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”

Who we are is predetermined and beyond our control

http://shakespeare.mit.edu/
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/
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
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
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)

What is considered a “serious“ condition?
Definition is problematic and subjective
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
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.
The Decision-Making Process of Genetically At-Risk Couples Considering Preimplantation Genetic Diagnosis: Initial Findings from a Grounded Theory Study

PHASES OF THE DECISION-MAKING PROCESS

Identify  Contemplate  Resolve  Engage
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$

"...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

*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
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)

### Germline mosaicism

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Should PGD be available/used for ADPKD?

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

KDIGO
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
Simple cysts are extremely rare in childhood.

In children with a 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 healthy (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 (hypomorphomic) mutation

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

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.
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**

**HNF1B**

**Coverage**

**RPC**

**Ratio RPC**
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 NGS-Panel: 100% coverage > 20x
Exome sequencing: ~ 90% coverage >20x
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- 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!"
Thank you very much for your attention

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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