Using old and new DNA sequencing technology to identify the genetic causes of hyperinsulinism

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MRC Medical Research Council

100 years of life-changing discoveries

The Wellcome Trust
Our aim: A fast, accurate genetic diagnosis for every patient

Because:
- A genetic diagnosis guides treatment
- A genetic diagnosis defines the risk of hyperinsulinism for siblings and future offspring
A genetic diagnosis guides treatment

Jack
- Diagnosed at 1 day
- Diazoxide unresponsive
- Homozygous ABCC8 mutation
- Diffuse disease
- Sub-total pancreatectomy

George
- Diagnosed at 1 day
- Diazoxide unresponsive
- Heterozygous ABCC8 mutation
- Focal lesion confirmed by PET-CT scan
- Keyhole lesionectomy
A genetic diagnosis defines the risk for siblings and future offspring.

Jack

Diffuse

Heterozygous
Heterozygous

Homozygous

Risk to sibs 1/4

George

Focal

Heterozygous
No mutation

Heterozygous

Low recurrence risk 1/1200
A genetic diagnosis defines the risk for siblings and future offspring

Simon (HNF4A)                                      Emily (GLUD1)

½ risk for siblings and offspring

low risk for siblings (<5%) but ½ risk for offspring
The ABCC8 gene is on chromosome 11.

Two copies of the ABCC8 gene:
- One inherited from mother
- One inherited from father
• Each cell contains 23 pairs of chromosomes.

• Chromosomes consist of tightly compacted DNA.
DNA: the genetic code

The information in DNA (Deoxyribonucleic acid) is stored as a code made up of four chemical bases: adenine (A), guanine (G), cytosine (C), and thymine (T).

The order of these bases (A,G,C,T) is the genetic code.

If you unravel your DNA, it would stretch from here to ?
The human genome

- The human genome is made up of 3 billion bases of DNA
- An instruction manual to create and maintain a human being from conception to the end of life

If you typed your genome sequence at 1 base per second, how long would it take?
A gene is a segment of DNA containing the code used to synthesize a protein.

Humans have approximately 20,000 genes.
The ABCC8 gene contains 39 exons and 38 introns

- Exons are the ‘coding’ part of the gene
  - They are the ingredients needed for the cake
- Introns are the ‘non-coding’ part of the gene
  - They are the cooking utensils needed to make the cake but won’t be part of the cake

The ABCC8 gene codes for a protein called SUR1 (Sulphonylurea Receptor 1)
The SUR1 protein controls insulin secretion
Every human genome differs by 3-4 million variants

Variants can have no effect, they define characteristics like eye colour or they may cause disease (mutation)

There are different types of mutations, e.g. missense, splicing, small deletions or whole gene deletions
When things go wrong...

- **No mutation**: Decorate with jam, cream and icing sugar
- **Missense mutation**: Decorate with **ham**, cream and icing sugar
- **Small deletion**: Decorate with --- ------ --- icing sugar
- **Splicing mutation**: Bake the cake with the mixing spoon left in
- **Gene deletion**: No cake!
ABCC8 mutations cause hyperinsulinism
How do we test for ABCC8 mutations?

1. Extract DNA from blood
2. PCR amplify the coding regions of the ABCC8 gene
3. Sequence coding regions of the ABCC8 gene (10,000 bases)

Reference

Patient
Laboratory tests are semi-automated.

DNA extraction

DNA is extracted using a robot.

DNA is stored in 2D barcoded tubes.

All details stored in password-protected database.
Genetic testing for patients with CHI

ABCC8/KCNJ11 Sanger sequencing test (1-2 weeks)

- Paternal mutation
  - PET-CT scan
  - Lesionectomy

- Homozygous mutation
  - Medical management or sub-total pancreatectomy
Using old and new DNA sequencing technology to identify the genetic causes of hyperinsulinism
Improved DNA sequencing technology

1977  2000  2010

Radioactive  Fluorescent  Next generation
From one gene to (nearly) all genes

- Sanger sequencing
  - Test one gene at a time
  - Output 0.5 million bases per day
  - Cost £1000 per million bases

- Next generation sequencing
  - Test 20,000 genes at once
  - Output 5 billion bases per day
  - 20p per million bases
Next generation sequencing of all CHI genes

1. Patient DNA
2. Fragment DNA
3. Capture genes using RNA baits
4. Sequence captured DNA using Next Generation Sequencer
Genetic testing for patients with CHI

**ABCC8/KCNJ11**
Sanger sequencing test (1-2 weeks)

- **Paternal mutation**
  - PET-CT scan
  - Lesionectomy

- **Homozygous mutation**
  - Medical management or sub-total pancreatectomy

- **No mutation**
  - NGS test for all known causes (ABCC8, KCNJ11, HNF4A, GLUD1, HADH, GCK, INSR, TRMT10A)
Genetic subtypes in patients with HI
Genetic testing for patients with CHI

ABCC8/KCNJ11 Sanger sequencing test (1-2 weeks)

PET-CT scan
- Lesionectomy

Paternal mutation

Homozygous mutation

Medical management or sub-total pancreatectomy

No mutation

NGS test for all other known causes

Persistent HI

Test all 20,000 genes by exome/genome sequencing
Exome sequencing

- Most mutations (>85%) are located within the protein-coding parts (exons) of the genome

- The exons represent ~1% of the genome and can be enriched ("captured") from genomic DNA by hybridisation

- Sequencing all the exons = exome sequencing
Genome sequencing

- Sequencing 3,000,000,000 letters of each person’s genetic code
- Sequencing of the first human genome (“finished” in 2003) cost an estimated ~ $3 billion
- Cost of sequencing has dropped to $1000
Uses of new DNA sequencing technology

1) Test all known CHI genes in one test (targeted NGS)

2) Identify new genetic causes of CHI (exome or genome sequencing)

3) Find new CHI causing mutations in non-coding DNA of known genes
Focal hyperinsulinism is due to a paternal $K_{ATP}$ mutation and somatic patUPD of 11p15.

Heterozygous + Paternal UPD in fetal pancreas → Focal lesion
Genetic testing results for focal CHI

1) Sanger sequencing of *KCNJ11* and *ABCC8* identified mutations identified in 35/39 confirmed focal cases

2) Dosage analysis by MLPA detected partial gene deletions in 2/39 cases

3) Two patients with focal disease but no mutation
The hunt for intronic mutations causing focal hyperinsulinism

- Sequence the entire ABCC8 gene (116,000 bases) by next generation sequencing

![Diagram of chromosome 11 and the ABCC8 gene with introns (green) and exons (purple)]
ABCC8 long range PCR
### Results – ABCC8 variants

**Focal hyperinsulinism**

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Shared</th>
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</thead>
<tbody>
<tr>
<td><strong>Heterozygous variants</strong></td>
<td></td>
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<tr>
<td></td>
<td>90</td>
<td>225</td>
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<td><strong>Exclude variants in 1000 genomes or dbSNP132</strong></td>
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<td><strong>Exclude indels in homopolymer tracts</strong></td>
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<td><strong>Predicted to create cryptic splice site</strong></td>
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**c.1033-1013A>G**
Father’s blood sample shows abnormal splicing
Further testing

- *ABCC8* mutation identified in 3 additional focal cases (12%) and one diffuse case
- This is a founder mutation in patients from the Republic of Ireland
- This discovery allowed a couple whose first child died of CHI to have a prenatal test in their next pregnancy
Thank you: 2102 probands from 77 countries