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Molecular genetics of the transcription factor GLIS3 identifies its dual function in beta cells and neurons

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ABSTRACT

The GLIS family zinc finger 3 isoform (GLIS3) is a risk gene for Type 1 and Type 2 diabetes, glaucoma and Alzheimer's disease endophenotype. We identified GLIS3 binding sites in insulin secreting cells (INS1) (FDR $q < 0.05$; enrichment range 1.40–9.11 fold) sharing the motif wrGTTCCCArTAGs, which were enriched in genes involved in neuronal function and autophagy and in risk genes for metabolic and neuro-behavioural diseases. We confirmed experimentally *Glis3*-mediated regulation of the expression of genes involved in autophagy and neuron function in INS1 and neuronal PC12 cells. Naturally-occurring coding polymorphisms in *Glis3* in the Goto-Kakizaki rat model of type 2 diabetes were associated with increased insulin production *in vitro* and *in vivo*, suggestive alteration of autophagy in PC12 and INS1 and abnormal neurogenesis in hippocampus neurons. Our results support biological pleiotropy of GLIS3 in pathologies affecting β-cells and neurons and underline the existence of trans-nosology pathways in diabetes and its co-morbidities.

1. Introduction

Genome-wide association studies (GWAS) for common inherited human diseases have shed light on novel candidate genes [1], which often lack functional characterisation to understand their role in disease pathogenesis. Growing evidence from GWAS data supports the involvement of common risk loci in diseases characterised by distinct pathophysiological features, suggesting that genes at these loci contribute to shared disease etiology through mechanisms of cross-phenotype association and pleiotropy [2,3]. This phenomenon is illustrated with common risk loci in autoimmune and immune-mediated inflammatory diseases [4,5], and recently extended to genetic variants shared in cancer and blood triglycerides and low-density lipoprotein cholesterol [6].

The GLIS family zinc finger 3 isoform (GLIS3) is among the most replicated GWAS signals for diabetes mellitus and related metabolic

Abbreviations: AD, Alzheimer's disease; BN, Brown Norway; ChIPseq, genome-wide chromatin immunoprecipitation sequencing; DAVID, Database for Annotation, Visualisation and Integrated Discovery; G3BS, Glis3-binding sites; GK, Goto-Kakizaki; GLIS3, GLIS family zinc finger 3 isoform; GO, Gene Ontology; GWAS, Genome-wide association studies; IPA, Ingenuity Pathway Analysis; T2D, type 2 diabetes

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traits. It is one of the few identified GWAS risk genes for both type 1 [7–9] and type 2 [10–12] diabetes. GWAS have shown that common variants within GLIS3 itself are also associated with cerebrospinal fluid Tau [13], a biomarker in Alzheimer's disease, glaucoma [14] and serum levels of the thyroid stimulating hormone [15]. Patients with mutations in GLIS3 exhibit neonatal diabetes and congenital hypothyroidism [16,17], but they also show a much broader spectrum of clinical manifestations, including hepatic, renal and cardiac diseases and skeletal abnormalities [16–18]. These data strongly suggest that genetic variations and mutations in GLIS3 have strong cross phenotypic effects in distinct organs.

GLIS3 is a component of Krüppel-like zinc finger transcriptional regulators that share a highly conserved five-C_2H_2-type zinc finger. It is expressed in many organs where it controls gene transcription through Glis3-binding sites (G3BS) in regulatory regions of target genes [19]. GLIS3 plays a critical role in the development and function of pancreatic β-cells, as shown for many type 2 diabetes GWAS genes [20]. Consistent with its primary etiological role in diabetes, it regulates fasting glucose and insulin [12,21] and glucose-stimulated insulin release [22]. *Glis3* disruption in mice causes neonatal diabetes and hypothyroidism [23-25]. *Glis3*-null pups are hyperglycemic and die prematurely. *Glis3* mutant mice exhibit small islets, with strongly decreased number of β- and δ-cells. Several studies indicate that *Glis3* indirectly regulates expression of key transcription factors (Pdx1, NeuroD1, MafA) required for the development of the endocrine pancreas and the function of mature β-cells [23,25,26] and is implicated in β-cell survival [27].

Despite the key role of GLIS3 in diabetes etiology and in endocrine pancreas development, molecular and cellular mechanisms mediating its function remain largely unknown. To understand diabetes-related mechanisms regulated by GLIS3 in β-cells, we carried out genome-wide chromatin immunoprecipitation sequencing (ChIPseq) of G3BS in INS1 cells, which derive from rat pancreatic β-cells, followed by molecular and physiological studies (Fig. 1). Results suggest that G3BS are enriched for GWAS loci associated with metabolic diseases and neuropathologies and that GLIS3 regulates the expression of genes involved in the function of endocrine pancreas and neurons, thus suggesting cross-phenotype associations of the GLIS3 locus. Further functional studies indicate that GLIS3 exhibits dual biological roles in β-cells and neurons possibly through differential expression of autophagy genes, which provide evidence for the involvement of mechanisms of biological pleiotropy in GLIS3 function.

2. Materials and methods

2.1. GLIS3 chromatin immuno-precipitation and sequencing

About 1×10^7 rat insulinoma 832/13 INS1 cells (gift from M LeGall, INSERM U872, Paris, France) were used. Proteins and DNA were cross-linked by addition of 0.4% formaldehyde (Sigma Aldrich, Saint Quentin Fallavier, France). Cells were washed with phosphate buffered saline (Sigma Aldrich, Saint Quentin Fallavier, France). Chromatin was collected by centrifugation and fragmented to generate DNA fragments of 200 bp. Immunoprecipitation was performed with anti-GLIS3 antibody (Abcam, 51268, Cambridge, UK). DNA sequencing was performed on a genome analyzer GA-IIx (Illumina, Saffron Walden, UK). We obtained over 20 M and about 37 M reads in IP and Input, respectively. Raw sequence data have been deposited in ArrayExpress (http://www.ebi.ac.uk/arrayexpress/) under the accession number E-MTAB-5454.

Sequence alignment was performed using Bowtie. Peak calling performed with MACS2 (https://github.com/taoliu/MACS/wiki/Install-macs2) identified 299 peaks with sizes ranging from 134 nt to 1656 nt. Peak sequences were searched for DNA motifs using Dimont, RSAT (Peak-motifs), GimmeMotifs and completeMotifs [28]. RSAT was used on the 299 sequences restricted to the 200 bp around the peak summits. Motifs were considered as significant when binomial significance ≥ 10. Twelve tools were considered (Mdmodule, MEME,

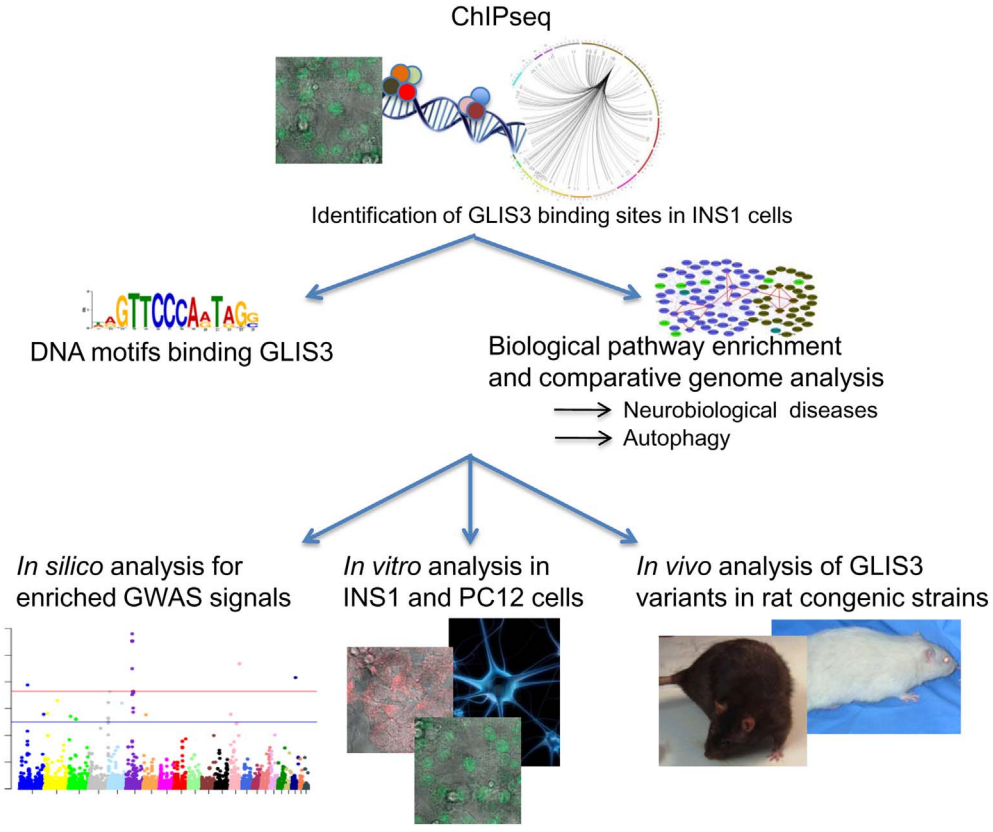

Fig. 1. Outlined experimental design and methods applied to the identification of GLIS3 target genes in INS1 insulin secreting cells and the characterisation of it function.

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