Whole exome sequencing in a diagnostic setting; experiences of clinical geneticists and patients

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NGS course Genomic Resequencing 2013
Why whole exome sequencing?

‘All’ coding sequences of a human genome (>200,000 exons), sequenced and analyzed in one experiment

• Higher chance of finding the underlying genetic cause
• Compared to genome sequencing; less un-interpretive data
• Chance for incidental findings; reduced by a first step of filtering
Which diseases in 2012?

Total of 350 patients (550 exomes)

- Intellectual Disability
- Movement disorders
- Hereditary blindness
- Hereditary deafness
- Ox. Phos. Disorders
- Oncogenetics
Final workflow

DNA

Exome sequencing

Filter known genes

De novo analysis

Confirmation of diagnosis?

yes

Report

no

Exome analysis

(Candidate) gene

Unsolicited finding

Discussions in bi-weekly meeting

Committee

Report

Informed consent procedure

Counselling by clinical geneticist

Patient referral

Genome diagnostics

Clinicians

Scientists

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Pre-test counselling procedure (1)

• Written information leaflet
Pre-test counselling procedure (2)

- Face to face counselling by clinical geneticist
  - (Possible) cause of the disease
  - No significant findings
- VUS
- Unsolicited findings
Pre-test counselling procedure (3)

- Signing of the informed consent by the patient and/or the legal representative
All individuals must sign dedicated informed consent form.

All individuals must agree with the entire procedure.

All individuals must agree to be informed about unsolicited findings.

The unsolicited findings will be assessed by an independent committee of experts.
Reactions of patients or parents

Intellectual Disability

- Many parents interested
- Waiting list
- Desperate in order to find the cause of the ID in their child
Reactions of patients/parents

Gene package groups

“ We just really want to know the genetic cause and the recurrence risk. “

“Great, after this test you can tell me everything about me! “

“ Completely ridiculous this incidental committee. I just want to know every little thing you detect in his DNA. “
Reactions of patients/parents

Gene package groups

“I am way too scared for an incidental finding. I am just sure you will find a genetic form of cancer.”

“I thought it was for science purposes. I myself don’t have the urgent question of finding the genetic cause of my handicap.”

“We will wait a few more years; perhaps the procedure will change over time and we can have an opt out for incidental findings in our daughter.”
Pre-test counselling procedure (4)

- Blood sample & a complete application form to Genome Diagnostics laboratory
  - Detailed description of the phenotype
    - Congenital abnormalities
    - Age of onset
    - Additional features?
  - Inheritance pattern
Intellectual Disability (*de novo* strategy)

- Severe intellectual disability (IQ<50)
- No etiological or syndromic diagnosis
- Negative family history
- Normal karyotype and genomic profile (250K/2.7M arrays)

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### Potential diagnostic yield

<table>
<thead>
<tr>
<th>Diagnosis based on</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Known ID genes</td>
<td>10</td>
</tr>
<tr>
<td>Known X-linked genes</td>
<td>3</td>
</tr>
<tr>
<td>Novel ID genes</td>
<td>3</td>
</tr>
<tr>
<td>Candidate ID genes</td>
<td>19*</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>16-35%</strong></td>
</tr>
</tbody>
</table>

*Of note, based on experience with testing for mutations in candidate genes it may be expected that at least 11 to 13 of these 19 genes will be true ID genes.

J. de Ligt *et al*. NEJM 2012
Diagnostic exome sequencing in persons with severe intellectual dissability
Heterogeneous diseases; gene package filter

- Movement disorders ~150 genes
- Hereditary blindness ~150 genes
- Hereditary deafness ~100 genes
- OXPHOS disorders ~200 genes
- Oncogenetics ~100 genes
### Heterogeneous diseases; diagnostic yield

<table>
<thead>
<tr>
<th>Package</th>
<th>Blindness</th>
<th>Deafness</th>
<th>Movement</th>
<th>OXPHOS</th>
<th>Oncogenetics</th>
</tr>
</thead>
<tbody>
<tr>
<td># pts sequences</td>
<td>29</td>
<td>51</td>
<td>51</td>
<td>47</td>
<td>48</td>
</tr>
<tr>
<td>analyzed</td>
<td>25</td>
<td>33</td>
<td>38</td>
<td>43</td>
<td>35</td>
</tr>
<tr>
<td>Causal mutations*</td>
<td>11</td>
<td>5</td>
<td>7</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Diagnostic yield</td>
<td>44%</td>
<td>18%</td>
<td>20%</td>
<td>23%</td>
<td>2%</td>
</tr>
</tbody>
</table>

* Deleterious mutations or known pathogenic mutations

**Total diagnostic yield:** **20-25%**

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Hereditary blindness

- 78 adults
- 6 children (< 10 years)

- Majority of patients with non-syndromal vision loss
  - 8 patients AD-inheritance
  - 19 patients AR-inheritance
  - 54 patients sporadic
  - 3 X-linked, other
Hereditary blindness

- 9 / 84 patients; no exome sequencing (11%)
- 4 children <18-years
Hereditary blindness

84 patients with vision loss

75 patients consent

39 exomes analysed

9 patients no consent

36 exomes pending
Hereditary blindness

- 84 patients with vision loss
  - 75 patients consent
  - 39 exomes analysed
    - 20 patients pathogenic mutation(s) in known gene
    - 8 patients possible pathogenic mutation(s)
    - 11 patients no mutation
  - 9 patients no consent
    - 36 exomes pending

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Patient blindness group

• 66-year old female

• Retinitis pigmentosa since the age of 25 years
• Nightblindness
• Optic disc pallor
• Pigmentary retinopathy with typical bone spicule appearance

• asthma/bronchitis
• psoriasis
• reumatism
• no hearing loss
Patient blindness group

- no previous genetic test
Patient blindness group

Result WES

- One variant with unknown pathogenicity in IMPG2
- Predicted to be pathogenic
- IMPG2 mutations cause autosomal recessive RP (type56)

Questions
- Does the phenotype fit with RP type 56?
- Did we miss a second IMPG2 mutation with exome analysis?
Patient blindness group

Post-test counselling

- Consultation of ophthalmologist: eye fundus phenotype fits RP type 56
- Explanation of test result; IMPG2 mutation is likely to be the cause of vision loss if a 2\textsuperscript{nd} mutation can be found

Sanger sequencing IMPG2
- A second missense mutation in IMPG2 was found (known pathogenic)
Patient blindness group

- no previous genetic test
Patient blindness group

Variant with predicted pathogenicity in COL11A1:

Stickler Syndrome and the Vitreous Phenotype: Mutations in COL2A1 and COL11A1

Allan J. Richards, Annie McNinch, Howard Martin, Kim Oakhill, Harjeet Rai, Sarah Waller, Becky Treacy, Joanne Whittaker, Sarah Meredith, Arabella Poulson, and Martin P. Sneed

A Report on 10 New Patients With Heterozygous Mutations in the COL11A1 Gene and a Review of Genotype–Phenotype Correlations in Type XI Collagenopathies

Marja Majava, Kristien P. Hoornaert, Debrah Bartholdi, Mieke C. Bouma, Katelijne Bouman, Marta Carrara, Koenraad Devriendt, Jane Hurst, George Kitsos, Dunja Niedrist, Michael B. Petersen, Debbie Shears, Irene Stolte-Dijkstra, J.M. Van Hagen, Leena Ala-Kokko, Minna Männikkö, and Geert R. Mortier

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Patient blindness group

Post-test counselling

- Explanation of test result; *COL11A1* variant is a possibly cause of vision loss
- Features of Stickler / Marshall syndrome?
  - Short nose, depressed nasal bridge, no cleft palate
  - No hearing loss
- Segregation study needed in family members
- Consultation of and/or referral to various medical specialists
Which diseases in 2012?

In 2013:
* Immune deficiencies
* MCA
* Neuromuscular disorders
* Kidney disorders
* Metabolic diseases
* Undiagnosed diseases
Concluding remarks

• Whole exome sequencing provides a high chance of finding the underlying genetic cause in heterogeneous diseases

• Accurate counselling by a clinical geneticist is essential

• Implementation of whole exome sequencing requires a multidisciplinary approach, in the pre- and post testing phase

• Whole exome sequencing leads to reverse phenotyping