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CASE REPORT

A novel *GATA6* mutation in a child with congenital heart malformation and neonatal diabetes

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Key Clinical Message

Diabetes in neonates is a monogenetic disease and genetic analysis is warranted to allow best treatment, prognosis, and genetic counseling. Transcription factor mutations may have a variable expression and different organs may be involved.

Keywords

Exocrine pancreas dysfunction, GATA6, heart malformation, intrauterine growth retardation, neonatal diabetes, transcription factor mutation.

Introduction

Children with diabetes mellitus requiring permanent insulin treatment from the neonatal period onwards need to be offered genetic screening in order to identify single-gene mutations. A precise genetic diagnosis may not only influence treatment, prognosis and genetic counseling but also provides further insight in the beta cell function and pancreas development. We describe a neonate presenting with intrauterine growth retardation, pancreatic endocrine and exocrine dysfunction as well as severe cardiac malformations. Exome sequencing revealed a novel de novo heterozygous frameshift mutation (c.968dupA; p.Tyr323 fs) in exon 2 of *GATA6*, leading to a premature stop signal and a truncated protein. Although GATA6 has been reported in relationship with neonatal diabetes, pancreatic aplasia, and cardiac malformations, the cardiac malformations are more often reported in relationship with *GATA4*, sharing a high degree of homology with *GATA6*. In this case, the mutation was only identified in *GATA6*. This finding expands the spectrum of mutations in *GATA6* linked to pancreatic dysfunction and the cardiac malformations suggesting, furthermore, the critical role of *GATA6* in pancreas and cardiac organogenesis in the human.

Although type 1 diabetes mellitus, or auto immune diabetes, remains the most frequent form of diabetes in childhood, this diagnosis needs to be carefully reevaluated when hyperglycemia is associated either with morbid obesity in the adolescent age (type 2 diabetes mellitus) or with a very early onset (before 6 months of life) and/or with a highly positive family history (monogenic forms of diabetes including neonatal diabetes or mitochondrial diabetes) [1–3].

Contrary to the classical multifactorial type 1 and type 2 diabetes mellitus, the monogenic forms result from mutations in single gene. These single-gene mutations have contributed to our understanding of the insulin secretion, insulin effect and more recently to our knowledge on pancreatic development. Besides, they have therapeutic and prognostic implications as in some situations insulin treatment can be replaced by oral drug therapy and better metabolic outcome.

Neonatal diabetes can be permanent (hyperglycemia starts within the first few months (<6 months) after birth and does not resolve over time) or transient (treatment can be stopped after 3–6 months). When an abnormality is detected in the imprinted region of chromosome 6, this suggests a transient form of neonatal diabetes, whereas in the permanent form, the role of ATP sensitive potassium channels (*KCNJ11* [MIM 600937] and *ABCC8* [MIM 600509]) in the beta cell has been extensively described [4]. More recently, some gene mutations in transcription factors (TFs) involved in pancreatic development (leading to pancreas agenesis or hypoplasia) have been identified playing a role in the development of neonatal diabetes including *PDX1* (MIM 600733) [5], *PTF1A* (MIM 607194) [6], and *GATA4* (MIM 600576) [7]. Although many cases remain unresolved, it remains relevant to screen for a genetic form of diabetes mellitus, if the child is diagnosed with diabetes before the age of 7–8 months. In a recent review, 171 out of 795 cases with unknown origin of their NDM were screened for a GATA6 mutation. The screening showed in 29 cases, a mutation in relationship with different clinical phenotypes [8].This genetic analysis should not be limited to those with a positive family history as de novo events might arise. Disease treatment, long-term outcome, as well as genetic counseling may be influenced by this issue.

We report here a novel mutation in exon 2 of *GATA6* (MIM 601656) leading to permanent diabetes, exogenous pancreas insufficiency, and congenital heart defects.

The Regional Ethical Committee for Medical Research approved this study, which was performed according to the Helsinki Declaration. We obtained written informed consent from the parents for genetic testing.

The boy is the first child of healthy parents. There is no diabetes known in either families. He was born small for gestational age (38 6/7 weeks) without any identified maternal cause and a normal pregnancy. His bodyweight was 1560 g, his height 38 cm and head circumference 27.5 cm and an APGAR score of 8 (1 min) 9 (5 min) and 9 (10 min). A small placenta was found without other abnormalities. Shortly after arrival in the neonatal ward, the child developed hyperglycemia without any infections or other explanation and with low insulin levels (glucose: 290 mg/dL (16.1 mmol/L); insulin: 2.9 mU/L). Further analysis (ultrasound) revealed only the pancreas head and tail but no body, abnormal mesenteric veins, without further malrotation, no brain abnormalities, but cardiac malformations (large muscular as well as a perimembraneous ventricular septal defect, an atrial septal defect (MIM 108800), valvular (MIM 265500), and supravalvular pulmonary stenosis). Further analysis revealed very low elastase level in the feces (<50 mcg/g) suggesting an exogenous pancreatic insufficiency as well. Intravenous insulin treatment was started and within the first few weeks replaced by continuous subcutaneous insulin infusion ensuring as physiological as possible administration of insulin and fast return home. Within his first year of life, corrective cardiac surgery was performed. For the exogenous pancreatic insufficiency, enzyme replacement was given. Insulin treatment remained necessary, confirming the diagnosis of permanent neonatal diabetes mellitus (MIM 606176). His current development is excellent and he has achieved all psychomotor development milestones as well as growth within the normal reference range at the age of 2 years. His metabolic control with continuous subcutaneous insulin infusion is good, without major acute complications.

Taking the intrauterine growth retardation, pancreatic endocrine and exocrine dysfunction, and cardiac malformations into account, genetic testing was proposed for the child and both his parents. Whole exome sequencing in both the proband and parents was performed at Hudson Alpha Institute for Biotechnology (Huntsville, AL) using Roche–NimbleGen Sequence Capture EZ Exome v2 kit (Roche NimbleGen, Inc., Madison, WI) and paired-end 100nt sequencing on the Illumina HiSeq [9–13].

Standard whole exome sequencing analysis [13] did not reveal any likely causative mutation in the child. We, therefore, next identified regions of low coverage among a set of genes previously defined as potential monogenic diabetes candidate genes [13]. In a region of poor coverage in exon 2 of *GATA6*, we detected one single read in the proband with a 1-bp insertion predicted to encode a frameshift of the protein at residue 323 (c.968dupA; p.Tyr323 fs). Sanger sequencing confirmed the presence of a de novo heterozygous mutation in the child with absence of the mutation in both parents (Fig. 1). This de novo mutation has not been reported previously.

Recent studies by Bonnefond [14] and Yorifuji [15] suggest a high degree of variability in the clinical manifestation of GATA6 haploinsufficiency. The great diversity in the phenotypic spectrum between carriers of GATA6-mutations suggests the existence of modifier genes. Two recent studies in mice have shown that double knockout of Gata4 and Gata6 [16, 17] caused complete pancreatic agenesis in contrast to single-gene knockout Gata4 or Gata6 that did not significantly affect normal pancreas

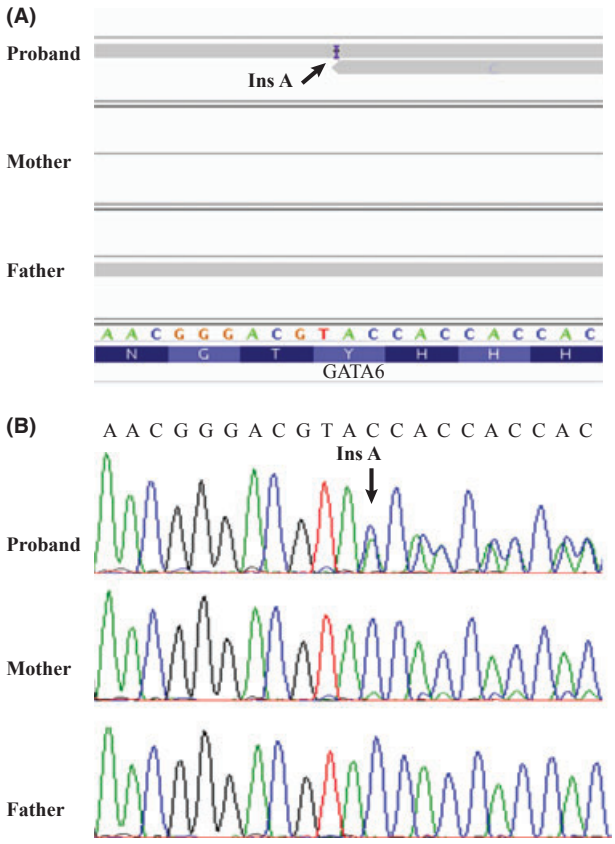

Figure 1. De novo mutation in exon 2 of GATA6 detected in the proband. (A) An *IGV* snapshot of a region in exon 2 of GATA6, which was poorly covered in whole exome sequencing (with 2, 0, and 1 reads in the proband, mother and father, respectively) and shows a 1-bp insertion (*arrow*, Ins A) in one single read in the proband. (B) Chromatograms of Sanger sequencing show the insertion in heterozygous status (*arrow*, Ins A) in the proband that is not found in the parents.

morphogenesis. Therefore, we searched for potentially functional modifier mutations in putative candidate genes such as GATA4 and GATA5 (MIM 611496) but found no obvious candidate mutations.

The *GATA* family of transcription factors represents a group of evolutionary conserved zinc finger TFs involved in development and differentiation of eukaryotic organisms. In vertebrates, this family of TFs is split into two subgroups encompassing in total six members including the hematopietic (*GATA1/2/3*) and cardiac groups (*GATA4/5/6*) [18]. *GATA4/5/6* are expressed in tissues of endodermal and mesodermal origin [19] including among others gut, lung, heart, and pancreas. *GATA6* shows a high temporal and functional overlap with *GATA4* during cardiac development [20]. This TF includes an N-terminal transcription activator domain as well as two tandem GATA zinc fingers acting together as DNA-binding domain.

Currently, the Human Gene Mutation Database (Professional release 2012.3) contains 25 clinically relevant mutations for *GATA6*. Different studies have related *GATA6* mutations to congenital cardiac defects including ventricular septal defect [21, 22], tetralogy of Fallot [20, 23], and persistent truncus arteriosus [24]. Furthermore, two recent studies were able to delineate the implication of heterozygous mutations in the coding sequence of *GATA6* in pancreatic agenesis in conjunction with cardiac defects based on whole exome sequencing of individuals [25, 26].

Interestingly, on the basis of the analysis of the whole exome of two nonconsanguineous sisters, Bonnefond and collaborators [14] identified a truncating mutation in *GATA6* that has a distinct phenotypic penetrance on the level of pancreatic development (pancreatic agenesis/hypoplasia) in the two sisters, while both sisters show heart anomalies. Allen and collaborators [25] revealed in their study a high proportion of GATA6 mutations (15 individuals) in a panel of 27 patients with pancreatic agenesis. In addition, among these individuals, 14 of 15 showed cardiac malformations.

In this study, we report a novel de novo heterozygous mutation (c.968dupA; p.Tyr323 fs) in the coding sequence of *GATA6* located in the N-terminal transcription activator domain of GATA6 resulting in a truncated protein. The individual is affected by cardiac malformations and pancreatic hypoplasia requiring insulin treatment and enzyme replacement therapy. These observations are in the same phenotypic range as reported in recent studies [14, 25].

Moreover, the clinical features we observe in this case confirm the relevance of *GATA6* haploinsufficiency in human for pancreatic developmental perturbations. It is well-known that haploinsufficiency of TF genes is not uncommon in human developmental disorders [26]. Furthermore, these authors suggested that the increased probability for such TF genes to show functionally relevant fluctuations in their RNA levels might not be buffered sufficiently by the expression of only one healthy allele.

The clinical relevance of the de novo mutation described in this study that leads to a premature termination codon has not been described so far. Even though this study does not focus on a functional study of the observed insertion, we think that the mutation we observe here, is likely to have a functional impact comparable to those reported by Allen et al. [25] and Bonnefond et al. [14]. Genetic screening of children developing diabetes mellitus before 6 months has increased our knowledge on the pathophysiology of insulin secretion and insulin action and should become current practice in all these children, ensuring personalized medical care.

Web Resources

The URLs for data presented herein are as follows: Online Mendelian Inheritance in Man (OMIM), http://www.omim.org.

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Conflict of Interest

None declared.

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