Gene of the month: *GLIS1-3*

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ABSTRACT

The *GLIS 1–3* genes belong to a family of transcription factors, the Krüppel-like zinc finger proteins. The GLIS proteins function primarily as activators of transcription (GLIS 1 and 3), while GLIS 2 functions as a repressor. Collectively, the GLIS proteins are involved in a variety of diseases in several organs ranging from Alzheimer's disease, facial dysmorphism, neonatal diabetes mellitus, breast and colon cancers and leukaemia. In particular, loss-of-function mutations in *GLIS2* are responsible for an autosomal recessive cystic kidney disease called nephronophthisis, which is characterised by tubular atrophy, interstitial fibrosis and corticomedullary cysts. Of diagnostic value in current practice are the presence of *GLIS 3* and *1* fusions with *PAX8* in almost 100% of hyalinising trabecular tumours of the thyroid gland. This enables its separation from papillary thyroid cancer.

INTRODUCTION

Krüppel-like zinc finger proteins (named after the *Drosophila* segmentation gene, *Krüppel*) constitute a large family of transcription factors. These proteins are characterised by containing two or more Cys2-His2-type zinc fingers with an intervening conserved consensus sequence. Krüppel-like zinc finger proteins are further categorised into subfamilies based on the number of zinc finger motifs, sequence homology between the zinc fingers, and the presence of specific repressor and activation domains.[1] Two subfamilies that are closely related and act as repressors and activators of transcription are glioma-associated oncogene (Gli) and Zinc finger protein of the cerebellum.[1] The GLI-Similar (GLIS) proteins (1-3) are named after their similarity to the Gli subfamily of Krüppel-like zinc finger proteins.

GLIS GENE LOCATION

GLIS1: is located on chromosome 1p32.3.
GLIS2: *GLIS2* maps to chromosome 16p13.3.
GLIS3: The *GLIS3* gene maps to chromosome 9p24.2.

GLIS FAMILY OF PROTEINS

The GLIS proteins are linked via a highly homologous DNA binding domain consisting of five Cys2His2-type zinc finger motifs. This mediates the interaction of GLIS1-3 with GLIS-binding sequences located in regulatory regions of target genes. The zinc finger domains of GLIS1 and GLIS3 demonstrate the highest homology indicating evolutionary closeness.[2]

Transcriptional activation or repression (mainly GLIS2) by GLIS proteins is mediated through several co-activators or co-repressors that interact with the transactivation domain at their C-terminus of the proteins.

GLIS proteins can undergo a number of post-translational modifications, such as ubiquitination, methylation, phosphorylation and sumoylation allow of which impact protein localisation, stability, interactions and transcriptional activity.[3–5]

GLIS1-3 proteins have also been demonstrated in stem/progenitor cells.[3]

GLIS1-3 are implicated in a wide array of diseases in several organs. Some of the more common disease entities related to each of the 3 GLIS proteins are highlighted further.

GLIS1

Central nervous system

A novel locus associated with an increased risk of Alzheimer's disease has been identified involving Aβ42 near *GLIS1* on 1p32.3.[6]

Single nucleotide polymorphism (rs797906) of *GLIS1* has been linked to an increased risk of late-onset Parkinson's disease.[7]

Skin

GLIS1 is not expressed in the normal epidermis. Yet, GLIS1 mRNA appears to be enhanced in psoriatic skin, in the suprabasal layer, probably suggesting its role in the aberrant differentiation observed in psoriatic epidermis.[8]

Thyroid gland

Hyalinising trabecular tumour (HTT) and *GLIS* genes are closely linked. Along with the distinctive *PAX8-GLIS3* fusion that is solely present in HTT, studies also show an overexpression of GLIS1 protein with about 7% showing a *PAX8-GLIS1* fusion. However, a single case of papillary thyroid carcinoma also showed the *PAX8-GLIS1* fusion (from 220 cases), suggesting that the latter may not be exclusive to HTT.[9]

Breast cancer

Overexpression of GLIS1 and CUX1 (a homeobox gene protein implicated in tumour suppression and progression) together has proved to play a key role in the autocrine activation of the wingless related integration site (WNT)/β-catenin pathway in breast cancer and stimulating tumour cell migration and invasion.[10]

Oestrogen receptor-negative breast cancers with increased expression of GLIS1 have a worse prognosis and seem to be resistant to irradiation.[11]

Colon cancer

Adenomatous polyposis coli (*APC*) is a tumour suppressor gene, the inactivation of which plays a

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pivotal role in initiating the adenoma–carcinoma pathway for colorectal carcinomas. Yet, there is a subset of *APC*-mutant-negative CRCs which show an activation of the WNT signalling pathway by GLIS1.[12]

Leukaemia

Mutations in *GLIS1* were also detected in recurrent, hyperploid acute lymphoblastic leukaemia.[13]

GLIS2 IN DISEASE

Embryologically, the proteins encoded by GLIS2 are expressed in the kidney, suggesting its role in kidney morphogenesis as well as in the neural tube and peripheral nervous system, likely promoting neuronal differentiation.

GLIS2 MUTATIONS

Kidney

Loss-of-function mutations in *GLIS2* are responsible for an autosomal recessive cystic kidney disease called nephronophthisis, which is the most common genetic cause of end-stage renal disease in the first three decades of life. GLIS2 arrests apoptosis and fibrosis and helps maintain normal kidney architecture. Mutations in *GLIS2* result in nephronophthisis, which is characterised by tubular atrophy, interstitial fibrosis and corticomedullary cysts which replace normal renal parenchyma.[14] The disease is also associated with extrarenal manifestations like retinal degeneration, cerebellar vermis hypoplasia (Joubert syndrome), occipital encephalocele (Meckel-Gruber syndrome), hepatic fibrosis, situs inversus, bronchiectasis and skeletal defects.[15]

Glis2 and cancer

1. Colon: *GLIS2* has now been recognised as an oncogene in colon cancer through a *p53*-mediated transcription regulation.[16]
2. Stomach: High expression of GLIS2 has been linked to chemoresistance and poor prognosis in gastric cancers. Its expression has been intimately related to tumour type, grade, pathological as well as clinical stage, and microsatellite instability.[17] Conversely, low expression of GLIS2 makes gastric cancer more radiosensitive. In the future, molecular studies for *GLIS2* may prognosticate the precise therapy for gastric cancer.[18]
3. Leukaemia: Acute megakaryoblastic leukaemia, a subtype of acute myeloid leukaemia, has recently been identified to possess a *CBFA2T3-GLIS2* (or *ETO2-GLIS2*) gene fusion, in 20%–30% of patients, which is associated with poor prognosis.[19]

GLIS3 IN DISEASE

GLIS3 is expressed in early embryogenesis and plays a role in the development of the pancreas, thyroid, kidney, liver, eyes and heart. Loss-of-function mutations can occur in any of these organs with varying severity. Abnormalities associated with *GLIS3* mutations include intrauterine growth retardation, developmental delay, development of polycystic kidneys, congenital glaucoma, hepatic cholestasis, osteopaenia, atrial septal defects and minor facial dysmorphisms (depressed nasal bridge, bilateral low-set ears, long philtrum, large anterior fontanelle and elongated and up-slanted palpebral fissures).[1 2]

In summary, the key diseases impacted in *GLIS3* mutations are as follows.

Endocrine organs

Considering that endocrine pancreas and thyroid arise from the same embryonic sheet, loss-of-*GLIS3*-function mutations are associated with hyperglycaemia, hypoinsulinaemia and hypothyroidism.

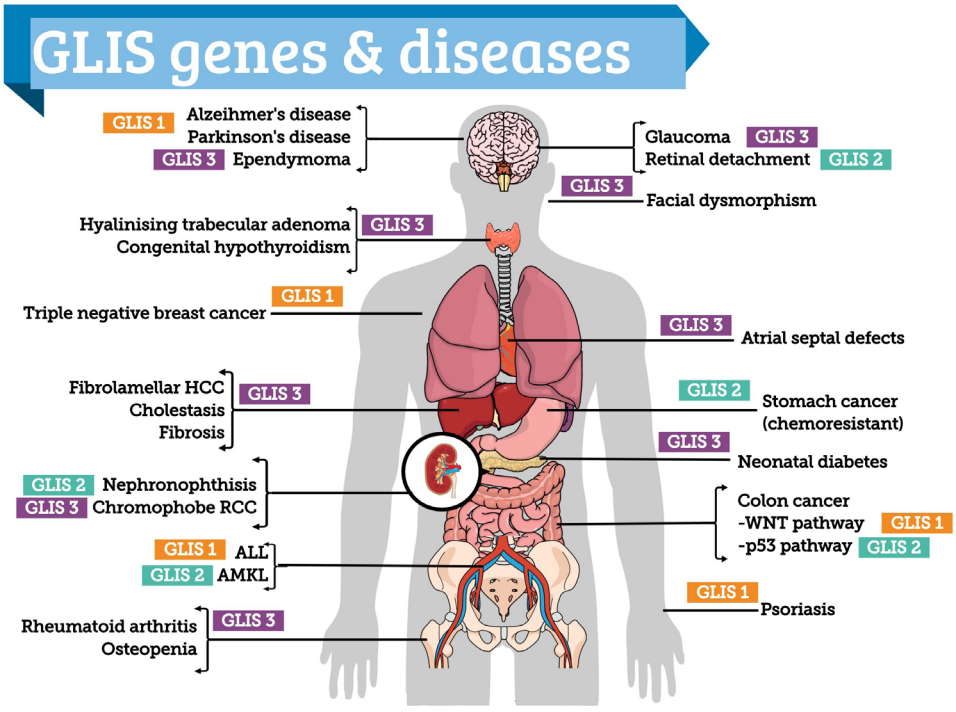

Figure 1 Schematic representation of the disease spectrum caused by *GLIS1-3*. ALL, acute lymphoblastic leukaemia (hyperdiploid); AMKL, acute megakaryoblastic leukaemia; HCC, hepatocellular carcinoma; RCC, renal cell carcinoma; WNT, wingless related integration site.

1. In the pancreas, GLIS3 is a key regulator in pancreatic β-cell generation and maturation, insulin gene expression and duct morphogenesis. GLIS3 dysfunction has been shown to greatly reduce the size of the pancreatic islets and the number of β cells causing neonatal diabetes.
2. GLIS3 expression is only seen in follicular cells in the thyroid gland. Thus, loss-of-function mutations show low blood levels of T3 and T4 with an elevated thyroid stimulating hormone (TSH). Yet, the perplexing spectrum of morphological variations seen with the mutation in *GLIS3* can range from aplasia, hypoplasia, and perifollicular and stromal fibrosis all the way to a normal-looking thyroid gland.[2 20]
3. Autosomal recessive inherited syndrome with loss-of-function mutations in *GLIS3* is associated with neonatal diabetes and congenital hypothyroidism, both leading to significantly reduced life expectancy. This syndrome can show a wide range of organ involvement, including the liver (hepatic fibrosis and cholestasis), eyes (glaucoma) and kidney (multiple cysts).[21]
4. Other: *GLIS3* mutations can also manifest as intrauterine growth retardation, developmental delay, facial dysmorphism, osteopaenia, atrial septal defects, polycystic kidney disease and infertility. Single-nucleotide polymorphisms in *GLIS3* have been associated with an increased risk of developing diabetes (types 1 and 2), glaucoma, autoimmune diseases, rheumatoid arthritis and neurological disorders like Alzheimer's disease.[2]

GLIS3 in cancers/tumours

1. Breast: It has been suggested that *GLIS3* plays a role in the aberrant activation of the WNT/B-catenin pathway in breast carcinogenesis, specifically for triple-negative breast tumours. Rami *et al* compared GLIS3 mRNA expression in normal breast tissue versus breast tumour tissue where there was significantly greater expression of the GLIS3 (four times more) in the tumour.[22] The more advanced the disease, the greater the gene expression, suggesting a potential relationship between the two.[22]
2. Thyroid: A recent case series by Marchiò *et al* showed that HTT showed a consistent *PAX8-GLIS3* fusion, suggesting that this can be used as an ancillary technique in differentiating it from its morphological mimics.[23] *PAX8-GLIS3/GLIS1* fusions are thought to occur in 100% of all HTTs of the thyroid gland.[9 24]
3. Liver: Recent studies have shown interchromosomal translocations between *GLIS3* and *CLPTM1L* in fibrolamellar hepatocellular carcinoma, a rare type of paediatric liver cancer, with limited therapeutic options.[25]
4. Others: Increased GLIS3 expression has been seen in ependymomas[26] and chromophobe renal cell carcinoma.[27]

In summary, the GLIS family of genes and proteins has an impact on several organs and causes a diverse range of diseases, which are summarised in figure 1.

Take home messages

- The GLIS family of proteins belong to the Krüppel-like zinc finger proteins.
- They are transcription factors that function as activators and repressors.
- The *GLIS* genes are involved in several diseases and developmental abnormalities.
- Autosomal recessive inheritance of *GLIS2* results in cystic kidney disease called nephronophthisis.
- Autosomal recessive inheritance of *GLIS3* is associated with neonatal diabetes and congenital hypothyroidism.
- *GLIS1/3* fusions with *PAX8* are found in almost 100% of hyalinising trabecular tumours of the thyroid.

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REFERENCES

1 Kim Y-S, Lewandoski M, Perantoni AO, *et al*. Identification of Glis1, a novel Gli-related, Kruppel-like zinc finger protein containing transactivation and repressor functions. *J Biol Chem* 2002;277:30901–13.
2 Jetten AM. GLIS1-3 transcription factors: critical roles in the regulation of multiple physiological processes and diseases. *Cell Mol Life Sci* 2018;75:3473–94.
3 Scoville DW, Kang HS, Jetten AM. GLIS1-3: emerging roles in reprogramming, stem and progenitor cell differentiation and maintenance. *Stem Cell Investig* 2017;4:80.
4 Ramachandran H, Herfurth K, Grosschedl R, *et al*. Sumoylation blocks the ubiquitin-mediated degradation of the nephronophthisis gene product Glis2/NPHP7. *PLoS One* 2015;10:e0130275.
5 ZeRuth GT, Williams JG, Cole YC, *et al*. Hect E3 ubiquitin ligase itch functions as a novel negative regulator of Gli-similar 3 (Glis3) transcriptional activity. *PLoS One* 2015;10:e0131303.
6 Deming Y, Li Z, Kapoor M, *et al*. Genome-Wide association study identifies four novel loci associated with Alzheimer's endophenotypes and disease modifiers. *Acta Neuropathol* 2017;133:839–56.
7 Song W, Chen YP, Huang R, *et al*. Glis1 rs797906: an increased risk factor for late-onset Parkinson's disease in the Han Chinese population. *Eur Neurol* 2012;68:89–92.
8 Nakanishi G, Kim Y-S, Nakajima T, *et al*. Regulatory role for Krüppel-like zinc-finger protein Gli-similar 1 (Glis1) in PMA-treated and psoriatic epidermis. *J Invest Dermatol* 2006;126:49–60.
9 Nikiforova MN, Nikitski AV, Panebianco F, *et al*. Glis rearrangement is a genomic hallmark of Hyalinizing trabecular tumor of the thyroid gland. *Thyroid* 2019;29:161–73.
10 Vadnais C, Shooshtarizadeh P, Rajadurai CV, *et al*. Autocrine activation of the Wnt/β-catenin pathway by CUX1 and Glis1 in breast cancers. *Biol Open* 2014;3:937–46.
11 Shimamoto K, Tanimoto K, Fukazawa T, *et al*. GLIS1, a novel hypoxia-inducible transcription factor, promotes breast cancer cell motility *via* activation of WNT5A. *Carcinogenesis* 2020:bgaa010.
12 Xicola RM, Manojlovic Z, Augustus GJ, *et al*. Lack of APC somatic mutation is associated with early-onset colorectal cancer in African Americans. *Carcinogenesis* 2018;39:1331–41.
13 Chen C, Bartenhagen C, Gombert M, *et al*. Next-generation-sequencing of recurrent childhood high hyperdiploid acute lymphoblastic leukemia reveals mutations typically associated with high risk patients. *Leuk Res* 2015;39:990–1001.
14 Attanasio M, Uhlenhaut NH, Sousa VH, *et al*. Loss of Glis2 causes nephronophthisis in humans and mice by increased apoptosis and fibrosis. *Nat Genet* 2007;39:1018–24.
15 Hildebrandt F, Zhou W. Nephronophthisis-associated ciliopathies. *J Am Soc Nephrol* 2007;18:1855–71.
16 Yao J, Lei P-J, Li Q-L, *et al*. Glis2 promotes colorectal cancer through repressing enhancer activation. *Oncogenesis* 2020;9:57–71.
17 Yuan J, Tan L, Yin Z, *et al*. Glis2 redundancy causes chemoresistance and poor prognosis of gastric cancer based on co-expression network analysis. *Oncol Rep* 2019;41:191–201.
18 Sun H, Gu J, Li Z, *et al*. Low expression of Glis2 gene might associate with radiosensitivity of gastric cancer. *J Oncol* 2019;2019:1–10.
19 Thirant C, Ignacimouttou C, Lopez CK, *et al*. ETO2-GLIS2 hijacks transcriptional complexes to drive cellular identity and self-renewal in pediatric acute megakaryoblastic leukemia. *Cancer Cell* 2017;31:452–65.

20 Rurale G, Persani L, Marelli F. *GLIS3* and Thyroid: A Pleiotropic Candidate Gene for Congenital Hypothyroidism. *Front Endocrinol* 2018;9:730.
21 Taha D, Barbar M, Kanaan H, *et al*. Neonatal diabetes mellitus, congenital hypothyroidism, hepatic fibrosis, polycystic kidneys, and congenital glaucoma: a new autosomal recessive syndrome? *Am J Med Genet A* 2003;122A:269–73.
22 Rami F, Baradaran A, Kahnamooi MM, *et al*. Alteration of Glis3 gene expression pattern in patients with breast cancer. *Adv Biomed Res* 2016;5:44.
23 Marchiò C, Da Cruz Paula A, Gularte-Merida R, *et al*. PAX8-GLIS3 gene fusion is a Pathognomonic genetic alteration of hyalinizing trabecular tumors of the thyroid. *Mod Pathol* 2019;32:1734–43.
24 Nikiforova MN, Nikiforov YE, Ohori NP. Glis rearrangements in thyroid nodules: a key to preoperative diagnosis of hyalinizing trabecular tumor. *Cancer Cytopathol* 2019;127:560–6.
25 Xu L, Hazard FK, Zmoos A-F, *et al*. Genomic analysis of fibrolamellar hepatocellular carcinoma. *Hum Mol Genet* 2015;24:50–63.
26 Lukashova-v Zangen I, Kneitz S, Monoranu C-M, *et al*. Ependymoma gene expression profiles associated with histological subtype, proliferation, and patient survival. *Acta Neuropathol* 2007;113:325–37.
27 Yusenko MV, Kovacs G. Identifying CD82 (KAI1) as a marker for human chromophobe renal cell carcinoma. *Histopathology* 2009;55:687–95.