Genetics of CHI

Sian Ellard

Consultant Clinical Scientist and Professor of Genomic Medicine

University of Exeter Medical School

EXETER CLINICAL LABORATORY INTERNATIONAL

EXCELLING IN SCIENCE AND SERVICE

Genetic testing for CHI in the UK

Dr Jayne Houghton
Royal Devon & Exeter Hospital

Dr Sarah Flanagan
University of Exeter

3029 patients referred from 82 countries

Exciting new partnership ensures that genetic testing is available for all

CHI Congenital Hyperinsulinism International

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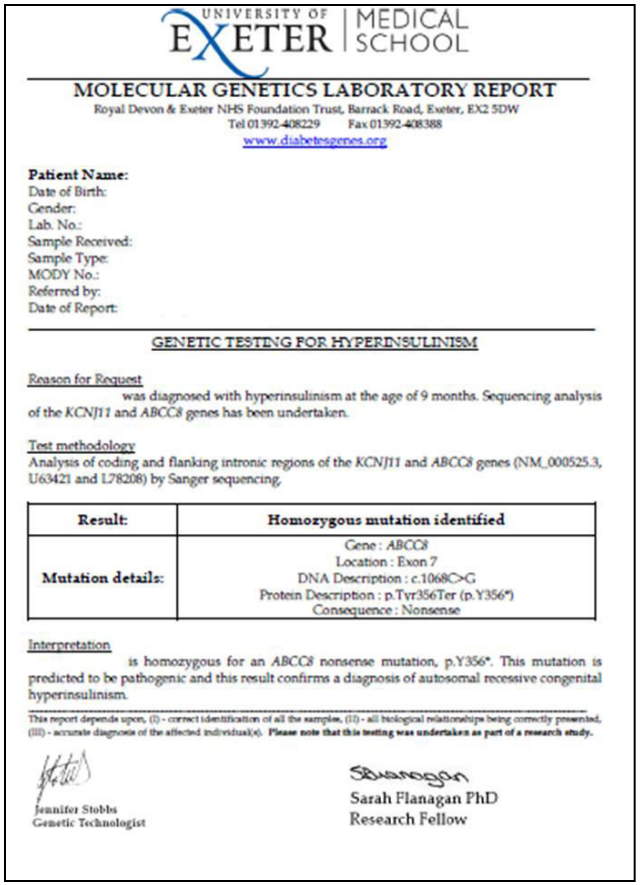
Our aim:

A fast, accurate and comprehensive genetic diagnosis for every patient



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MOLECULAR GENETICS LABORATORY REPORT

Royal Devon & Exeter NHS Foundation Trust, Barrack Road, Exeter, EX2 5DW
Tel 01392-408229 Fax 01392-408388
www.diabetesgenes.org

Patient Name:
Date of Birth:
Gender:
Lab. No.:
Sample Received:
Sample Type:
MODY No.:
Referred by:
Date of Report:

GENETIC TESTING FOR HYPERINSULINISM

Reason for Request

was diagnosed with hyperinsulinism at the age of 9 months. Sequencing analysis of the *KCNJ11* and *ABCC8* genes has been undertaken.

Test methodology

Analysis of coding and flanking intronic regions of the *KCNJ11* and *ABCC8* genes (NM_000525.3, U63421 and L78208) by Sanger sequencing.

Result:	Homozygous mutation identified
Mutation details:	Gene : *ABCC8* Location : Exon 7 DNA Description : c.1068C>G Protein Description : p.Tyr356Ter (p.Y356*) Consequence : Nonsense

Interpretation

is homozygous for an *ABCC8* nonsense mutation, p.Y356*. This mutation is predicted to be pathogenic and this result confirms a diagnosis of autosomal recessive congenital hyperinsulinism.

This report depends upon, (i) - correct identification of all the samples, (ii) - all biological relationships being correctly presented, (iii) - accurate diagnosis of the affected individual(s). **Please note that this testing was undertaken as part of a research study.**

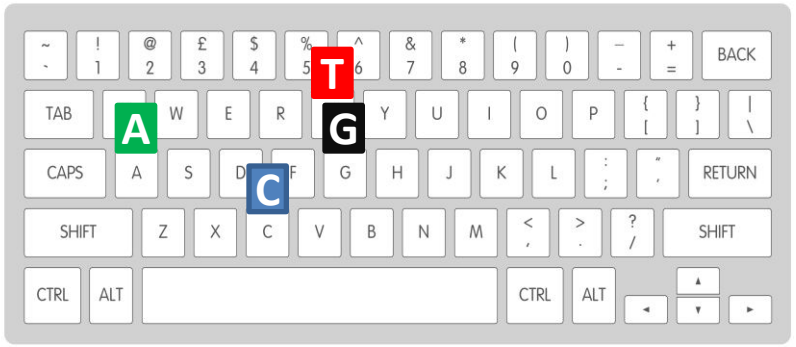
Jennifer Stobbs
Genetic Technologist

Sarah Flanagan PhD
Research Fellow

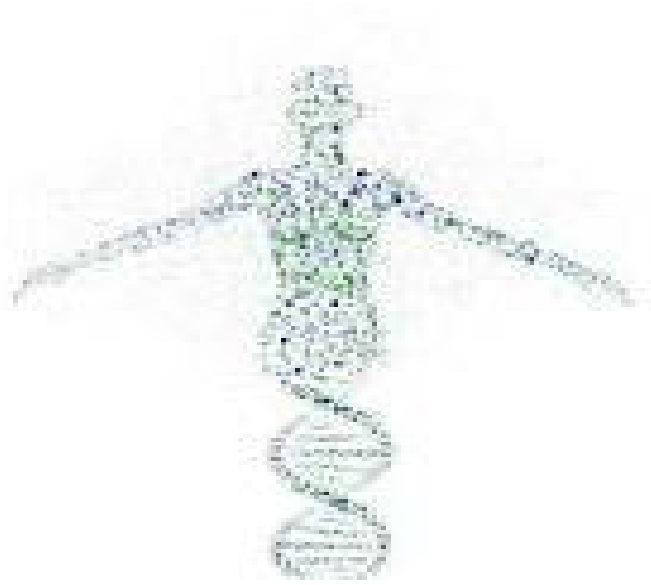
The Human Genome

- An instruction manual to create, maintain and repair a human being from conception to the end of life
- The human genome is made up of 3 billion bases of DNA (ATGC)
- DNA is organised into 23 pairs of chromosomes (one copy from mum and one copy from dad)

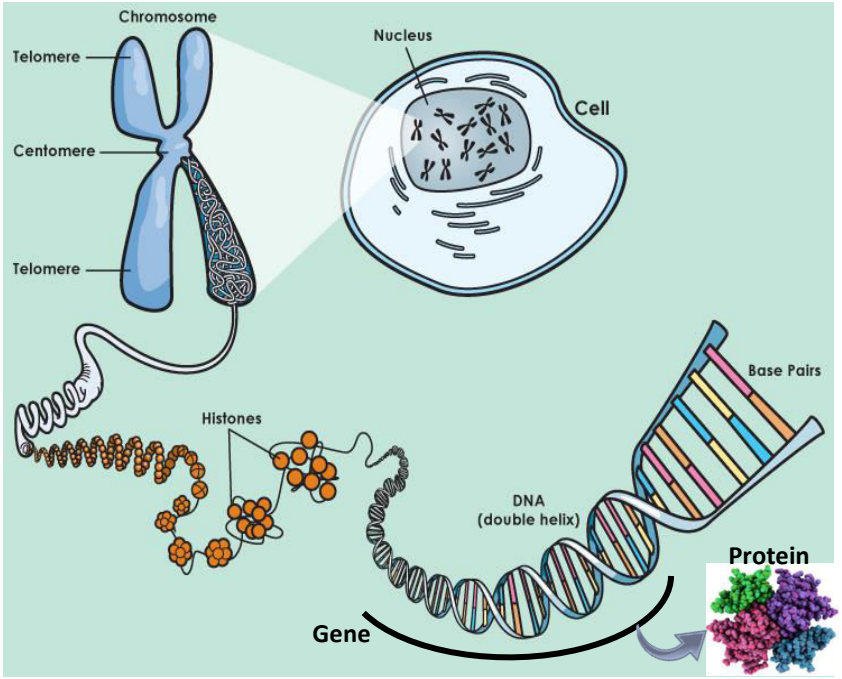
There are ~3,000,000,000 base pairs in the human genome



60 characters per min, 8 hours per day, 50 years



Genes code for proteins

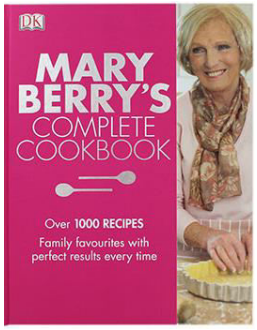


- A gene is a segment of DNA containing the code used to synthesize a protein (e.g *INS* gene encodes the Insulin protein).
- Humans have approximately 20,000 genes

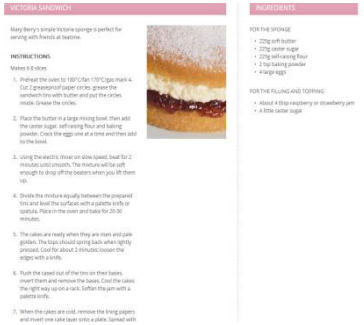
Nucleus

Chromosomes

Chromosome



Gene



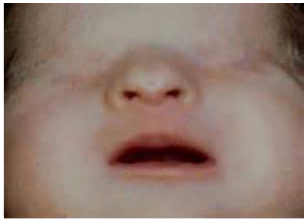
Protein

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 or pathogenic variant)

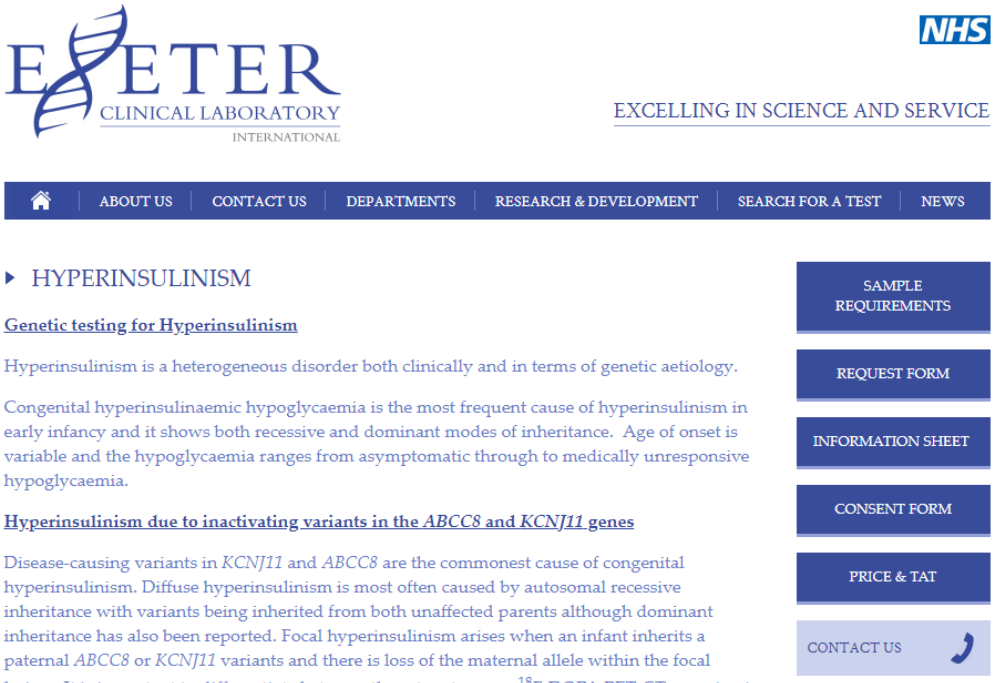
- There are different types of pathogenic variants, e.g. missense, splicing, small deletions or whole gene deletions



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!

The Exeter lab tests for 15 different genetic causes of congenital hyperinsulinsim



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NHS

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HYPERINSULINISM

Genetic testing for Hyperinsulinism

Hyperinsulinism is a heterogeneous disorder both clinically and in terms of genetic aetiology.

Congenital hyperinsulinaemic hypoglycaemia is the most frequent cause of hyperinsulinism in early infancy and it shows both recessive and dominant modes of inheritance. Age of onset is variable and the hypoglycaemia ranges from asymptomatic through to medically unresponsive hypoglycaemia.

Hyperinsulinism due to inactivating variants in the *ABCC8* and *KCNJ11* genes

Disease-causing variants in *KCNJ11* and *ABCC8* are the commonest cause of congenital hyperinsulinism. Diffuse hyperinsulinism is most often caused by autosomal recessive inheritance with variants being inherited from both unaffected parents although dominant inheritance has also been reported. Focal hyperinsulinism arises when an infant inherits a paternal *ABCC8* or *KCNJ11* variants and there is loss of the maternal allele within the focal lesion. It is important to differentiate between these two types as ^{18}F-DOPA PET-CT scanning is recommended for patients with a paternally inherited variant to locate a possible focal lesion within the pancreas as lesionectomy or partial pancreatectomy can cure focal hyperinsulinism. Loss of heterozygosity can be detected using microsatellite markers within the chromosome 11p15 region. Diffuse hyperinsulinism is treated medically where possible with sub-total pancreatectomy only as a last resort since 75% of patients then develop iatrogenic diabetes.

First line urgent testing for *ABCC8* and *KCNJ11* gene variants is available with a result issued in 1-2 weeks, followed by a 10 gene next generation sequencing test if no variant is found.

Hyperinsulinism-Hyperammonaemia Syndrome due to dominant variants in the *GLUD1* gene

Hyperinsulinism-hyperammonemia syndrome is caused by heterozygous gain-of-function variants in the *GLUD1* gene. Patients usually present outside the neonatal period and a consistent feature is the presence of hyperammonaemia with plasma ammonium levels being persistently raised. The variants are located in the GTP and ATP-binding domains of the enzyme which are encoded by exons 6, 7, 10, 11 and 12. The majority of cases (~80%) are due to

SAMPLE REQUIREMENTS

REQUEST FORM

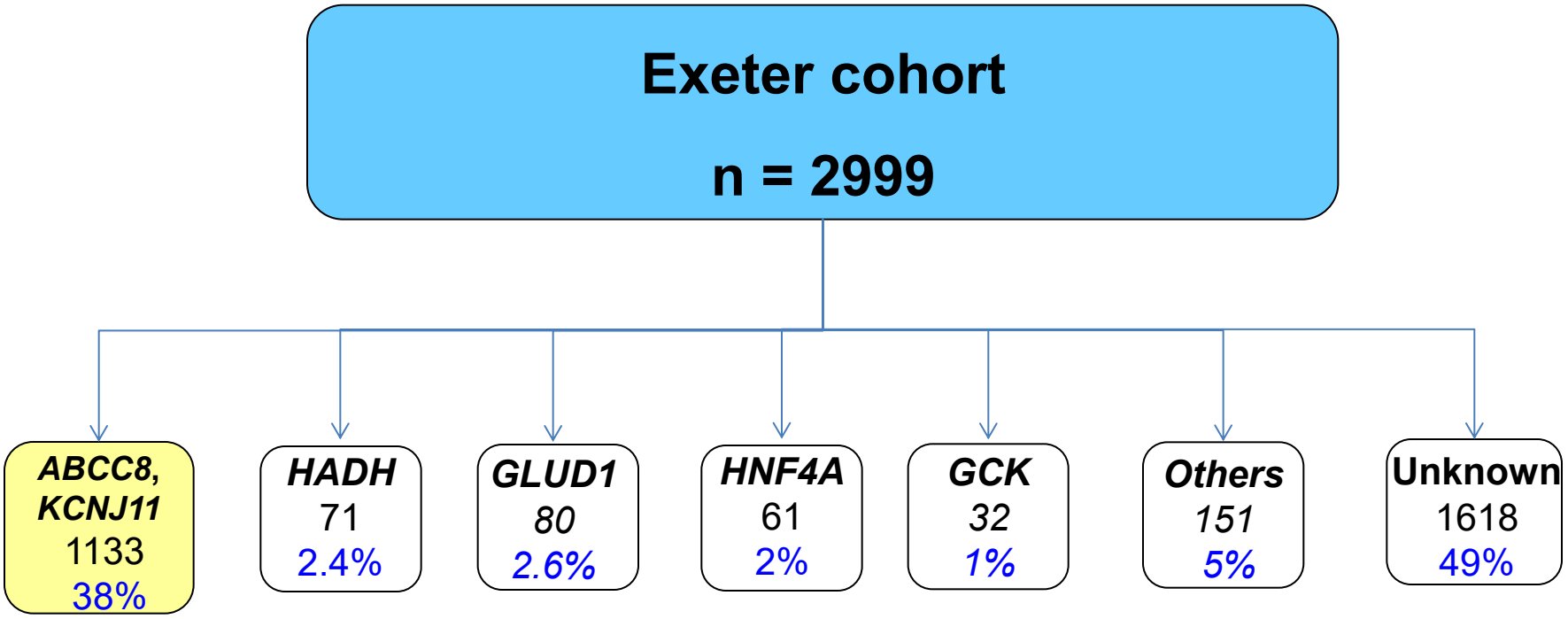
INFORMATION SHEET

CONSENT FORM

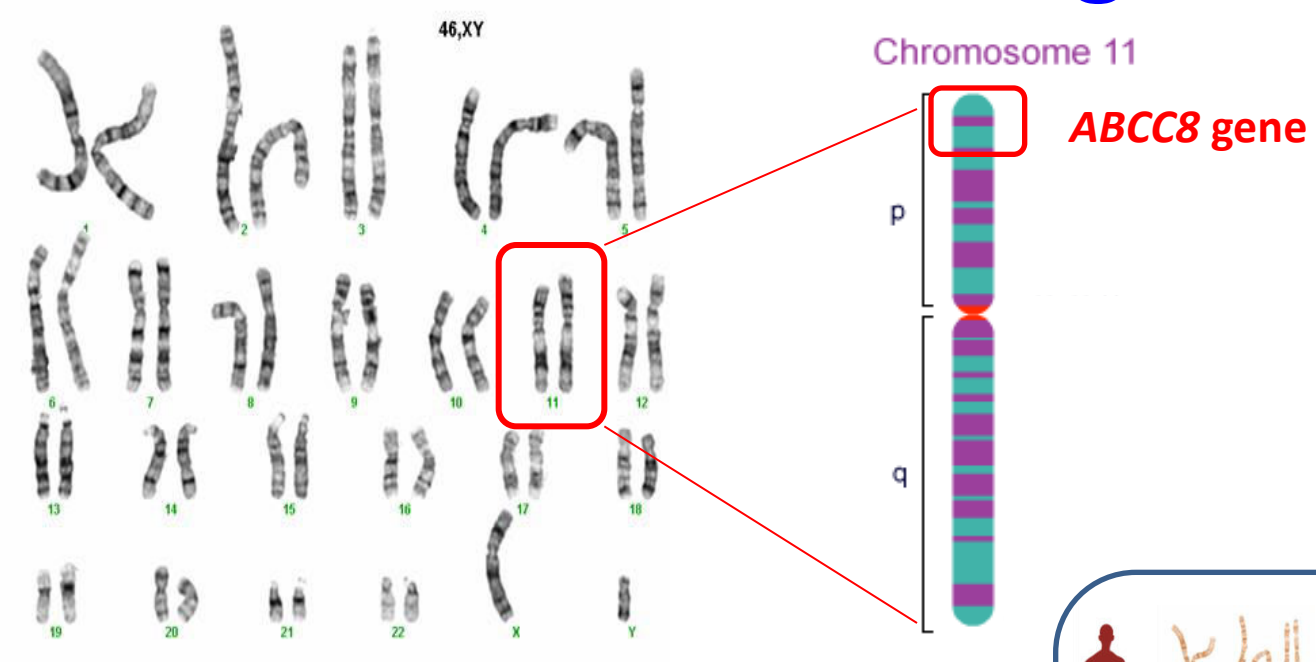
PRICE & TAT

CONTACT US

Changes in the *ABCC8* and *KCNJ11* genes are the most common cause of congenital hyperinsulinism



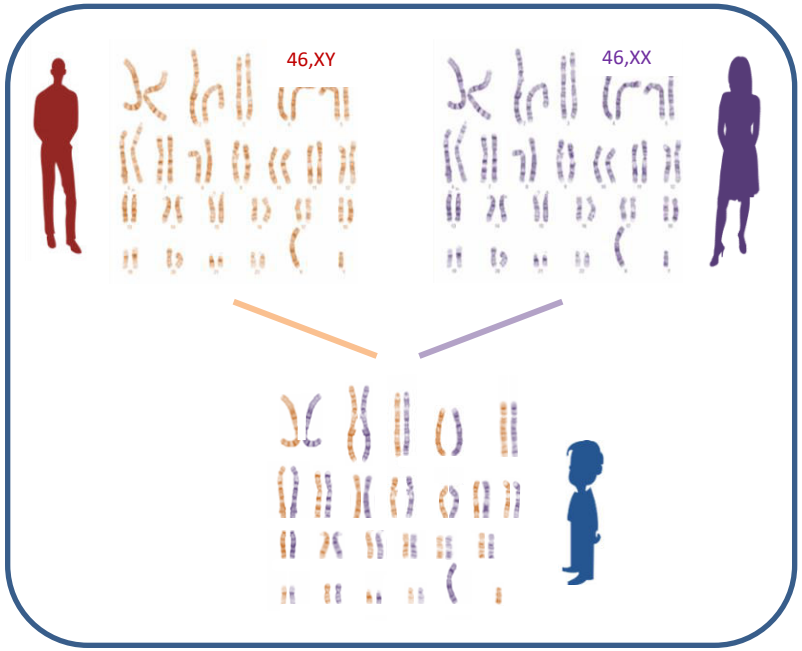
The ABCC8 gene



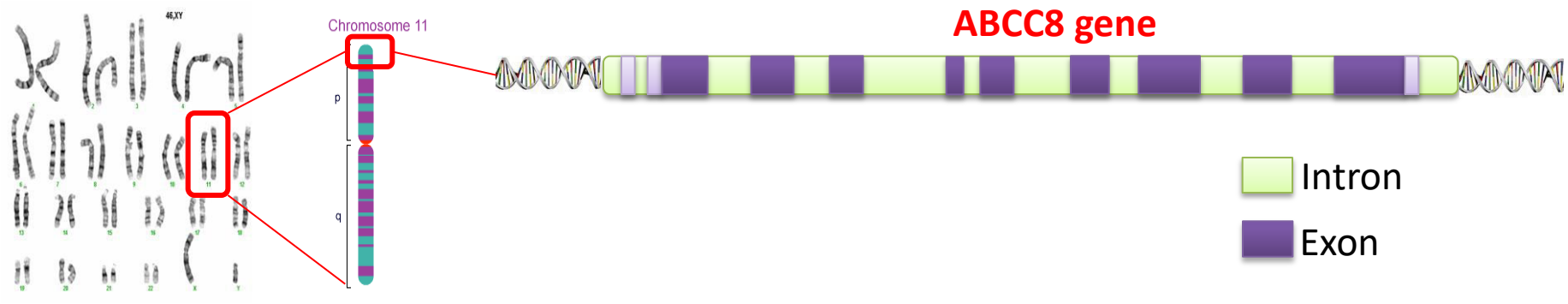
The *ABCC8* gene is on chromosome 11

Two copies of the *ABCC8* gene:

- One inherited from mother
- One inherited from father



The ABCC8 gene

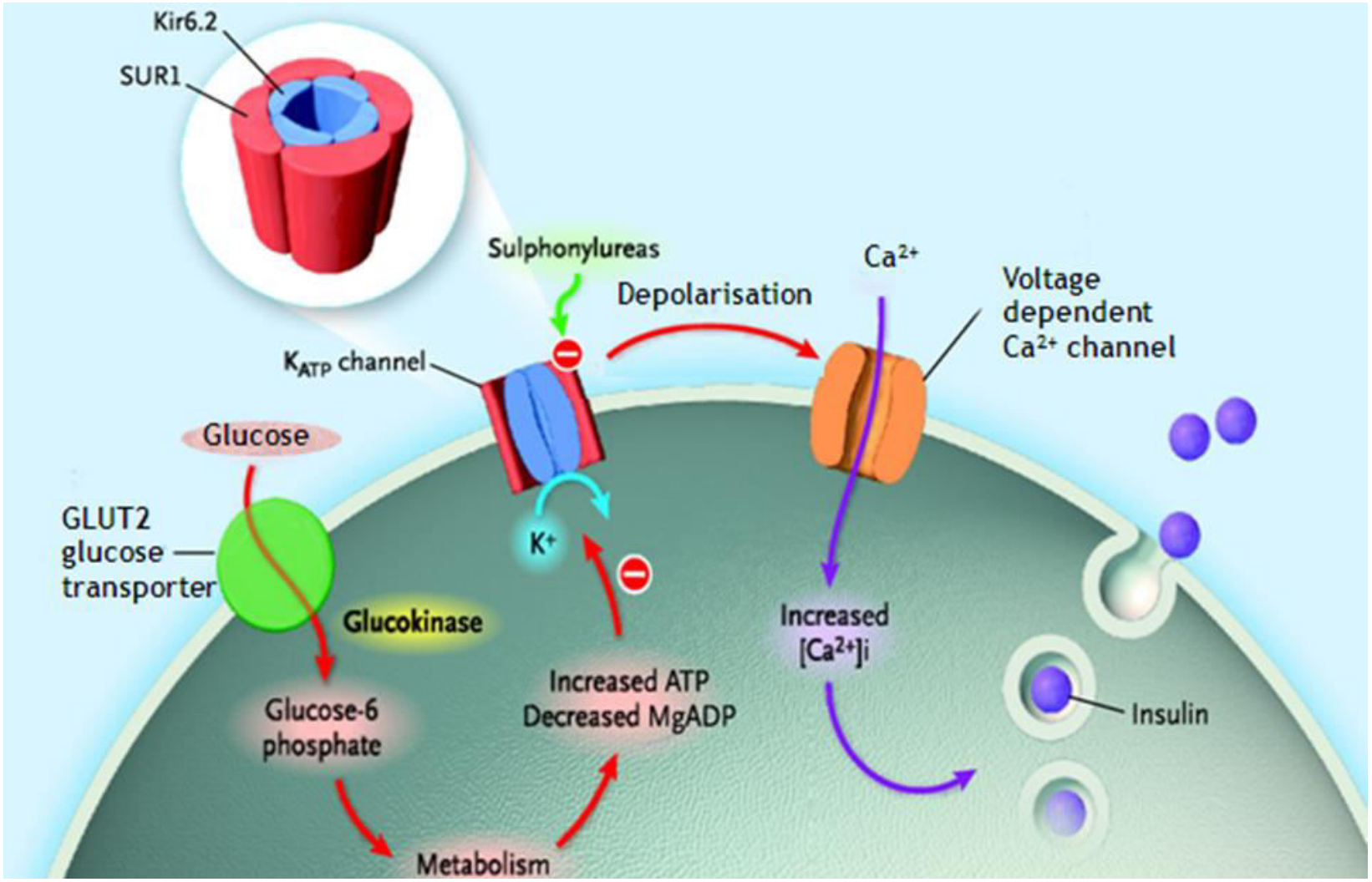


The *ABCC8* gene contains 39 exons and 38 introns

- 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)

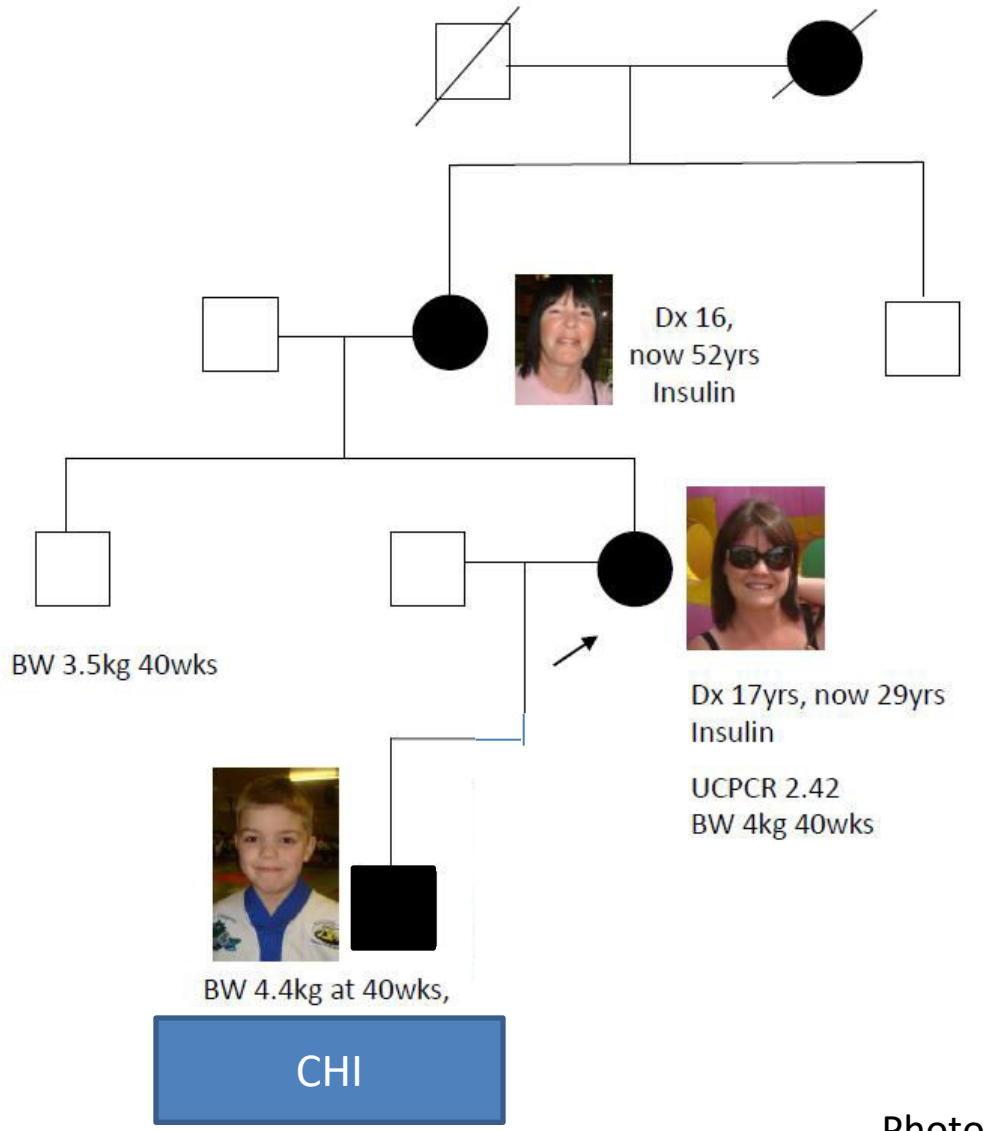
Changes in the *ABCC8* gene can cause the pancreatic beta cell to secrete too much insulin



Why is it important to understand the genetic cause of hyperinsulinism?

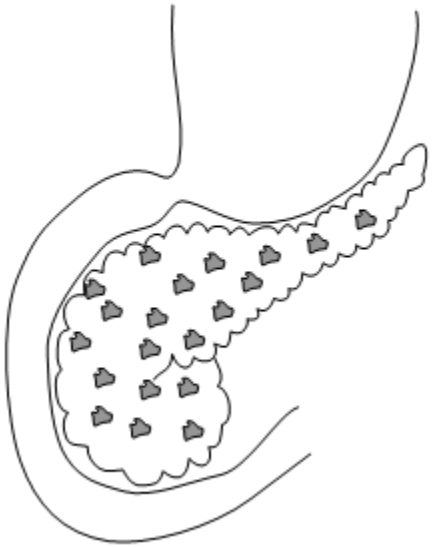
A genetic diagnosis can provide important information on treatment response and disease progression

HNF4A

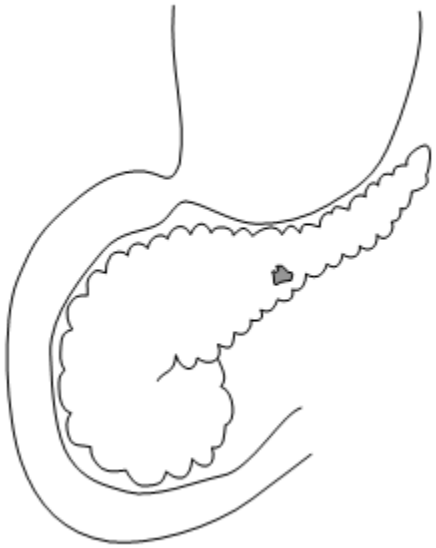


Photos shown with permission

Changes in the *ABCC8* and *KCNJ11* genes can cause focal or diffuse pancreatic disease



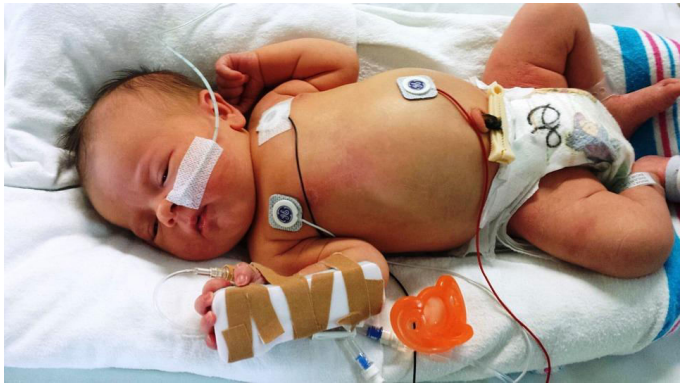
Diffuse disease (50%)



Focal disease (50%)

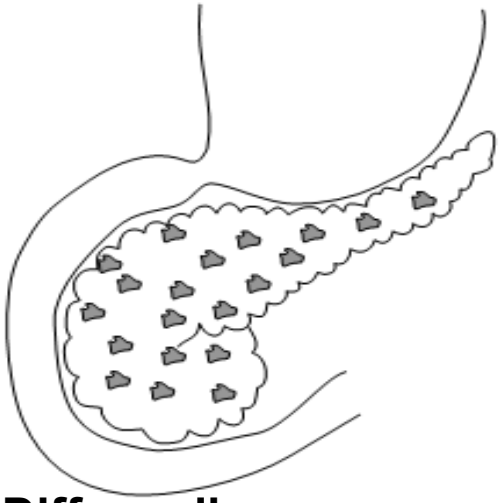
A genetic diagnosis guides treatment

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

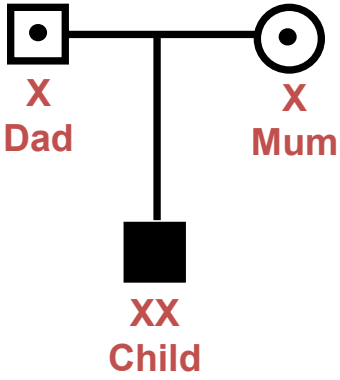


- Diagnosed at 1 day
- Diazoxide unresponsive
- Heterozygous *ABCC8* mutation
- Focal lesion confirmed by PET-CT scan
- Keyhole lesionectomy

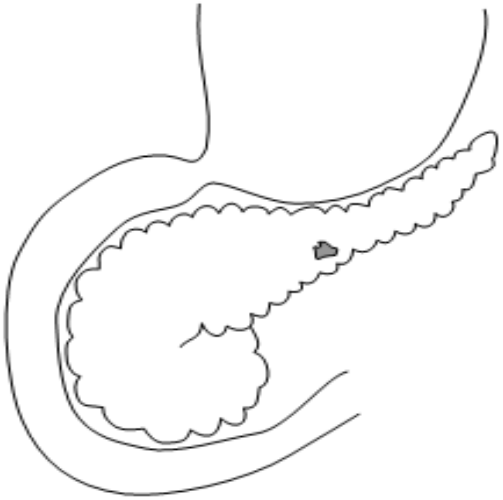
The amount of pancreas affected is determined by the genetics



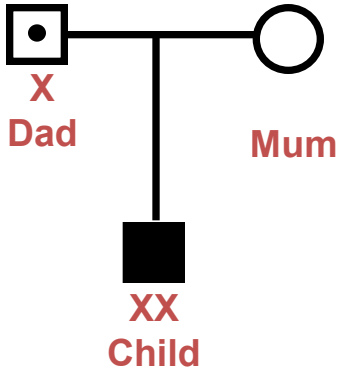
Diffuse disease

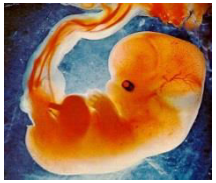


One change inherited from each parent



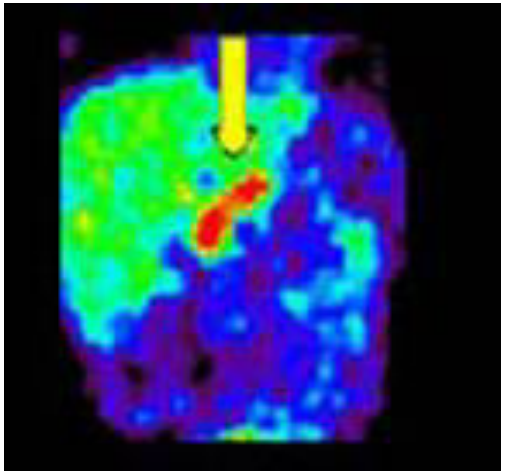
Focal disease



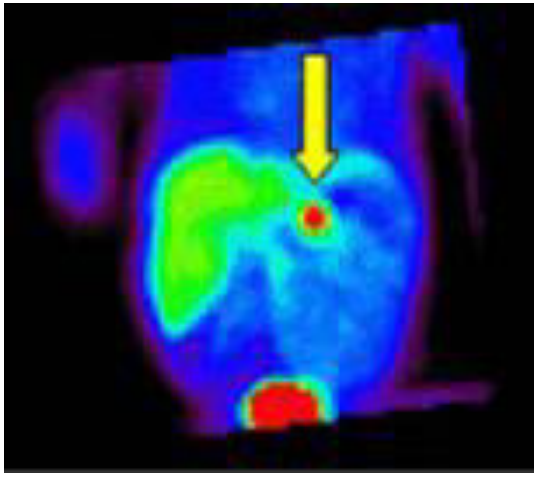


One change inherited from dad, one change occurring spontaneously

PET-CT scans confirm the genetic findings



Diffuse disease



Focal disease

Islets convert ^{18}F-L-dopa to dopamine by dopa decarboxylase

Ribeiro et al (2007) Eur J. Nucl Med Mol Imaging

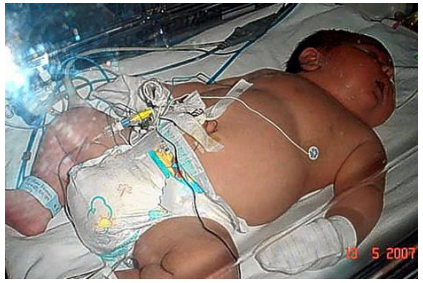
A genetic diagnosis also defines the risk for siblings and future offspring

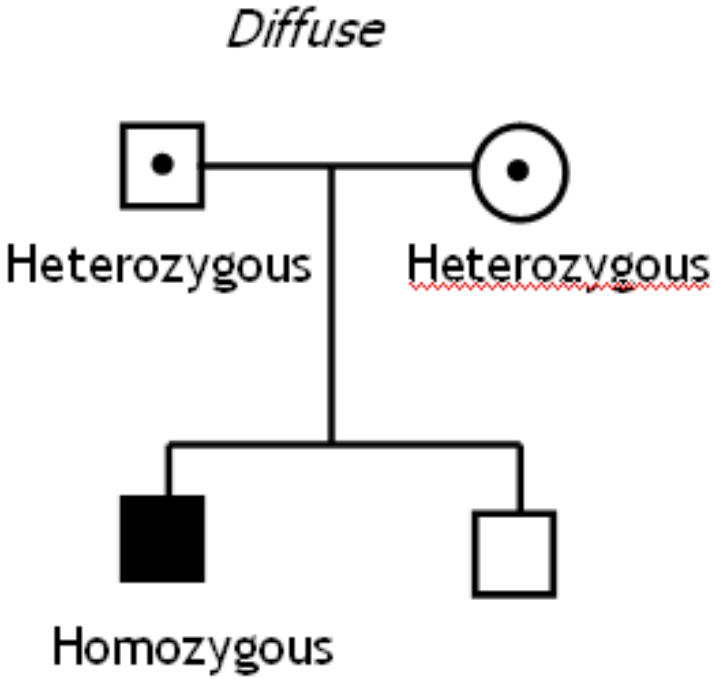
- Likelihood that another sibling will have CHI
- Risk for patient's future children of having CHI
- Allows for prenatal testing

Management post pregnancy

- Paediatrician at delivery
- Monitor baby for hypoglycaemia
- May need to start treatment

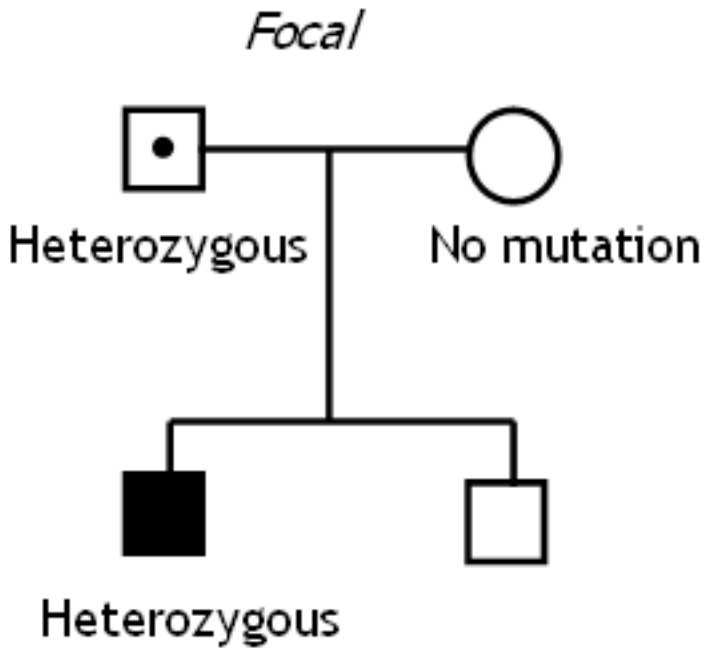
A genetic diagnosis defines the risk for siblings and future offspring





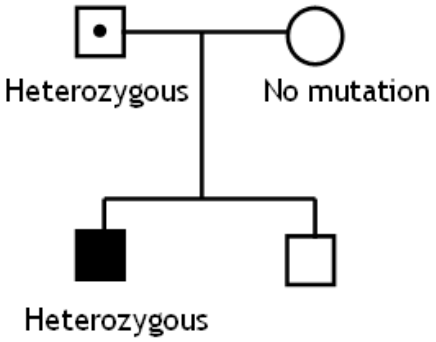
- Likelihood that his brother will have CHI?
- Risk for his children having CHI?
- Chance that his brother is a carrier?

A genetic diagnosis defines the risk for siblings and future offspring



- Risk that his brother will have CHI?
- Risk for his children having CHI?
- Chance that his brother is a carrier?

Focal hyperinsulinism is due to a paternal K_{ATP} mutation and somatic patUPD of 11p15



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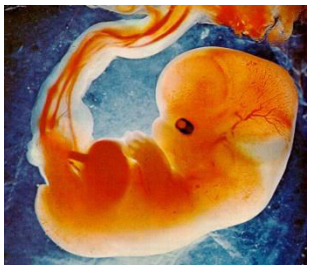
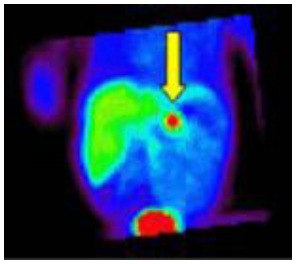
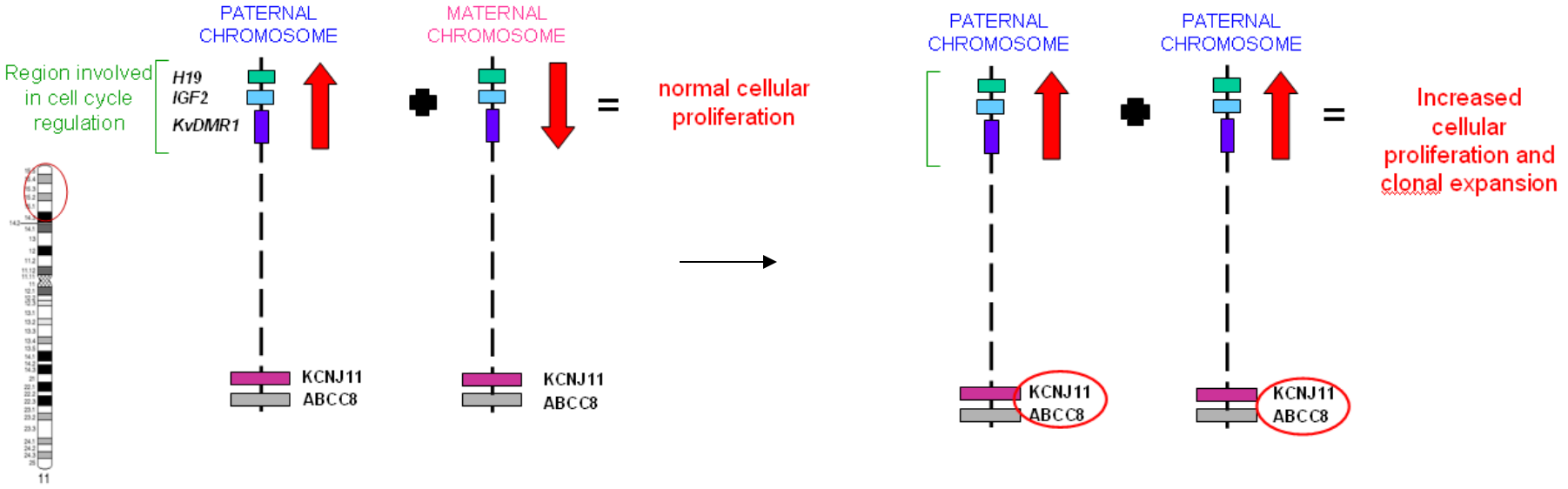


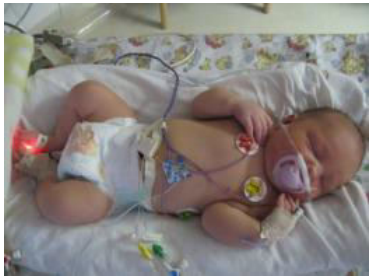
Paternal UPD in fetal pancreas (1/600 pregnancies)



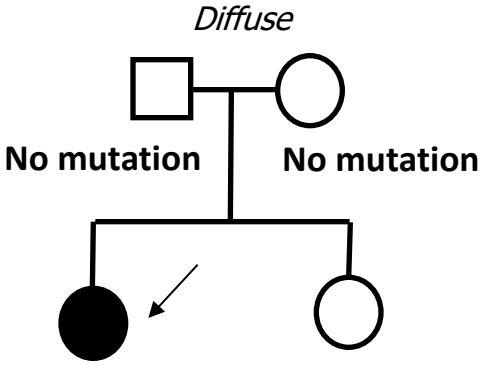
Focal lesion



A genetic diagnosis defines the risk for siblings and future offspring



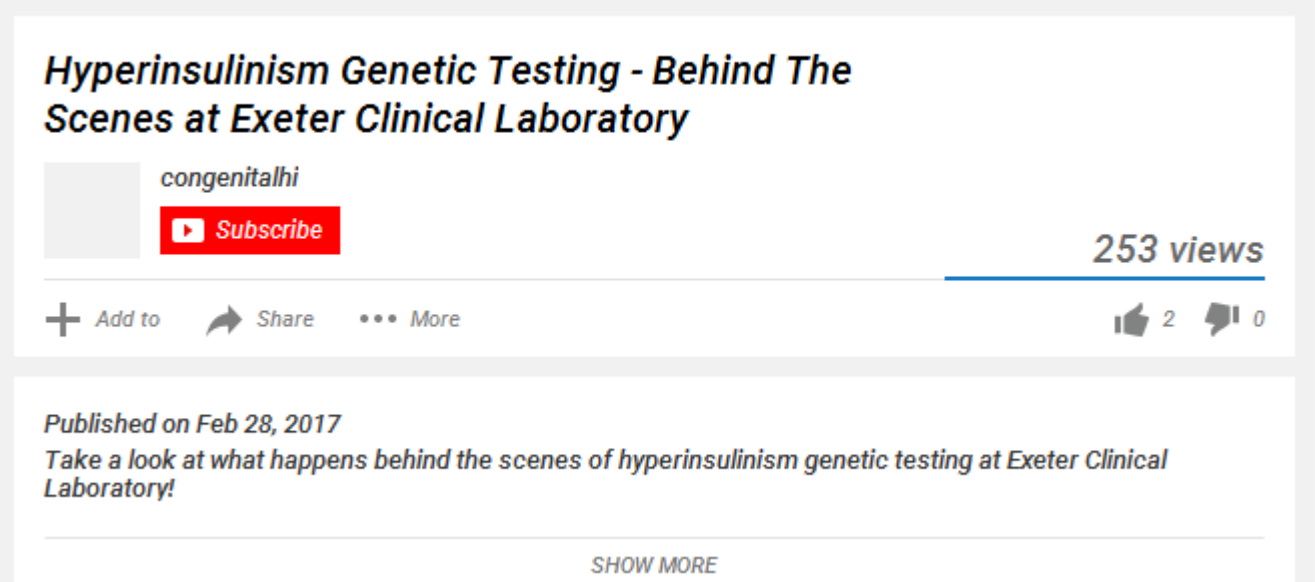
- *De novo* or “new” mutation
- Low risk for siblings (<5%)
- 50% risk for offspring



Heterozygous *ABCC8* missense mutation

How does the genetic testing work?

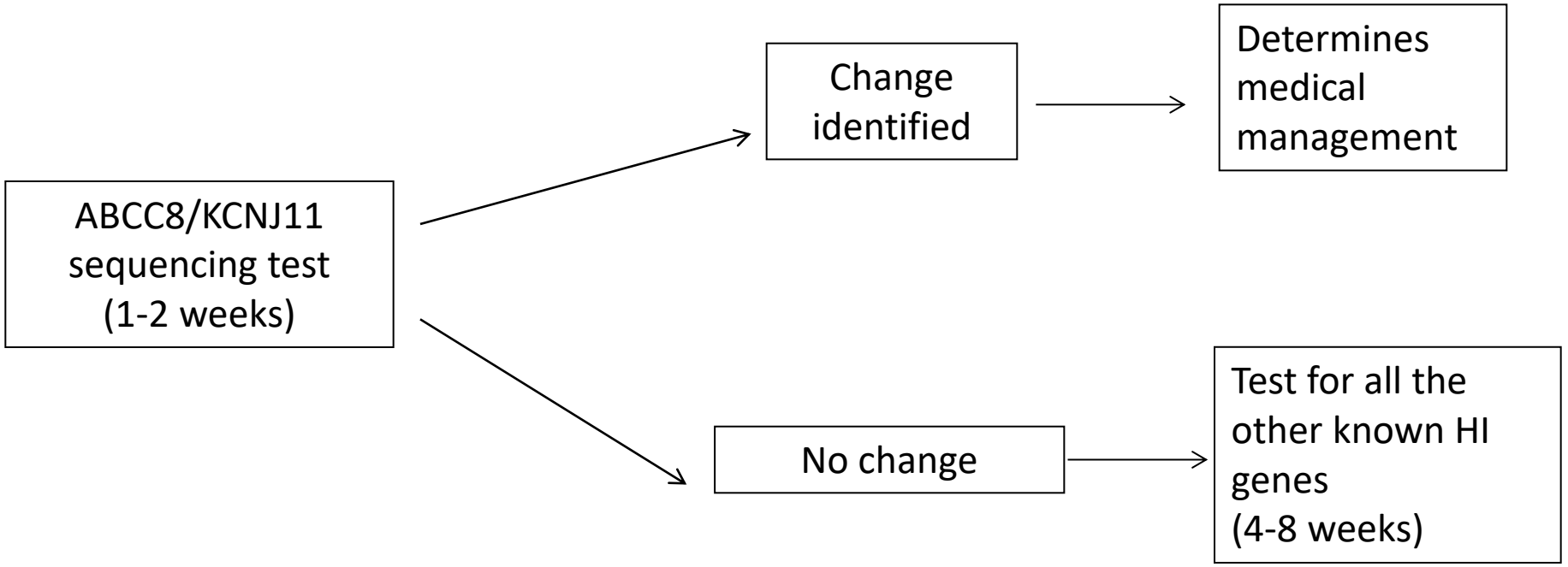
Behind the scenes in the Exeter Laboratory



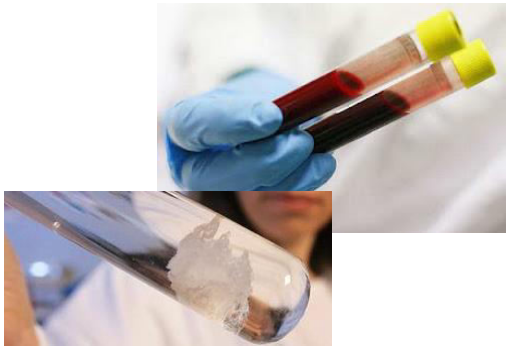
https://www.youtube.com/watch?v=EvZeCynYuYc

Congenital Hyperinsulinism International

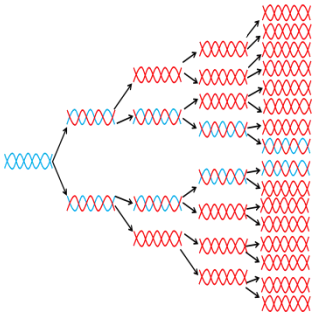
Aim: A **fast**, accurate and comprehensive genetic test for every patient



Rapid testing for the *ABCC8* and *KCNJ11* genes



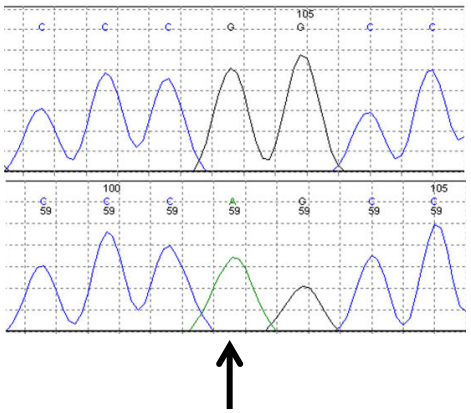
Extract DNA from blood



PCR amplify the coding regions of the *ABCC8* and *KCNJ11* genes

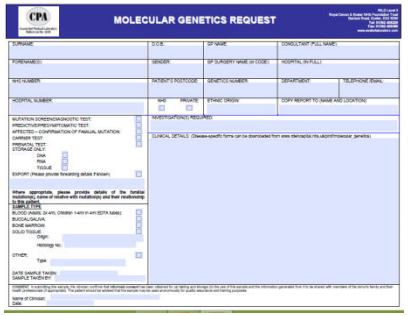
Reference

Patient



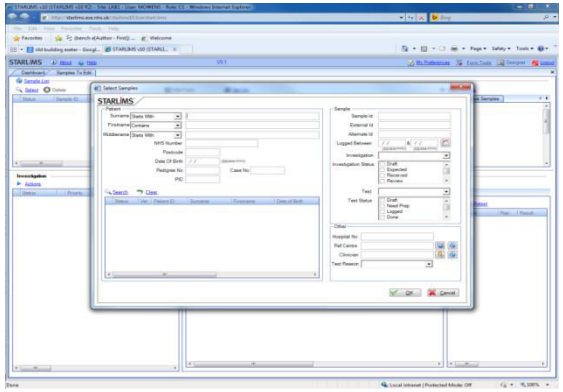
Read the sequence of the *ABCC8* and *KCNJ11* genes (11,000 bases)

Aim: A fast, **accurate** and comprehensive genetic test for every patient



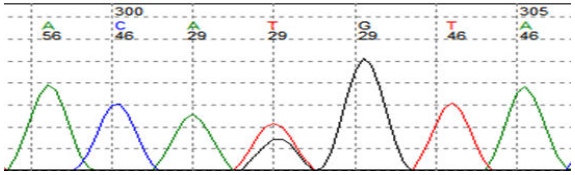
DNA extraction

Barcodes to label blood and DNA samples



Robotic DNA extraction, PCR and sequencing set-up

LIMS with automatic data upload from sequence analysis software



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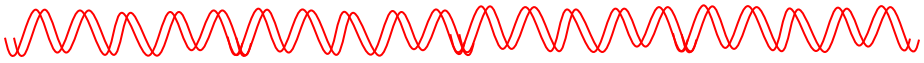
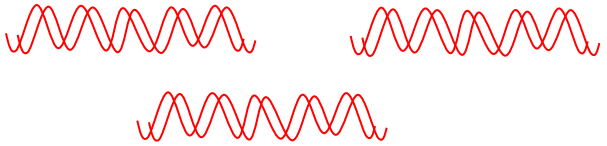
Next generation sequencing of all CHI genes

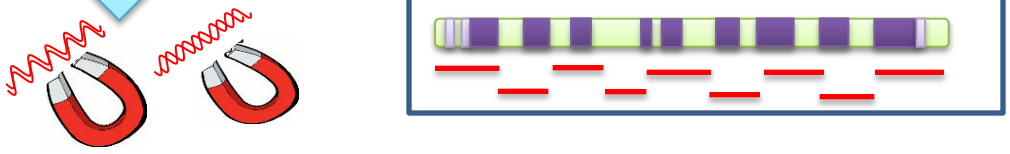


Patient DNA

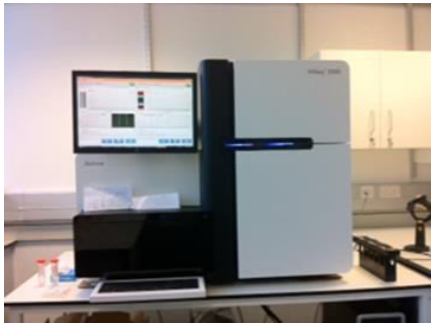
Fragment DNA

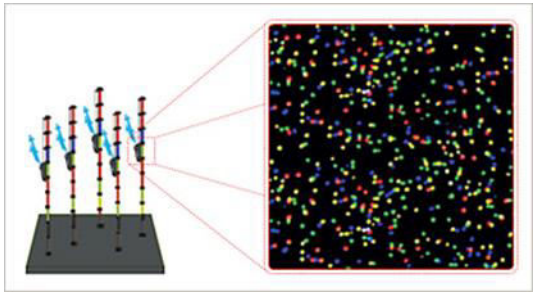


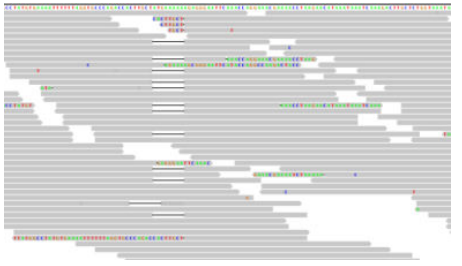
Capture coding regions of HI genes using RNA baits



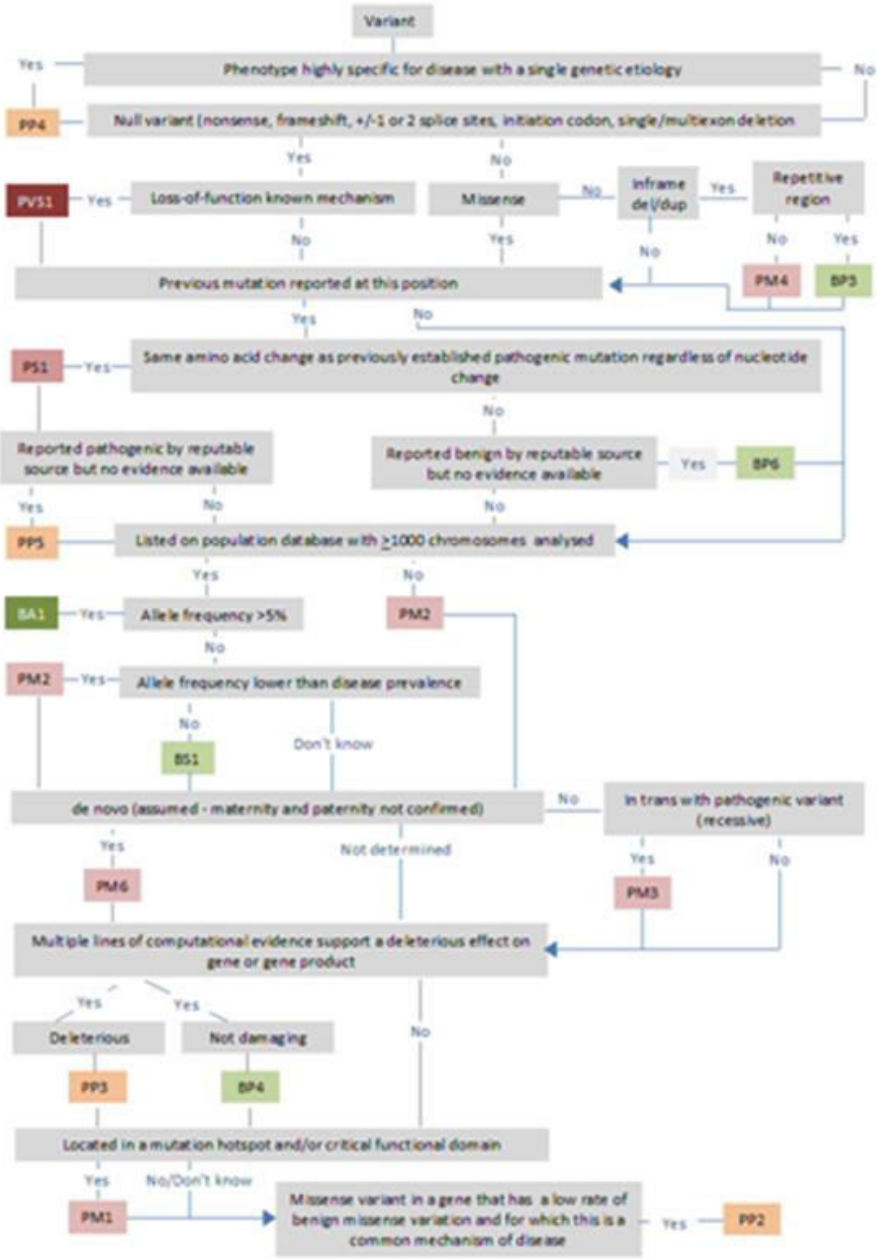
Sequence captured DNA using
Next Generation Sequencer







Interpreting genetic variants



A complex process!

Classify as:

- Pathogenic
- Likely pathogenic
- Uncertain significance
- Likely benign
- Benign

Difficulties in interpreting genetic variants

MUTATION UPDATE

Human Mutation

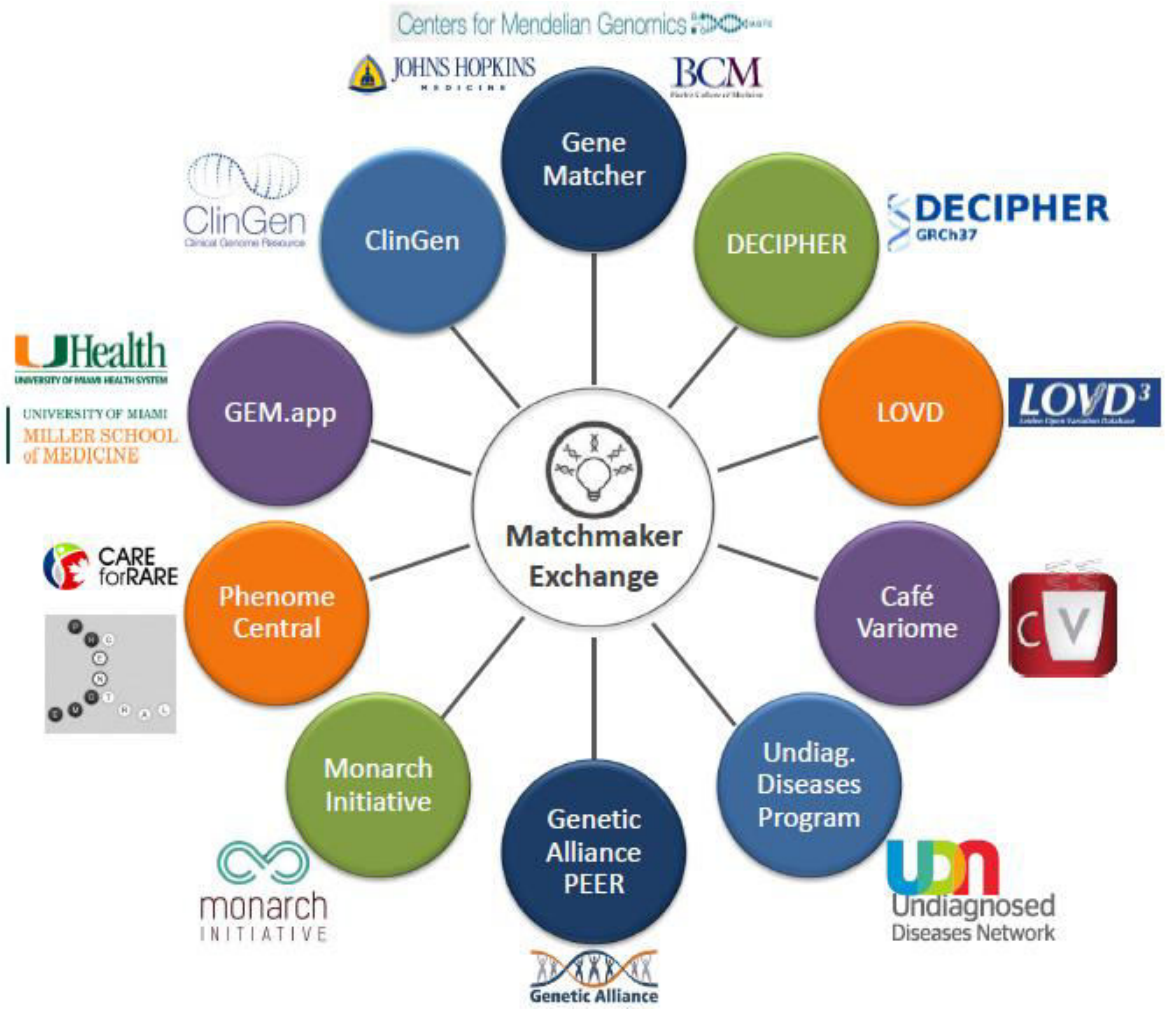
OFFICIAL JOURNAL HGVS HUMAN GENOME VARIATION SOCIETY www.hgvs.org

Update of Mutations in the Genes Encoding the Pancreatic Beta-Cell K_{ATP} Channel Subunits Kir6.2 (*KCNJ11*) and Sulfonylurea Receptor 1 (*ABCC8*) in Diabetes Mellitus and Hyperinsulinism

Sarah E. Flanagan,[1] Séverine Clauin,[2] Christine Bellanné-Chantelot,[2] Pascale de Lonlay,[3] Lorna W. Harries,[1] Anna L. Gloyn,[4] and Sian Ellard[1]*

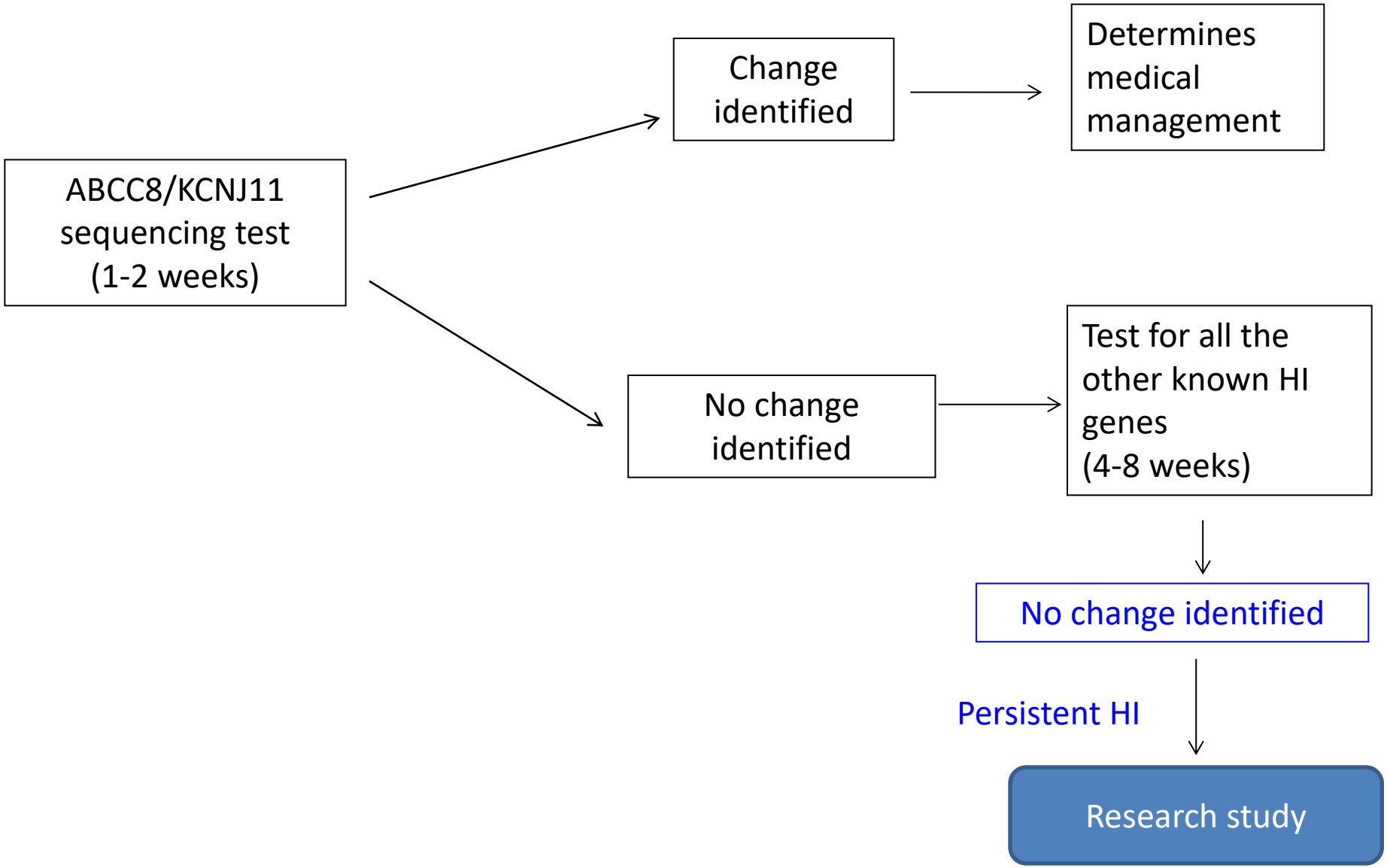
- 265 different mutations reported in patients with hyperinsulinism or neonatal diabetes (2008)
- Update in progress: anticipate nearly 1000 variants (2018)

Global sharing of variant data across countries

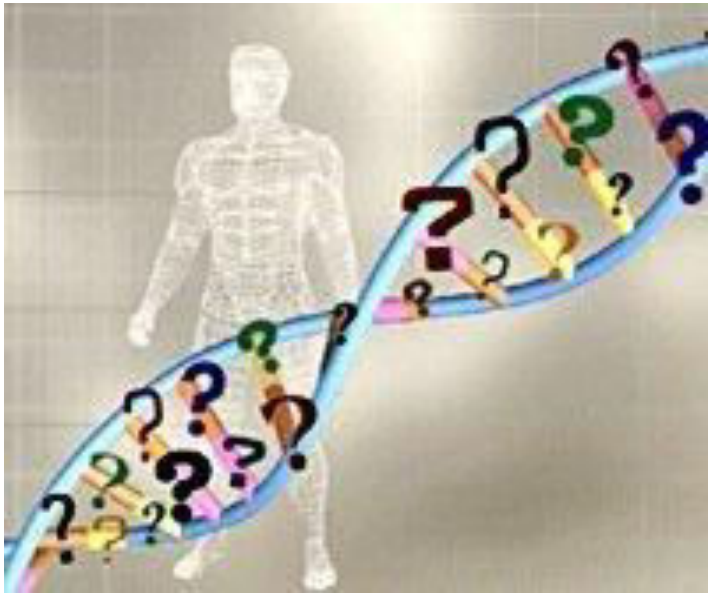


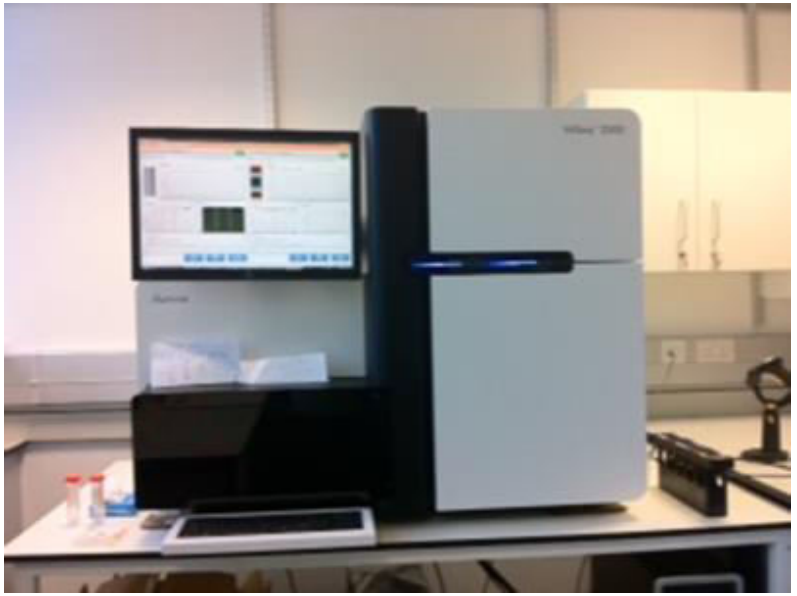
CHI Congenital Hyperinsulinism International

Aim: A fast, accurate and **comprehensive** genetic test for every patient



Genome sequencing to find new hyperinsulinism genes



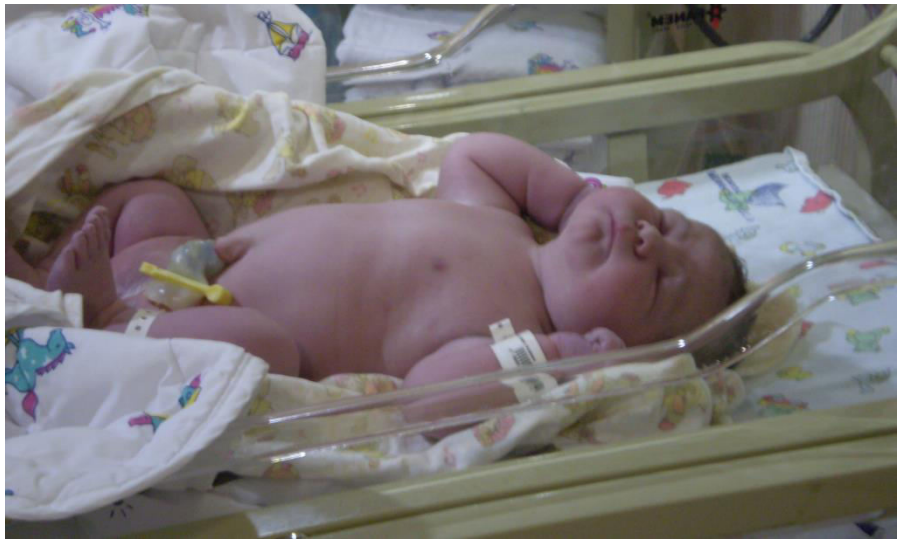


Sequence 3 billon base pairs (incl. ~20,000 genes) simultaneously instead of one at a time

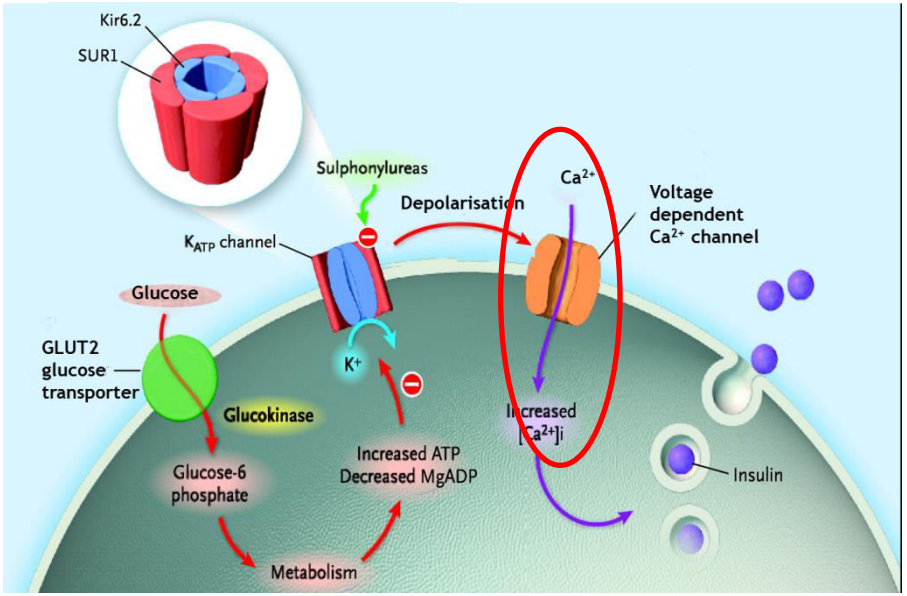
Change in a calcium channel gene found to be the cause of hyperinsulinism in a patient from Brazil

Baby M

- 4.5Kg (37/40)
- Hyperinsulinaemic hypoglycaemia at birth
- Diazoxide (13mg/kg/day)
- Heart problems
- Developmental delay



Birth



3 yrs

6 yrs

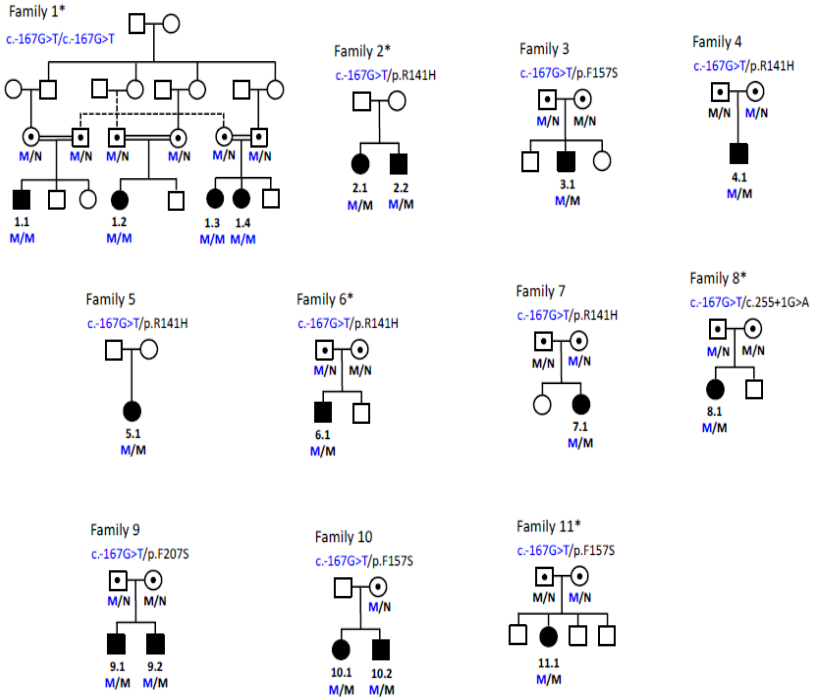
Photographs shown with permission courtesy of Dr Filippo Vairo, Brazil

Changes in the *PMM2* gene identified in 17 children with congenital hyperinsulinsim

CLINICAL RESEARCH www.jasn.org

Polycystic Kidney Disease with Hyperinsulinemic Hypoglycemia Caused by a Promoter Mutation in Phosphomannomutase 2

Oscar Rubio Cabezas,* Sarah E. Flanagan,† Horia Stanescu,‡ Elena García-Martínez,§ Richard Caswell,† Hana Lango-Allen,† Montserrat Antón-Gamero,§ Jesús Argente,*‖¶** Anna-Marie Bussell,† Andre Brandli,†† Chris Cheshire,‡ Elizabeth Crowne,‡‡ Simona Dumitriu,‡ Robert Drynda,§§ Julian P Hamilton-Shield,‡‡ Wesley Hayes,‖ Alexis Hofherr,¶¶ Daniela Iancu,‡ Naomi Issler,‡ Craig Jefferies,*** Peter Jones,§§ Matthew Johnson,† Anne Kesselheim,‡ Enriko Klootwijk,‡ Michael Koettgen,¶¶ Wendy Lewis,††† José María Martos,‡‡‡ Monika Mozere,‡ Jill Norman,‡ Vaksha Patel,‡ Andrew Parrish,† Celia Pérez-Cerdá,§§§ Jesús Pozo,* Sofia A Rahman,‖‖ Neil Sebire,‖‖ ‖‖ Mehmet Tekman,‡ Peter D. Turnpenny,¶¶¶ William van't Hoff,‖ Daan H.H.M. Viering,‡ Michael N. Weedon,† Patricia Wilson,‡ Lisa Guay-Woodford,**** Robert Kleta,‡‖‖ ‖‖ Khalid Hussain,‖‖†††† Sian Ellard,† and Detlef Bockenhauer‡‖‖ ‖‖

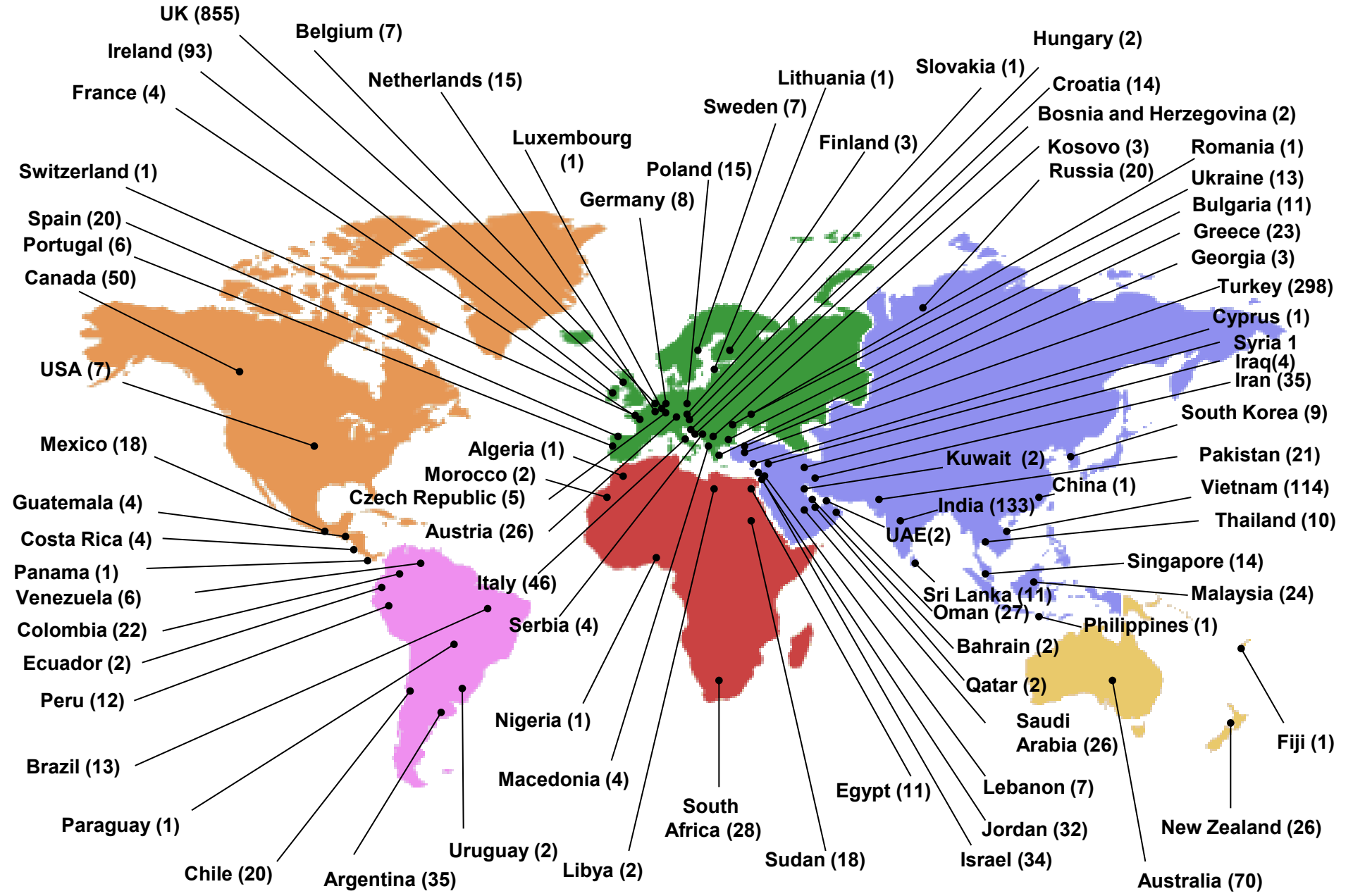


February 2017

Summary

- Congenital hyperinsulinism can be caused by changes in many different genes
- It is important to find the genetic cause in newly diagnosed patients as this can guide clinical management
- A genetic diagnosis is possible for about 50% of patients
- Research is underway to try and find answers for the remainder of patients

Thank you!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Belgium (7)
Ireland (93)
Netherlands (15)
France (4)
Lithuania (1)
Slovakia (1)
Hungary (2)
Sweden (7)
Croatia (14)
Bosnia and Herzegovina (2)
Luxembourg (1)
Finland (3)
Kosovo (3)
Romania (1)
Switzerland (1)
Poland (15)
Russia (20)
Ukraine (13)
Germany (8)
Bulgaria (11)
Spain (20)
Greece (23)
Portugal (6)
Georgia (3)
Canada (50)
Turkey (298)
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Iraq(4)
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South Korea (9)
Mexico (18)
Algeria (1)
Kuwait (2)
Pakistan (21)
Morocco (2)
China (1)
Guatemala (4)
Czech Republic (5)
India (133)
Vietnam (114)
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UAE(2)
Thailand (10)
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Sri Lanka (11)
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Oman (27)
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Bahrain (2)
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Qatar (2)
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Nigeria (1)
Saudi Arabia (26)
Fiji (1)
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