Marked intrafamilial variability of exocrine and endocrine pancreatic phenotypes due to a splice site mutation in GATA6

Radka Savova, Elisa De Franco, Charles Shaw-Smith, Ralitza Georgieva, Maia Konstantinova, Margarita Archinkova, Emilia Panteleeva, Anna Kaneva, Rumen Marinov, Sian Ellard and Andrew Hattersley

**ABSTRACT**

The objective of this study was to describe the clinical characteristics of syndromic neonatal diabetes in a family with a GATA6 mutation. A girl, currently aged 12 years 3 months, was born with intrauterine growth retardation: weight 1600 g (−4.3 SDS) at term. After birth, foramen ovale and patent ductus arteriosus (PDA) were diagnosed by echocardiography. Diabetes was diagnosed on the 9th day after birth. Exocrine pancreatic insufficiency was clinically diagnosed at about 2 years of age and pancreatic agenesis was revealed later by magnetic resonance imaging. Her father had undergone surgery during infancy for PDA and had developed insulin dependent diabetes at 12 years of age. Ultrasound revealed a thin pancreas with normal length and anatomical structure. He has subclinical exocrine pancreatic insufficiency, low insulin needs and no late complications of diabetes up to the age of 40 years. Sequencing of GATA6 identified a heterozygous splicing mutation, 1136-2A>G, in the girl and her father. Testing of the paternal grandparents showed that the mutation was likely to have arisen de novo in the father. Identification of a GATA6 mutation explains the cardiac anomalies and diabetes in this family. This case highlights the marked intrafamilial variability of both exocrine and endocrine pancreatic phenotypes in patients with GATA6 mutations.

**Introduction**

Genome sequencing technologies have helped to establish the etiology of diabetes mellitus (DM) in a small group of pediatric patients presenting non-autoimmune mechanisms of impaired insulin secretion. Some of them are diagnosed in the neonatal period or during early infancy. More than 20 gene mutations causing permanent (PNDM) or transient (TNDM) neonatal diabetes mellitus have been identified [1].

The transcription factor GATA6 has been intensively studied after the discovery of its role in the development of the pancreas. GATA 4/5/6 subfamily presents primary endodermal and mesodermal transcription factors, expressed in the primitive endoderm and extra-embryonic tissues for supporting the growing epiblast [2–4] and endodermal differentiation [5]. During organogenesis, GATA4/5/6 ensure the anatomical and functional development of the pancreas, heart, blood vessels, hepatobiliary system, urogenital tract, thyroid gland as well as the brain [6,7]. GATA 4/6 have a leading role in the transcriptional network hierarchy in pancreatic multipotent progenitors arisen from the foregut endoderm [8,9].

In humans, GATA4 mutations were initially discovered to cause familial congenital cardiac septal defects [10] and have been found in some individuals from screened series of patients with congenital non-syndromic cardiac defects.[11–13] Further studies have shown that GATA4/6 haploinsufficiency can also cause a combination of pancreatic agenesis/hypoplasia, cardiac and hepatobiliary defects [14–16].

In this report we describe the clinical characteristics of syndromic neonatal diabetes in a family, father and daughter, with a GATA6 mutation. The genetic finding has already been reported [16]. Here, the clinical features are described in details and, especially, the marked phenotypic variability between the two affected family members.

**Subjects and methods**

This study included two family members, father and daughter, reportedly carrying a GATA6 mutation [16]. The girl patient, currently aged 12 years 3 months was born with intrauterine growth retardation with weight...
1600 g (−4.3 SDS), length 41 cm (−4.8 SDS), following a normal full-term pregnancy of a healthy mother and a father diagnosed with type 1 diabetes at the age of 12 years.

Written informed consent to participate in this study was obtained for both subjects. The study was approved by the Ethics committee at the University Pediatric Hospital–Sofia.

**Genetic testing**

Molecular genetic analysis was performed by screening the coding sequence and ~50 bp of flanking sequence of GATA6 using Sanger sequencing [16]. Sequencing reactions were run on an ABI3730 capillary machine (Applied Biosystems, Warrington, U.K.) and analysed using Mutation Surveyor v3.98 (SoftGenetics, State College, PA) (GATA6 nucleotide reference NM_005257.3).

**Results and discussion**

**Clinical case**

The first ultrasound cardiac investigation of the girl revealed foramen ovale, small patent ductus arteriosus (PDA) and peripheral stenosis of both branches of the pulmonary artery. At the age of 9 days, her condition worsened with dehydration, weight loss up to 1200 g, hyperglycemia of 30 mmol/L without ketonuria and hematocrit decrease. At 10 days, she was hospitalized with some degree of insulin deficiency and malnutrition. C-peptide was low: 88.2 pmol/L (normal 196–960 pmol/L). Ultrasound investigation revealed a hypoplastic thin pancreas but normal length and anatomical structure. Fecal elastase measured at his last visit was less than 15 μg/g feces (normal > 200 μg/g), suggesting subclinical pancreatic insufficiency.

In the girl, the ligation of PDA was performed at 20 months of age because of clinically significant signs of large left to right shunting, marked left ventricular volume overload and congestive heart failure. After surgery, the specific medical therapy was stopped and the cardiac dimensions gradually normalized. During the first 3 years of life, the main clinical issues were iron-deficient anemia, hypoproteinemia, mildly elevated transaminases and subclinical biliary stasis, perhaps all attributable to hepatic dysfunction and/or steatosis hepatitis due to some degree of insulin deficiency and malnutrition (Table 1).

Breast milk enriched in proteins and starch was given in the first few months after birth. Unusually large amount of carbohydrates, up to 20–22 g/kg b.w. (normal 12–14 g/kg), was necessary to keep weight gain with a corresponding insulin dose of 0.5 U/kg b.w. Follow-ups of C-peptide were recorded at 3 months (79.6 pmol/L) and 2 years (19.4 pmol/L). Clinical signs of exocrine pancreatic insufficiency appeared after 2 years of age: abdominal distension, bulky stools 5–7 times daily with steatorrhoea with up to 50% of ingested fat. No weight loss was seen and the insulin dose was stable (0.8–0.9 U/kg b.w.). Unfortunately, we could not investigate fecal

**Table 1. Laboratory signs of hepatic function.**

| Age          | ASAT a U/L | ALAT b U/L | GGT c U/L | Alkaline phosphatase U/L | Total bilirubin (μmol/L) (n < 17) | Direct bilirubin (μmol/L) (n = 0–3.5) |
|--------------|------------|------------|-----------|--------------------------|-----------------------------------|---------------------------------------|
| 3 months     | 76 (n < 61)| 39 (n < 50)| 316 (n = 4–163) | 1626 (n = 124–341) | 49.0                              | 15.4                                  |
| 1.1 years    | 32 (n < 50)| 42 (n < 36)| 12 (5–31)  | 875 (108–317)            |                                   |                                       |
| 2 years      | 73         | 73         | 12         | 876                      |                                   |                                       |
| 2.4 years    | 60         | 74         | 11         | 838                      |                                   |                                       |
| 3 years      | 182        | 117        | 14         | 323                      |                                   |                                       |
| 3.5 years    | 28         | 31         | 12         | 314                      |                                   |                                       |

aAlanine aminotransferase.
bAspartate aminotransferase.
cGamma-glutamyl transpeptidase.
Note: n, normal (reference) value.
elastase at the time. Ultrasound revealed initially a small pancreas with 3.5 mm length. Replacement therapy similar to that of patients with cystic fibrosis was initiated at age of 2 years 2 months: 1000 lipase units/kg b.w. per meal, Vitamin A 2000 U/d, Vitamin D 800 U/d and Vitamin E 50 U/d. At the age of 4 years, magnetic resonance imaging (MRI) was performed and confirmed pancreatic agenesis. Fecal elastase measured at her last visit was less than 15 μg/g feces (normal > 200 μg/g).

**Current status**

The girl takes a normal traditional diet and receives three to four injections of short-acting insulin analog for meals and long-acting insulin analog at bedtime with a total daily dose of 0.8 U/kg b.w. The dose of Creon is 75,000–100,000 U daily. At the last examination, the plasma levels of fat-soluble vitamins under daily supplemental therapy were near the lower reference: Vitamin A, 0.24 mg/L (0.3–0.8 mg/L); Vitamin E (tocopherol), 7.9 mg/L (5–18 mg/L) and 25-OH-Vitamin D, 19.42 ng/mL (sufficiency level 30–100 ng/mL).

At present (12 years 3 months of age), the girl’s height is 151.5 cm (−0.06 SDS), weight 37 kg (−0.83 SDS) (Figure 1). The expected final height according to the parent’s height is 162.5 ± 5 cm.

She has normal pubertal (Tanner stage 3) and intellectual development with good school performance and normal physical activity. She maintains good glycemic control with HbA1c 7%–8% (53.1–63.93 mol/mol). The persisting small foramen ovale is followed by cardiologists.

![Figure 1. Growth chart of the girl since birth.](image)
Genetic testing

Agenesis of pancreas had not been suspected up to 2 years of age because of detectable C-peptide and no clear signs of exocrine pancreatic insufficiency. A first series of DNA analyses excluded mutations in the KCNJ11, ABCC8 and INS genes as well as biallelic mutations in GCK. After MRI at 4 years of age, PDX1 and PTF1A gene mutations were excluded as well.

Further analysis revealed a heterozygous GATA6 splicing mutation, c.1136-2A>G. This mutation affects the conserved splice acceptor site of intron 2 (c.1136-2A>G) and is predicted to cause aberrant splicing (Figure 2).

Testing of parental samples revealed that the mutation had been inherited from her father; therefore, very likely to cause congenital heart defect as well as diabetes in both patients. The mutation was not found in the DNA sample from the paternal grandparents of the girl, indicating that it has arisen de novo in the father.

Thus, in the family examined in this study, the autosomal dominant transmission of a GATA6 mutation is associated with a congenital heart defect (PDA) in the two relatives, pancreatic agenesis and PNDM in the daughter and pancreatic hypoplasia and juvenile-onset diabetes in the father. In a cohort of 795 neonatal diabetes patients, 24 (3%) have been reported to have GATA6 mutations, 21 of them with complete absence or marked hypoplasia of the pancreas, including our patient [16]. The most frequent extra-pancreatic features among the patients with GATA6 mutations are congenital heart defects (83%), but additional extra-pancreatic features are reported, too. McMillan et al. [17] report a case of neonatal diabetes and pancreatic hypoplasia resulting from a de novo mutation of the GATA6 gene and several associated anomalies including truncus arteriosus, gallbladder agenesis, an inguinal hernia and protein-losing enteropathy. This illustrates the variable phenotype associated with mutations of this gene [17,18]. Some of the patients have late onset diabetes, but others have isolated cardiac defects only [16].

Our familial case of GATA6 haploinsufficiency presents the main clinical characteristics of the syndrome: diabetes and cardiac defect [15–21]. Among familial cases, variable penetrance of the diabetes phenotype has been reported regarding age of onset, severity of diabetes
and exocrine insufficiency, whilst the cardiac phenotype is highly penetrant [16,22,23]. The family described here highlights the variable clinical presentation of the diabetic phenotype, with pancreatic agenesis and PNDM in the proband and hypoplasia, juvenile onset diabetes and subclinical exocrine insufficiency in the father. It is of interest to discuss if the hypoplastic 3.5 mm pancreas initially existed in the girl, being subjected to apoptosis later on in the absence of GATA6 supportive transcription factor. The same low levels of C-peptide and fecal elastase in the daughter and in the father do not reflect real pancreatic endocrine and exocrine functional capacity in each patient. The proband is our first case with PNDM out of 4150 children with diabetes registered in the Clinic of Diabetes at the University Pediatric Hospital – Sofia since 1970, including five children with TNDM.

Conclusions

Genetic analysis is important in patients with neonatal or late onset syndromic diabetes. Identification of the genetic defect guides treatment and allows prognosis, assessment of recurrence risk and prenatal diagnosis. Identification of a GATA6 mutation in the family described here explains the cause of the syndromic neonatal and juvenile onset diabetes, although the mechanism underlying the phenotypic variability of the disease is still unknown. GATA 6 mutations could be suspected at any age beyond infancy in diabetic patients with inherited cardiac anomaly like in the father.

Acknowledgment

ATH and SE are Wellcome Trust Senior Investigators and ATH is an NIHR Senior Investigator.

Disclosure statement

No potential conflict of interest was reported by the author(s).

References

[1] Rubio-Cabezas O, Ellard S. Diabetes mellitus in neonates and infants: genetic heterogeneity, clinical approach to diagnosis, and therapeutic options. Horm Res Paediatr. 2013;80:137–146.
[2] Frankenberg S, Gerbe F, Bessonnard S, et al. Primitive endoderm differentiates via a three-step mechanism involving Nanog and RTK signaling. Dev Cell. 2011;21 (6):1005–1013.
[3] Cai KQ, Capo-Chichi CD, Rula ME, et al. Dynamic GATA6 expression in primitive endoderm formation and matura tion in early mouse embryogenesis. Dev Dyn. 2008;237 (10):2820–2829.
[4] Koutsourakis M, Langeveld A, Patient R, et al. The transcription factor GATA6 is essential for early extraembryonic development. Development. 1999;126:723–732.
[5] Morrisey E, Tang Z, Sigrist K., et al. GATA6 regulates HNF4 and is required for differentiation of visceral endoderm in the mouse embryo. Genes & Dev. 1998;12:3579–3590.
[6] Molkentin JD. The zinc-finger-containing transcription factors GATA-4, -5, and -6: ubiquitously expressed regulators of tissue-specific gene expression. J Biol Chem. 2000;275:38949–38952.
[7] Chao CS, McKnight KD, Cox KL, et al. Novel GATA6 mutations in patients with pancreatic agenesis and congenital heart malformations. PLoS ONE. 2015 [cited 2017 Apr 30];10(2):e0118449. DOI:10.1371/journal.pone
[8] Jennings RE, Berry AA, Kirkwood-Wilson R, et al. Development of the human pancreas from foregut to endocrine commitment. Diabetes. 2013;62:3514–3522.
[9] Carrasco M, Delgado I, Soria B, et al. GATA4 and GATA6 control mouse pancreas organogenesis. J Clin Invest. 2012;122(10):3504–3515.
[10] Garg V, Kathiriya IS, Barnes R, et al. GATA4 mutations cause human congenital heart defects and reveal an interaction with TBX5. Nature. 2003;424(6947):443–447.
[11] Lin X, Huo Z, Liu X, et al. A novel gata6 mutation in patients with tetralogy of fallot or atrial septal defect. J Hum Genet. 2010;55:662–667.
[12] Maitra M, Koenig SN, Srivastava D, et al. Identification of GATA6 sequence variants in patients with congenital heart defects. Pediatr Res. 2010;68(4):281–285.
[13] Kodo K, Nishizawa T, Furutani M, et al. GATA6 mutations cause human cardiac outflow tract defects by disrupting semaphorin-plexin signaling. Proc Natl Acad Sci USA. 2009;106:13933–13938.
[14] Shaw-Smith C, De Franco E, Allen HL, et al. GATA4 mutations are a cause of neonatal and childhood-onset diabetes. Diabetes. 2014;63(8):2888–2894.
[15] Allen HL, Flanagan SE, Shaw-Smith C, et al. International pancreatic agenesis consortium. GATA6 haploinsufficiency causes pancreatic agenesis in humans. Nat Genet. 2011;44:20–22.
[16] De Franco E, Shaw-Smith C, Flanagan SE, et al. International NDMC. GATA6 mutations cause a broad phenotypic spectrum of diabetes from pancreatic agenesis to adult-onset diabetes without exocrine insufficiency. Diabetes. 2013;62:993–997.
[17] McMillan T, Girgis R, Sellers E. Neonatal diabetes and protein losing enteropathy: a case report. BMC Med Genet. 2016 [cited 2017 Apr 30];17:32. DOI:10.1186/s12881-016-0296-0
[18] Yau D, De Franco E, Flanagan S, et al. Case report: maternal mosaicism resulting in inheritance of a novel GATA6 mutation causing pancreatic agenesis and neonatal diabetes mellitus. Diagnostic Pathol. 2017 [cited 2017 Oct 24];12:1. DOI:10.1186/s13000-016-0592-1
[19] Catli G, Abaci A, Flanagan SE, et al. A novel gata6 mutation leading to congenital heart defects and permanent neonatal diabetes: a case report. Diabetes Metab. 2013;39 (4):370–374.
[20] Gong M, Simaitė D, Kühnen P, et al. Two novel GATA6 mutations cause childhood-onset diabetes mellitus, pancreas malformation and congenital heart disease. Horm Res Paediatr. 2013;79(4):250–256.
[21] Suzuki S, Nakao A, Sarhat AR, et al. A case of pancreatic agenesis and congenital heart defects with a novel GATA6 nonsense mutation: evidence of haploinsufficiency due to nonsense-mediated mRNA decay. Am J Med Genet A. 2014;164A(2):476–479.

[22] Yorifuji T, Kawakita R, Hosokawa Y, et al. Dominantly inherited diabetes mellitus caused by gata6 haploinsufficiency: variable intrafamilial presentation. J Med Genet. 2012;49(10):642–643.

[23] Bonnefond A, Sand O, Guerin B, et al. GATA6 inactivating mutations are associated with heart defects and, inconsistently, with pancreatic agenesis and diabetes. Diabetologia. 2012;55:2845–2847.