

Familial *GATA6* mutation causing variably expressed diabetes mellitus and cardiac and renal abnormalities

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Summary

A 26-year-old man presented with a combination of permanent neonatal diabetes due to pancreatic aplasia, complex congenital heart disease, central hypogonadism and growth hormone deficiency, structural renal abnormalities with proteinuria, umbilical hernia, neurocognitive impairment and dysmorphic features. His older brother had diabetes mellitus due to pancreatic hypoplasia, complex congenital heart disease, hypospadias and umbilical hernia. Their father had an atrial septal defect, umbilical hernia and diabetes mellitus diagnosed incidentally in adulthood on employment screening. The proband's paternal grandmother had a congenital heart defect. Genetic testing of the proband revealed a novel heterozygous missense variant (Chr18:g.19761441T>C, c.1330T>C, p.Cys444Arg) in exon 4 of *GATA6*, which is class 5 (pathogenic) using American College of Medical Genetics and Genomics guidelines and is likely to account for his multisystem disorder. The same variant was detected in his brother and father, but not his paternal grandmother. This novel variant of *GATA6* likely occurred *de novo* in the father with autosomal dominant inheritance in the proband and his brother. The case is exceptional as very few families with monogenic diabetes due to *GATA6* mutations have been reported to date and we describe a new link between *GATA6* and renal pathology.

Learning points:

- Monogenic diabetes should be suspected in patients presenting with syndromic features, multisystem congenital disease, neonatal-onset diabetes and/or a suggestive family history.
- Recognition and identification of genetic diabetes may improve patient understanding and empowerment and allow for better tailored management.
- Identification of a genetic disorder may have important implications for family planning.

Background

GATA6 (GATA-binding protein 6) is a member of a small family of zinc finger transcription factors that is important in the regulation of cellular differentiation and organogenesis. *GATA6* is expressed during embryogenesis of organs derived from the endo- and mesoderm, including the heart, pancreas, gut, lung and liver (1). Pathogenic (disease-causing) variants are associated

with multisystem congenital defects, particularly cardiac defects and pancreatic agenesis/hypoplasia (1). The present case is one of only a handful of reported *GATA6* kindreds with diabetes mellitus from pancreatic aplasia/hypoplasia. It represents one of the largest kindreds, illustrating an autosomal dominant, variably expressive pattern of inheritance. Features unique to this family

include dysmorphic facial features and proteinuria with renal biopsy findings.

Case presentation

A 26-year-old man born to non-consanguineous parents was referred to the Endocrinology Service for management of poorly controlled diabetes mellitus. He had dysmorphic features, including a long and narrow face, malar flush, reduced elbow extension, fixed flexion deformities of fourth and fifth fingers bilaterally, arachnodactyly, dolichostenomelia (height 166.5 cm, arm span 176 cm) and an umbilical hernia.

He had a complex past history:

- Pancreatic agenesis with permanent neonatal diabetes mellitus and exocrine insufficiency.
- Double outlet right ventricle, pulmonary stenosis, patent ductus arteriosus and ventricular septal defect.
- Central hypogonadism and growth hormone deficiency.
- Bifid left pelvicalyceal system, right hydrocele and right epididymal cyst.
- Unexplained proteinuria with minor focal increase in mesangial cellularity on renal biopsy (Fig. 1).
- Neurocognitive impairment on neuropsychology assessment at age 18 years.

A detailed family history (Fig. 2) revealed his older brother, IV.1, had diabetes mellitus due to pancreatic hypoplasia, truncus arteriosus, bicuspid aortic valve, hypospadias and umbilical hernia. Their deceased father, III.3, was reported to have a similar physical appearance to the proband, as well as atrial septal defect, umbilical hernia and a positive result for diabetes on employment screening, although he did not seek medical attention for this. He died at age 45 years from a myocardial infarction. Autopsy revealed severe triple-vessel disease, which in the absence of a smoking history, may have been explained by untreated diabetes; the pancreas was presumed to have undergone autolysis. The proband's deceased paternal grandmother also had an incidentally detected 'hole in the heart' and five other relatives had diabetes mellitus.

Given the striking combination of clinical features and family history, genetic testing was arranged to seek an explanation for his multisystem disorder. He indicated that the information could help his family, particularly his brother, IV.1, who had previously stated he would not have children as he did not want to pass on his pancreatic and cardiac disorders. His neonatal diabetes and positive family history were suggestive

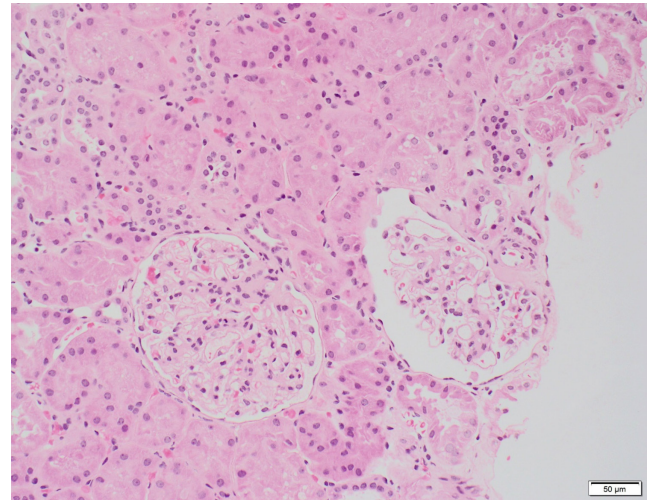


Figure 1

Proband's renal biopsy (haematoxylin and eosin (H&E) stain, magnified 200×) showing mild focal increase in mesangial cellularity and occasional focal increase in mesangial matrix within borderline enlarged glomeruli. Ultrastructural examination by electron microscopy showed more prominent mesangial deposition but only one glomerulus was captured. Immunofluorescence examination showed only weak interactions of capillary loops with IgG and C1q. Overall, the features were not consistent with diabetic nephropathy and were instead suggestive of an immune-mediated glomerulonephritis without being typical of any specific subtype.

of monogenic diabetes, whilst his concomitant heart disease and the extant literature pointed specifically to a *GATA6* mutation.

Investigation

Sanger sequencing of the *GATA6* gene was performed using genomic DNA extracted from peripheral leukocytes using standard procedures. This revealed a novel heterozygous missense variant (Chr18:g.19761441T>C, c.1330T>C, p.Cys444Arg) in exon 4 of *GATA6*. This was classified as a pathogenic (class 5) variant in accordance with the American College of Medical Genetics and Genomics (ACMG) guidelines (2).

In silico modelling (Fig. 3) indicated that the change from the wild-type cysteine amino acid to the mutant arginine amino acid induced by this variant is likely to impact adversely on the function of the *GATA6* protein. Arginine is a larger amino acid, which disturbs the interaction with zinc in this critical zinc finger domain of the protein, known to bind DNA. Arginine is also positively charged which causes repulsion and destabilisation of the domain. It also protrudes from the core of the protein and is unable to form the same hydrogen bond as the wild-type amino acid.

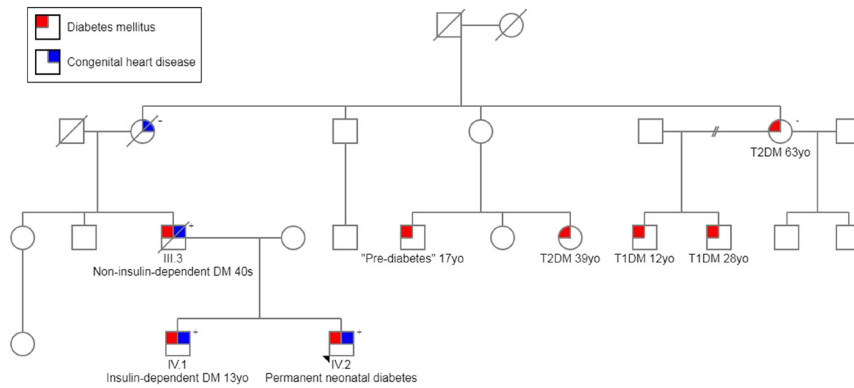


Figure 2

Pedigree showing affected family members with diabetes mellitus and/or congenital heart disease. *GATA6* mutation status indicated by symbols: + mutation present; – mutation absent. DM, diabetes mellitus; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; yo, years old.

Outcome and follow-up

The knowledge of this genetic diagnosis has allowed the proband to better understand the aetiology of his multisystem disorder and empowered him to play a larger role in his care. Previously a frequent non-attender of clinic appointments, his therapeutic relationship with the various medical specialties involved in his care as well as adherence to treatment have improved.

Just as importantly, identification of the causative variant in the proband allowed for cascade testing of his family. Genetic testing in his brother and their parents was undertaken after genetic counselling and consent, which revealed absence of the *GATA6* variant in the mother; presence of the *GATA6* variant in the deceased father and presence of the *GATA6* variant in the brother. It is likely that this novel *GATA6* variant arose *de novo* in the father and was inherited in an autosomal dominant fashion in the proband and his brother. Their different clinical presentations highlight the variable expressivity of *GATA6* mutations.

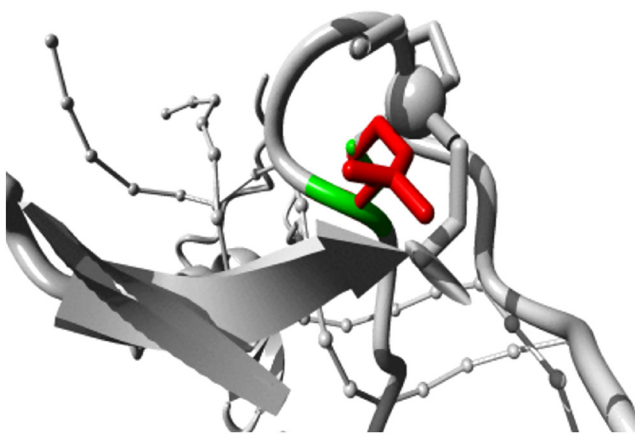


Figure 3

In silico modelling showing the *GATA6* protein with the wild-type amino acid (cysteine) in green and the mutant amino acid (arginine) in red. HOPE web application used, available at <http://www.cmbi.ru.nl/hope/>.

IV.1, who is of normal intellect, works full time and is in a stable relationship with a long-term partner, was previously reticent about having children as he did not want to pass on his then suspected genetic multisystem disorder to his offspring. Confirmation that he inherited the familial *GATA6* variant has given him the opportunity to consider reproductive technologies such as *in vitro* fertilisation (IVF) with preimplantation genetic diagnosis (PGD) to select for unaffected embryos and mitigate the 50% inheritance risk of this severe multisystem disorder.

Discussion

This case of neonatal-onset diabetes due to a genetic cause that was not identified until the affected proband was an adult is instructive in the general principles underlying clinical genetic testing in endocrinology.

Genetic spectrum of diabetes

Genetic forms of diabetes are often thought to be limited to the traditional forms of monogenic diabetes such as Mature Onset Diabetes of the Young (MODY); however, diabetes may also be a component of syndromic disorders due to pathogenic variants in well-defined genes. A critical clue in the proband was the neonatal onset of his diabetes. Permanent neonatal diabetes is most commonly due to mutations in *KCNJ11*, *ABCC8*, *INS* and *GCK* (3). Neonatal diabetes specifically related to pancreatic agenesis arises from mutations in multiple genes including *PDX1*, *PTF1A*, *HNF1B*, *EIF2AK3*, *RFX6* and *GATA6* (4). Only the latter gene is currently implicated in the combined phenotype of pancreatic agenesis and congenital heart disease (5) and enabled a successful single-gene approach in the present case using Sanger sequencing.

GATA6 was first implicated in neonatal diabetes in 2011 when heterozygous loss-of-function variants were



Table 1 The eight reported *GATA6* kindreds with diabetes mellitus from pancreatic agenesis/hypoplasia.

Reference	Inheritance	<i>GATA6</i> mutation by cDNA and protein nomenclature	Diabetes presentation	Cardiac abnormalities	Other endocrine and congenital abnormalities	Features in mutation positive relatives
De Franco <i>et al.</i> (6)	Paternal	c.969 C>A, p.Y323X	Pancreatic agenesis	ASD, PDA		Father with adult-onset DM and VSD
De Franco <i>et al.</i> (6)	Paternal	c.1036_1042del, p.T346PfsX44	Pancreatic agenesis	TOF	Hypothyroidism, mild learning difficulties	Father with adult-onset DM
De Franco <i>et al.</i> (6)	Paternal	c.1136-2A>G, not reported	Pancreatic agenesis	PDA	Hepatic dysfunction	Father with childhood-onset DM
De Franco <i>et al.</i> (6)	Paternal	c.1406G>A, p.G469E	Pancreatic agenesis	None	Hypothyroidism, hepatomegaly, diaphragmatic hernia, mild-moderate developmental delay, hemiplegia	Father with adult-onset DM
De Franco <i>et al.</i> (6)	Maternal (mosaic)	c.1303-1G>T, not reported	Permanent neonatal DM (no pancreatic imaging)	Congenital heart defect (unspecified)	Hypothyroidism, neurocognitive deficit (unspecified)	Mother with congenital heart defect (unspecified) mosaic for mutation 20% in leukocytes
Bonnefond <i>et al.</i> (7)	Maternal	c.1504_1505del, p.Lys502Aspfs*5	Pancreatic agenesis	VSD	Gallbladder agenesis, bicornuate uterus, neonatal pancytopenia, severe IUGR	Mother with mild cardiac valvular abnormalities
Yorifuji <i>et al.</i> (1)	Maternal	c.1504_1505delAA, p.K562Dfs*5	Pancreatic hypoplasia diagnosed in adolescence	TOF		Sister with PDA, gallbladder agenesis, proctorrhagia
Yau <i>et al.</i> (8)	Maternal (mosaic)	c.635_660del, p.Pro212fs	Pancreatic agenesis	PDA, PS	Gallbladder agenesis, diaphragmatic hernia, intestinal malrotation	Mother with PDA, ASD, adult-onset DM
This case report	Paternal	c.1330T>C, p.Cys444Arg	Pancreatic agenesis	Double outlet RV, PS, PDA, VSD	Hypogonadism, growth hormone deficiency, umbilical hernia, bifid left pelvic/cecal system, right hydrocele, right epididymal cyst, proteinuria, dysmorphic features, mild-moderate neurocognitive impairment	Unaffected mother mosaic for mutation 11% in peripheral leukocytes Sibling with multiple cardiac abnormalities Father with adult-onset DM, ASD, umbilical hernia, dysmorphic features Brother with childhood-onset DM, truncus arteriosus, bicuspid AV, hypospadias, umbilical hernia

ASD, atrial septal defect; DM, diabetes mellitus; PDA, patent ductus arteriosus; PS, pulmonary stenosis; RV, right ventricle; TOF, tetralogy of Fallot; VSD, ventricular septal defect.



found in 15 of 27 individuals with pancreatic agenesis (5). Following multiple reports of *de novo* cases, only seven familial cases of *GATA6*-related diabetes mellitus have been previously described (1, 6, 7, 8) and the present family represents one of the largest (Table 1). The *GATA6* phenotypic spectrum includes neonatal-, childhood- and adult-onset diabetes, exocrine pancreatic insufficiency, pancreatic agenesis or hypoplasia, various cardiac malformations, gallbladder agenesis/biliary atresia, hypothyroidism, hypopituitarism and pituitary agenesis, intestinal malrotation, hernias, colonic perforation, structural kidney abnormalities, neurocognitive deficits and seizures (5, 6, 9). Other features are unique to this family. Dysmorphic facial features as observed in this case have not been described. There has been one report of proteinuria in an infant with a *GATA6*-truncating variant (4) and another child with a *GATA6* deletion and structural renal abnormalities (9). However, to our knowledge, our case is the only report detailing renal histology in an individual with a *GATA6* mutation and proteinuria. It is tempting to speculate that *GATA6* may be implicated in both microscopic and macroscopic renal development. As *GATA6* overexpression in rat glomerular mesangial cells results in cell cycle arrest (10), loss-of-function *GATA6* variants could plausibly result in the mesangial cell proliferation observed on the proband's renal biopsy.

Approach to genetic testing

Recognising a potential genetic disorder and accurately diagnosing it through the appropriate genetic test may pay dividends across the healthcare of an individual and their family. Whether genetic testing should be pursued should reflect how likely a disorder is to be genetic and how likely a genetic result would be to alter management of the individual and/or their family. It should only be performed after comprehensive phenotyping using history, examination and preliminary investigations to determine the likelihood of finding a genetic disorder and identify candidate genes.

It is impossible to know all genetic disorders within endocrinology, but features such as young-onset and/or aggressive disease or the development of multiple rare disorders within an individual or one or more related rare disorders within multiple family members is suggestive of a genetic cause. A strong autosomal dominant family history of sulphonylurea-sensitive diabetes mellitus in lean family members with glycosuria immediately brings to mind monogenic diabetes due to a mutation in *HNF1A*. Monogenic diabetes due to *GATA6* mutations are much

rarer but the phenotype of pancreatic agenesis, congenital heart disease and other congenital malformations should raise the suspicion of some sort of genetic disorder worthy of further evaluation.

Though next-generation sequencing (NGS) may be the modality typically used to evaluate monogenic diabetes, *GATA6* is one of the special cases where Sanger sequencing is preferable. *GATA6* contains a ~300bp region with high guanine-cytosine content, which is difficult to amplify because of its high stability with formation of secondary structures and higher melting points, and Sanger sequencing using tailored PCR settings can circumvent this issue.

Utility of genetic testing

The indications for genetic testing in endocrinology include differentiation of clinical disorders; disease monitoring; therapeutic guidance; prognostication and predictive testing of family members. In our case, though the genetic test results did not directly lead to a change in medical treatment for the proband, the knowledge of the diagnosis empowered the patient to take a greater part in his care and improved adherence. In addition, genetic testing with successful identification of the culprit variant allowed for cascade testing and family planning. Even though IV.1 was previously reticent about having children due to the risk of passing on his multisystem disorder, the identification of his genetic condition has given him the opportunity to consider reproductive technologies that would not have been available without the genetic test result.

Declaration of interest

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

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Patient consent

Written informed consent has been obtained from the patient for publication of this article.

Author contribution statement

Y T D and S M C D were involved in the conception, literature review and drafting of this case report. S M C D arranged genetic testing of the



proband and relatives and performed *in silico* protein modelling. L M was responsible for the renal biopsy histopathological assessment. N K oversaw genetic testing of the family. All authors contributed to the final manuscript.

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References

- 1 Yorifuji T, Kawakita R, Hosokawa Y, Fujimaru R, Yamaguchi E & Tamagawa N. Dominantly inherited diabetes mellitus caused by *GATA6* haploinsufficiency: variable intrafamilial presentation. *Journal of Medical Genetics* 2012 **49** 642–643. (<https://doi.org/10.1136/jmedgenet-2012-101161>)
- 2 Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, *et al.* Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine* 2015 **17** 405–424. (<https://doi.org/10.1038/gim.2015.30>)
- 3 Naylor RN, Greeley SA, Bell GI & Philipson LH. Genetics and pathophysiology of neonatal diabetes mellitus. *Journal of Diabetes Investigation* 2011 **2** 158–169. (<https://doi.org/10.1111/j.2040-1124.2011.00106.x>)
- 4 Tuhan H, Catli G, Anik A, Ozmen D, Turkmen MA, Bober E & Abaci A. Neonatal diabetes mellitus due to a novel mutation in the *GATA6* gene accompanying renal dysfunction: a case report. *American Journal of Medical Genetics: Part A* 2015 **167A** 925–927. (<https://doi.org/10.1002/ajmg.a.36984>)
- 5 Allen HL, Flanagan SE, Shaw-Smith C, De Franco E, Akerman I, Caswell R, International Pancreatic Agenesis Consortium, Ferrer J, Hattersley AT & Ellard S. *GATA6* haploinsufficiency causes pancreatic agenesis in humans. *Nature Genetics* 2011 **44** 20–22. ([doi:10.1038/ng.1035](https://doi.org/10.1038/ng.1035))
- 6 De Franco E, Shaw-Smith C, Flanagan SE, Shepherd MH, International NDM Consortium, Hattersley AT & Ellard S. *GATA6* mutations cause a broad phenotypic spectrum of diabetes from pancreatic agenesis to adult-onset diabetes without exocrine insufficiency. *Diabetes* 2013 **62** 993–997. (<https://doi.org/10.2337/db12-0885>)
- 7 Bonnefond A, Sand O, Guerin B, Durand E, De Graeve F, Huyvaert M, Rachdi L, Kerr-Conte J, Pattou F, Vaxillaire M, *et al.* *GATA6* inactivating mutations are associated with heart defects and, inconsistently, with pancreatic agenesis and diabetes. *Diabetologia* 2012 **55** 2845–2847. (<https://doi.org/10.1007/s00125-012-2645-7>)
- 8 Yau D, De Franco E, Flanagan SE, Ellard S, Blumenkrantz M & Mitchell JJ. Case report: maternal mosaicism resulting in inheritance of a novel *GATA6* mutation causing pancreatic agenesis and neonatal diabetes mellitus. *Diagnostic Pathology* 2017 **12** 1. (<https://doi.org/10.1186/s13000-016-0592-1>)
- 9 Bui PH, Dorrani N, Wong D, Perens G, Dipple KM & Quintero-Rivera F. First report of a de novo 18q11.2 microdeletion including *GATA6* associated with complex congenital heart disease and renal abnormalities. *American Journal of Medical Genetics: Part A* 2013 **161A** 1773–1778. (<https://doi.org/10.1002/ajmg.a.35974>)
- 10 Nagata D, Suzuki E, Nishimatsu H, Yoshizumi M, Mano T, Walsh K, Sata M, Kakoki M, Goto A, Omata M, *et al.* Cyclin A downregulation and p21(cip1) upregulation correlate with *GATA-6*-induced growth arrest in glomerular mesangial cells. *Circulation Research* 2000 **87** 699–704. (<https://doi.org/10.1161/01.RES.87.8.699>)

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