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

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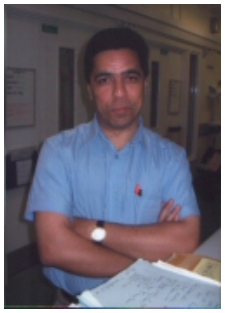

Prof Khalid Hussain, London

norchi
northern congenital hyperinsulinism service

Great Ormond Street Hospital

MRC Medical Research Council
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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

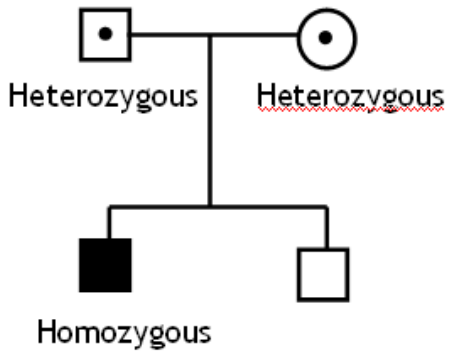
- Diagnosed at 1 day
- Diazoxide unresponsive
- Heterozygous *ABCC8* mutation
- Focal lesion confirmed by PET-CT scan
- Keyhole lesionectomy

A genetic diagnosis defines the risk for siblings and future offspring

Jack

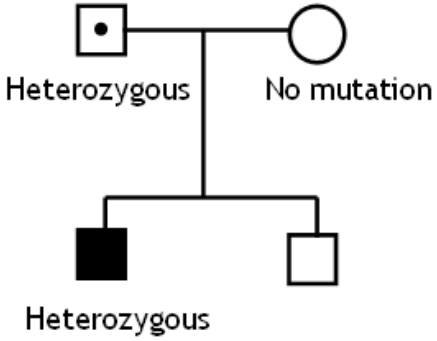
George

Diffuse



Risk to sibs 1/4

Focal



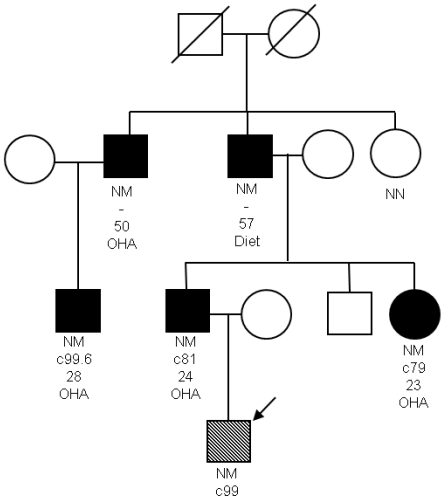
Low recurrence risk

1/1200

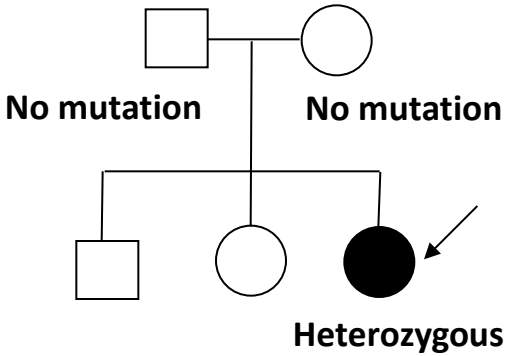
A genetic diagnosis defines the risk for siblings and future offspring

Simon (HNF4A)

Emily (GLUD1)

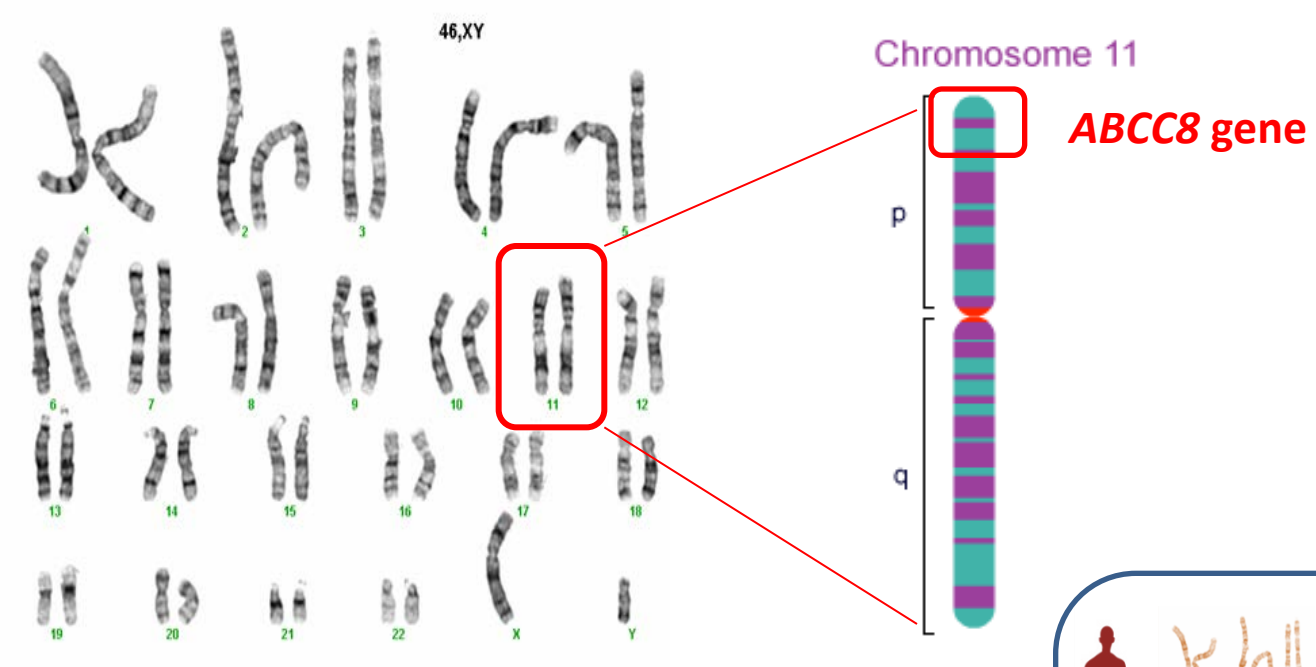


½ risk for siblings and offspring



low risk for siblings (<5%) but ½ risk for offspring

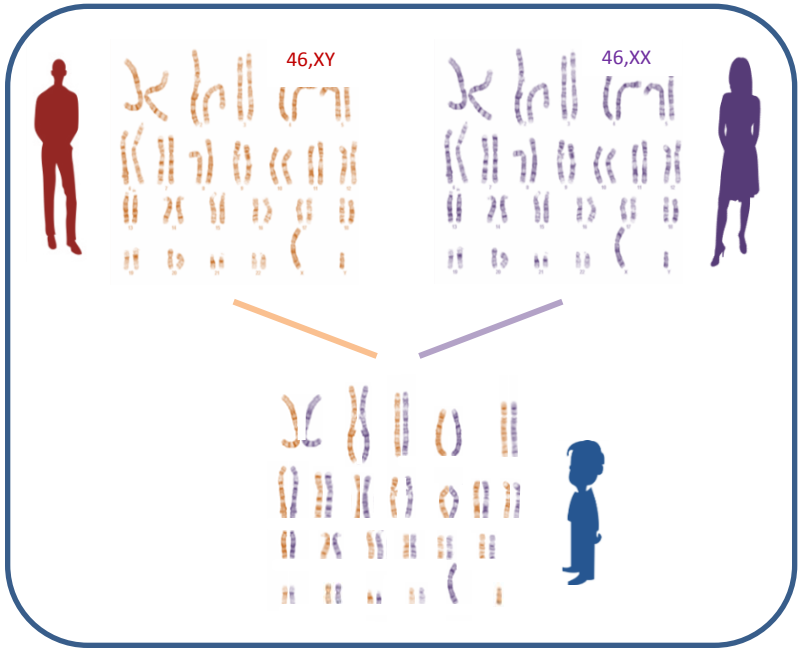
The *ABCC8* gene



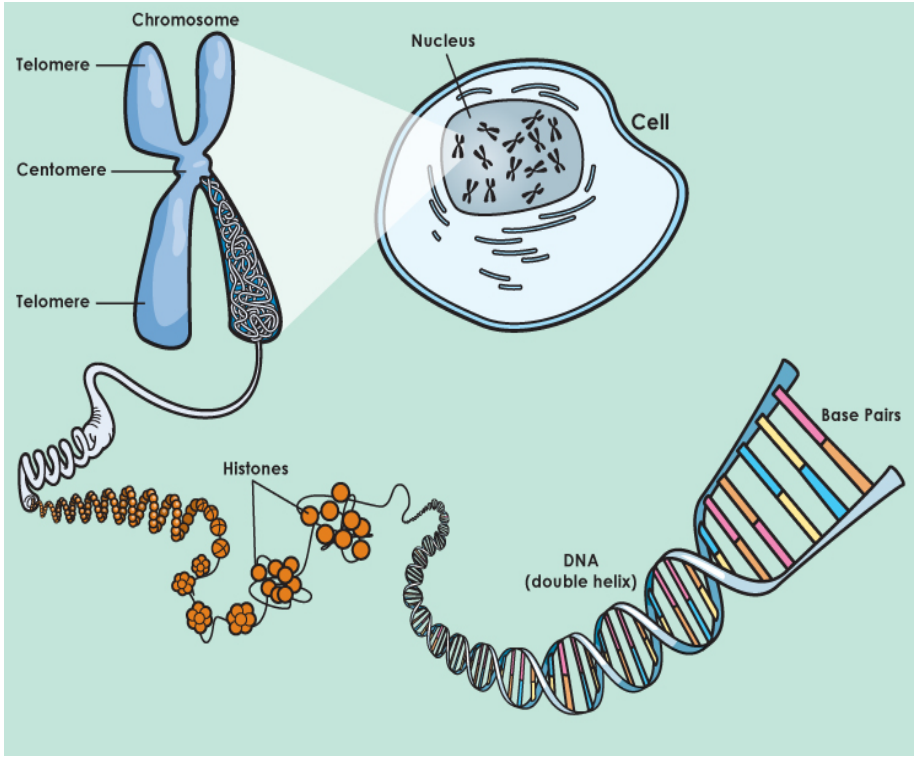
The *ABCC8* gene is on chromosome 11

Two copies of the *ABCC8* gene:

- One inherited from mother
- One inherited from father



From chromosomes to DNA

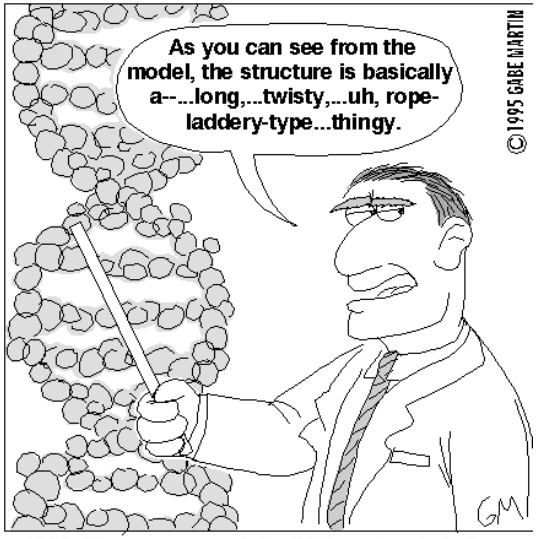


- Each cell contains 23 pairs of chromosomes.
- Chromosomes consist of tightly compacted DNA.

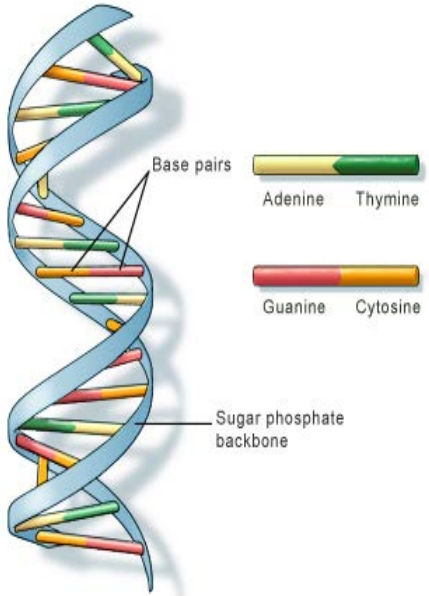
DNA: the genetic code

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.



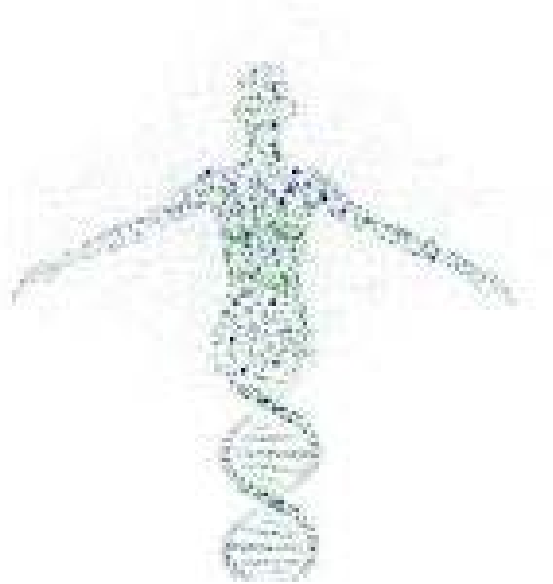
1953: The structure of the DNA molecule is first described.



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

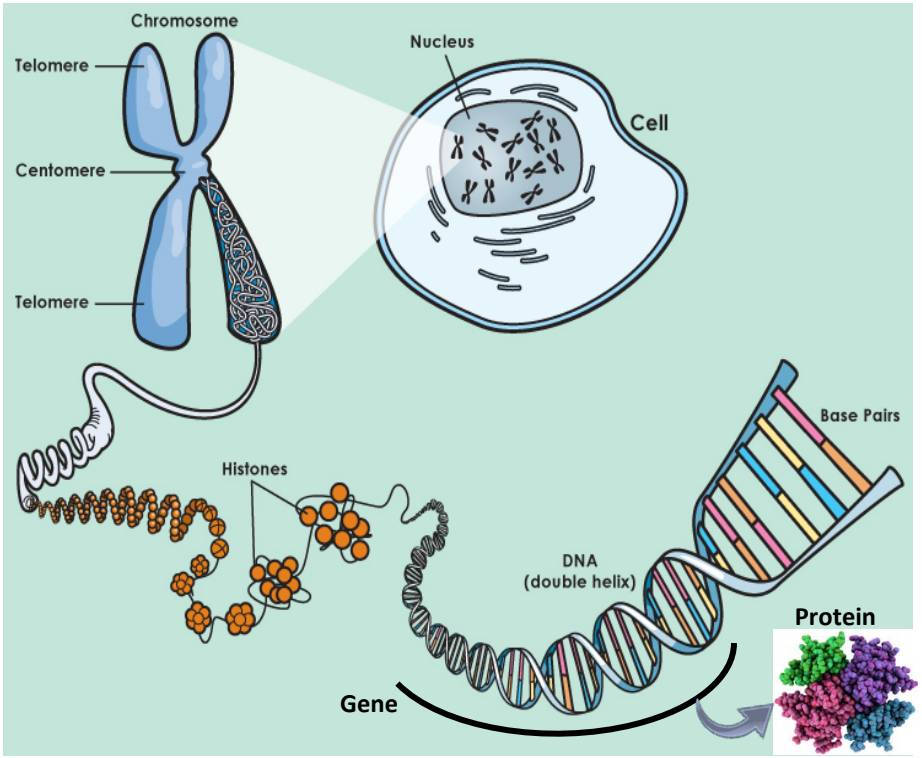
The human genome

- 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



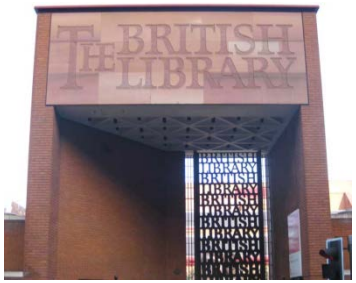
If you typed your genome sequence at 1 base per second, how long would it take?

Genes to proteins



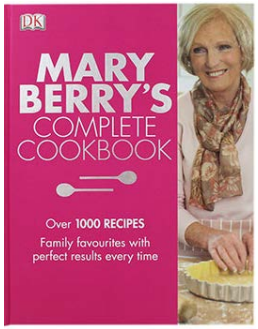
- A gene is a segment of DNA containing the code used to synthesize a protein.
- Humans have approximately 20,000 genes

Nucleus

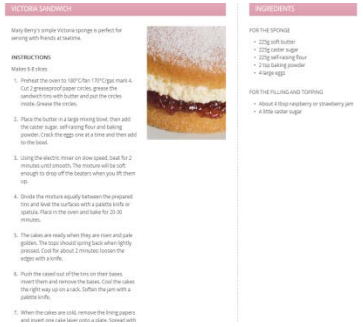


Chromosomes

Chromosome

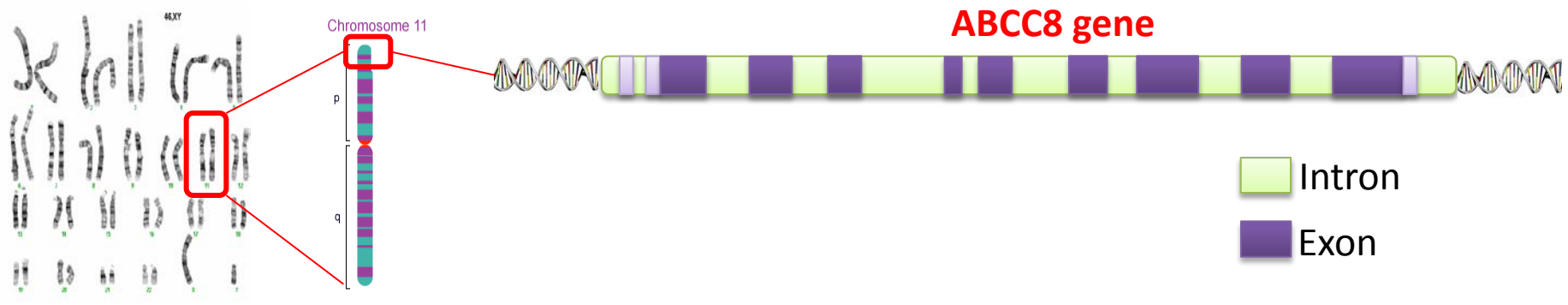


Gene



Protein

The *ABCC8* gene

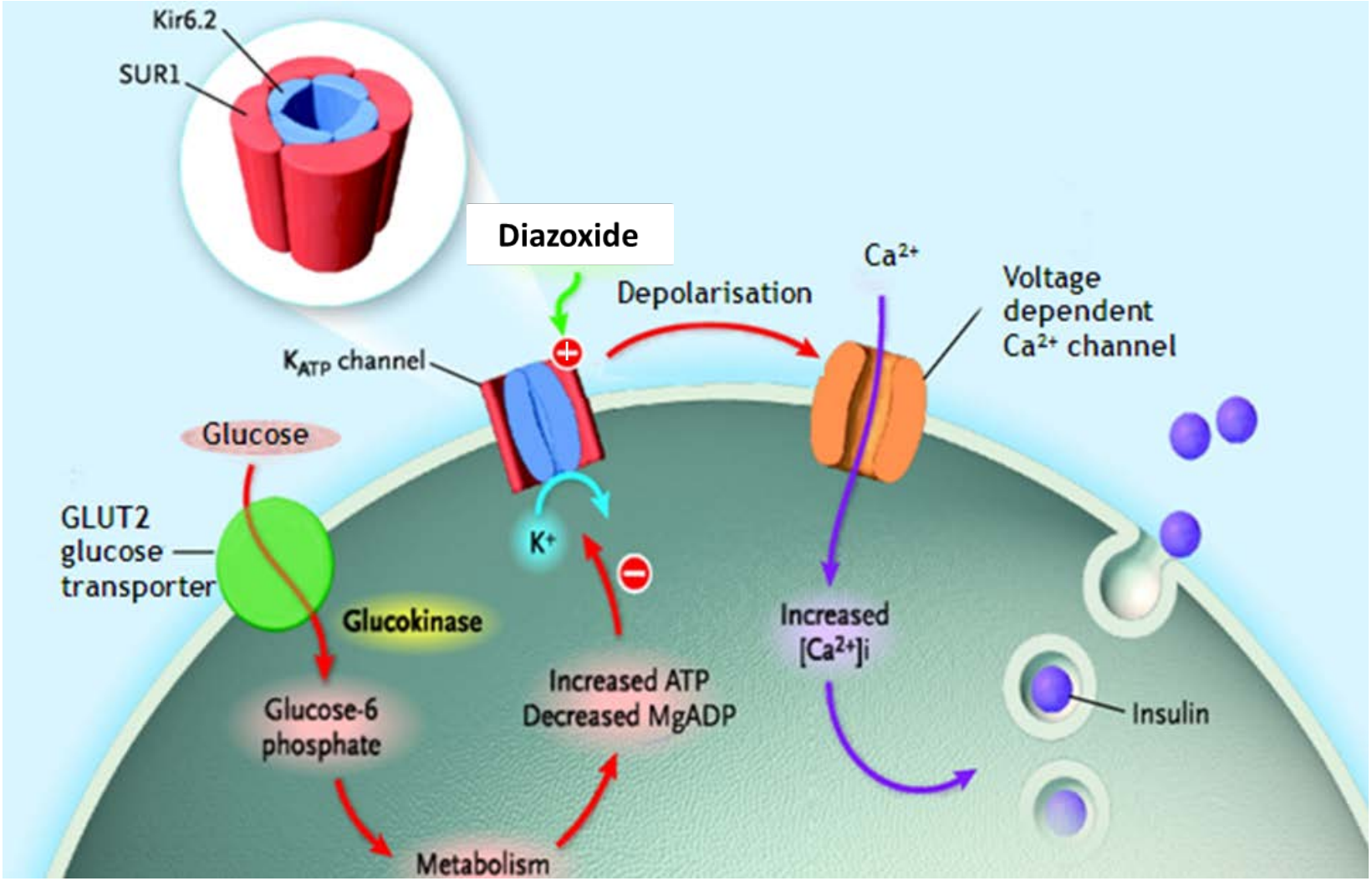


The *ABCC8* gene contains 39 exons and 38 introns

- 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



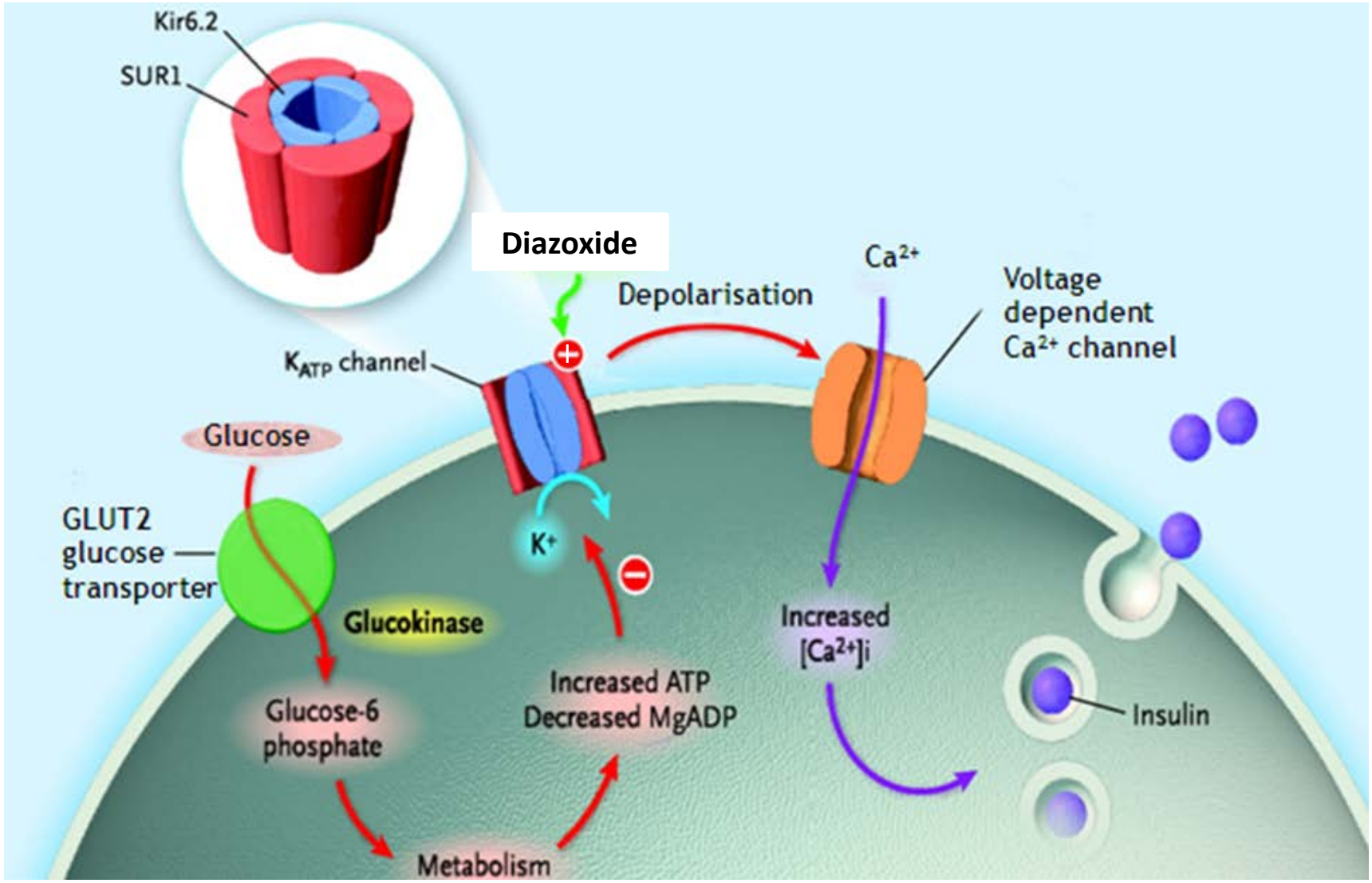
When things go wrong…

- 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

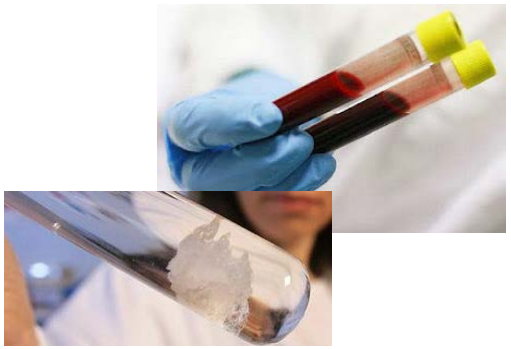
When things go wrong…

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!

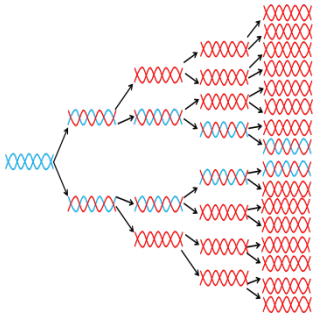
ABCC8 mutations cause hyperinsulinism



How do we test for *ABCC8* mutations ?



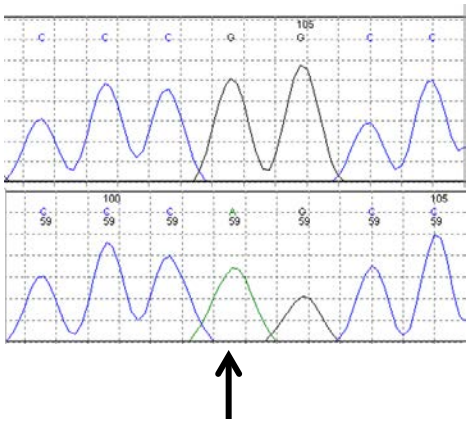
Extract DNA from blood



PCR amplify the coding regions of the *ABCC8* gene

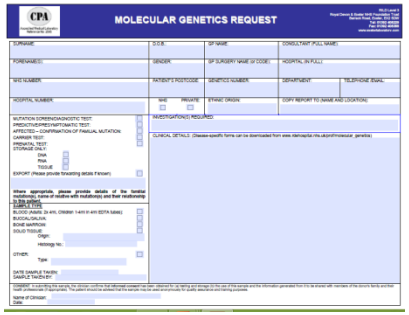
Reference

Patient



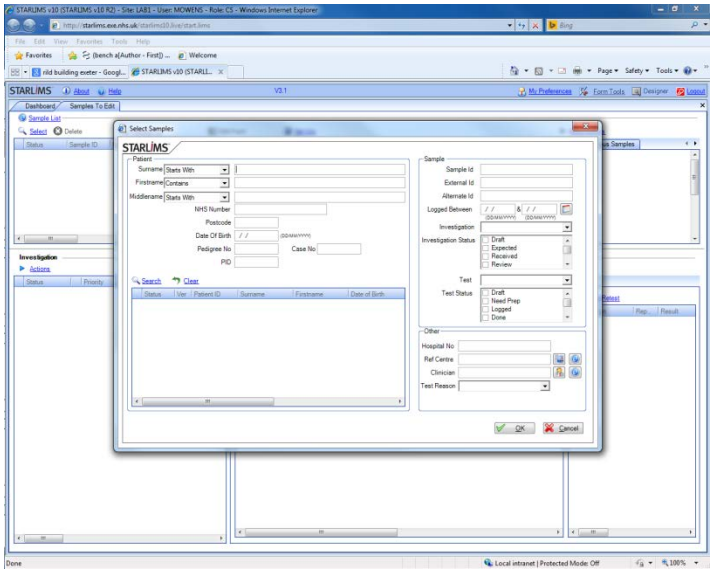
Sequence coding regions of the *ABCC8* gene (10,000 bases)

Laboratory tests are semi-automated



DNA extraction

DNA is extracted using a robot



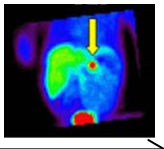
DNA is stored in 2D barcoded tubes

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

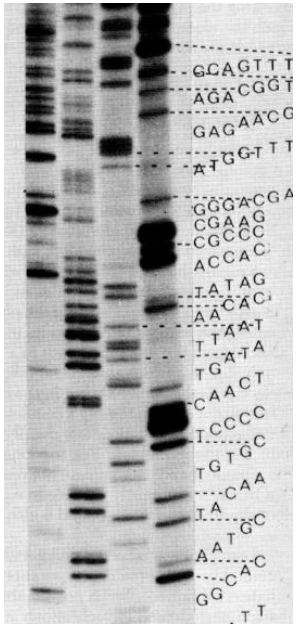
Homozygous mutation

Medical management or sub-total pancreatectomy

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

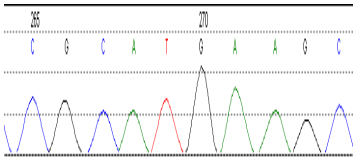
Improved DNA sequencing technology

1977



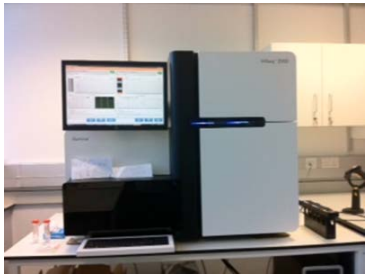
Radioactive

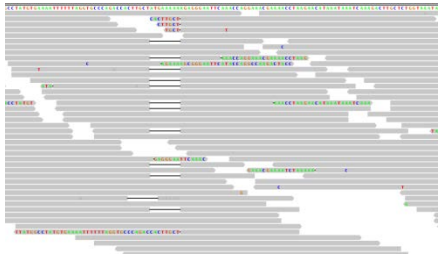
2000



Fluorescent

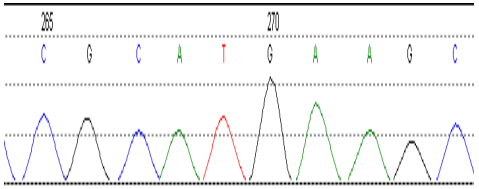
2010

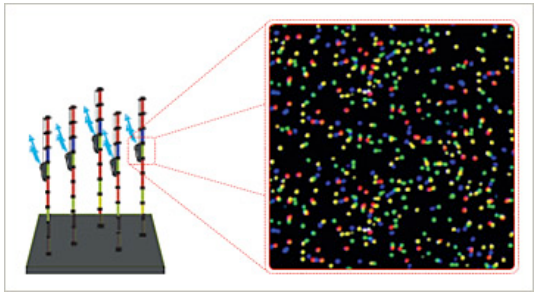




Next generation

From one gene to (nearly) all genes





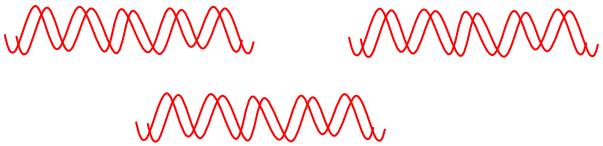
- Sanger sequencing
- Test one gene at a time
- Output 0.5 million bases per day
- Cost £1000 per million bases

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

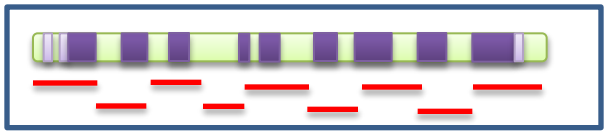
Next generation sequencing of all CHI genes

Patient DNA

Fragment DNA



Capture genes using RNA baits



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)

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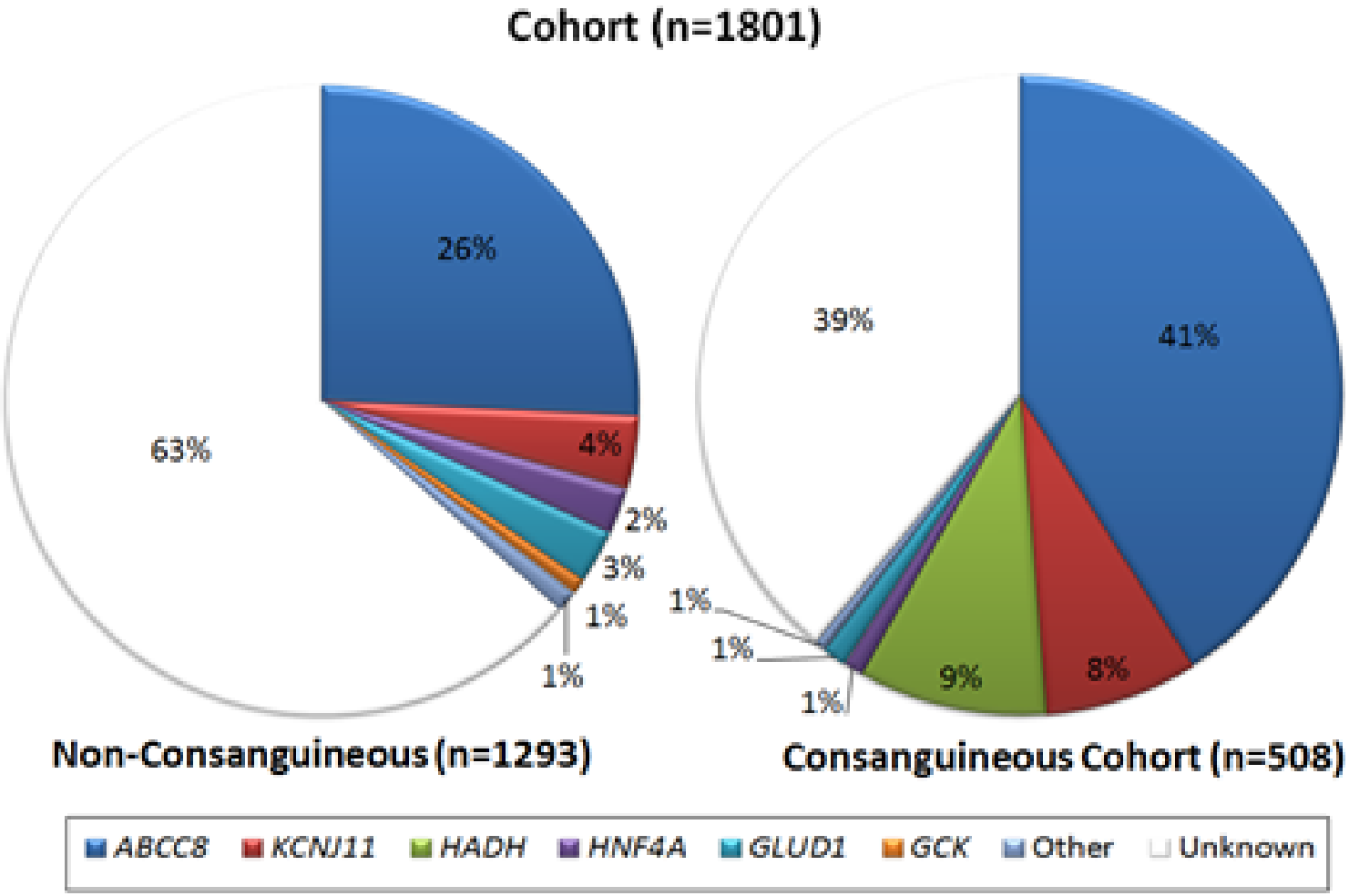
Genetic subtypes in patients with HI



Genetic testing for patients with CHI

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

Paternal mutation → PET-CT scan → Lesionectomy

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No mutation → NGS test for all other known causes

Persistent HI → Test all 20,000 genes by exome/genome sequencing

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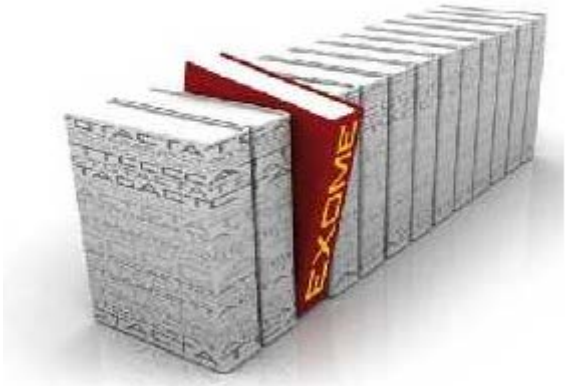
Exome sequencing

- 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

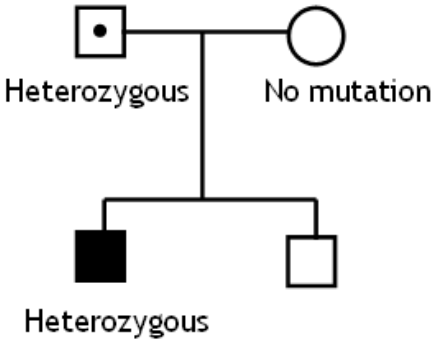
Uses of new DNA sequencing technology

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



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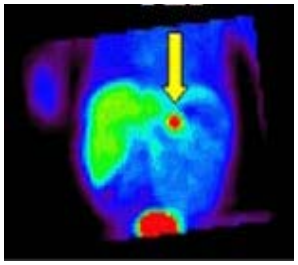
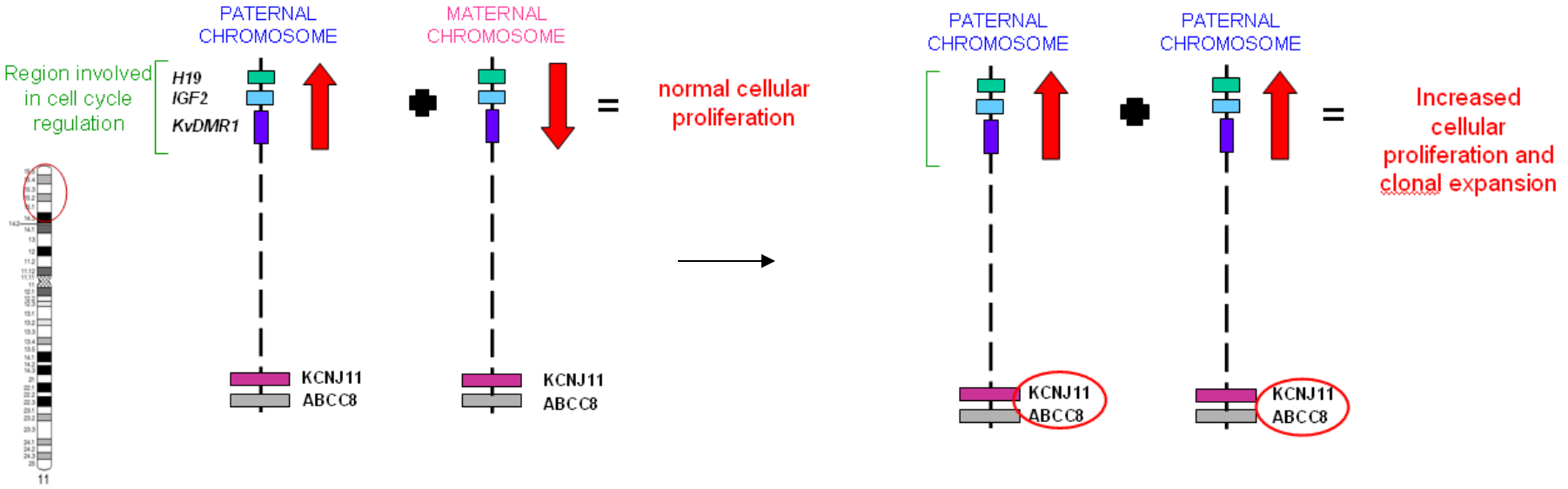
Paternal UPD in fetal pancreas

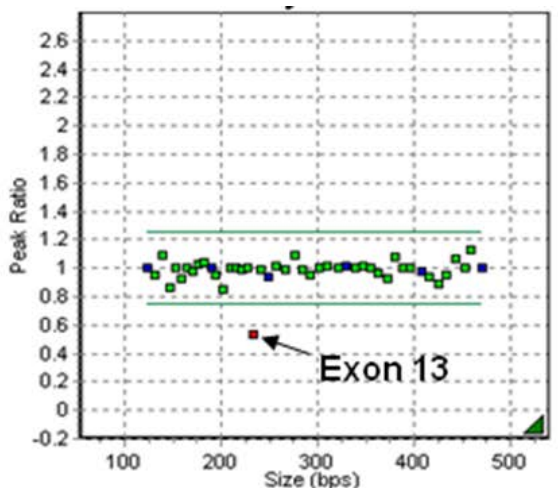


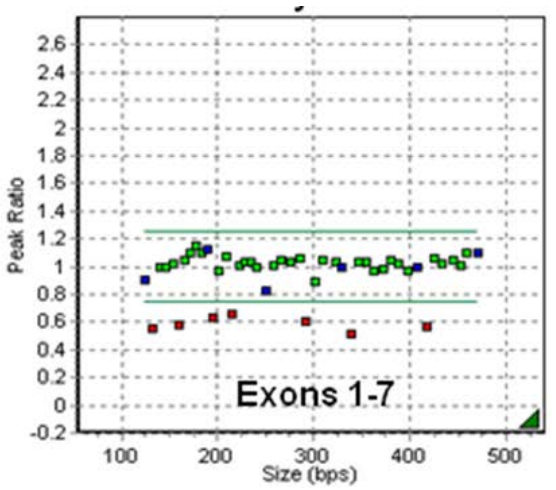
Focal lesion



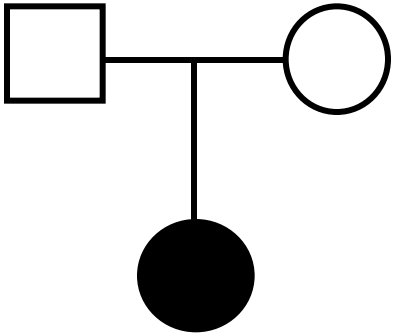
Genetic testing results for focal CHI

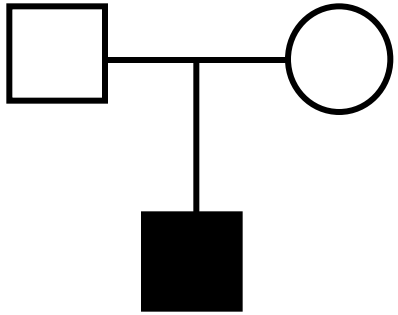
1) Sanger sequencing of *KCNJ11* and *ABCC8* identified mutations identified in 35/39 confirmed focal cases
2) Dosage analysis by MLPA detected partial gene deletions in 2/39 cases
3) Two patients with focal disease but no mutation

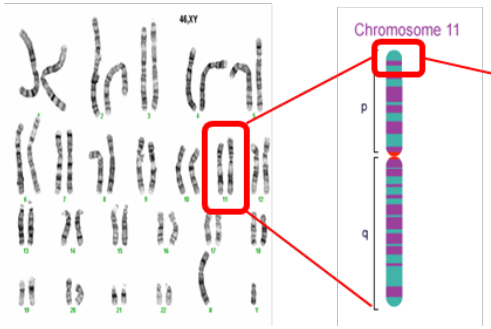




The hunt for intronic mutations causing focal hyperinsulinism





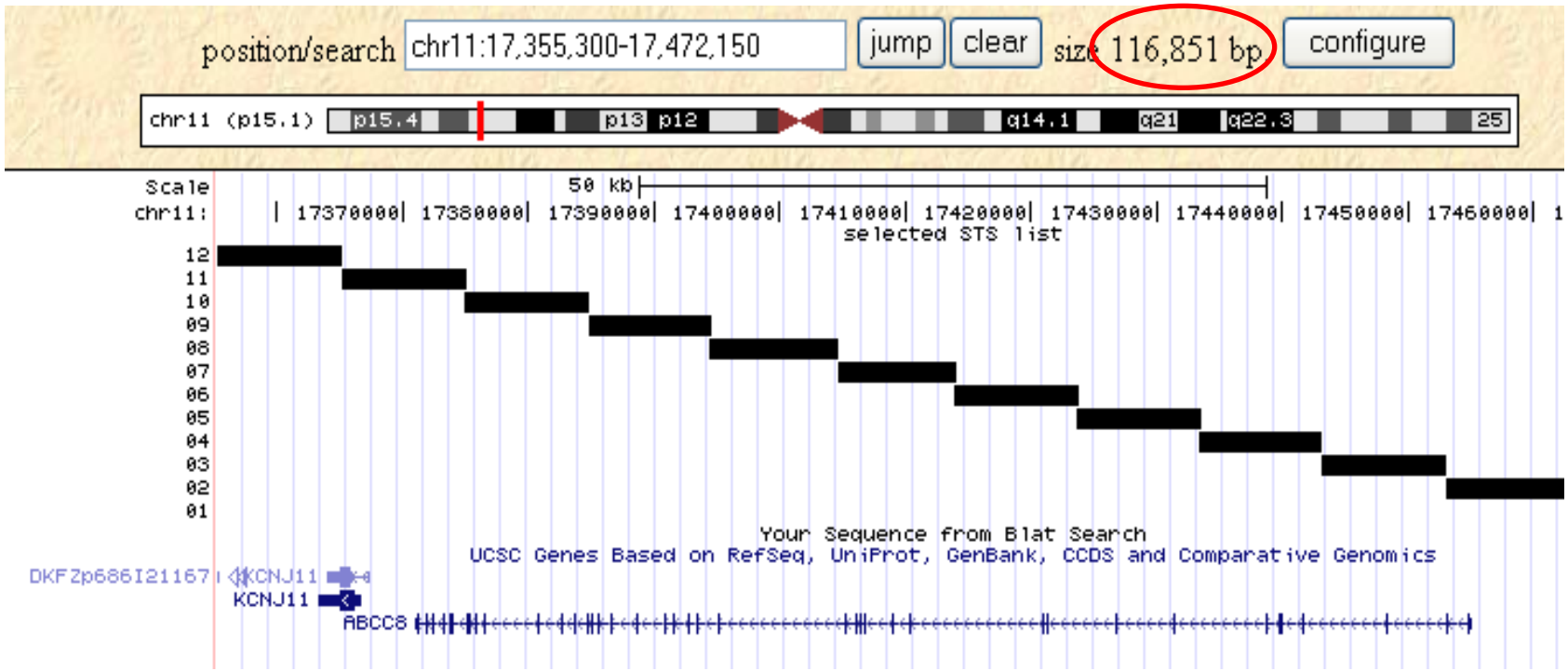


Intron ~106,000 bases

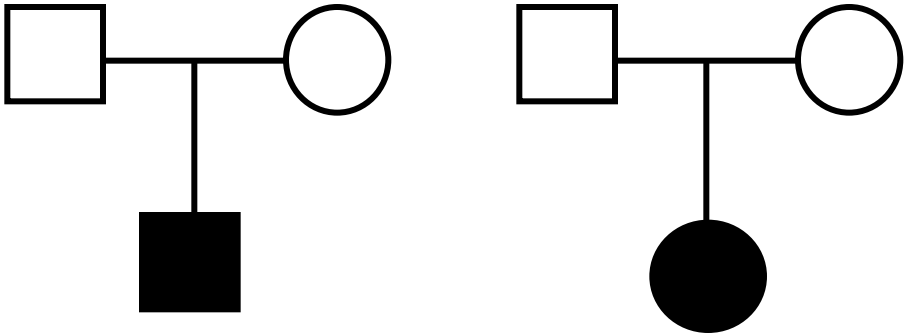
Exon ~10,000 bases

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

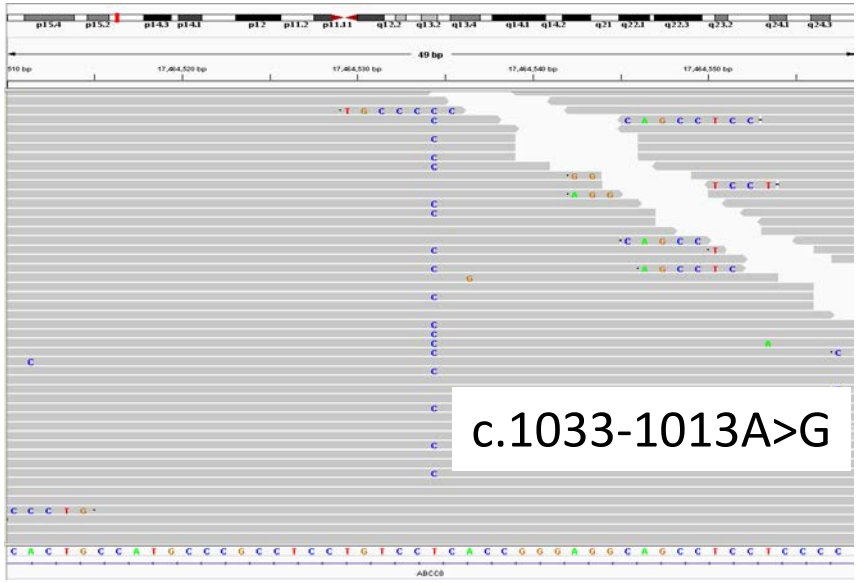
ABCC8 long range PCR



Results – *ABCC8* variants

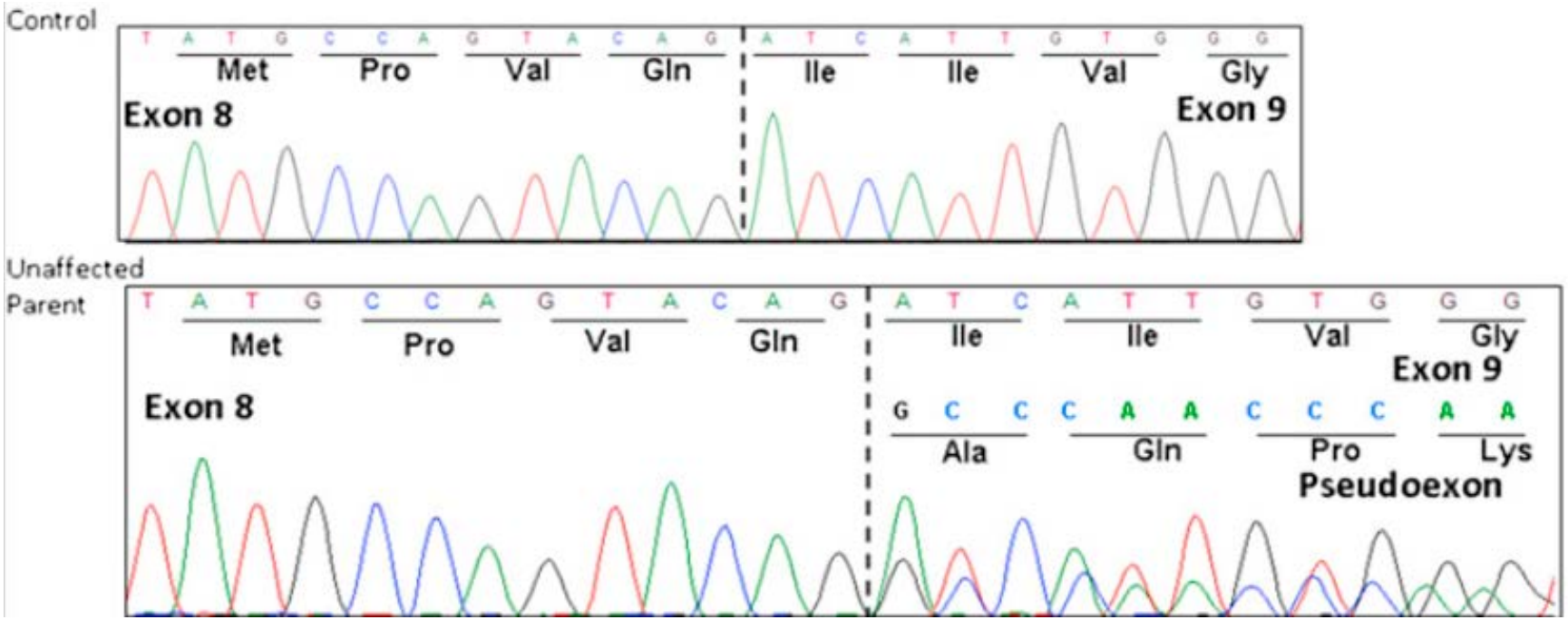


Focal hyperinsulinism



	Patient 1	Patient 2	Shared
Heterozygous variants	90	225	83
Exclude variants in 1000 genomes or dbSNP132	9	10	9
Exclude indels in homopolymer tracts	3	4	**1**
Predicted to create cryptic splice site	**1**	**1**	**1**

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

REPORT

Next-Generation Sequencing Reveals Deep Intronic Cryptic *ABCC8* and *HADH* Splicing Founder Mutations Causing Hyperinsulinism by Pseudoexon Activation

AJHG 2013

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Thank you: 2102 probands from 77 countries