The role of HNF1B in human pancreas development and diabetes
Ranna El-Khairi1,2, Andrew Hattersley5, Ludovic Vallier1,2,3
Wellcome Trust: MRC Cambridge Stem Cell Institute, University of Cambridge, UK
Wellcome Trust Sanger Institute, Hinxton, UK
Department of Surgery, University of Cambridge, UK
Institute of Biomedical and Clinical Science, University of Exeter Medical School, UK

Introduction
- Differentiation of human pluripotent stem cells (PSCs) along the pancreatic and hepatic lineages presents a unique tool to explore the roles of specific genes in human pancreas and liver development and function.
- Disease-specific PSCs can be used as an in vitro platform for identification of new genes and pathways that contribute to diabetes pathogenesis and identification of novel therapeutic targets.

HNF1B and endoderm development
- HNF1B → Homeodomain-containing transcription factor.
- Widely distributed in embryonic tissues – kidney, pancreas, liver, biliary tract, genital-urinary tract...
- Expressed in early embryonic development at anterior definitive endoderm stage.
- Key member of network of transcription factors controlling differentiation of the pancreatic progenitor cells that assemble the exocrine and endocrine pancreases.

HNF1B gene mutation phenotypes
- In mice: Heterozygous mutations → no phenotype
- Homozygous mutations → embryonic lethal
- In humans: Heterozygous mutations → HNF1B-associated disease / RCAD / MODY5

Clinical Phenotype includes:
- Diabetes: β-cell dysfunction, hepatic insulin resistance.
- Pancreatic hypoplasia, exocrine insufficiency.
- Liver dysfunction and cholestasis.
- Renal developmental disease and genitourinary abnormalities.

Aims and objectives
1) To establish an in vitro model of HNF1B-associated diabetes
   a) Derivation of HNF1B mutant human PSC lines
      - HNF1B knock out NEES lines using CRISPR-Cas9 nucleae system
      - PSCs from patients with HNF1B mutations
   b) Pancreatic and hepatic differentiation and functional characterisation of HNF1B mutant cell lines.
2) To determine the molecular mechanisms by which HNF1B gene mutations cause pancreatic hypoplasia and diabetes.

Methods
CRISPR-Cas9 assisted bi-allelic targeting in human PSCs

![Image of PCR screening of targeted clones. Example of a homozygous clone with insertion of puromycin and neomycin resistance markers highlighted in yellow boxes.]

Collection of patient samples
- Human Induced Pluripotent Stem Cells Initiative (HiPSC) → UK national iPSC resource funded by the Wellcome Trust and MRC.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Condition</th>
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</table>

Table: Skin biopsy samples collected from patients with monogenic forms of diabetes.

Results
- Homozygous knockout of HNF1B results in failure of pancreatic endoderm development in vitro.

- CRISPR-Cas9 knockout human iPSCs
  - mRNA expression of pluripotency (OCT4), definitive endoderm (SOX17), dorsal foregut (HNF1B, FOXA2), pancreatic progenitor cell (PDX1, HNF6) markers in a human iPSC line chemically induced to undergo pancreatic differentiation, assessed by qRT-PCR in undifferentiated (UD) PSCs and at Day 3, 6, 9, 12 of differentiation. All genes were normalised to the housekeeping gene, PEG6. Bars represent mean ± standard error of the mean (SEM).

Conclusions
- HNF1B has an essential role in pancreatic progenitor cell specification.
- Genetically engineered PSCs and iPSCs derived from patients with known genetic defects can be used to generate in vitro models of human disease.
- Targeted differentiation to a relevant mature cell type can be used to study the functional molecular and cellular consequences of a defined genetic defect.

![Image of flow cytometry analyses showing expression of SOX17 in definitive endoderm cells (Day 3) and PDX1 in pancreatic progenitor cells (Day 12).]