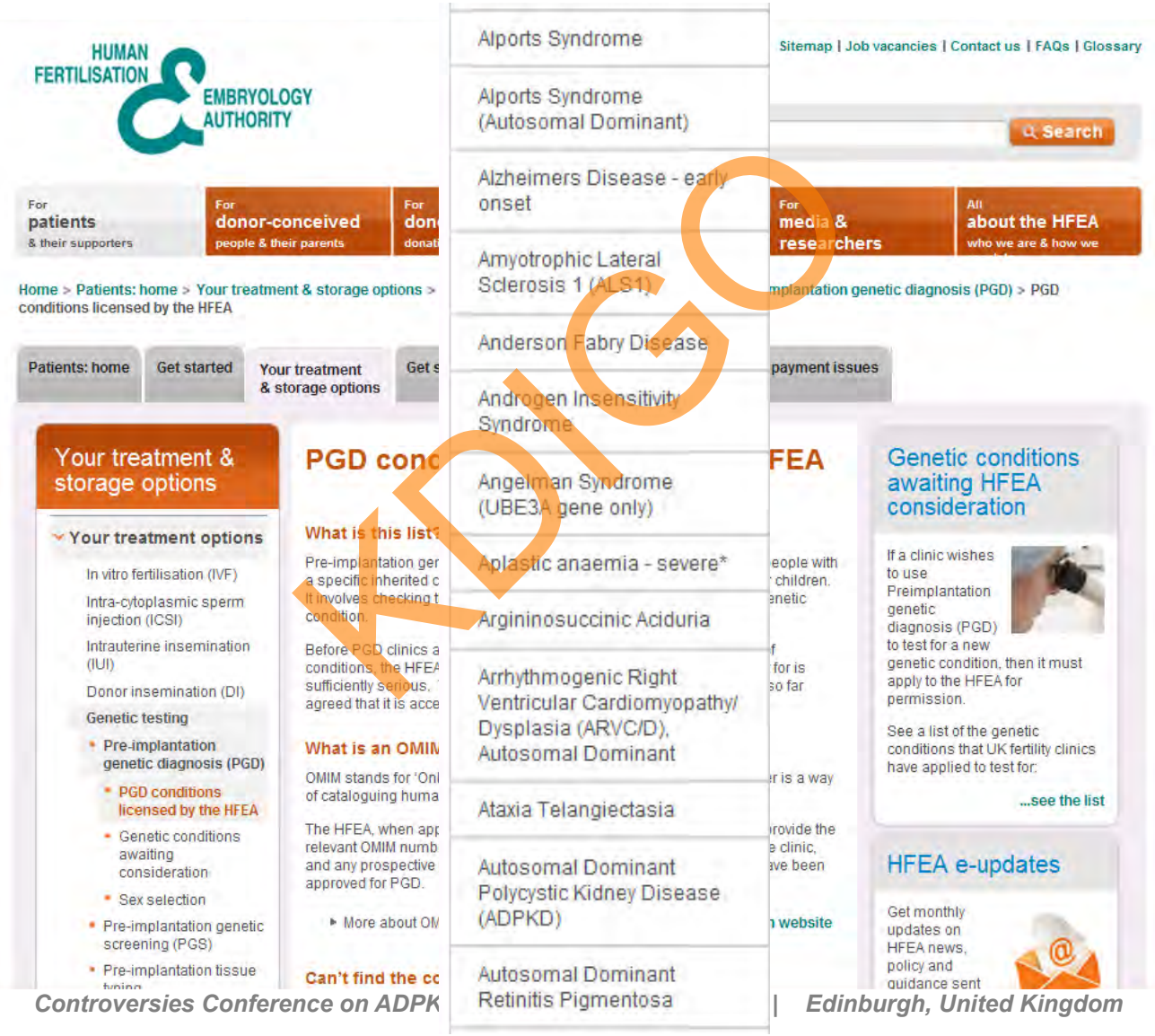
(1) Wer Zellen eines Embryos in vitro vor seinem intrauterinen Transfer genetisch untersucht (Präimplantationsdiagnostik), wird mit Freiheitsstrafe bis zu einem Jahr oder mit Geldstrafe bestraft.

(2) Besteht auf Grund der genetischen Disposition der Frau, von der die Eizelle stammt, oder des Mannes, von dem die Samenzelle stammt, oder von beiden für deren Nachkommen das hohe Risiko

Germany (from February 2014 on)

- Only specific centers will be authorized (only PGD on pluripotent cells allowed)
- Ethical commissions will decide on each individual case (not only considering the disease, but also the individual situation of the couple)

Current status of PGD in the UK



Use of preimplantation genetic diagnosis for serious adult onset conditions: a committee opinion

Ethics Committee of the American Society for Reproductive Medicine
American Society for Reproductive Medicine, Birmingham, Alabama

Amato et al.,
Fertil Steril 2013

What is considered a "serious" condition?

Definition is problematic and subjective

- Arguments pro PGD:

  - Right to reproductive choice
  - Earlier than other techniques for prenatal diagnosis
  - Preventing transmission of genetic disorders
  - Potential social benefits of reducing overall burden of disease (growing lifetime cost for chronic medical conditions)

Use of preimplantation genetic diagnosis for serious adult onset conditions: a committee opinion

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Amato et al.,
Fertil Steril 2013

- Arguments against PGD:

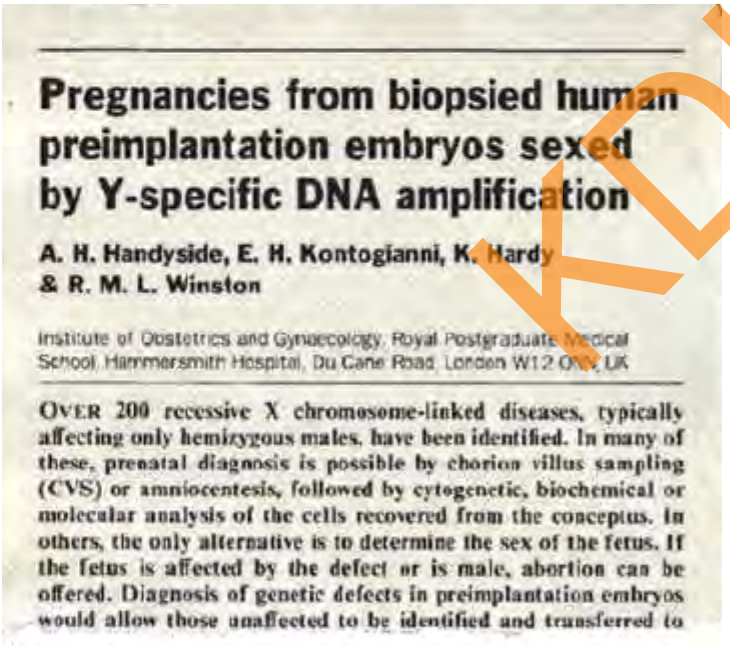
  - Expense
  - IVF: No certainty of live birth, increased risk of multiple pregnancy, ovulation hyperstimulation syndrome (1%)
  - Concern of misdiagnosis
  - Beginning of a slide down a “slippery slope“ toward unacceptable uses of genetic technology (“designer baby“ concern)
  - Affected individuals may live healthy lives for several decades before disease may become an active concern
  - Embryo selection risks devaluing certain lives

nature Vol 464|15 April 2010

OPINION

Let parents decide

Twenty years on from the first pregnancies after preimplantation genetic diagnosis, **Alan Handyside** argues that informed prospective parents are largely good guides to the use of the thriving technology.



Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification

A. H. Handyside, E. H. Kontogianni, K. Hardy & R. M. L. Winston

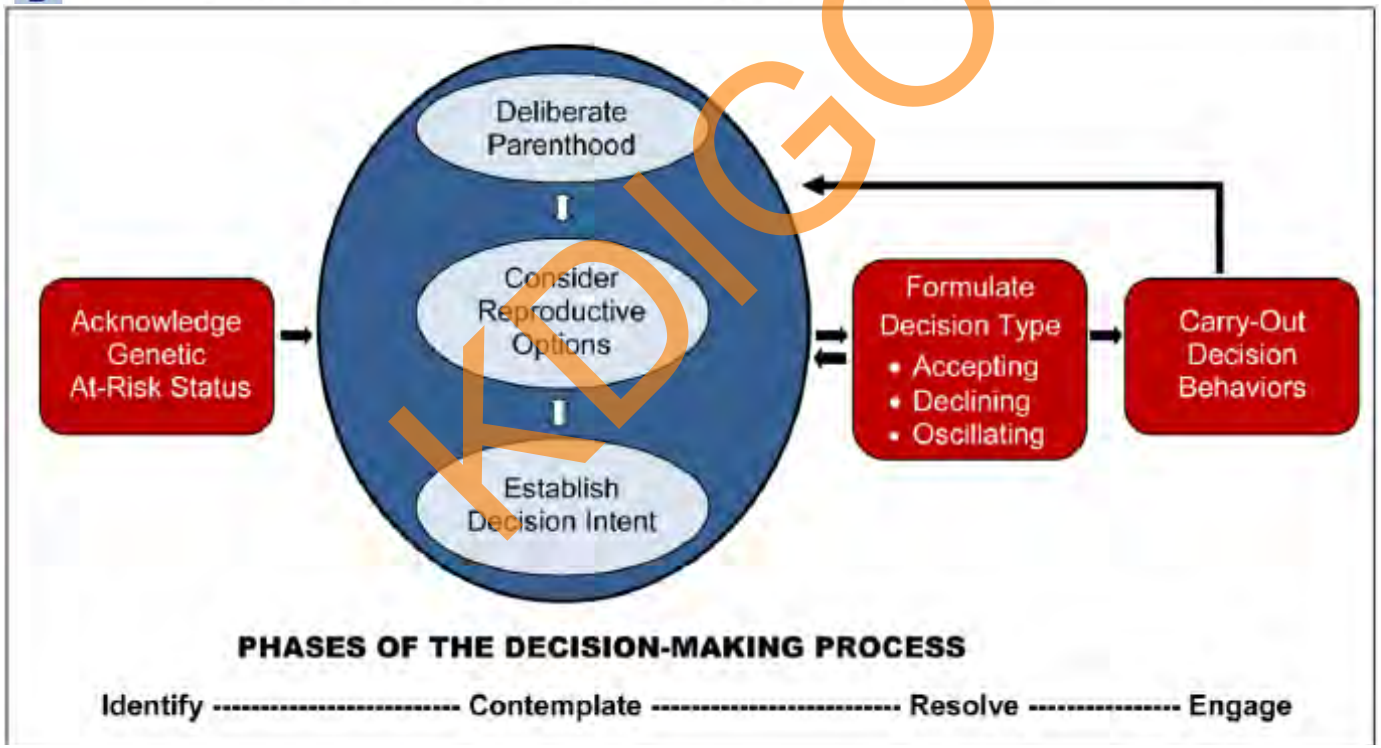
Institute of Obstetrics and Gynaecology, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK

OVER 200 recessive X chromosome-linked diseases, typically affecting only hemizygous males, have been identified. In many of these, prenatal diagnosis is possible by chorion villus sampling (CVS) or amniocentesis, followed by cytogenetic, biochemical or molecular analysis of the cells recovered from the conceptus. In others, the only alternative is to determine the sex of the fetus. If the fetus is affected by the defect or is male, abortion can be offered. Diagnosis of genetic defects in preimplantation embryos would allow those unaffected to be identified and transferred to

Happy family: British parents Shanana (left) and Raj Hashmi were given permission in 2003 to select an embryo with tissue that matched that of their son Zain, terminally ill with β thalassaemia.

Published in final edited form as:
Soc Sci Med. 2012 May ; 74(10): 1536–1543. doi:10.1016/j.socscimed.2012.02.003.

The Decision-Making Process of Genetically At-Risk Couples Considering Preimplantation Genetic Diagnosis: Initial Findings from a Grounded Theory Study



Impact of costs associated with PGD

PGD is not covered financially under the majority of private and public health insurance institutions in many countries

- Costs IVF per cycle: 9,000-12,500 US$
- Costs PGD per cycle: 2,500-6,000 US$

A Qualitative Inquiry of the Financial Concerns of Couples Opting to Use Preimplantation Genetic Diagnosis to Prevent the Transmission of Known Genetic Disorders

Kathryn T. Drazba · Michele A. Kelley · Patricia E. Hershberger

J Genet Counsel 2013

"…opportunity to avoid passing on a genetic disorder was paramount to the cost of PGD, but costs major barrier…"

Technical challenges of preimplantation genetic diagnosis (PGD)

- Very small DNA content of single blastomere cell (6 pg)
- Establishment of multiplex RT-PCR is cumbersome and requires detailed optimization
- Sample contamination (→ strict quality control)
- Allele drop-out (one of the two alleles for given gene fails to amplify), i. e. false negative result for autosomal dominant disorders may lead to transfer of an affected embryo

  ⇒ Therefore, highly recommended to perform additional linkage analysis and type several closely linked polymorphisms/markers (in one multiplex PCR)

What are the challenges of PGD in ADPKD?

Preimplantation Genetic Diagnosis in Genomic Regions with Duplications and Pseudogenes: Long-Range PCR in the Single-Cell Assay

HGVS
HUMAN GENOME VARIATION SOCIETY
www.hgvs.org

PKD1 gene-specific long-range PCR (LR-PCR) must be coupled for linkage analysis with short-range genetic markers on single cells

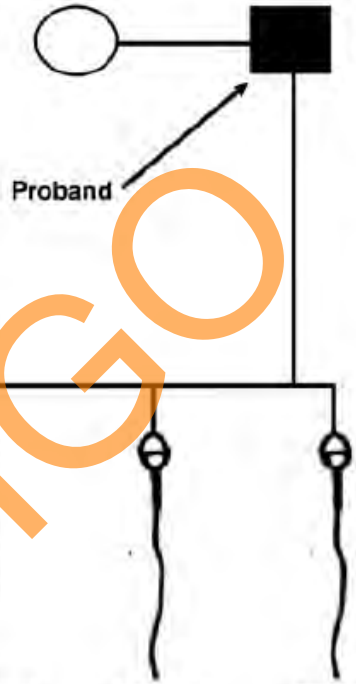
Zeevi et al.,
Hum Mutat 2013

PKD1 gene homologous to six different pseudogene copies

Further challenges of PGD in ADPKD: *de novo* mutations and mosaicism

Linkage analysis with family members not possible in this patient with ***de novo* mutation** (i.e., no other affected family member available)

→ Affected haplotype/allele in the patient must be identified in single gametes (single sperm)



Marker	Position Chr. 16 (hg19)	Allele A size (bp)	Allele B size (bp)
PKD1-AC21	876259	308	296
PKD1-TG1	950626	187	185
D16S525	1031160	272	260
PKD1-AC1	1926725	248	242
PKD1 c.701delC	2168060	C	*delC*
D16S291	2339890	161	153
D16S3124	2447585	168	175
D16S633	2457102	118	120
D16S3082	3117181	193	212
D16S2618	3252609	126	128
D16S3027	4050872	281	271

Allele A size (bp)	Allele B size (bp)	Allele B size (bp)
308	296	296
187	185	185
272	260	260
248	242	242
C	C	*delC*
161	153	153
168	175	175
118	120	120
193	212	212
126	128	128
281	271	271
(*n* = 11)	(*n* = 2)	(*n* = 3)

Germline mosaicism

Should PGD be available/used for ADPKD?

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Amato et al.,
Fertil Steril 2013

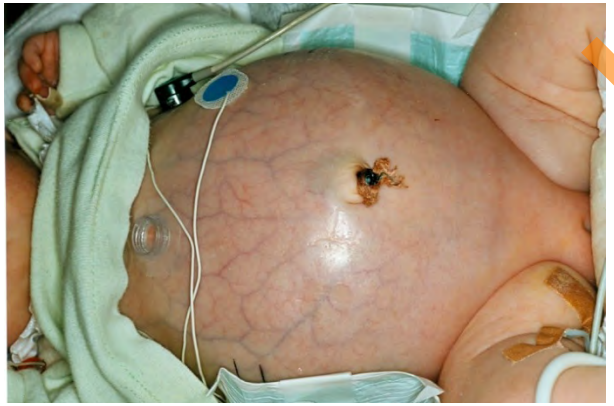
Definition "serious condition"

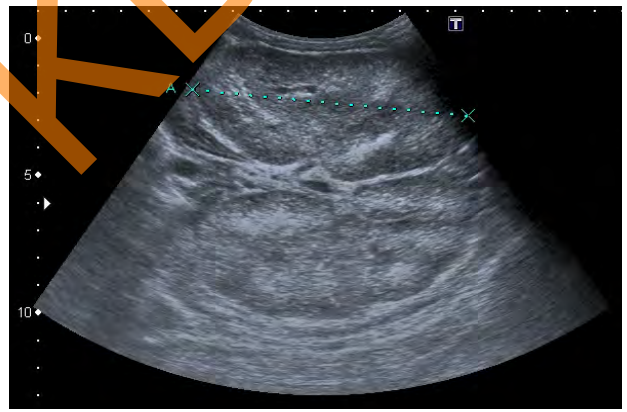
How variable can the disease be expressed?

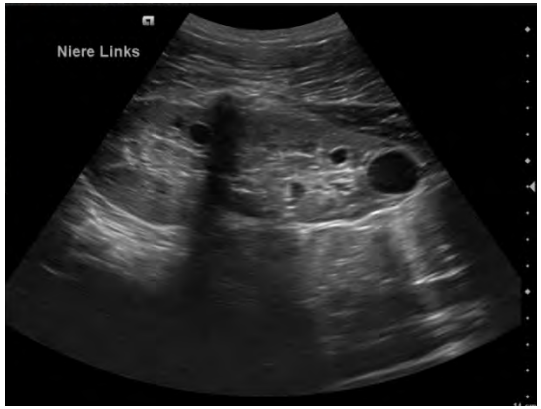
What is the severe end of the clinical spectrum?

Childhood ADPKD

- Small proportion (2%) of ADPKD patients presents with clinical symptoms < 15 years of age
- Among these are babies with significant peri-/neonatal morbidity and mortality (mimicking severe ARPKD)
- In paediatric nephrology, total number of patients with early-onset ADPKD equals number of ARPKD patients



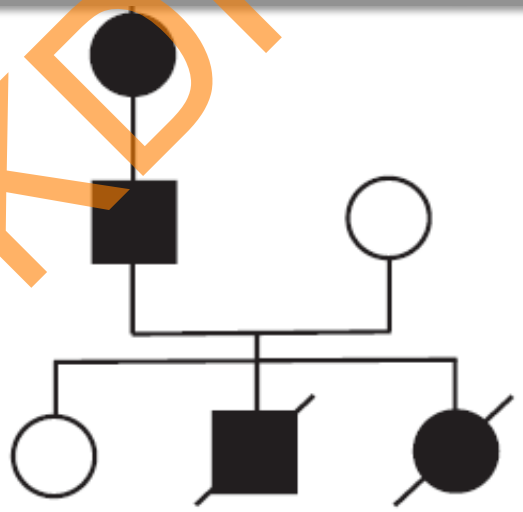




Childhood ADPKD

- ADPKD families with early-manifesting offspring: High recurrence risk for further children with similar phenotype
  ⇒ Common familial modifying background

Delineation of early & severe forms essential for understanding of ADPKD



Diagnostic evaluation of childhood ADPKD

- Simple cysts are extremely rare in childhood
- In children with positive family history of ADPKD, finding of one cyst can thus be considered diagnostic
  ⇒ Usually, in these cases currently no need for genetics

- Professional organizations (e.g., American Academy of Pediatrics, German Society of Human Genetics) recommend that for adult-onset conditions for which interventions are unavailable genetic testing is inappropriate in <u>healthy</u> (asymptomatic) children until children reach adulthood.

What are the current indications for genetic testing in childhood ADPKD?

- Child with PKD, positive family history and practically no doubt about ADPKD:

  As long as no treatment available or other benefit from early knowledge of genotype, usually no indication for genetic testing

- Child with PKD and negative family history:

  More complex and sophisticated to decide if and which genetic testing may make sense

Childhood ADPKD

- Reasons for negative family history of ADPKD:
  - Spontaneous *de novo* mutation in *PKD1* or *PKD2*
  - Incompletely penetrant (hypomorphic) mutation

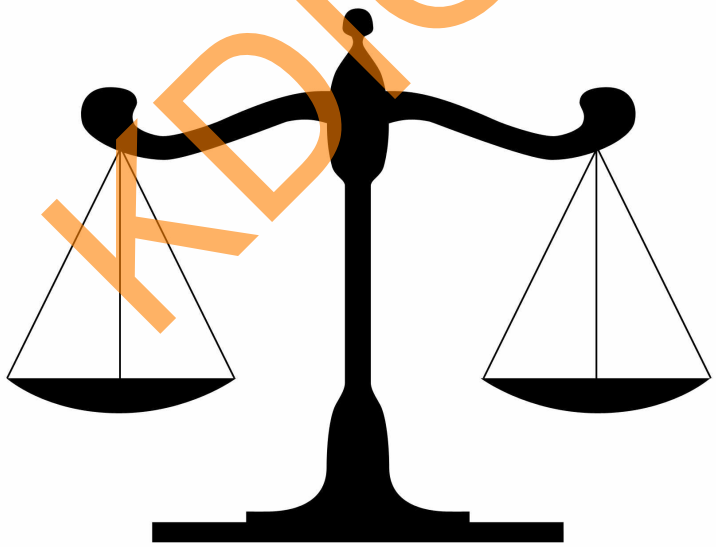
Incompletely penetrant *PKD1* alleles suggest a role for gene dosage in cyst initiation in polycystic kidney disease

Sandro Rossetti[1], Vickie J. Kubly[1], Mark B. Consugar[1], Katharina Hopp[2], Sushmita Roy[3], Sharon W. Horsley[4], Dominique Chauveau[5], Lesley Rees[3], T. Martin Barratt[3], William G. van't Hoff[3], W. Patrick Niaudet[6], Vicente E. Torres[1] and Peter C. Harris[1,2]

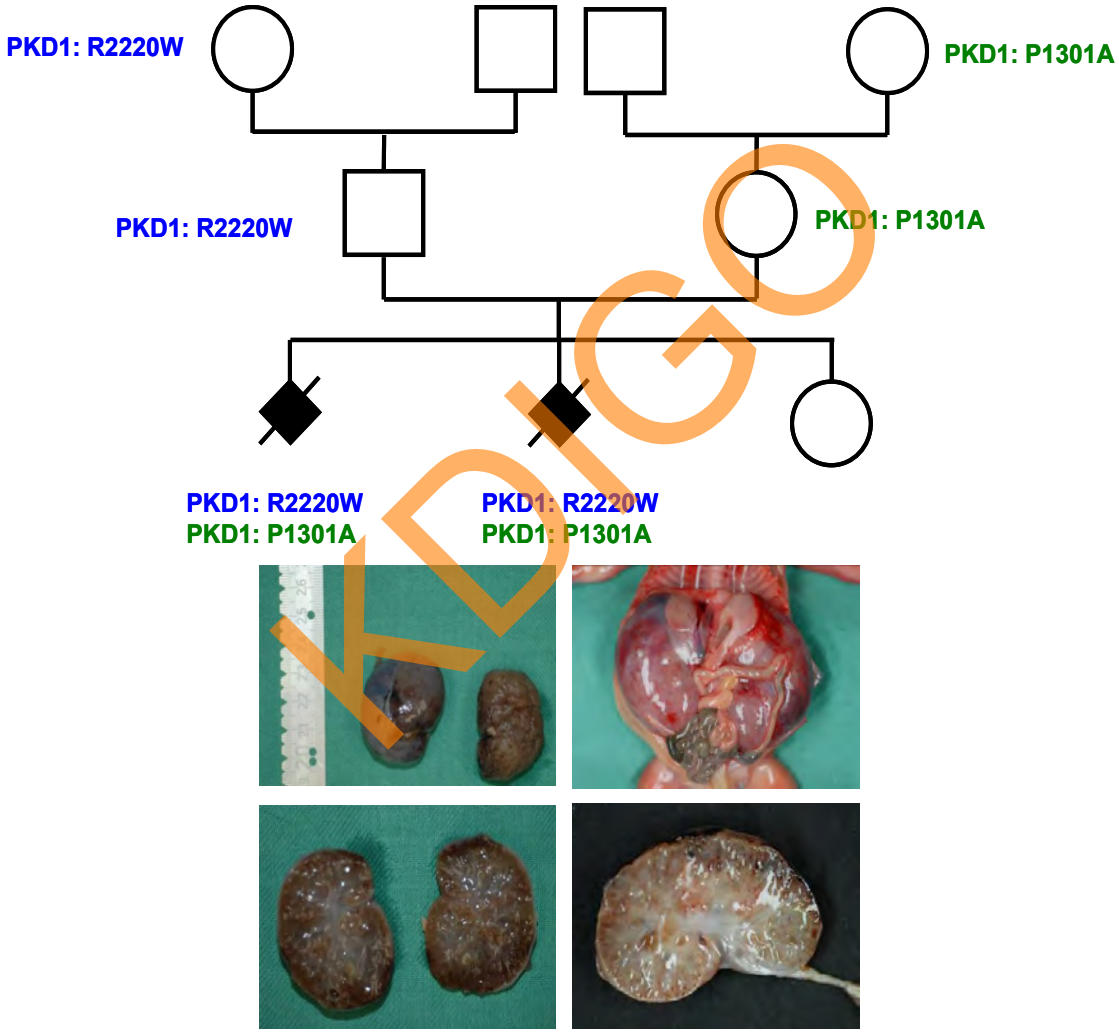
Rossetti et al.,
Kidney Int 2009

Dosage-sensitive network and second-site modifiers in polycystic kidney disease

Some severely affected patients bear second-site modifiers (mutations in more than one allele and/or gene)



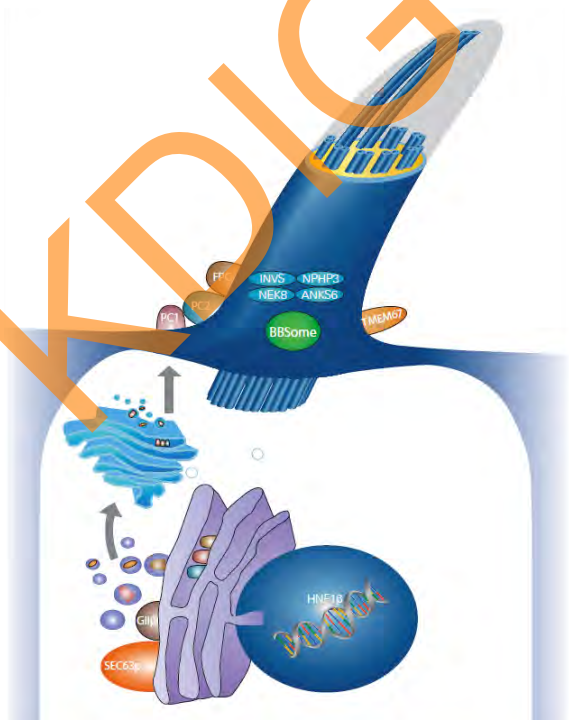
ADPKD genes can also be inherited in a recessive way



own unpublished data

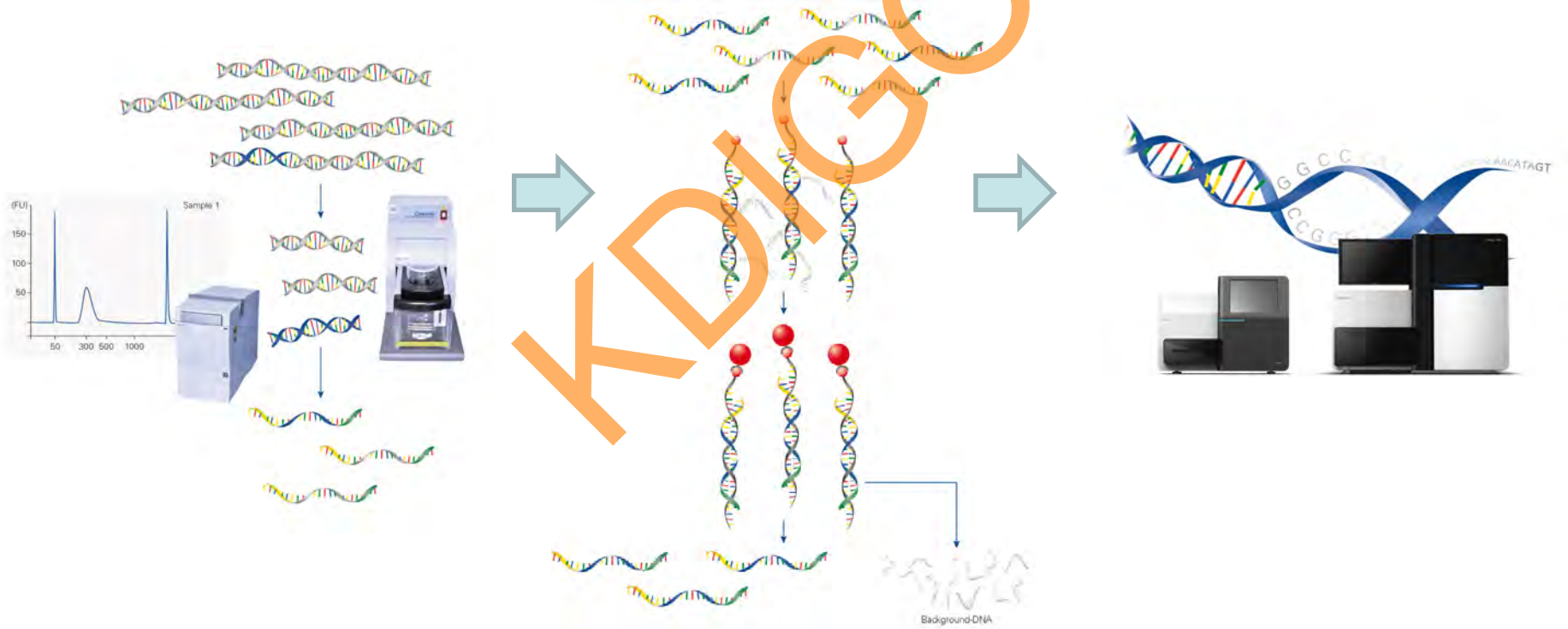
Polycystic kidney disease becomes increasingly complex

Mutations in other genes can mimic polycystic kidney disease ("Phenocopies")



Targeted Next-Generation Sequencing (NGS)

Efficient genetic diagnostics (detection rates ↑)
by parallel analysis of all genes that may have to be discussed
(allows better interpretation of changes and avoids pitfalls)

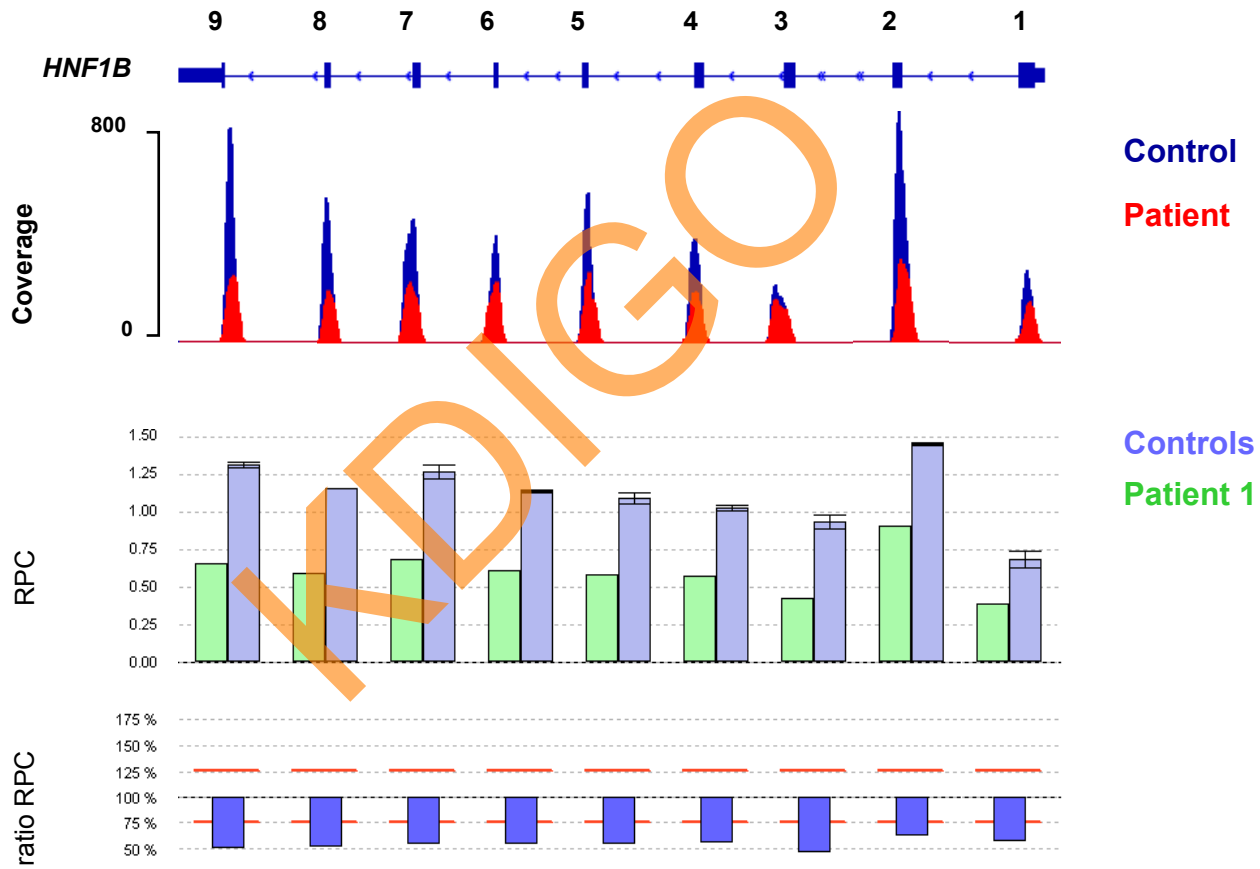


NGS workflow of targeted sequence capture approach

Detection of copy number variations (CNVs) by targeted NGS approach

Absolute coverage

Relative coverage



What is the role of exome sequencing for diagnosis of childhood PKD?

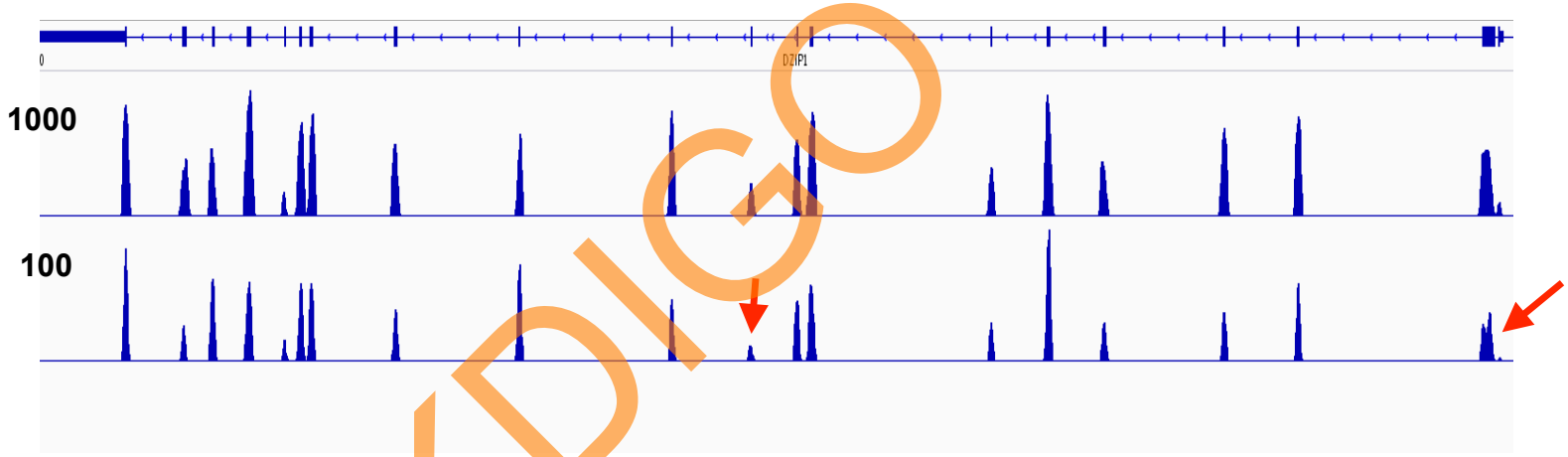
- For routine diagnostics, whole-exome sequencing (WES) is currently not the method of choice
- For some research samples of interest
- Disadvantages/challenges of exome sequencing:
  - CNVs (deletions/duplications) less likely to detect
  - WES kits “off-the-shelf“ products (rebalancing not possible)

Comparison exome sequencing versus "customized" NGS panel (targeted approach)

Customized Panel V5

Absolute coverage

NimbleGen Exome V2



Customized NGS-Panel: 100% coverage > 20x

Exome sequencing: ~ 90% coverage >20x

What is the role of exome sequencing for diagnosis of childhood PKD?

- For routine diagnostics, whole-exome sequencing (WES) is currently not the method of choice
- For some research samples of interest
- Disadvantages/challenges of exome sequencing:
  - CNVs (deletions/duplications) less likely to detect
  - WES kits "off-the-shelf" products (rebalancing not possible)
  - *PKD1* is not sufficiently captured by current WES designs
  - Interpretation of data/changes is challenging
  - Detailed informed consent of patients/parents prior WES crucial
- For diagnostics, specific "customized" NGS panels (targeted approach) currently better than exome sequencing

Future prospects

Paradigm shift towards preventive, more individualized medicine

⇒ Ultimate goal: Risk score

"Nobody uses crystal balls anymore!"

BIOSCIENTIA
HUMAN GENETICS

Center for
Human Genetics
Ingelheim

Thank you very much
for your attention

ALBERT-LUDWIGS-
UNIVERSITÄT FREIBURG

Deutsche
Forschungsgemeinschaft
DFG

Deutsche
Nierenstiftung

PKD
FOUNDATION
For Research in Polycystic
Kidney Disease

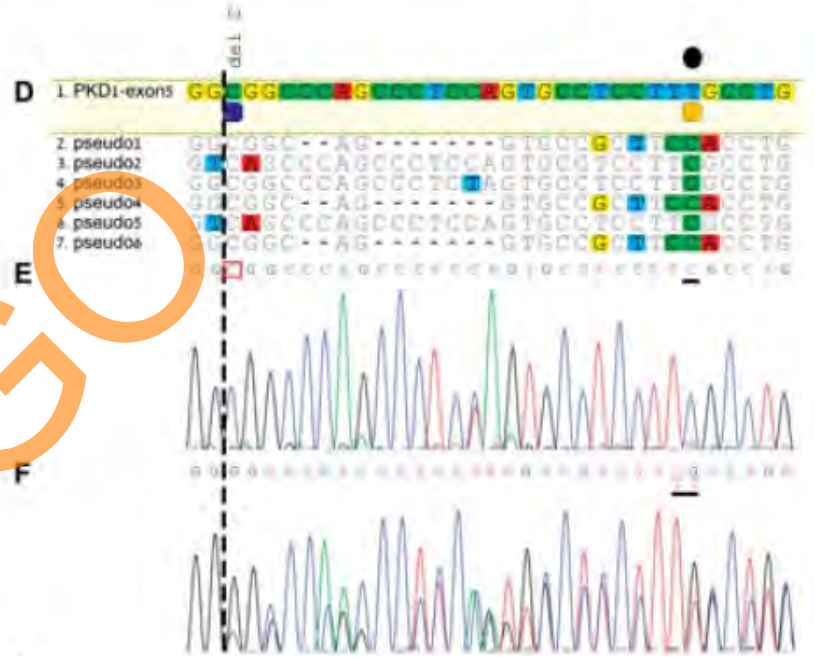
carsten.bergmann@bioscientia.de
carsten.bergmann@uniklinik-freiburg.de

Challenges of PGD in ADPKD

Sequence alignment of mutation locus and its pseudogene copies

Without prior *PKD1*-gene specific LR-PCR: Pseudogenic contamination

Prior *PKD1*-gene specific LR-PCR eliminates pseudogenic contamination



Preimplantation genetic diagnosis for ADPKD

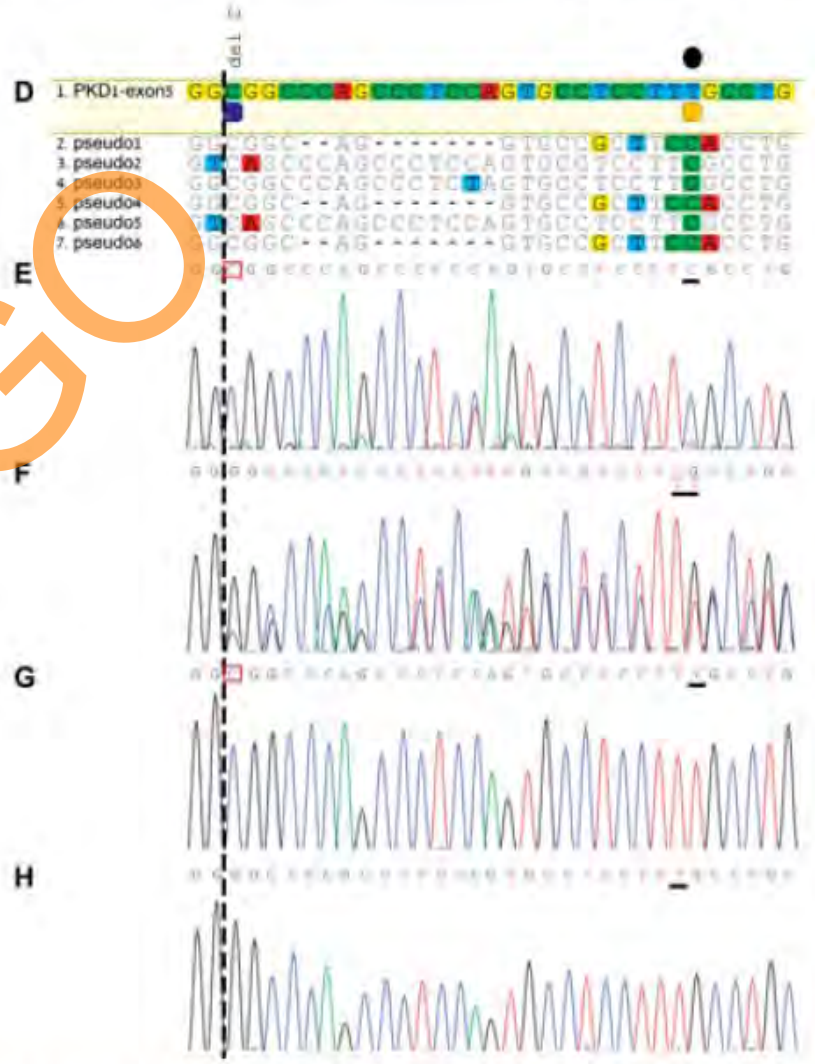
Sequence alignment of mutation locus and its pseudogene copies

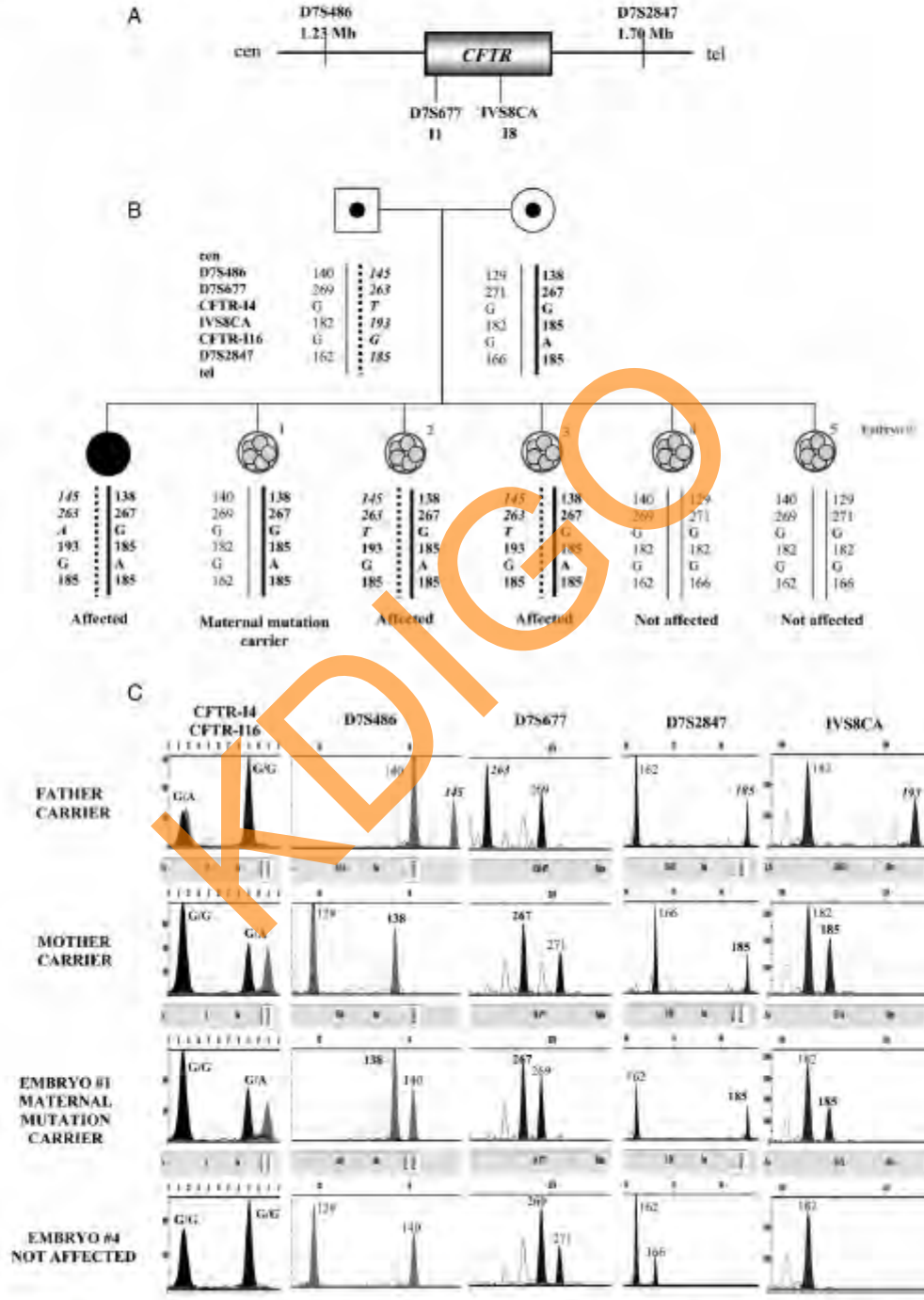
Without prior *PKD1*-gene specific LR-PCR: Pseudogenic contamination

Prior *PKD1*-gene specific LR-PCR eliminates pseudogenic contamination

Sequencing of a single sperm of patient after gene-specific LR-PCR: WT

Sequencing of a single sperm of patient after gene-specific LR-PCR: Mutation

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IVS8CA
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D7S2847
tel
Affected
Maternal mutation carrier
Affected
Affected
Not affected
Not affected
C
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CFTR-I16
D7S486
D7S677
D7S2847
IVS8CA
FATHER CARRIER
MOTHER CARRIER
EMBRYO #1 MATERNAL MUTATION CARRIER
EMBRYO #4 NOT AFFECTED