Next generation sequencing –

What can I tell the patient?

Helger Yntema, PhD
Department of Human Genetics
Radboud University Nijmegen Medical Centre
Email: h.yntema@gen.umcn.nl
Ideal genetic test

A single analysis of all genes involved in a disease

Next-generation sequencing (NGS)

Use of NGS:

• targeted sequencing
• exome sequencing
• whole genome sequencing

Increasing risk on incidental/unsolicited findings
Outcome – what can I tell the patient?

- Cause of the genetic disease
- Possible cause of genetic disease (family studies needed)
- No significant findings (for now)

➢ Report clinically relevant findings
➢ Report variants of unknown significance
➢ Not much different from standard genetic testing
Outcome - Should I tell the patient?

- Genetic information on other diseases

  unsought for findings
  accidental findings
  (co-)incidental findings
  unsolicited findings
Unsolicited findings are not new

- Imaging techniques reveal cancer
- Linkage analysis reveals non-paternity
- Genome wide CNV detection reveals deletion with cancer gene

- Genomide sequencing increases the amount of unsolicited findings
- Targeted sequencing and/or analysis decreases the risk
Unsolicited findings

Positive
- genetic predisposition to disease that can be treated/prevented
- carrier of recessive condition; reproductive choices

Negative
- untreatedable conditions
- problems with health insurance/employers/mortgage
Right not to know

Patient might want to know some but not all incidental findings

My desire to be well-informed is currently at odds with my desire to remain sane.
Whole genome sequencing

Protection
- risks of genome wide testing

Improvement
- diagnostic possibilities

➢ Genome wide testing involves consent for unsolicited findings
Exome sequencing procedure Nijmegen

1. DNA-sample
2. Exome sequencing
3. Filter known genes
4. Confirmation diagnosis?
   - yes: Report
   - no: Exome analysis
5. Candidate gene
6. Report

Informed consent

Human Genetics Nijmegen
All individuals must agree with the entire procedure.

All individuals must understand the possibility of unsolicited findings and agree to be informed when significant impact on health.
Who decides what is clinically relevant for the patient?
In a 5-year-old boy the test for genetic causes of his ataxia shows that he has a mutation causing Long QT syndrome, a cardiac disease in which ventricular arrhythmia may result in recurrent syncopes, seizure, or sudden death. The ventricular arrhythmia could be prevented by medication or implantation of a defibrillator.

Do you tell the parents?

Adopted from Couzin-Frankel (2011) Science 331:662
A woman with retinitis pigmentosa is tested to find the genetic cause of her blindness. The test shows that she carries a mutation in BRCA1. The mutation raises the risk of breast and ovarian cancer and can be passed to any children she may have.

Would you find her and tell her?
A young woman with deafness receives a positive report from exome sequencing. The lab also found that she carries APOE4, which raises her risk of Alzheimer’s. The disease remains unpreventable, though some measures may delay it.

Would you tell her?

Adopted from Couzin-Frankel (2011) Science 331:662
All individuals must agree with the entire procedure.

Unsolicited findings will be assessed by independent expert committee.

All individuals must understand the possibility of unsolicited findings and agree to be informed when significant impact on health.
Independent expert committee

• Clinical molecular geneticist (NOT the one detecting the mutation)

• Clinical geneticist (NOT referring clinician)

• Molecular geneticist (research)

• Social Worker

• Law representative

• Ethics representative

• (Medical doctor with knowledge of the disease involved)

➢ Decision on whether or not the referring clinician will be informed
Important role for informed consent!

Remains difficult, even with consent for reporting of unsolicited findings.
What should be reported to the patient?

- report clinically relevant findings
- avoid reporting of variants of unknown significance

**COMMENTARY**

Deploying whole genome sequencing in clinical practice and public health: Meeting the challenge one bin at a time

Jonathan S. Berg, MD, PhD¹, Muin J. Khoury, MD, PhD², and James P. Evans, MD, PhD¹

➢ Sort variants in predetermined “bins” and report only those likely to be deleterious
<table>
<thead>
<tr>
<th>Criteria:</th>
<th>Clinical Utility</th>
<th>Clinical Validity</th>
<th>Unknown Clinical Implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bins:</td>
<td>Bin 1&lt;br&gt;Medically actionable incidental information</td>
<td>Bin 2A&lt;br&gt;Low risk incidental information</td>
<td>Bin 3&lt;br&gt;All other loci</td>
</tr>
<tr>
<td>Examples:</td>
<td>BRCA1/2&lt;br&gt;MLH1, MSH2&lt;br&gt;FBN1&lt;br&gt;NF1</td>
<td>PGx variants and common risk SNPs</td>
<td>Bin 2B&lt;br&gt;Medium risk incidental information</td>
</tr>
<tr>
<td>Estimated number of genes/loci:</td>
<td>10s</td>
<td>10s (eventually 100s – 1000s)</td>
<td>1000s</td>
</tr>
<tr>
<td></td>
<td>~20,000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Alleles that would be reportable (YES) or not reportable (NO) in a clinical context

<table>
<thead>
<tr>
<th>Variants</th>
<th>Known deleterious</th>
<th>Presumed deleterious</th>
<th>VUS</th>
<th>Presumed benign</th>
<th>Known benign</th>
</tr>
</thead>
<tbody>
<tr>
<td>Known deleterious</td>
<td>YES</td>
<td>YES/NO</td>
<td>YES/NO</td>
<td>YES/NO</td>
<td>N/A</td>
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<tr>
<td>Presumed deleterious</td>
<td>YES</td>
<td>N/A</td>
<td>YES/NO</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>VUS</td>
<td>NO</td>
<td>N/A</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Presumed benign</td>
<td>NO</td>
<td>N/A</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Known benign</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
</tr>
</tbody>
</table>

N/A: not applicable; VUS: Variant of uncertain significance

1 Reporting through decision making with an appropriate provider if elected by the patient.
2 By definition, variants in genes with unknown implications could not be considered deleterious.
3 By definition, SNPs or PGx variants will either be present or absent.
4 Variants in genes with unknown clinical implications would not be reported; however, they may serve as an important substrate for research, potentially uncovering new disease genes.

Fig. 1. Proposed system for “binning” of incidental WGS results
Unsolicited findings in diagnostic exome sequencing

• ~500 cases (gene package; interpretation not complete) + 100 ID trio’s

• No risk for unsolicited findings in gene package?

  ATM is in onco-package and movement disorders

  Carriers for recessive diseases not reported (carrier frequency < 1%)

• Only one unsolicited finding
Case

- No *de novo* changes explaining the ID
- *de novo* missense change in *RB1*
- Classification: *UV!*
- Presumed deleterious when detected in affected child (A. vd Hout, pers comm)
Considerations

• RB1 variant not identified previously (literature and personal communication)
• Variant of unknown significance

Outcome

Advice to inform the parents
• Risk for RB is very small considering the age of the patient
• Small risk for osteosarcoma at adolescent age
• Quick recognition of prognostic value (actionable)
Only a yes/no option for disclosure of unsolicited findings might be too easy..........
Nijmegen now

DNA-sample

Exome sequencing

filter known genes

Confirmation diagnosis?

yes

no

Report

Informed consent


Wish national ethics committees

NO unsolicited findings
Choices (Bredenoord et al.)

1. **Standard default package**
   Life-saving data and data of immediate clinical utility that entail a significant health problem

2. **Extra package 1**
   Data of potential or moderate clinical utility

3. **Extra package 2**
   Data of reproductive significance (including information for woman at risk of being in early menopause)

4. **Extra package 3**
   Data of personal or recreational significance

- Opt out for default package / Opt in voor extra packages
- Take into account the possibilities of the laboratory/health care system
The ACMG recommends that for any evaluation of clinical sequencing results, all of the genes and types of variants in the Table should be examined and the results reported to the ordering clinician. The conditions listed in the Table are those that the Working Group and external
<table>
<thead>
<tr>
<th>Phenotype</th>
<th>MIM - Disorder</th>
<th>PMID - GeneReviews Entry</th>
<th>Age of Onset</th>
<th>Gene</th>
<th>MIM - Gene</th>
<th>Inheritance*</th>
<th>Variants to Report*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hereditary Breast and Ovarian Cancer</td>
<td>604370, 612555</td>
<td>20301425</td>
<td>Adult</td>
<td>BRCA1</td>
<td>113705</td>
<td>AD</td>
<td>KP &amp; EP</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BRCA2</td>
<td>600185</td>
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<td>Li-Fraumeni Syndrome</td>
<td>151623</td>
<td>20301488</td>
<td>Child/adult</td>
<td>TP53</td>
<td>191170</td>
<td>AD</td>
<td>KP &amp; EP</td>
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<tr>
<td>Peutz-Jeghers Syndrome</td>
<td>175200</td>
<td>20301443</td>
<td>Child/adult</td>
<td>STK11</td>
<td>602216</td>
<td>AD</td>
<td>KP &amp; EP</td>
</tr>
<tr>
<td>Lynch Syndrome</td>
<td>120435</td>
<td>20301390</td>
<td>Adult</td>
<td>MLH1</td>
<td>120436</td>
<td>AD</td>
<td>KP &amp; EP</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MSH2</td>
<td>609309</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MSH6</td>
<td>600678</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PMS2</td>
<td>600259</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Familial adenomatous polyposis</td>
<td>175100</td>
<td>20301519</td>
<td>Child</td>
<td>APC</td>
<td>611731</td>
<td>AD</td>
<td>KP &amp; EP</td>
</tr>
<tr>
<td>MYH-Associated Polyposis; Adenomas, multiple colorectal, FAP type 2; Colorectal adenomatous polyposis, autosomal recessive, with pilomatrixomas</td>
<td>608456, 132600</td>
<td>23035301</td>
<td>Adult</td>
<td>MUTYH</td>
<td>604933</td>
<td>AR**</td>
<td>KP &amp; EP</td>
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<tr>
<td>Von Hippel Lindau syndrome</td>
<td>193300</td>
<td>20301636</td>
<td>Child/adult</td>
<td>VHL</td>
<td>608537</td>
<td>AD</td>
<td>KP &amp; EP</td>
</tr>
</tbody>
</table>
Future directions

- Best practices for informed consent, return of results, integration with medical record

- Major discussion point: report of incidental findings in children

- Learn from patient experiences

- Learn from laboratory experiences
Acknowledgements

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