Emerging roles of GLIS3 in neonatal diabetes, type 1 and type 2 diabetes

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Abstract

GLI-similar 3 (GLIS3), a member of the Krüppel-like zinc finger protein subfamily, is predominantly expressed in the pancreas, thyroid and kidney. *Glis3* mRNA can be initially detected in mouse pancreas at embryonic day 11.5 and is largely restricted to β cells, pancreatic polypeptide-expressing cells, as well as ductal cells at later stage of pancreas development. Mutations in *GLIS3* cause a neonatal diabetes syndrome, characterized by neonatal diabetes, congenital hypothyroidism and polycystic kidney. Importantly, genome-wide association studies showed that variations of *GLIS3* are strongly associated with both type 1 diabetes (T1D) and type 2 diabetes (T2D) in multiple populations. GLIS3 cooperates with pancreatic and duodenal homeobox 1 (PDX1), v-maf musculoaponeurotic fibrosarcoma oncogene family, protein A (MAFA), as well as neurogenic differentiation 1 (NEUROD1) and potently controls insulin gene transcription. GLIS3 also plays a role in β cell survival and likely in insulin secretion. Any perturbation of these functions may underlie all three forms of diabetes. GLIS3, synergistically with hepatocyte nuclear factor 6 (HNF6) and forkhead box A2 (FOXA2), controls fetal islet differentiation via transactivating neurogenin 3 (NGN3) and impairment of this function leads to neonatal diabetes. In addition, GLIS3 is also required for the compensatory β cell proliferation and mass expansion in response to insulin resistance, which if disrupted may predispose to T2D. The increasing understanding of the mechanisms of GLIS3 in β cell development, survival and function maintenance will provide new insights into disease pathogenesis and potential therapeutic target identification to combat diabetes.

Key Words

- ▶ GLIS3
- ▶ neonatal diabetes
- ▶ type 1 diabetes
- ▶ type 2 diabetes
- ▶ insulin
- ▶ NGN3

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Introduction

Type 1 (T1D) and type 2 diabetes (T2D) share some clinical and pathologic similarities, presenting with hyperglycemia resulting from islet β cell dysfunction and/or defect of insulin action. Hyperglycemia associated with both T1D and T2D may lead to similar chronic clinical complications. However, genome-wide association studies (GWAS) indicate that their susceptibility loci are mostly distinct. To date, more than 50 and 100 susceptibility loci

are found to be associated with T1D and T2D, respectively (Todd 2010, Bonnefond & Froguel 2015, Yang & Chan 2016). Remarkably, only a limited number of them are associated with both T1D and T2D. These include: insulin, GLI-similar 3 (*GLIS3*), RAS guanyl nucleotide-releasing protein 1 (*RASGRP1*), cordon-bleu WH2 repeat protein (*COBL*), renalase and breast cancer anti-estrogen resistance 1 (*BCAR1*) (Basile *et al.* 2014, Yang & Chan 2016).

Within these shared susceptibility loci, insulin and *GLIS3* are the only two known monogenic diabetes genes (Yang & Chan 2016).

GLIS3 is a member of the Krüppel-like zinc finger protein subfamily that was named due to its similarity to *Drosophila* regulatory protein Krüppel (Kim *et al.* 2003). Mutations of *GLIS3* cause a neonatal diabetes syndrome characterized by neonatal diabetes, congenital hypothyroidism and polycystic kidney (Senee *et al.* 2006), which can be phenocopied in *Glis3*-deficient mice (Kang *et al.* 2009b, Watanabe *et al.* 2009, Yang *et al.* 2011). GWAS have also identified that variations of *GLIS3* are associated with a number of other diseases. For example, *GLIS3* rs1571583 is associated with elevated serum thyroid-stimulating hormone (Porcu *et al.* 2013). *GLIS3* rs514716 is a risk variant for the levels of cerebrospinal fluid tau and ptau 181, biomarkers for Alzheimer's disease (Cruchaga *et al.* 2013). *GLIS3* rs736893 is associated with primary angle closure glaucoma (Khor *et al.* 2016). In addition, increased expression of GLIS3 has been observed in several types of tumors, such as ependymoma (Lukashova-v Zangen *et al.* 2007), proneural glioblastoma (Cooper *et al.* 2010), chromophobe renal cell carcinoma (Yusenko & Kovacs 2009), breast cancer (Rami *et al.* 2016) and metastatic melanoma (Jayachandran *et al.* 2016). GLIS3, interacting with its co-activator WW domain containing transcription regulator 1 (WWTR1), plays a critical role in maintaining proper renal functions (Kang *et al.* 2009a). A recent report showed that GLIS3 is also involved in the early postnatal spermatogenesis in mice (Kang *et al.* 2016a). In the present review, we will focus on GLIS3 and diabetes, summarizing its roles in monogenic neonatal diabetes (Table 1), T1D and T2D (Table 2), as well as the underlying mechanisms explored in animal models.

Molecular characteristics of GLIS3

GLI-similar subfamily contains three Krüppel-like zinc finger proteins, such as GLIS1–3. They share a highly homologous zinc finger domain (ZFD) consisting of five Cys₂/His₂-type zinc finger motifs. The ZFD in mouse GLIS3 exhibits 93% and 59% identities with that in mouse GLIS1 and GLIS2, respectively (Kim *et al.* 2003). The full length of mouse *Glis3* gene (NM_175459) contains 11 exons and encodes a protein of 935 amino acids with a calculated molecular weight 99.7 kDa. Part of exon 4 encodes the five ZFDs, and all of them are required for the binding to target DNA (Fig. 1A). The ZFDs are well conserved between rodents and human. A potential bipartite nuclear

localization signal (NLS) is located between Arg645 and Lys661, which overlaps with the fifth zinc finger. Interestingly, deletion and mutation analyses revealed that the nuclear localization of GLIS3 does not require the putative NLS, whereas the fourth zinc finger is completely required. C-terminal truncated GLIS3 can localize to the nucleus, but loses its transactivational activity, suggesting that the transactivation domain (TAD) of GLIS3 resides at its C-terminus (Fig. 1A) (Beak *et al.* 2008).

RNA blot hybridization detected two dominant transcripts of human *GLIS3* gene, long (7.5 kb) and smaller (0.8–2.0 kb), in multiple tissues. The 7.5 kb transcript is highly expressed in the pancreas, kidney and thyroid; in contrast, the smaller transcript is strongly expressed in the heart, liver and skeletal muscle (Senee *et al.* 2006). A number of alternatively spliced variants of human *GLIS3* gene that encode different protein isoforms have been reported. However, only two major variants that encode GLIS3 protein isoforms a and b have been determined. These two variants share the last 9 exons. Compared to the variant 1 (NM_001042413), the variant 2 (NM_152629) harbors a distinct 5' untranslated region (UTR) and lacks an in-frame portion of the 5' coding region, resulting in a shorter N-terminus in isoform b (Fig. 1B). These alternatively spliced variants of human *GLIS3* gene may contribute to the heterogeneities of clinical presentations.

GLIS3 may function as a cell-type-dependent transactivator or repressor. Early study showed that GLIS3 may bind to the GLI consensus sequence GACCACCCAC, suggesting a cross-talk between the GLIS3 and GLI signaling pathways (Kim *et al.* 2003). To identify the specific binding site for GLIS3, Beak and coworkers combined PCR and electrophoretic mobility shift assay (EMSA) to screen a mixture of 60 bp oligonucleotides that contain 22 random nucleotides. They reported that (G/C) TGGGGGGT(A/C) is the optimal DNA-binding site for GLIS3 (Beak *et al.* 2008). Subsequently, an endogenous consensus GLIS3 response element (GLIS3RE), G(T/C) CCCC(T/A)GCTGTGA(A/G), was identified in the promoter/enhancer regions of insulin (Yang *et al.* 2009), neurogenin 3 (*Ngn3*) (Yang *et al.* 2011) and cyclin d2 (*Ccnd2*) (Yang *et al.* 2013) in islet progenitors or adult β cells.

The temporal and spatial expression of GLIS3 in mouse pancreas

The pancreas has both exocrine and endocrine functions. The exocrine pancreas, consisting of acinar and ductal cells, involves the secretion of digestive enzymes that aid

Table 1 Clinical characteristics of neonatal diabetes syndrome in patients with *GLIS3* mutation.

Mutation	Ethnicity	Family/ patient	NDM	CH	PKD	Other features	Alive/dead	Reference
p.625fs703Stop ^a	Arabia	1/3	Y	Y	Y ^b	Liver fibrosis, IUGR, facial dysmorphism, congenital glaucoma	Died at 10 d, 6 and 16 months	Senee et al. (2006)
p.Cys536Trp	Arabia	1/1	Y	N	N	IUGR, hyperactivity	Alive (6.8 years)	Dimitri et al. (2015)
p.Gly311Alafs	Pakistani	1/1	Y	Y	Y	Right sensorineural deafness, skeletal disease	Alive (2.5 years)	Dimitri et al. (2015)
p.His561Tyr	Kurds	1/1	Y	Y	Y	Liver fibrosis, facial dysmorphism, congenital glaucoma, patent ductus arteriosus	Alive (4.5 years)	Dimitri et al. (2015)
p.Arg589Trp/ exons 1–11 del ^c	Caucasian	1/1	Y	N	N	IUGR, choanal atresia, hiatus hernia	Alive (36 years)	Dimitri et al. (2015)
426 kb deletion Chr9:4176077– 4601776	Arabia	1/1	Y	Y	N	IUGR, congenital glaucoma	Alive (2 years)	Senee et al. (2006)
149 kb deletion Chr9:4249915– 4398572	French	1/2	Y	Y	N	IUGR	Alive (14 and 22 years)	Senee et al. (2006)
Exons 1–2 deletion	Arabia	2/2	Y	Y	Y	Insulin resistance or high insulin sensitivity, ostium secundum atrial, septal defect, facial dysmorphism, congenital glaucoma	Alive (6 years and 7 months)	Dimitri et al. (2015)
Exons 1–4 deletion	Caucasian	1/2	Y	Y	Y	Exocrine insufficiency, liver fibrosis, IUGR, bilateral sensorineural deafness, facial dysmorphism, pancreatic cyst	Alive (6 years and 20 months)	Dimitri et al. (2011, 2015)
Exons 3–4 deletion	Turkish	1/1	Y	Y	Y	IUGR, liver fibrosis, facial dysmorphism	Died at 6 months	Dimitri et al. (2015)
Exons 5–9 deletion	Arabia	1/1	Y	Y	Y	IUGR, liver fibrosis, facial dysmorphism	Alive (4.7 years)	Dimitri et al. (2015)
Exons 9–11 deletion	African– American	1/1	Y	Y	Y	IUGR, liver fibrosis, facial dysmorphism, skeletal disease, congenital glaucoma	Died at 6 years	Dimitri et al. (2015)
Exons 10–11 deletion	Yemeni	1/1	Y	Y	Y	IUGR, liver fibrosis, exocrine insufficiency, facial dysmorphism, congenital glaucoma	Alive (3 years)	Dimitri et al. (2015)
p.Phe857Tyr ^{c,d}	Chinese	1/1	Y	N	N/A	Liver and kidney failure	Died at 1.5 years	Cao et al. (2016)

^aEqual to p.779fs858Stop in the updated version of human *GLIS3* gene (NM_001042413); ^bthe patient who died at 10 day of age did not present PKD; ^cindicates heterozygous mutation. All others are homozygous mutations in *GLIS3*; ^dit is unclear if this mutation was causative; whether this patient developed PKD was not documented, although we note that the patient died of liver and kidney failure.

CH, congenital hypothyroidism; IUGR, intrauterine growth restriction; NDM, neonatal diabetes mellitus; N, no; PKD, polycystic kidney disease; Y, yes.

nutrient digestion. The endocrine pancreas (also called islet), containing five types of hormone-expressing cells such as α , β , δ , ϵ and pancreatic polypeptide (PP), that is responsible for the regulation of glucose homeostasis. Both pancreatic exocrine and endocrine cells arise from a common origin of progenitor cells in the gut endoderm. Pancreas specification becomes morphologically evident around embryonic day (E) 8.5–E9.0 with the expression of pancreatic and duodenal homeobox 1 (PDX1). Pancreatic progenitors adopt either tip identity that develops to an acinar phenotype or trunk identity that is bipotential for the ductal and endocrine cell fate ([Shih et al. 2013](#)). Mouse

pancreas development contains a primary transition from E9.5 to E12.5 and a secondary transition from E12.5 to birth. During the primary transition, a subset of progenitor cells initiate the expression of NGN3, which marks the onset of endocrine cell differentiation, whereas the subset of trunk epithelial cells that do not express NGN3 develop into ductal cells. During the secondary transition, endocrine hormone-expressing cells are rapidly expanded in the developing pancreas starting from E13.5 ([Oliver-Krasinski & Stoffers 2008](#), [Shih et al. 2013](#)).

GLIS3 is predominantly expressed in the pancreas, thyroid and kidney, with modest expression in the heart,

Table 2 Variations of *GLIS3* associated with common T1D and T2D.

Study type	Sample size	Ethnicity	Major findings	Effect size*	Reference
Variations of <i>GLIS3</i> and T1D					
GWAS/meta-analysis	7514 cases/9045 controls	European	<i>GLIS3</i> rs7020673 associated with T1D	0.88, 0.97	Barrett et al. (2009)
GWAS	858 general population children/851 first-degree relatives of T1D patients	Non-Hispanic White	<i>GLIS3</i> rs7020673 associated with the development of islet autoimmunity	0.77 ^a	Steck et al. (2014)
GWAS	4574 cases/1207 controls, validated in 765 cases/423 controls	T1D genetics consortium, Germans	<i>GLIS3</i> rs7020673 contributes to the prediction of T1D	N/A	Winkler et al. (2014)
GWAS replication analysis	23 cases/39 controls (related individuals); 68 cases/61 controls (unrelated individuals)	Pakistani	<i>GLIS3</i> rs7020673 associated with T1D	N/A	Kiani et al. (2015)
GWAS	706 cases/863 controls	Japanese	<i>GLIS3</i> A908V associated with resistance to T1D	0.046 ^b	Awata et al. (2013)
Variations of <i>GLIS3</i> and T2D					
GWAS meta-analysis	46,186 non-diabetic participants and follow-up in 76,558 additional subjects	European	<i>GLIS3</i> rs7034200 associated with fasting glucose and impaired β cell function	0.018 ^c	Dupuis et al. (2010)
GWAS	6784 middle-aged participants	Danish	<i>GLIS3</i> rs7034200 associated with reduced glucose-stimulated β cell function	-0.02 ^d -0.031 ^c	Boesgaard et al. (2010)
GWAS replication analysis	3410 T2D patients and 3412 controls	Chinese	<i>GLIS3</i> rs7034200 associated with T2D	1.103	Hu et al. (2010)
GWAS meta-analysis	Over 6000 healthy children and adolescents	European and Australians	<i>GLIS3</i> rs7034200 associated with impaired β cell function and altered fasting glucose	-0.023 ^d	Barker et al. (2011)
GWAS	1678 cases/1584 controls	South Asians	<i>GLIS3</i> rs7034200 associated with T2D	1.03	Rees et al. (2011)
GWAS	3210 unrelated individuals	Chinese Han	<i>GLIS3</i> rs7034200 associated with fasting glucose, β cell function and T2D	1.27	Liu et al. (2011)
GWAS meta-analysis, replication analysis	Meta-analysis: 6952 cases/11,865 controls; <i>in silico</i> replication: 5843 cases/4,574 controls; <i>de novo</i> replication: 12,284 cases/13,172 controls	East Asians	<i>GLIS3</i> rs7041847 associated with T2D	1.1	Cho et al. (2012)
GWAS	2632 cases/2050 controls	Japanese	<i>GLIS3</i> rs7034200 borderline associated with T2D	1.09	Fujita et al. (2012)
GWAS replication analysis	3548 DPP participants	Multiple ethnics	No impact of <i>GLIS3</i> rs7034200 on diabetes incidence or interaction with preventive interventions	N/A	Florez et al. (2012)
GWAS meta-analysis	1329 participants in 329 families	Hispanic-American	<i>GLIS3</i> rs2380949 associated with insulin clearance	N/A	Goodarzi et al. (2013)
GWAS replication analysis	5315 cases/2064 controls	Japanese	<i>GLIS3</i> rs7041847 confers susceptibility to T2D	1.03	Sakai et al. (2013)
GWAS	8569 cases/8923 controls	Chinese Han, East Asians	<i>GLIS3</i> rs10814916 associated with the risk of T2D	1.11	Li et al. (2013)
GWAS meta-analysis	5974 non-diabetic subjects	Koreans	<i>GLIS3</i> rs7034200 associated with impaired β cell function	-0.02 ^d	Hong et al. (2014)
Matched case-control	160 T2D/160 controls, 195 impaired glucose regulation/195 controls	Chinese Han	<i>GLIS3</i> rs7034200 associated with the risk of T2D	1.625	Dou et al. (2016)

*Effect size is presented as odds ratio unless specified; ^ahazard ratio; ^bper-allele effect; ^cper-allele effect; ^d-coefficient. N/A, not available.

Figure 1

Schematic diagrams of mouse and human *GLIS3* gene and protein structures. (A) Mouse *Glis3* gene and protein structures. The full-length mouse *Glis3* gene contains 11 exons and encodes a protein of 935 aa. The ZFD is encoded by part of exon 4 and is responsible for the binding to the consensus GLIS3RE of its target genes such as insulin, *Ngn3* and *Ccnd2*. TAD is localized at its C-terminus. CUL3 and ITCH promote GLIS3 polyubiquitination and degradation via the proteasomal pathway. SUFU, interacting with GLIS3 through the conserved VYGHF motif at N-terminus of GLIS3, was shown to inhibit the association of CUL3 with GLIS3, thereby protecting GLIS3 protein from proteolytic degradation. (B) Human *GLIS3* gene and protein structures. Human *GLIS3* gene have two major variants that encode GLIS3 protein isoforms a and b that share the last 9 exons. The variant 1 encodes the longest isoform a with 930 aa. The variant 2 harbors a distinct 5' UTR and lacks an in-frame portion of the 5' coding region, resulting in a shorter N-terminus in isoform b with 775 aa, compared to variant 1.

lung, brain, liver, skeletal muscle and testis (Kim *et al.* 2003, Senec *et al.* 2006, Yang *et al.* 2009). RT-PCR and *in situ* hybridization showed that *Glis3* mRNA can be initially detected at E11.5 and significantly increases at E12.5 in mouse pancreata (Kang *et al.* 2009b). Recently, Jetten and coworkers generated a GLIS3-enhanced green fluorescent protein (EGFP) knock-in mouse model and reported that GLIS3 protein was undetectable during the primary transition (E10.5–12.5), instead it was first detected at E13.5 in the nucleus of bipotent progenitors of the pancreas development (Kang *et al.* 2016b). Several possibilities might explain this discrepancy in the expression of GLIS3 mRNA and protein. These include delayed protein synthesis and accumulation due to post-transcriptional regulation and GLIS3 protein may be unstable in the pancreata between E11.5 and E12.5. However, distinct sensitivity of the approaches used in these experiments could more likely lead to this apparent difference. In *Glis3*-deficient mouse pancreas, the expression of NGN3, a direct downstream target of GLIS3,

was drastically reduced at both mRNA and protein levels at E12.5, which reveals that *Glis3* gene is functionally important in mouse endocrine pancreas development at early as E12.5, immediately before the secondary transition begins (Yang *et al.* 2011).

In the GLIS3-EGFP knock-in mice, GLIS3-EGFP mainly co-expresses with PDX1 and sex-determining region Y-box 9 (SOX9) in the trunk domains, but not in the tip domains at E13.5. This is consistent with the observation that GLIS3 is predominantly expressed in endocrine and preductal progenitors (Kang *et al.* 2016b). At later stages, GLIS3 is largely restricted to β cells and PP-expressing cells, as well as ductal cells. GLIS3 protein is also detected in a few δ cells, but neither in α and ϵ cells nor in acinar cells (Kang *et al.* 2016b), which is in agreement with the reports that exocrine pancreas was unaffected in *Glis3*-deficient mice (Kang *et al.* 2009b, Watanabe *et al.* 2009, Yang *et al.* 2011). We, however, note that some patients with *GLIS3* mutations present exocrine pancreas insufficiency (Table 1). The reason

for this discrepancy remains unclear and likely due to species difference.

Interacting partners of GLIS3

A previous high-throughput yeast two-hybrid screening suggested that GLIS3 may interact with suppressor of fused homolog (SUFU), a tumor suppressor and a negative regulator of hedgehog signaling (Rual *et al.* 2005). Subsequently, co-immunoprecipitation assay confirmed that the SUFU was able to interact with GLIS3 through the conserved VYGHF motif at N-terminus of GLIS3. Functional study showed that the SUFU repressed the activation of the insulin promoter mediated by GLIS3 (ZeRuth *et al.* 2011). In addition, the E3 ubiquitin ligase scaffolding protein Cullin 3 (CUL3) promotes GLIS3 polyubiquitination and degradation via the 26S proteasomal pathway. SUFU was shown to inhibit the association of CUL3 with GLIS3, thereby protecting GLIS3 protein from proteolytic degradation and enhancing the stability of GLIS3 protein (Fig. 1A) (ZeRuth *et al.* 2011).

Combining gel-enhanced liquid chromatography mass spectrometry and yeast two-hybrid analysis, they further reported that the ITCH protein, an itchy E3 ubiquitin protein ligase, interacted with GLIS3 through the WW domains with a PPxY motif located at N-terminus of GLIS3 and enhanced GLIS3 proteasomal degradation to reduce the GLIS3 stability. ITCH significantly increased GLIS3 protein turnover to inhibit the transactivation of GLIS3, resulting in the reduced expression of downstream targets including insulin (ZeRuth *et al.* 2015).

Long non-coding RNAs (lncRNAs) are non-protein coding transcripts longer than 200 nucleotides. lncRNAs are usually lineage specific and regulate multiple biological functions (Knoll *et al.* 2015). In an integrated sequence-based transcriptome and chromatin maps of human islets and β cells, Ferrer and coworkers identified a β cell-specific lncRNA *HI-LNC25*. They found that the mRNA expression of *GLIS3* was significantly reduced in the *HI-LNC25*-knockdown human β cells, suggesting that *GLIS3* is a regulatory target of *HI-LNC25* (Moran *et al.* 2012). It, however, remains to be determined whether *HI-LNC25*-*GLIS3* regulation correlates with the pathophysiology of diabetes as the expression of *HI-LNC25* was unchanged in the islets from T2D donors (Moran *et al.* 2012).

GLIS3 and neonatal diabetes syndrome

In 2003, Taha and coworkers first described a rare clinical syndrome in a consanguineous Saudi Arabian family

characterized by permanent neonatal diabetes, congenital hypothyroidism, polycystic kidneys, intrauterine growth retardation, facial anomalies, congenital glaucoma and hepatic fibrosis. Three affected children died at 10 days, 6 and 16 months of life, respectively (Taha *et al.* 2003, Senee *et al.* 2006). In 2006, Senee and coworkers identified a homozygous insertion (2067insC) in *GLIS3* that underlies the neonatal diabetes syndrome in one family. In addition, they reported that affected individuals harbor homozygous large fragment (426 and 149 kb) deletions affecting the 5' UTR of the *GLIS3* gene in two other families (Senee *et al.* 2006). Recently, Dimitri and coworkers reported additional patients who carried homozygous or compound partial *GLIS3* gene deletions or mutations. In addition to neonatal diabetes, congenital hypothyroidism and polycystic kidneys, they presented a broad spectrum of clinical phenotypes. These include craniosynostosis, hiatus hernia, atrial septal defect, splenic cyst, choanal atresia, sensorineural deafness and exocrine pancreatic insufficiency (Dimitri *et al.* 2011, 2015). They further described facial dysmorphism including bilateral low-set ears, depressed nasal bridge, elongated and upslanted palpebral fissures, persistent long philtrum with a thin vermilion border of the upper lip in some patients (Dimitri *et al.* 2016). Notably, one patient with compound heterozygous mutations in *GLIS3* (p.Arg589Trp/exons 1–11 del) presenting with neonatal diabetes, but not congenital hypothyroidism or renal cysts, survived to adulthood. Interestingly, all patients present with neonatal diabetes, but with variable insulin sensitivity that is not associated with genotypes. For example, the patients with homozygous exons 1–2 deletion in *GLIS3* may present with insulin resistance or high insulin sensitivity (Dimitri *et al.* 2015). Therefore, insulin therapy should be started with careful attention for this type of neonatal diabetes. The clinical features associated with the mutations in *GLIS3* identified to date are summarized in Table 1.

As stated previously, human *GLIS3* gene has multiple transcripts with differential expression profiles, which may contribute to the variability in phenotype (Senee *et al.* 2006). In addition, the severity of impaired function of *GLIS3* mutants may also cause the variable phenotypes. For example, a homozygous insertion (2067insC) in *GLIS3* leads to either a frameshift and a truncated protein that completely loses its transactivities (Senee *et al.* 2006, Yang *et al.* 2009) or likely no protein expression as a result of nonsense-mediated mRNA decay (Lykke-Andersen & Jensen 2015), whereas the compound heterozygous mutations (p.Arg589Trp/exons

1–11 del) might partially affect the function of GLIS3 (Dimitri *et al.* 2015).

GLIS3 and type 1 diabetes

Common T1D is an autoimmune disorder that arises from the action of a combination of genetic and environmental factors (Herold *et al.* 2013). More than 50 regions of the human genome have been identified to confer the susceptibility for common T1D (Yang & Chan 2016). Barrett and coworkers first identified the association between a *GLIS3* variant rs7020673 and common T1D in European populations. They found that the region of strong linkage disequilibrium at chromosome 9p24.2 that harbors only a single gene, *GLIS3* (Barrett *et al.* 2009). This association was replicated recently in Pakistani T1D patients (Kiani *et al.* 2015).

The human leukocyte antigen (HLA) is the major susceptibility locus for T1D, with an estimated 30–50% of the genetic risk of this disease (Noble *et al.* 1996). Winkler and coworkers assessed the predictive power of 40 non-HLA gene single nucleotide polymorphisms (SNP) associated with T1D. They found that the combination of *GLIS3* rs7020673 and other eight SNPs significantly improved the prediction accuracy of T1D, compared to that provided by HLA alone (Winkler *et al.* 2014). However, *GLIS3* rs7020673 alone exhibited nominal association with the development of islet autoimmunity (IA) and failed to predict the progression from IA to T1D (Steck *et al.* 2014).

Interestingly, Awata and coworkers reported that low frequency *GLIS3* A908V variant was associated with the resistance to T1D in Japanese population. The underlying mechanism remains elusive. Given that *GLIS3* mRNA is also moderately expressed in human thymus, they proposed that the protective variant *GLIS3* 908V might more efficiently induce central or peripheral immune tolerance than wild-type *GLIS3* 908A (Awata *et al.* 2013). Additional functional studies are needed to prove or disprove this hypothesis.

GLIS3 and type 2 diabetes

Shortly after the report that the *GLIS3* variant was strongly associated with common T1D, Dupuis and coworkers first identified the *GLIS3* variant rs7034200 as one of nine newly identified loci associated with fasting glucose in T2D trait, but not associated with body mass index (BMI), blood pressure or lipid profile in participants

of European descent (Dupuis *et al.* 2010). This finding has been replicated in Danes (Boesgaard *et al.* 2010), Chinese (Hu *et al.* 2010, Liu *et al.* 2011, Dou *et al.* 2016) and South Asians (Rees *et al.* 2011), with a borderline association with T2D in a Japanese population (Fujita *et al.* 2012). Furthermore, the glucose-raising allele *GLIS3* rs7034200 A was also strongly associated with impaired β -cell function both in non-diabetic adults (Dupuis *et al.* 2010, Hong *et al.* 2014) as well as in healthy children and adolescents (Barker *et al.* 2011), which suggests an age-independent effect of this locus on inter-individual differences in glucose levels from childhood onward. It is worth mentioning that *GLIS3* rs7034200, in common with other previously identified T2D risk loci, had no significant impact on diabetes incidence or interaction with preventive interventions such as metformin or lifestyle modifications in individuals at high risk of diabetes in the Diabetes Prevention Program (Florez *et al.* 2012).

In addition, two other *GLIS3* variants, rs7041847 and rs10814916, were reported to confer the susceptibility to T2D in East Asians (Cho *et al.* 2012, Sakai *et al.* 2013) and in Chinese (Li *et al.* 2013), respectively. In contrast to *GLIS3* rs7034200, a risk SNP for T2D in multi-ethnic populations, *GLIS3* rs7041847, appears to confer T2D susceptibility only in East Asians. It would be of interest to address whether *GLIS3* rs7041847 contributes to underlie the unique epidemic characteristics in East Asians, e.g., higher rates of T2D at lower average BMI, compared to those in Europeans.

Notably, *GLIS3* rs2380949 was found to be associated with insulin clearance in Hispanic Americans (Goodarzi *et al.* 2013), which suggests that *GLIS3* may play a dual role in regulating both insulin production and clearance. *GLIS3* variants associated with T2D and related traits are summarized in Table 2.

The molecular mechanisms that underlie the association between GLIS3 and diabetes

These association studies between *GLIS3* variants and diabetes in people are intriguing. However, the underlying mechanisms were largely unknown until *Gli3* global and β cell-specific knockout mice were generated. To gain insight into the role of *GLIS3* in diabetes, three groups independently generated *Gli3*-deficient mice with different strategies (Kang *et al.* 2009b, Watanabe *et al.* 2009, Yang *et al.* 2011) and identified three direct downstream targets of *GLIS3*, such as NGN3 (Yang *et al.* 2011), insulin

(Yang *et al.* 2009, 2013, ZeRuth *et al.* 2013) and CCND2 (Yang *et al.* 2013), in the pancreatic progenitors and β cells. These studies reveal that GLIS3 is required not only for fetal islet differentiation but also for the maintenance of adult β cell function.

GLIS3 is required for proper islet differentiation by transactivating *Ngn3*

Recapitulating the phenotype of neonatal diabetes syndrome in patients with *GLIS3* mutations (Senee *et al.* 2006), *Glis3*-deficient (*Glis3*^{-/-}) mice died with severe hyperglycemia and ketoacidosis within the first few days of life (Kang *et al.* 2009b, Watanabe *et al.* 2009, Yang *et al.* 2011). The total islet area of *Glis3*^{-/-} neonatal mice was only ~15% of that in *Glis3*^{+/+} littermates. Moreover, the size of islets was much smaller and were poorly organized morphologically in the neonatal *Glis3*^{-/-} mice. Immunostaining indicated that the numbers of all types of endocrine cells were markedly reduced in the neonatal *Glis3*^{-/-} mice (Yang *et al.* 2011). The mice also developed hypothyroidism and polycystic kidney, whereas exocrine development was unaffected (Kang *et al.* 2009b, Yang *et al.* 2011), which is supported by the recent report that GLIS3 protein was undetectable in mouse acinar cells (Kang *et al.* 2016b).

Proper NGN3 expression is crucial for pancreatic endocrine cell fate determination (Shih *et al.* 2013). A number of positive regulators of NGN3, such as PDX1 (Miyazaki *et al.* 2004, Oliver-Krasinski *et al.* 2009), HNF6 (Jacquemin *et al.* 2000), SOX9 (Lynn *et al.* 2007), FOXA2 (Lee *et al.* 2001) and hepatocyte nuclear factor 1 β (HNF1 β) (Maestro *et al.* 2003) have been identified in the developing pancreas. The expression of NGN3 was drastically reduced in the embryonic pancreata of *Glis3*^{-/-} mice both at mRNA and protein levels, compared to that in *Glis3*^{+/+} mice, whereas GLIS3 overexpression significantly upregulated the *Ngn3* mRNA in pancreatic ductal cells. Combining the *in vitro* luciferase report assay and EMSA, Yang and coworkers found that GLIS3 directly bound to the promoter/enhancer of *Ngn3* and activated its transcription, which was confirmed by chromatin immunoprecipitation in embryonic pancreata. They further reported that GLIS3 physically interacted with HNF6 as well as FOXA2 and synergistically transactivated *Ngn3* (Yang *et al.* 2011), the endocrine lineage-defining transcription factor (Shih *et al.* 2013).

GLIS3 controls insulin gene transcription

A number of cis-acting regulatory sequences in the insulin promoter region, which bind the key β cell-restricted or ubiquitous transcription factors, have been identified to confer the transcriptional control of insulin expression. For example, PDX1, MAFA and NEUROD1 control insulin gene transcription through their binding to A boxes, C elements and E boxes, respectively (Hay & Docherty 2006). Early studies in rat insulinoma 832/13 cells revealed that stable overexpression of GLIS3 enhanced the expression of *Ins2*, whereas knockdown of GLIS3 drastically reduced the expression of *Ins1* and *Ins2*. They further identified a GLIS3RE in the insulin gene promoter and found that GLIS3 physically and functionally interacts with PDX1, MAFA and NEUROD1 to control insulin gene transcription (Yang *et al.* 2009). Subsequent study showed that mutations in the GLIS3RE markedly attenuated the insulin promoter activation by a combination of PDX1, MAFA and NEUROD1, indicating that the direct binding of GLIS3 to the insulin promoter is required for the interaction of its partners with the promoter (ZeRuth *et al.* 2013). In agreement with these *in vitro* findings, Yang and coworkers found that tamoxifen-mediated β cell-specific inactivation of *Glis3* in mice markedly downregulates insulin expression, leading to fulminant diabetes and death (Yang *et al.* 2013). Thus, both *in vitro* and *in vivo* data demonstrated that GLIS3 is a potent transactivator of the insulin gene.

Interestingly, the mRNA level of glucose transporter 2 (*Glut2*), the key β cell glucose transporter, was significantly reduced in adult islets of β cell-specific *Glis3*-deficient mice, compared to that in the control mice (Yang *et al.* 2013). In addition, the mRNA expression of ATP-binding cassette transporter sub-family C member 8 (*Abcc8*) encoding sulfonylurea receptor 1 (SUR1) was also decreased in the pancreata of *Glis3*^{-/-} mice at postnatal day 3, compared to that in their wild-type littermates (Kang *et al.* 2009b). SUR1 is a sensor of intracellular levels of the nucleotides ATP and ADP to initiate insulin secretion in response to the sulfonylurea class of antidiabetic drugs (Yang & Chan 2016). These data suggested that in addition to controlling insulin gene transcription, GLIS3 may also play a role in insulin secretion in β cells. Given that the insulin production is markedly reduced by more than 80% (Yang *et al.* 2011), it is a challenge to assess the defect of insulin secretion *per se* in *Glis3*^{-/-} mice.

GLIS3 is required for obesity-induced β cell proliferation and mass expansion

In states of insulin resistance, such as obesity and pregnancy, the expansion of β cell mass in accordance with the requirements for insulin is critical for maintaining glucose homeostasis and preventing diabetes. Increasing β cell proliferation is the primary mechanism underlying this β cell mass expansion, although neogenesis may also be involved (Ackermann & Gannon 2007). Failure of β cell mass expansion may be associated with T2D. Consistent with an autosomal recessive inheritance observed in humans, heterozygous *Glis3*-mutant (*Glis3*^{+/-}) mice remained euglycemic while on a regular chow diet. However, when they were challenged with a high-fat diet (HFD), adult mice developed overt diabetes with hypoinsulinemia. Histology showed that *Glis3*^{+/-} mice failed β cell mass expansion in response to HFD. Mechanistically, it has been shown that GLIS3 controls β cell proliferation in response to HFD feeding partly by direct regulation of *Ccnd2* mRNA transcription (Yang *et al.* 2013).

CCND2 is essential for postnatal pancreatic β cell growth (Georgia & Bhushan 2004, Kushner *et al.* 2005) and compensatory mass expansion in response to insulin resistance (Georgia *et al.* 2010). Interestingly, GWAS

identified that the variant rs11063069 G in *CCND2* confers susceptibility to T2D (Morris *et al.* 2012), whereas the variant rs76895963 G in *CCND2* reduces risk of T2D by half and is correlated with increased *CCND2* expression (Steinthorsdottir *et al.* 2014). Further research is needed to identify additional potential targets of GLIS3 to fully elucidate the molecular mechanisms that underlie reduced GLIS3 and defective β cell mass expansion.

GLIS3 may protect pancreatic β cells against apoptosis

The loss of β cell mass is a critical feature for both T1D and T2D. Pro-inflammatory cytokines, such as interleukin 1 β (IL1 β) and interferon γ (IFN γ), and the saturated nonesterified fatty acid palmitate, may contribute to the β cell loss in T1D and T2D, respectively (Donath *et al.* 2005, Santin & Eizirik 2013). An *in vitro* study showed that *Glis3* knockdown in INS-1 cells increased basal and inflammatory cytokine (IL1 β +IFN γ)- or palmitate-induced β cell apoptosis. Reduced GLIS3 expression was found to modulate the alternative splicing of the pro-apoptotic BH3-only protein Bim, promoting the expression of the pro-death variant BimS and β cell death (Nogueira *et al.* 2013). These data suggest that proper GLIS3 expression could be required for β cell survival. This notion was

Figure 2

Current understanding of the molecular actions of GLIS3 in diabetes. This figure summarizes the pathways by which ablation, loss-of-function mutations or functional impairment of *GLIS3* cause neonatal diabetes, as well as common T1D and T2D. See text for details. A full colour version of this figure is available at <http://dx.doi.org/10.1530/JME-16-0232>.

supported by a recent study in *Glis3*^{+/-} mice on NOD^k genetic background.

NOD^k mice harbor congenic replacement of the potent Idd1 major histocompatibility complex susceptibility locus, a genetic model used to dissect the components of diabetes susceptibility without spontaneous autoimmunity against islets. Compared to C57BL/10, NOD^k mice are more prone to islet β cell failure due to increased susceptibility to endoplasmic reticulum stress. Dooley and coworkers found that *Glis3*^{+/-} mice developed glucose intolerance and increased level of proinsulin secretion on NOD^k genetic background. These mice developed diabetes upon aging. They further reported that reduced GLIS3 expression results in enhanced susceptibility to apoptosis probably due to poor induction of a critical antiapoptotic factor, mesencephalic astrocyte-derived neurotrophic factor (MANF) during the unfolded protein response (Dooley *et al.* 2016).

Conclusions

Candidate gene studies and GWAS have identified over 50 and 100 susceptibility loci for common T1D and T2D, respectively (Todd 2010, Bonnefond & Froguel 2015, Yang & Chan 2016). Among the repertoire of susceptibility loci associated with diabetes, GLIS3 and insulin are the only two known monogenic diabetes genes whose variations are strongly associated with both common T1D and T2D (Yang & Chan 2016). These unique characteristics differentiate GLIS3 from other pancreatic transcription factors and highlight its roles in neonatal diabetes (Table 1) as well as in T1D and T2D (Table 2).

Glis3 global and β cell-specific knockout models, together with the data from β cell lines, provide the mechanistic basis for the genetic associations of the *GLIS3* gene and neonatal diabetes, common T1D and T2D (Fig. 2). GLIS3 potently controls insulin gene transcription (Yang *et al.* 2009, 2013, ZeRuth *et al.* 2013) as well as likely insulin secretion (Kang *et al.* 2009b, Yang *et al.* 2013) and β cell survival (Nogueira *et al.* 2013, Dooley *et al.* 2016), which perturbations may underlie all three forms of diabetes. Mutations or deletions in *GLIS3* impair the proper expression and/or function of GLIS3 and its direct downstream target NGN3, causing severely abnormal β cell development *in utero* and marked impairment in insulin production at birth, culminating in permanent neonatal diabetes (Yang *et al.* 2011). A new report further unveiled that the differential gene dosage of GLIS3 largely determines the expression levels of NGN3 and insulin, leading to variable phenotypes (Yang *et al.* 2016).

Moreover, studies in adult mouse models reveal that GLIS3 is required for the maintenance of proper adult β cell function. GLIS3 also plays an essential role in obesity-induced β cell proliferation via the transactivation of *Ccnd2* (Yang *et al.* 2013). These may underlie the GWAS finding that the *GLIS3* locus is also linked to T2D in people. Of note, the variations of *GLIS3* associated with T1D and T2D are unique and do not overlap, likely because they confer their susceptibility effects via different mechanisms. Continued research into *GLIS3* gene will provide new insights into disease pathogenesis and potential therapeutic target identification to combat the global epidemic of T1D and T2D.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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