

Recent International Research and Investigative Projects



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**Congenital Hypoglycemia Disorders: Hyperinsulinism and GSD, Ruth and Tristram Colket, Jr.
Translational Research Building at, The Children's Hospital of Philadelphia, April 14 and 15, 2016**



Aims

- ☐ **Overview of research at University College London/Exeter/Great Ormond Street Children's Hospital**

Genetics of HH

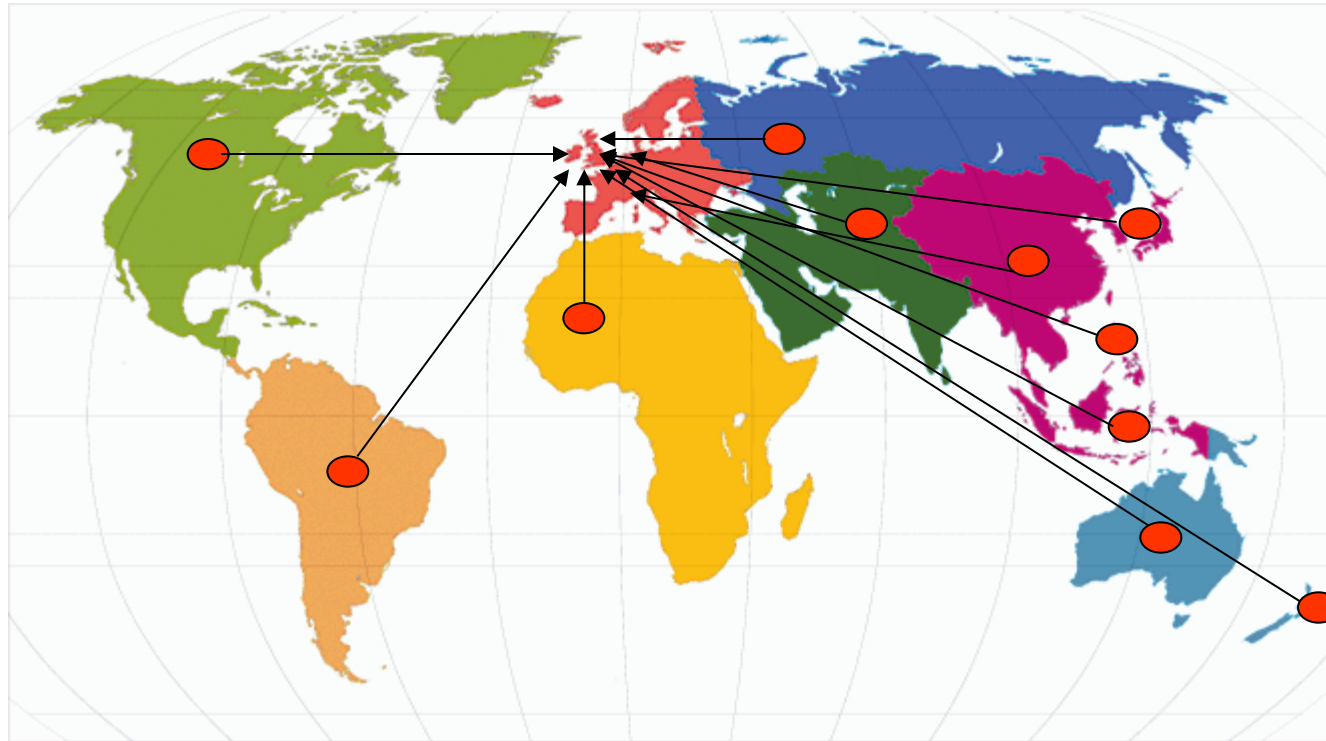
- ❑ Research collaboration between UCL/GOSH and Exeter
- ❑ Funded by The Medical Research Council
- ❑ Trying to understand the genetic basis of HH

**Professor
Sian Ellard**



**Dr. Sarah
Flanagan**

Genetics of HH



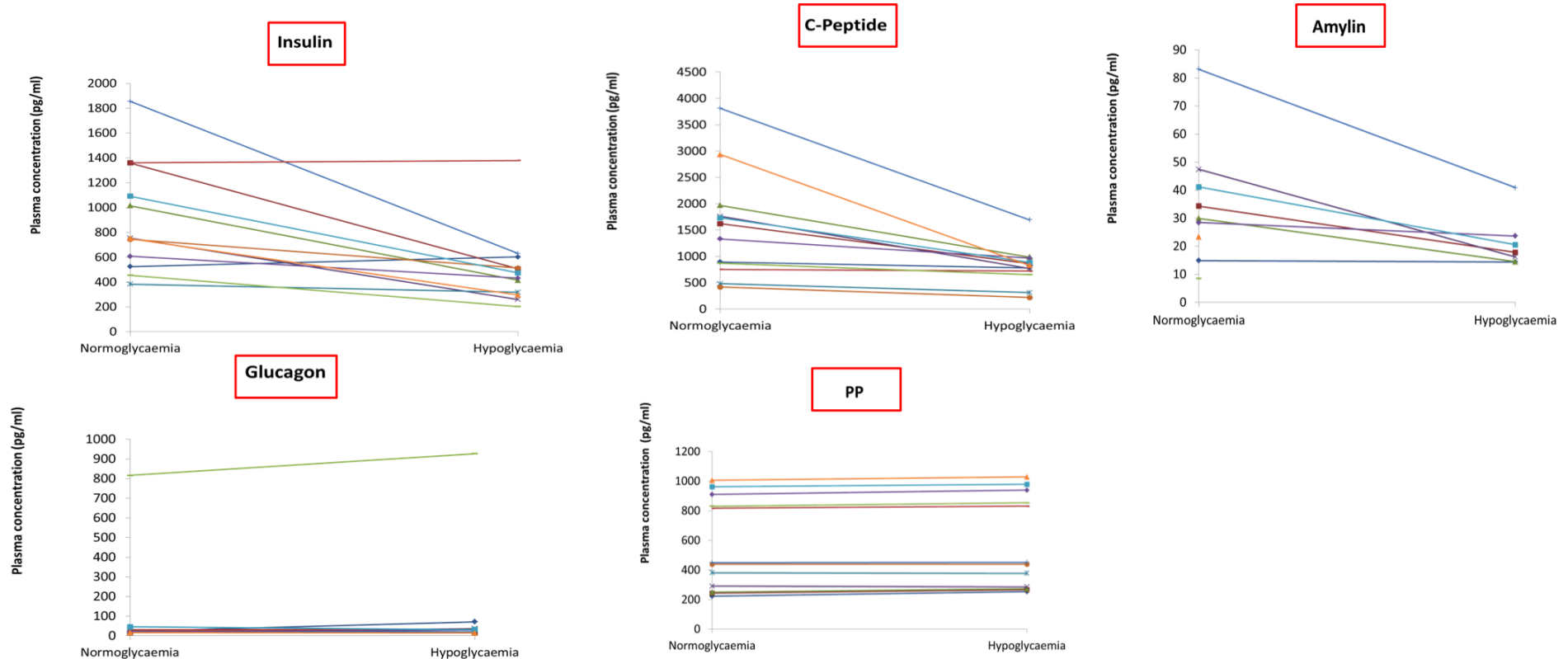
> 1500 DNA samples collected from all over the world for genetic testing

Investigating the role of pancreatic hormones **Insulin, Glucagon, Amylin, Pancreatic Polypeptide** in children with HH

Dr. Maria Güemes Hidalgo

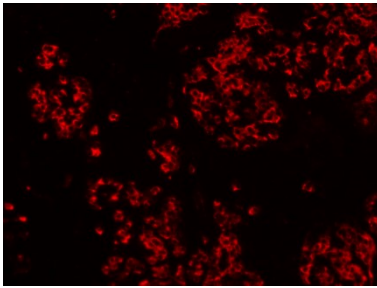
Pancreatic Hormones

1. Determining **hormone concentrations** in **fasting and feeding** state in **healthy** and **hypoglycaemic** children

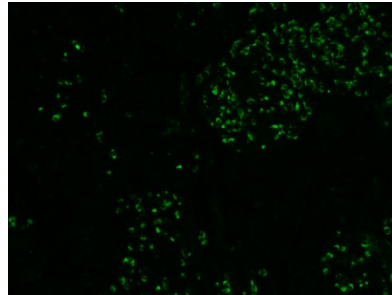


Pancreatic Hormones

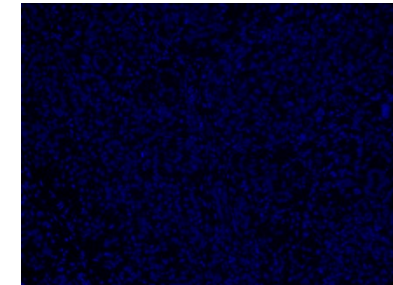
2. **Localisation and interrelationship** between hormones in normal, foetal and CHI (focal and diffuse) **pancreatic tissue** [IHC].
Correlation with clinical phenotype



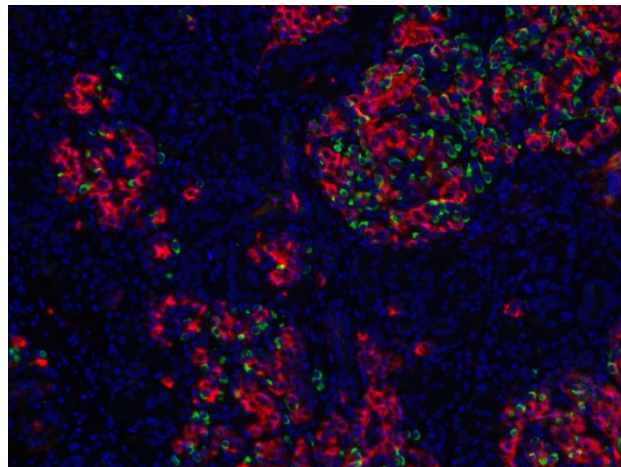
Primary antibody for Insulin (mouse, 1:100). Secondary antibody anti-mouse 595nm (red)



Primary antibody for Glucagon (rabbit, 1:50). Secondary antibody anti-rabbit 488nm (green)



DAPI staining of nuclei



There is no apparent co-localisation of glucagon and insulin

Note: Images correspond to human pancreatic section of **focal** CHI.
Picture augmentation: x10

Pancreatic Hormones

3. **qRT-PCR in pancreas** (focal disease, diffuse disease and in foetal tissue) to **quantify the expression** of these hormones. Correlation with clinical phenotype
4. **Cases** of abnormalities in glucose homeostasis to identify **novel pancreatic hormone pathways**

Future work

5. **Cell work** to characterise **amylin** and **PP** intracellular **pathways** in pancreatic tissue

Using iPSC to Understand CHI

Dr. Azizun Nessa

Using iPSC to Understand CHI

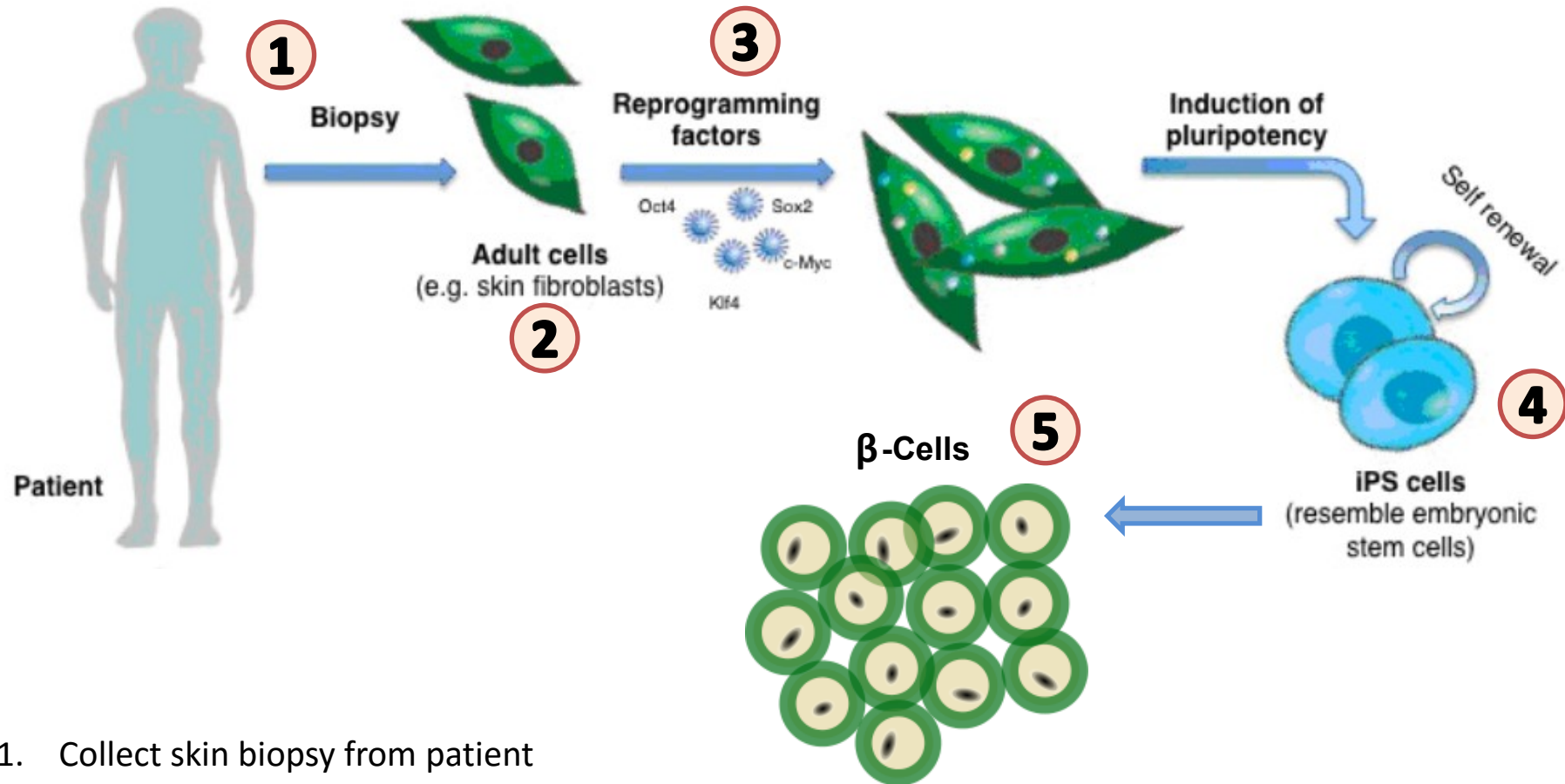
What are induced pluripotent stem cells (iPSC)?

- Induced pluripotent stem cells (iPSC) are a completely new method of trying to model a disease process and understanding the pathophysiology of a disease (Takahashi K, Nat Protoc. 2007).
- iPSC are derived from skin or blood cells that have been reprogrammed back into an embryonic-like pluripotent state that enables the development of an unlimited source of any type of human cell needed for therapeutic purposes.
- For example, iPSC can be manipulated into becoming beta islet cells to treat diabetes mellitus (Pagliuca et al., Cell 2014; Rezania A et al., Nature biotech 2014), blood cells to create new blood free of cancer cells for a leukaemia patient, or neurons to treat neurological disorders.

Aim

The aim of this pilot project is to develop iPSC derived β -cells from patients with CHI to understand the molecular basis of unregulated insulin secretion.

Method



1. Collect skin biopsy from patient
2. In the lab grow fibroblasts from the skin biopsy
3. Add special reprogramming factors to the fibroblasts
4. Reprogramming factors transform the fibroblasts into iPS cells
5. iPS cells can now be used to generate β -cells

Clinical Implications

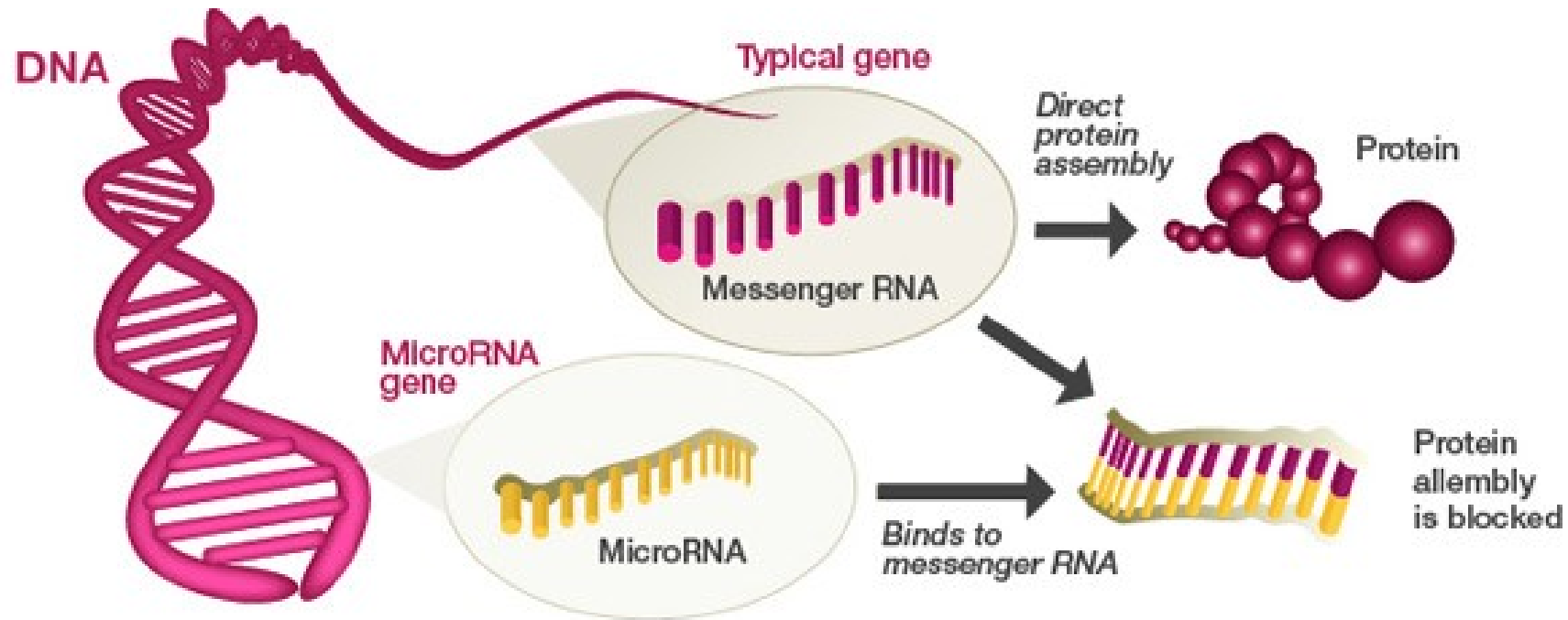
If our research proposal is successful then it will make a difference in the following ways:

- Give new insights into the normal and abnormal physiology of insulin secretion
- This will have major implications for more common diseases such as diabetes mellitus
- Allow a genetic diagnosis and an understanding of the cause of the child's hyperinsulinism
- Allow a prenatal diagnosis
- Develop new medical treatments
- Avoid a major operation in the form of a near total pancreatectomy

Understanding the Role of MicroRNAs in Insulin Regulation

Miss Bonita Cumming

What is microRNA



Role of microRNA



- MicroRNAs (miRNAs) are small RNA molecules found within our genomes. These are non-coding RNAs, i.e. they do not make proteins.
- miRNAs function to regulate the expression of specific protein-coding genes by targeting their messenger RNAs. They either directly inhibit translation, or mark them for degradation by other regulatory components in the cells.
- Since their discovery in 1993 these molecules have been implicated in various diseases including Alzheimer's, Parkinson's and several forms of cancer.

Rationale: Why Focus on MicroRNAs?



- Several genes have been linked to CHI. These genes make proteins that function in the regulation of insulin secretion
- Approximately 60% of CHI patients have no genetic diagnosis, and no disease-causing mutations have been identified within CHI-associated protein-coding genes.
- The aim of this research is to determine the expression of mRNA in tissue of CHI patients

Clinical Implications

- Identifying the expression of microRNAs, in pancreatic tissue will help us to understand if microRNA plays a role in insulin secretion in CHI patients.
- This might help us to develop more effective treatment methods for patients without genetic diagnoses.

Using CRISPR-Cas9 to understand the molecular mechanisms of CHI



Dr. Preetha Purushothaman

Using CRISPR-Cas9 to understand the molecular mechanisms of CHI



What is the CRISPR-Cas9 system?

- CRISPR stands for:
- Clustered regularly interspaced short palindromic repeats
- Microbial adaptive immune system
- Evolved to adapt and defend against foreign genetic material
- Several types of CRISPR systems identified in microbes.
- Type II CRISPR-Cas 9 system facilitates RNA-guided site-specific DNA cleavage
- Efficient and easy genome editing tool

Aim



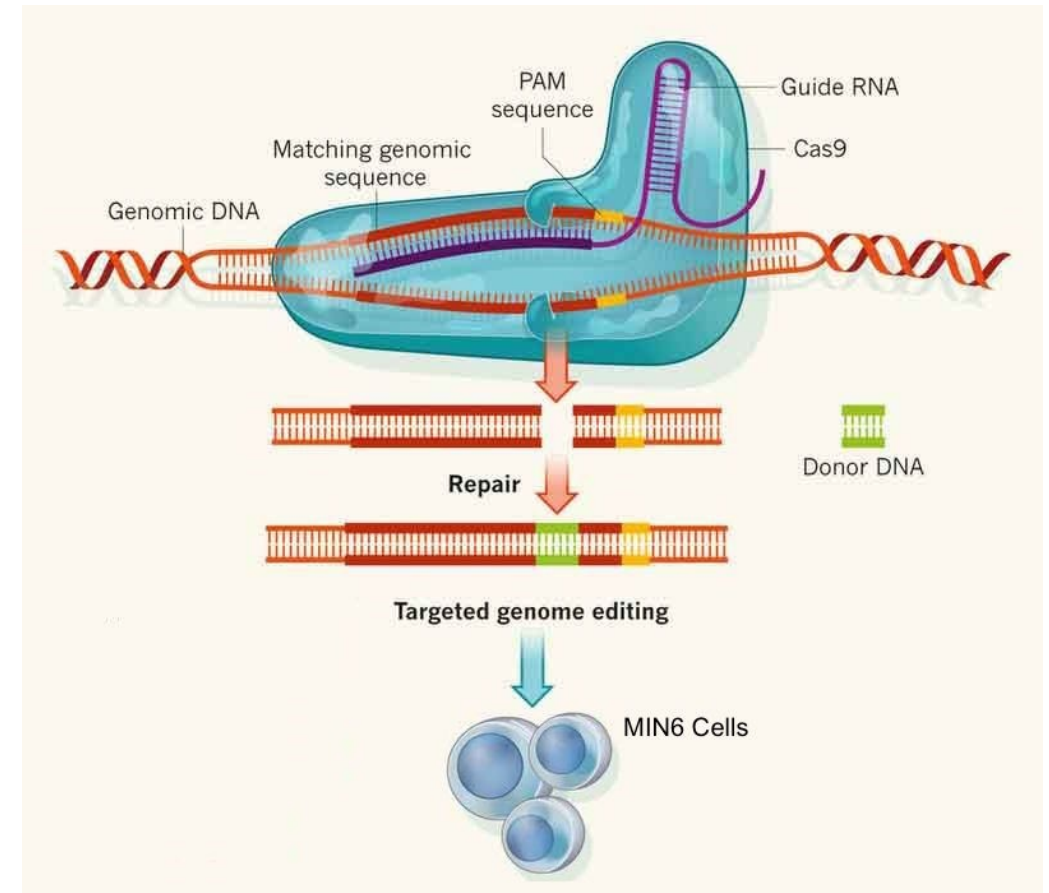
Use CRISPR-Cas9 technology to

- **Generate Knock Out (KO) models of CHI**
- **Specific mutations in known CHI genes**

Cultured cell lines will be used as a basic model of insulin secretion. This will allow us to enhance our existing knowledge of the molecular basis of unregulated insulin secretion.

Methods

- 1) Design guide RNA
- 2) Deliver sg RNA + Cas9 to cells
- 3) Complementary oligos synthesized
- 4) Cas9 creates double stranded DNA breaks
- 5) Cells have 2 repair pathways
 - a) Non homologous end joining (error prone)
 - b) Homologous recomb (error free, perfect repair)
6. Clone validation using PCR



Applications of CRISPR



- **Novel method to provide insight into the physiology of insulin secretion.**
- **Aid in identification of new genetic causes linked to CHI.**
- **May provide a platform for the development of novel therapies for children with CHI.**

Physiology and Pharmacology of Glucagon and Somatostatin in CHI



Dr Pratik Shah
Clinical Research Fellow

Long acting Somatostatin analogue (Lanreotide) in CHI



- To look at pharmacokinetics of Long Acting Somatostatin Analogue (Lanreotide) therapy in Hyperinsulinaemic Hypoglycaemia (HH)
- To understand Lanreotide molecular action via somatostatin receptors by Immunohistochemistry - to determine somatostatin receptors and glucagon receptor expression on the diffuse and focal islets (on those children who had previous pancreatectomy and treated with Lanreotide).

Understanding Pharmacokinetics of intravenous Glucagon



- Understanding Pharmacokinetics of intravenous Glucagon in children with Hyperinsulinaemic Hypoglycaemia
- Use of different doses of intravenous Glucagon and their glycaemic response

Measurement of pancreatic hormones Glucagon and Somatostatin



- Measurement of pancreatic hormones - Glucagon and Somatostatin in children with HH at the time of normoglycaemia and hypoglycaemia.
- To understand the pancreatic glucagon and Somatostatin secretion in HH and its role in glucose regulation

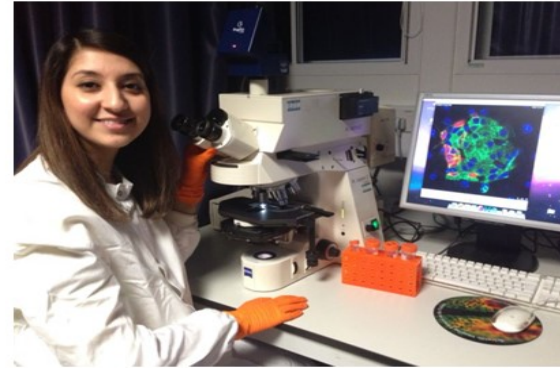
Acknowledgment: Colleagues



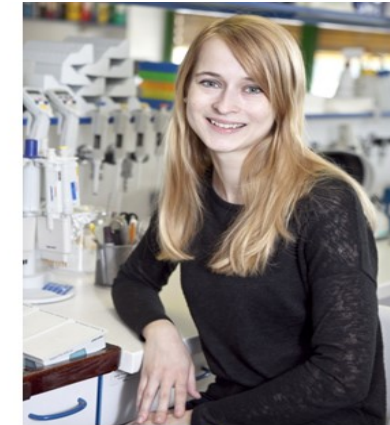
Azizun



Pratik



Sofia



Klara



Georgia



Maria



Malissa



Bonita

Thank you