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A novel mutation in *GATA6* causes pancreatic agenesis

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Abstract

Context—Heterozygous mutations in *GATA6* have been linked to pancreatic agenesis and cardiac malformations.

Objective—To describe a new mutation in *GATA6* in an infant with pancreatic agenesis, associated with truncus arteriosus and absent gallbladder.

Research Design and Methods—Clinical data were obtained from chart review. Gene sequencing was performed on genomic DNA.

Results—Our patient was a female diagnosed shortly after birth with a severe cardiac malformation, absent gallbladder, anomalous hepatic blood flow, unilateral hydronephrosis and hydroureter, neonatal diabetes and pancreatic exocrine insufficiency. Despite prolonged intensive management care, she expired at 2 months of age due to cardiac complications. Analysis of her genomic DNA revealed a novel missense mutation of *GATA6*.

Conclusions—The novel mutation described in this case extends the list of *GATA6* mutations causing pancreatic agenesis and cardiac malformations.

Introduction

Mutations in *GATA6*, a transcription factor containing two zinc-finger domains, are the most frequent cause of pancreatic agenesis, associated with several other congenital malformations (1). Studies in mice had shown the importance of *GATA6* and its relative, *GATA4*, in the development of the endocrine and exocrine pancreatic tissue, respectively (2, 3).

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A large cohort of patients with pancreatic agenesis were found to have *GATA6* mutations; missense mutations in exon 4, thought to affect the zinc finger domains, were showed to abolish its binding to a classical target, HNF4A (1), a transcription factor that is known to cause neonatal and later onset diabetes without exocrine insufficiency (1). Cardiac malformations are present in all cases of *GATA6* mutations reported to date (4), while it appears that the pancreatic defect is variable and neonatal diabetes is not present in all affected patients (5–8). Children carrying mutations in *GATA6* were also found to have other endocrine anomalies (pituitary agenesis, hypothyroidism), gastrointestinal malformations (intestinal malrotation, microcolon, gallbladder agenesis, biliary atresia, proctorrhagia), bicornuate uterus, neurodevelopmental anomalies and seizures (1, 5, 8–10). The frequency of these manifestations varies and it appears to be no correlation between the position of the mutation and a specific phenotype.

In this report, we describe a novel *GATA6* mutation in a neonate with a severe cardiac malformation, absent gallbladder, anomalous hepatic blood flow, hydronephrosis and pancreatic agenesis.

Methods

Clinical Data

Clinical information was obtained retrospectively by chart review. This study was approved by The Children's Hospital of Philadelphia Institutional Review Board.

DNA mutation analysis

Mutation analysis was completed by amplification of genomic DNA by Polymerase Chain Reaction and Sanger sequencing using the ABI Prism BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, CA). Non-coding exon 1 and coding exons 2–7 of *GATA6* along with intron/exon boundaries were included. Sequences were analyzed by Sequencher v5.0 software (Gene Codes Corp, MI) and compared to the published *GATA6* sequence NM_005257 (<http://www.ncbi.nlm.nih.gov/>).

Expression studies in 293T cells

A pCMV6-Entry expression vector containing human *GATA6* cDNA was obtained from Origene Technologies Inc. (Rockville, MD). Site-directed mutagenesis was performed using a QuikChange II XL Site-Directed Mutagenesis Kit (Stratagene, Santa Clara, CA). Wild-type and mutant *GATA6* were transfected into 293T cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) with 7 ug of expression plasmids [pCMV6-Entry-*GATA6* mutant (*GATA6* mut), pCMV6-Entry-*GATA6* [*GATA6* wild type (WT)], or pCMV6-Entry empty vector (EV)]. Cells were harvested after 48 h and lysed in a RIPA buffer (5mM EDTA, 50mM Tris pH 8.0, 150mM NaCl, 1mg/ml sodium deoxycholate) containing 1% NP-40, 1X protease inhibitor cocktail and 1X phosphatase inhibitor cocktail (Sigma-Aldrich, St. Louis, MO). Western blot analysis from whole cell extracts was performed using goat polyclonal *GATA6* (N-18) antibody (Santa Cruz Biotechnology, Dallas, TX). For the Western blot, loading protein quantity was standardized by equivalent β -actin bands.

Results and Discussion

The case was a female, born at 39 weeks gestation, small for gestational age (1760 g, <1th percentile), after an uncomplicated pregnancy and delivery. She was found to have a severe cardiac malformation (truncus arteriosus type III), left hydronephrosis and hydroureter, an absent gallbladder with no biliary atresia and a meconium plug. Her blood glucose level gradually increased, reaching a peak of 906 mg/dl (50 mmol/L) on day 2 of life, at which point insulin was started. She required an insulin drip, ranging from 0.01–0.05 U/kg/hr to maintain blood glucose levels below 200 mg/dl (11 mmol/L). The persistently high blood glucose levels and insulin requirement lead to the diagnosis of neonatal diabetes. This was later supported by undetectable C-peptide level (<0.5 ng/mL). Stool studies revealed a stool pH between 5 and 6 and reducing substances between 0.75% and 1%, suggestive of carbohydrate malabsorption. Serum trypsin level was undetectable at <3.5 ng/ml (reference range 10–57 ng/ml), fecal trypsin level was 1:80 (normal >1:10) and total fecal fat content was increased, consistent with exocrine pancreatic insufficiency. An abdominal CT at 1 month of life revealed minimal pancreatic tissue (questionable presence of a small portion of the pancreatic head) and no gallbladder. In addition to confirming the absence of the pancreas, the abdominal sonogram also revealed anomalous hepatic blood flow with dilated intrahepatic umbilical vein and patent ductus venosus. A subsequent HIDA scan showed normal intrahepatic biliary flow. Apart from the passage of a large meconium plug (noted to be 25 cm in length), there were no other intestinal anomalies. Barium enema revealed a normal diameter of the colon and there was no evidence of malrotation on x-ray. Thyroid function tests were normal; no other pituitary function testing was undertaken.

There was no family history of cardiac defects or pancreatic agenesis, but paternal aunt and other paternal family members had history of diabetes. Unfortunately, no further information was available on the extended family members. Both parents denied any personal history of diabetes or abnormal blood glucose levels, or further testing.

By 2 months of age, the child's had symptomatic congestive heart failure and was transferred to a specialized cardiac center for further management. She underwent single ventricle repair of the cardiac malformation, but postoperative course was complicated by open sternotomy, hemodynamic instability and gram negative rods sepsis. Despite prolonged intensive care stay, the child expired at 3 months of age.

Karyotype analysis and a FISH test for the 22q11 deletion revealed no anomalies. Genomic DNA sequencing for mutations in *IPF1* was negative. Several years after the initial presentation, the *GATA6* gene was sequenced after reports of similar phenotype in a cohort of children with pancreatic agenesis and cardiac malformations (1). Analysis of the *GATA6* gene revealed a novel heterozygous frameshift mutation, p.His204ValfsX97/ c. 606_609dupGTAC, in exon 2 (Figure 1). Unfortunately, genetic analysis could not be performed on her parents or other family members. Studies in 293T cells revealed that the mutated protein is expressed but it is prematurely truncated and predicted to lack the zinc finger DNA-binding domain (Figure 2).

This report extends the list of known *GATA6* mutations and the phenotype by describing an affected infant with severe cardiac malformation, absent gallbladder, hydronephrosis, abnormal hepatic blood flow and pancreatic agenesis. The novel mutation described here causes an early stop codon, abrogating the DNA-binding domain and leading to the formation of a shortened peptide (Figure 2).

Imaging studies suggested the questionable presence of the pancreatic head; this pancreatic remnant was not functional in view of the exocrine and endocrine insufficiency. This finding is also consistent with other reports that showed a variable pancreatic anatomical defect in children carrying *GATA6* mutations – ranging from complete absence to dorsal pancreatic aplasia to normal imaging (5, 8). The anomalous hepatic blood flow with dilated intrahepatic umbilical vein and patent ductus venous were not been previously reported in association with *GATA6* mutations. These could be consequence of her severe cardiac defect, of the mutation itself or independent of either. The hydronephrosis and hydroureter were reported in cases presenting with only cardiac malformations, and no pancreatic defect (11). A thorough investigation of renal and liver anatomy and function seems warranted in cases like this one.

Heterozygous mutations in *GATA6* have recently emerged as the most common cause of pancreatic agenesis and neonatal diabetes, most often associated with other congenital malformations. Sequencing of the *GATA6* gene is becoming an integral part of the current panel of genetic tests in these cases and should be undertaken in any patient with neonatal diabetes, particularly if other features are present, such as cardiac or biliary malformations. Furthermore, our case suggests that other anomalies of the kidney and liver could also be implicated and warrant further testing in children carrying mutations in *GATA6*.

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Figure 1.

Sanger sequencing performed on patient and control samples. Chromatograms depict consensus sequence and duplication of GTAC causing a frameshift and a stop codon 97 bases downstream.

Figure 2. Western blot analysis of HEK293T whole cell lysate transfected with plasmids expressing mutant GATA6, wild type GATA6 and empty vector. Probed using goat polyclonal anti-GATA6 antibody and mouse monoclonal β -actin.