New gene sequencing technology is changing diagnosis and management

Prof Eric Haan
SA Clinical Genetics Service
SA Pathology
A low throughput technology that is expensive

One gene at a time, one patient at a time
$500-1500 per gene per case

Cannot deal with genetic heterogeneity, because a number of genes have to be sequenced

e.g. deafness >100; retinitis pigmentosa >50; familial spastic paraplegia >40
Next generation (massively parallel) sequencing (NGS/MPS)

METHOD OF THE YEAR | SPECIAL FEATURE

The year of sequencing

In 2007, the next-generation sequencing technologies have come into their own with an impressive array of successful applications. Kelly Rae Chi reports.

In the toxicology building of North Carolina State University in Raleigh, Nigel Deighton, head of a small genome research facility, and a few others unpack the facility’s first next-generation sequencing machine, a 454 GS FLX, on loan from Roche Diagnostics for three months. They train for a few days, nebulize a colleague’s bacterial DNA and PCR-amplify “the living daylights out of it,” Deighton recalls. They load the bead-bound PCR products onto a plate with holes that are not visible to the naked eye, pop the plate into the

Sanger sequencing becomes the ‘old’ generation.

In 2007, researchers performed whole-genome human sequencing using old and new platforms. Researchers at Baylor College of Medicine and 454 Life Sciences sequenced James Watson’s genome in two months, for about $1 million. Two other personal genomes were sequenced: Craig Venter’s, at the Institute he founded, and that of a Chinese individual, at the Beijing Genomics Institute. The J. Craig Venter Institute used Sanger technology for sequencing Venter’s DNA, which cost an estimated $70 million and took sev-
Processing power of computers will double every two years

NIH National Human Genome Research Institute

genome.gov/sequencingcosts
Genetic diagnosis with NGS

Choose gene coverage

Gene 1
Gene 2
Gene 3
Gene n

Precise diagnosis or diagnostic group

Sequence all genes simultaneously

1-5,000 genes with known phenotypes in one go
<table>
<thead>
<tr>
<th>Neurological</th>
<th>Neuromuscular</th>
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<tbody>
<tr>
<td>Aicardi-Goutieres Syndrome</td>
<td>Joubert and Meckel</td>
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<td>Amiotrophic Lateral Sclerosis</td>
<td>Joubert Syndrome</td>
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<td>Ataxia with Oculomotor Apraxia</td>
<td>Leukoencephalopathy</td>
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<td>Autism</td>
<td>Lipodystrophy</td>
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<td>Basal Ganglia Calcification</td>
<td>Lissencephaly</td>
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<td>Cerebellar Hypoplasia</td>
<td>Lysosomal Disorders</td>
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<td>Cerebral Cavernous Malformations</td>
<td>Microcephaly</td>
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<td>Charcot Marie Tooth Disease Extended</td>
<td>Migraine</td>
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<td>Ciliopathies</td>
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<tr>
<td>Cockayne Syndrome</td>
<td>Neuromuscular</td>
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<td>Cornelia De Lange Syndrome</td>
<td>Neuronal Ceroid Lipofuscinoses</td>
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<td>Distal Hereditary Motor Neuropathy</td>
<td>Noonan Syndrome</td>
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<td>Dystonia Dyskinesia</td>
<td>Nuclear-Mito</td>
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<tr>
<td>Early Onset Familial Alzheimer Disease</td>
<td>Parkinson-Alzheimer-Dementia</td>
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<tr>
<td>Epilepsy</td>
<td>Polymicrogyria</td>
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<tr>
<td>FGFR-Related Craniosynostosis</td>
<td>Pontocerebellar Hypoplasia</td>
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<tr>
<td>Hemiplegia/Stroke</td>
<td>Rett-Angelman</td>
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<tr>
<td>Hereditary Neuropathies</td>
<td>Rubenstein-Taybi Syndrome</td>
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<td>Hirschsprung Disease</td>
<td>Septo-optic Dysplasia</td>
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<tr>
<td>Holoprosencephaly</td>
<td>Spastic Paraplegia</td>
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<tr>
<td>Intellectual Disability</td>
<td>XLID</td>
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Choosing the right sequencing test

Simple clinical phenotypes/confident diagnosis:

- **Single gene**  e.g. Cystic fibrosis, familial adenomatous polyposis
- **Focussed gene panel**  e.g. familial Alzheimer, auto-inflammatory disorders

Heterogeneous disorders and complex clinical phenotypes:

- **Broad panel**  that covers the diagnostic group  
  e.g. intellectual disability 400 genes; retinal dystrophy 127 genes
- **Clinical exome**  (~5,000 genes associated with known phenotypes)  
  e.g. patient with epilepsy and intellectual disability
- **Whole Exome Sequencing**  (~20,000 protein coding genes)  
  e.g. long differential with possibility of a novel gene cause
- **Whole Genome Sequencing**  e.g. long differential with possibility of a novel gene cause and/or a cause outside the exome
Decoding Massimo Damiani's rare genetic disease

DARS gene: Hypomyelination with brain stem and spinal cord involvement and leg spasticity

SPR gene: Dopa-responsive dystonia due to sepiapterin reductase deficiency
Treated with L-Dopa and 5-hydroxytryptophan

The First Child Saved By DNA Sequencing

XIAP gene: X-linked lymphoproliferative syndrome
Treated with cord blood cell transplant

Genome study solves twins' mystery condition

Sequencing ends years of speculation over children's rare disorder.

Erika Check Hayden

Two years ago, 13-year-old Alexis Beery developed a cough and a breathing problem so severe that her parents placed a baby monitor in her room just to make sure she would survive the night. Alexis would often cough so hard and so long that she would throw up, and had to take daily injections of adrenaline just to keep breathing. Yet doctors weren't sure what was wrong.

Genome sequencing suggested a new approach to treatment for twins Noah and Alexis Beery, shown here with their parents.

Life Technologies
Identifying the cause of ‘severe’ intellectual disability

Extrapolated diagnostic yield 60%

Gilissen et al. Nature 2014; 511, 344
Patient with clinical and biochemical features of GSD type III, VI, IX

Over 12 years, patient had repeated enzyme analysis, a liver biopsy and conventional sequencing of three GSD IX genes, without a definite diagnosis

NGS identified a homozygous pathogenic variant in the *PYGL* gene, finally confirming a diagnosis of GSD type VI

NGS made a diagnosis in weeks, at 10% of the combined cost of all the inconclusive tests performed over 12 years - $1,700 vs $17,000

The ‘diagnostic odyssey’ can become a ‘diagnostic stroll in the park’, which is time- and cost-effective and minimises invasive procedures
Variants of uncertain significance
An annoyingly common outcome

TEST RESULT
No clearly pathogenic variant detected
Variant of uncertain pathogenic potential detected: \textit{COL4A5} c.892-18T>G

SUMMARY
The \textit{COL4A5} c.892-18T>G sequence variant may affect the splicing of the \textit{COL4A5} transcript (in silico analysis shows the creation of a new acceptor splice site), however the pathogenic potential of this sequence change is \textit{uncertain}.

The diagnosis of Alport syndrome or TBMN has \textit{NOT} been confirmed.

Significance may become clear over time or after family studies
Family studies can be helpful

- Sudden death
- Long QT syndrome
- SCN5A mutation present

(d. = death, dx = diagnosis, VF = Ventricular Fibrillation, y = years, sleep)

[Family tree diagram showing genetic inheritance of sudden death and Long QT syndrome]
Incidental (secondary) findings: 1-3% of genome studies

<table>
<thead>
<tr>
<th>Gene</th>
<th>c-position</th>
<th>p-position</th>
<th>Zygosity</th>
<th>significance</th>
<th>Disease (according to OMIM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA2</td>
<td>c.1514T&gt;C</td>
<td>p.Ile505Thr</td>
<td>Heterozygous</td>
<td>Variant of uncertain clinical significance</td>
<td>Breast cancer</td>
</tr>
<tr>
<td>RYR1</td>
<td>c.7844G&gt;A</td>
<td>p.Arg2615His</td>
<td>heterozygous</td>
<td>Variant of uncertain clinical significance</td>
<td>Malignant hyperthermia susceptibility</td>
</tr>
<tr>
<td>SCN5A</td>
<td>c.659C&gt;T</td>
<td>p.Thr220Ile</td>
<td>Heterozygous</td>
<td>Variant of uncertain clinical significance</td>
<td>Brugada syndrome 1</td>
</tr>
<tr>
<td>APC</td>
<td>c.2586C&gt;G</td>
<td>p.Asn862Lys</td>
<td>Heterozygous</td>
<td>Variant of uncertain clinical significance</td>
<td>Familial adenomatous polyposis</td>
</tr>
</tbody>
</table>
Genetic testing outcomes

Maron, Maron and Semsarian. J Am Coll Cardiol 2012; 60: 705-15
Consent – things to discuss

• What will be done: gene/panel/WES/WGS

• Possibility of finding:
  • ≥1 pathogenic variant
  • nothing relevant – clinical diagnosis/inheritance unchanged!
  • variants of uncertain significance, incidental findings and variants causing recessive / X-linked disorders – and does the patient want to know about them
  • Non-paternity/maternity

• Implications of finding a disorder-causing variant – for tested individual and family (including for some types of insurance)

• Use of results and DNA for research
Genetic diagnosis made – does it help the patient?

• Provide or support a clinical diagnosis
• Determine/confirm inheritance pattern
• Allow prognostication/risk stratification
• May lead to improved management
• Reproductive options
Genetic diagnosis made – useful for relatives

Makes possible predictive testing to assess susceptibility:

• **if mutation present**, possibilities may include
  
  o surveillance for early diagnosis
  
  o preventative strategies
  
  o early management
  
  o genetic counselling, including reproductive options

• **if mutation not present**, risk becomes low for patient and offspring, and surveillance, if in place, can stop
Diagnostic technologies converge

A single platform for common genetic mechanisms (?)

- Single nucleotide variants
- Copy number variants (deletions/duplications)
- Repeats
- Epigenetic modifications
- RNA sequence variation (gene expression)
Genetic diagnosis the ‘old’ way

Decide on most likely diagnosis in the genetic differential, or most likely gene in the diagnostic group

Gene 1 → neg. → Gene 2 → neg. → Gene 3

Spending limits reached quickly!