Hyperinsulinism Genetics

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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
  - Risk to sibs 1/4

**George**
- **Focal**
  - Heterozygous
  - No mutation
  - Low recurrence risk 1/1200
A genetic diagnosis defines the risk for siblings and future offspring.

- **Jack**
  - Diffuse
  - Heterozygous
  - Risk to sibs 1/4

- **George**
  - Focal
  - Heterozygous
  - Low recurrence risk 1/1200

- **Gemma**
  - Diffuse
  - No mutation
  - Low risk for siblings (<5%) but 50% risk for offspring
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

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.
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?
GENOMIC MEDICINE: TRANSFORMING PATIENT CARE IN DIABETES
UNIVERSITY OF EXETER

Learn how developments in genomics are transforming our knowledge and treatment of conditions such as diabetes.

https://www.youtube.com/watch?feature=youtu.be&list=PLnuuR95Vzf8laNwS1rFxOXVtMNVTqxDC&v=iGA1CC4lB9A&app=desktop

https://www.futurelearn.com/courses/diabetes-genomic-medicine/1/steps/65986
• 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 (Sulphphonylurea Receptor 1)
The SUR1 protein controls insulin secretion.
When things go wrong...

- 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 \textit{ABCC8} mutations?

1. Extract DNA from blood.
2. PCR amplify the coding regions of the \textit{ABCC8} gene.
3. Sequence coding regions of the \textit{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 week)

  - Paternal mutation → PET-CT scan → Lesionectomy
  - Homozygous mutation → Medical management or sub-total pancreatectomy
  - Dominantly acting missense mutation
DNA sequencing to identify the genetic causes of hyperinsulinism

1977 Radioactive

2000 Fluorescent

2010 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. Fragment DNA
2. Capture genes using RNA baits
3. Sequence captured DNA using Next Generation Sequencer

Patient DNA
Genetic testing for all known HI genes

ABCC8/KCNJ11
Sanger sequencing test (~1 week)

- Paternal mutation → PET-CT scan → Lesionectomy
- Homozygous mutation → Medical management or sub-total pancreatectomy
- Dominantly acting missense mutation

No mutation → NGS test for all known HI genes
Genetic subtypes in patients with HI

Cohort (n=1801)

Non-Consanguineous (n=1293)
- ABCC8: 26%
- KCNJ11: 63%
- HADH: 4%
- HNF4A: 2%
- GLUD1: 1%
- GCK: 1%
- Other: 1%
- Unknown: 1%

Consanguineous Cohort (n=508)
- ABCC8: 39%
- KCNJ11: 41%
Gene discovery testing for patients with HI

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ABCC8/KCNJ11 Sanger sequencing test (~1 week)
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 $K_{\text{ATP}}$ mutation and somatic patUPD of 11p15.
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

- Chromosome 11
  - Intron: ~106,000 bases
  - Exon: ~10,000 bases
Found! An *ABCC8* variant deep in intron 8
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: 2239 probands from 78 countries