Characterization of a Type 2 diabetes-associated islet-specific enhancer cluster in *STARD10* by genome editing of EndoC-βH1 cells

Ming Hu[1], Paul J Gadue[2] and Guy A. Rutter[1]

[1]Section of Cell Biology and Functional Genomics, Department of Medicine, Imperial College London, London W12 0NN, United Kingdom.

[2]Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia; and Center for Cellular and Molecular Therapeutics, Children's Hospital of Philadelphia, PA. United States

Background: Genome-wide association studies (GWAS) have identified more than 100 genetic loci associated with type 2 diabetes. The majority of these are located in the intergenic or intragenic regions suggesting that the implicated variants may alter chromatin conformation. This, in turn, is likely to influence the expression of nearby or more remotely located genes to alter beta cell function. At present, however, detailed molecular and functional analyses are still lacking for most of these variants. We recently analysed one of these loci and mapped five causal variants in an islet-specific enhancer cluster within the *STARD10* gene locus. Here, we aimed to understand how these causal variants influence β-cell function by alteration of the chromatin structure of enhancer cluster.

GWAS studies identified 5 causal variants at *STARD10* gene locus

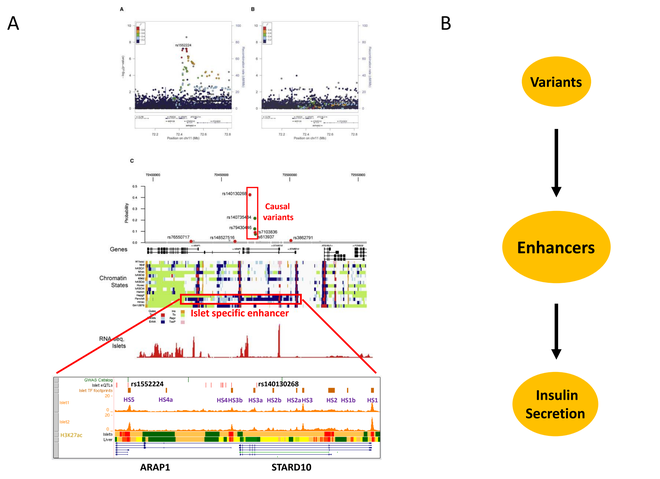


Fig.1 A. Variants with highest causal probabilities fall in a stretch-enhancer region (dark blue) active in islets and largely inactive in other cell types. B. Possible pathway variants may take to influence β cell function.

Result 1: Variants exhibit differential transcriptional binding potentials

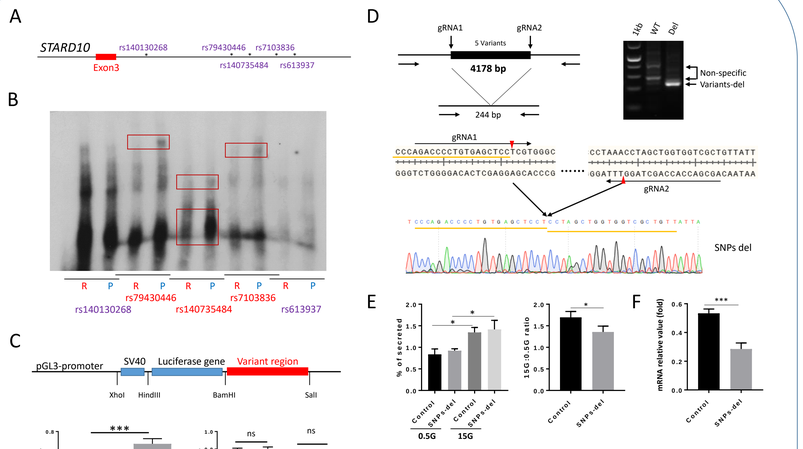


Fig.2 A. Diagram of variant region at *STRAD10* locus. B. EMSA assay showing differential protein bindings between risk (R) and protective (P) variants. C. Promoter-luciferase assay. D. Deletion of variant region in EndoC-βH1 cells by CRISPR-Cas9 mediated genome editing. F. Glucose stimulated insulin secretion (GSIS) assay. F. RQ-PCR analysis of *STARD10* gene expression.

Result 2: Variant region physically interacts with HS5 and HS1 regions containing multiple CTCF binding sites

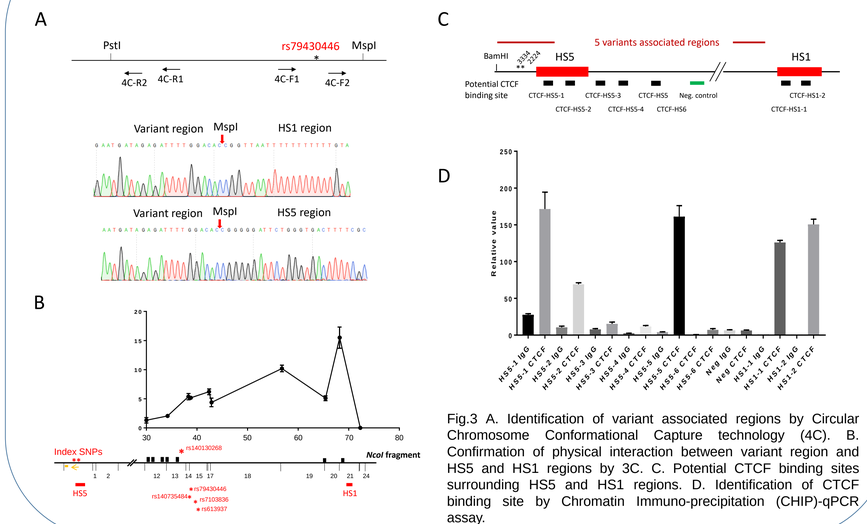


Fig.3 A. Identification of variant associated regions by Circular Chromosome Conformational Capture technology (4C). B. Confirmation of physical interaction between variant region and HS5 and HS1 regions by 3C. C. Potential CTCF binding sites surrounding HS5 and HS1 regions. D. Identification of CTCF binding site by Chromatin Immuno-precipitation (CHIP)-qPCR assay.

Result 3: Mutations of CTCF binding site impair insulin secretion

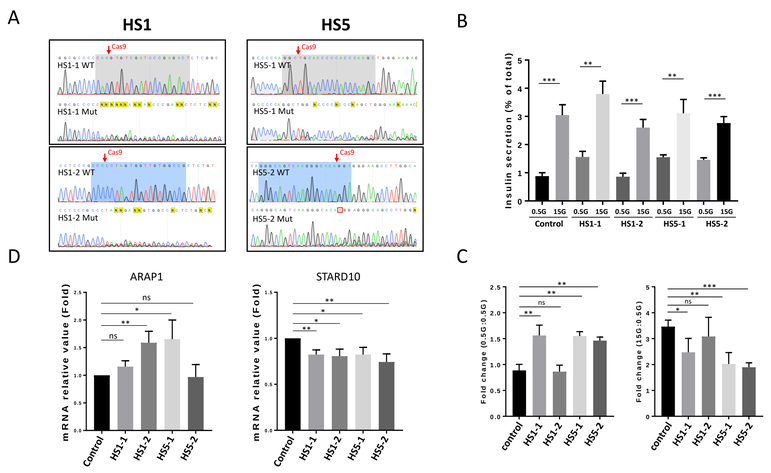


Fig.4 CRISPR-Cas9 mediated genome editing at CTCF-binding sites in HS1 and HS5 regions. A. DNA sequencing confirmation of mutations generated at CTCF binding sites (CTCF-BS) after genome editing in EndoC-βH1 cells. B. GSIS assay of CTCF-BS mutated cells. C. Fold changes of secreted insulin content at basal level (0.5 mM glucose) and after high glucose (15mM) stimulation. D. RQ-PCR analysis of *ARAP1* and *STARD10* gene expression.

Result 4: HS1b exhibits highest transcriptional activity and its deletion impairs insulin secretion

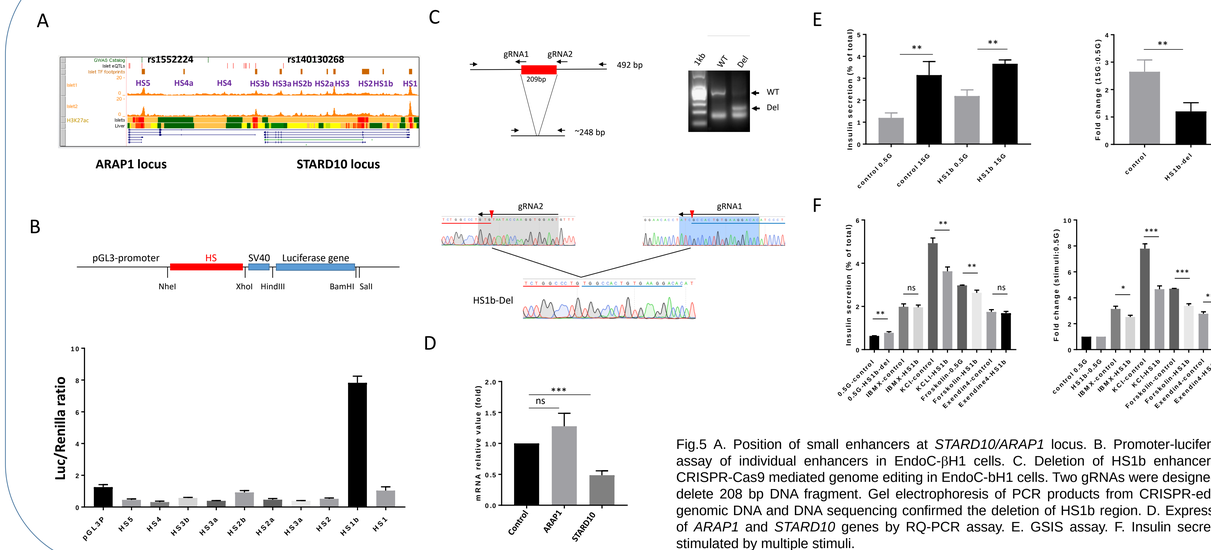
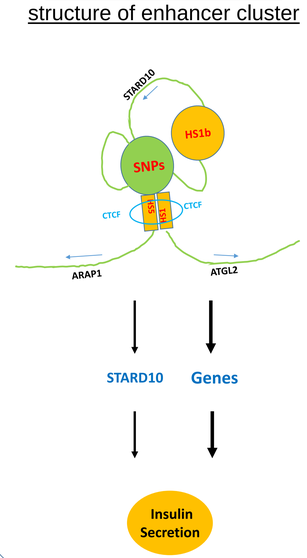


Fig.5 A. Position of small enhancers at *STARD10/ARAP1* locus. B. Promoter-luciferase assay of individual enhancers in EndoC-βH1 cells. C. Deletion of HS1b enhancer by CRISPR-Cas9 mediated genome editing in EndoC-bH1 cells. Two gRNAs were designed to delete 208 bp DNA fragment. Gel electrophoresis of PCR products from CRISPR-edited genomic DNA and DNA sequencing confirmed the deletion of HS1b region. D. Expression of *ARAP1* and *STARD10* genes by RQ-PCR assay. E. GSIS assay. F. Insulin secretion stimulated by multiple stimuli.

Model: variants affect 3D structure of enhancer cluster



Conclusion: 1. The causal variants are likely to influence the chromatin structure of enhancer cluster through CTCF binding sites.
2. Islet-specific enhancer cluster at *STARD10* locus regulates insulin secretion upon multiple stimuli.

Reference: Carrat, et al., *Am J Hum Gen*, (2017) 100: 238-256.
Parker SCJ, et al., PNAS, (2012) 110: 17921-17926.

wellcome
MRC Medical Research Council
Imperial College London