

## ORIGINAL ARTICLE

# GATA6 mutations: Characterization of two novel patients and a comprehensive overview of the GATA6 genotypic and phenotypic spectrum

Doris Škorić-Milosavljević<sup>1</sup>  | Fleur V. Y. Tjong<sup>1</sup> | Julien Barc<sup>1</sup> | Ad P. C. M. Backx<sup>2</sup> | Sally-Ann B. Clur<sup>2</sup> | Karin van Spaendonck-Zwarts<sup>3</sup> | Roelof-Jan Oostra<sup>4</sup> | Najim Lahrouchi<sup>1</sup> | Leander Beekman<sup>1</sup> | Regina Bökenkamp<sup>5</sup> | Daniela Q. C. M. Barge-Schaapveld<sup>6</sup> | Barbara J. Mulder<sup>1</sup> | Elisabeth M. Lodder<sup>1</sup> | Connie R. Bezzina<sup>1</sup> | Alex V. Postma<sup>3,4</sup>

<sup>1</sup>Department of Clinical and Experimental Cardiology, Amsterdam UMC, University of Amsterdam, Heart Centre, Amsterdam, The Netherlands

<sup>2</sup>Department of Pediatric Cardiology, Amsterdam UMC, Emma Children's Hospital, Amsterdam, The Netherlands

<sup>3</sup>Department of Clinical Genetics, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

<sup>4</sup>Department of Medical Biology, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

<sup>5</sup>Department of Pediatric Cardiology, Leiden University Medical Center, Leiden, The Netherlands

<sup>6</sup>Department of Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands

## Correspondence

Doris Škorić-Milosavljević, Amsterdam UMC, University of Amsterdam, Heart Centre, Department of Clinical and Experimental Cardiology, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands.  
Email: d.milosavljevic@amsterdamumc.nl

Alex V. Postma, Amsterdam UMC, University of Amsterdam, Department of Clinical Genetics, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands.  
Email: a.v.postma@amsterdamumc.nl

## Funding information

Hartstichting, Grant/Award Numbers: 2014T053, CVON 2014-18; Children's Heart Foundation

## Abstract

The first human mutations in *GATA6* were described in a cohort of patients with persistent truncus arteriosus, and the phenotypic spectrum has expanded since then. This study underscores the broad phenotypic spectrum by presenting two patients with de novo *GATA6* mutations, both exhibiting complex cardiac defects, pancreatic, and other abnormalities. Furthermore, we provided a detailed overview of all published human genetic variation in/near *GATA6* published to date and the associated phenotypes ( $n = 78$ ). We conclude that the most common phenotypes associated with a mutation in *GATA6* were structural cardiac and pancreatic abnormalities, with a penetrance of 87 and 60%, respectively. Other common malformations were gallbladder agenesis, congenital diaphragmatic hernia, and neurocognitive abnormalities, mostly developmental delay. Fifty-eight percent of the mutations were de novo, and these patients more often had an anomaly of intracardiac connections, an anomaly of the great arteries, and hypothyroidism, compared with those with inherited mutations. Functional studies mostly support loss-of-function as the pathophysiological mechanism. In conclusion, *GATA6* mutations give a wide range of phenotypic defects, most frequently malformations of the heart and pancreas. This highlights the importance of detailed clinical evaluation of identified carriers to evaluate their full phenotypic spectrum.

## KEYWORDS

congenital heart disease, *GATA6*, heart, mutation, pancreas, phenotypic spectrum

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2019 The Authors. *American Journal of Medical Genetics Part A* published by Wiley Periodicals, Inc.

## 1 | INTRODUCTION

Congenital heart disease (CHD) consists of structural abnormalities of the heart and/or the intrathoracic blood vessels and is the most common birth defect worldwide with an estimated prevalence of around 1% (van der Linde et al., 2011). Although the heritable nature of CHD is well-demonstrated (Oyen et al., 2009), the underlying genetic causes are incompletely understood. Aneuploidies and large copy number variations have been found in CHD patients, but usually in those with well-recognized clinical syndromes, like Down syndrome and DiGeorge syndrome (Fahed, Gelb, Seidman, & Seidman, 2013; Zaidi & Brueckner, 2017). The use of linkage analysis, positional cloning, sequencing of candidate genes, and next-generation sequencing (including whole-exome and whole-genome sequencing) has led to the implication of a large number of genes in CHD, highlighting its high genetic heterogeneity (Fahed et al., 2013; Zaidi & Brueckner, 2017).

A significant subset of these genes encodes cardiac transcription (co)factors (McCulley & Black, 2012), among which are the GATA transcription factors. Of this family of six zinc finger nuclear transcription factors, GATA4, 5, and 6 are important regulators of gene expression in developing organs derived from endoderm and mesoderm (Molkentin, 2000), including the heart, organs of the digestive and urogenital system, and the brain (Nemer & Nemer, 2003). GATA factors owe their name to the DNA sequence (5'-(T/A)GATA(A/G)-3') in enhancer and promoter regions of target genes they bind to (Brewer & Pizzey, 2006). The expression of GATA6 has a distinct spatiotemporal pattern shows overlap with that of GATA4 and 5 in the heart (Xin et al., 2006) and also in other tissues (Nemer & Nemer, 2003). Although GATA6-knockout mice all die before heart formation (E6.5-E7.5), due to defects in the early endoderm formation (Morrisey et al., 1998), conditional inactivation of GATA6 in murine neural-crest-derived vascular smooth-muscle cells resulted in a spectrum of cardiac outflow tract defects (64% persistent truncus arteriosus [PTA] and 36% double outlet right ventricle [DORV]; Lepore et al., 2006), underscoring the importance of GATA6 in normal heart development.

The initial discovery of human GATA6 mutations was in individuals with a severe CHD, namely PTA (Kodo et al., 2009). However, shortly after, GATA6 mutations were also found to be an important cause of pancreatic agenesis (Allen et al., 2011). Indeed, GATA6 is initially expressed in the early pancreatic epithelium (together with GATA4) and later becomes restricted to the endocrine cells of the pancreas (Decker, Goldman, Grasc, & Sussel, 2006; Ketola et al., 2004). In addition, mice conditionally overexpressing a Gata6/4-Engrailed fusion protein designed to function as a dominant negative transcriptional repressor show a strong reduction in pancreatic tissue volume (Decker et al., 2006). This model recapitulates the pancreatic phenotype in humans.

We present two patients, both with a de novo GATA6 mutation, who illustrate the phenotypic heterogeneity associated with mutations in this gene. In addition, we provide an overview of all the GATA6 mutations reported in the literature ( $n = 78$ ) to date and explore genotype-phenotype correlations.

## 2 | MATERIALS AND METHODS

### 2.1 | Clinical evaluation

Patient 1 and Patient 2 were recruited at two academic hospitals in the Netherlands (Amsterdam University Medical Centre and Leiden University Medical Centre, respectively). Of note, Patient 2 was previously included in an international neonatal diabetes cohort that was tested for mutations in GATA6 (De Franco et al., 2013). Detailed phenotypic data were obtained by medical history interviews and from clinical records. Written informed consent was obtained from the patient and/or parents. The study was approved by the appropriate medical ethical committee of the two hospitals.

### 2.2 | Genetic testing

#### 2.2.1 | Patient 1

Whole-exome sequencing was performed in Patient 1 and his unaffected parents on DNA isolated from peripheral blood leucocytes at the Beijing Genomics Institute (Shenzhen, China). Details on the sequencing process and bioinformatics pipeline can be found in the Supporting Information Methods section. Based on the pedigree and family history, de novo variants were prioritized. We also explored the option of autosomal recessive and autosomal X-linked inheritance. All the analysis was carried out in the software program R (R version 3.4.0; R core team, 2017).

#### 2.2.2 | Patient 2

Detailed description on the molecular genetic analysis of Patient 2 has been previously reported (De Franco et al., 2013). In brief, mutations in the genes *ABCC8*, *KCNJ11*, and *INS* and chromosome 6q24 abnormalities had been excluded before screening the coding and flanking sequence of GATA6 using Sanger sequencing. Subsequently, the de novo state of the identified GATA6 mutation was confirmed by microsatellite analysis.

### 2.3 | Literature search

We searched in PubMed using the terms "GATA6 Transcription Factor"[Mesh] AND "GATA6 protein, human" [Supporting Information Concept]. This resulted in 178 items. Suitable papers were selected based on the abstract. Inclusion criteria were: human subjects with specified GATA6 mutations (all ethnicities and phenotypes) where the full text was available. Large (case-control) cohorts as well as case reports were selected. We checked the references in these papers and screened papers that cited the included papers. Additionally, large CHD cohorts (Homsy et al., 2015; Jin et al., 2017; Sifrim et al., 2016; Zaidi et al., 2013) were checked for patients with GATA6 mutations. Lastly, we checked curated databases (e.g., Clinvar and OMIM) to identify any missing mutations. We included all rare variants with (minor allele frequency < 0.001) and/or those not found in ethnically

matched healthy controls (in case-control studies). Patients with trisomy 18 were excluded. Congenital cardiac defects were classified based on the International Pediatric and Congenital Cardiac Code (Franklin et al., 2017). Statistical comparisons were performed using Fisher's exact test, and Bonferroni corrected  $p$  values were used for determination of statistical significance ( $p = .05/n$  tests).

### 3 | RESULTS

#### 3.1 | Patient 1

##### 3.1.1 | Clinical description

Patient 1, a 32-year-old male born to Dutch healthy nonconsanguineous parents, had a complex cyanotic congenital heart defect, with features never described before in *GATA6* positive individuals (superior-inferior position of the ventricles with a ventricular septal defect [VSD] and pulmonary stenosis [PS]; Table 1 and Figure 1a-g). In addition, he was diagnosed with diabetes mellitus (DM) Type I during young adulthood. Discovery of the *GATA6* mutation initiated a renewed examination of the etiology of the DM and revealed pancreatic hypoplasia (Figure 1i,j). Other abnormalities are described in Table 1.

#### 3.1.2 | Genetic findings

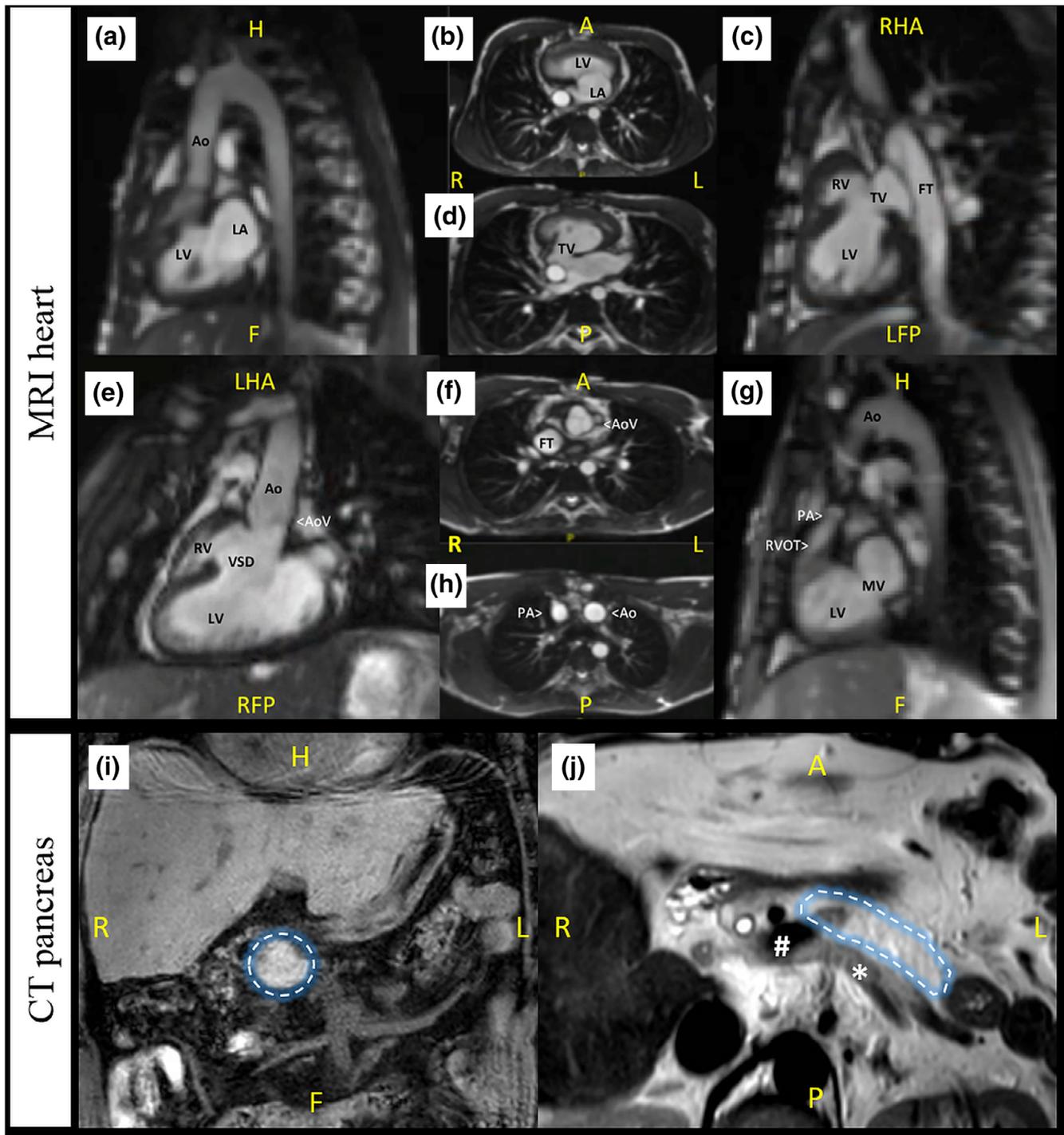
After applying the described filtering steps (see Supporting Information Methods section), eight variants were left that fitted a de novo inheritance (seven single nucleotide variants [SNVs] and one deletion; Table S1). After visual inspection of the Binary Alignment Map files, four variants were found in (one of) the parents and therefore discarded as de novo. The four remaining variants were tested by Sanger sequencing in the patient and his parents, two of which were confirmed to be truly de novo, namely *EGR3* p.(Glu347Asp) (NM\_004430) and *GATA6* p.(Arg456His) (NM\_005257). *EGR3*, early growth response 3, is a zinc-finger transcription factor, involved in proper muscle spindle development and knock-out mice show a severe neurological and musculo-skeletal phenotype including tremor, ataxia, abnormal gait, and scoliosis (Tourtellotte & Milbrandt, 1998), but no cardiac or pancreatic phenotype was described. Thus far, this gene was not implicated in human disease. Conversely, *GATA6* was clearly a strong candidate based on function and literature. The same mutation, p.(Arg456His) was previously identified in a patient with CHD, and other mutations at the same amino acid location, namely p.(Arg456Cys) and p.(Arg456Gly), were identified in another five patients (for phenotypes see Table S2; Allen et al., 2011; Jin et al., 2017; Sanyoura et al., 2018; Yu et al., 2014). Although the *GATA6*-p.(Arg456His) was not tested functionally, the *GATA6*-p.(Arg456Cys) showed significantly decreased transcriptional activity on the *WNT2* promoter (Allen et al., 2011). The deleteriousness of the variant found in

**TABLE 1** Overview of clinical phenotypes of patient 1 and patient 2

	Patient 1	Patient 2
Sex	M	M
Current age	32	11
Origin of parents	Dutch	Dutch
Family history	Negative for CHD/DM	Negative for CHD/DM
Inheritance	de novo	de novo
<i>GATA6</i> mutation	p.(Arg456His)	p.(Arg479Gly)
Cardiac phenotype	Dextrocardia; situs solitus; abnormal aligned AV valves; that is, the tricuspid valve was smaller and overrides a large VSD; superior-inferior position of the ventricles; hypoplastic right ventricle with a severe PS; aorta overriding the VSD; aorta and pulmonary artery in a side-by-side position; and large patent ductus arteriosus	Complete transposition of the great arteries with VSD; hypoplastic right ventricle with PS; and patent ductus arteriosus
Final corrective surgical treatment	Lateral tunnel Fontan age 13	One and a half ventricle repair (Rastelli) age 4
Pancreatic phenotype	Pancreatic hypoplasia with young adult-onset DM I (age 22)	Permanent child-onset DM I (age 5) after episodes of transient hyperglycaemia with exocrine pancreatic insufficiency
Treatment	Insulin	Insulin and pancreatic enzyme replacement
Other abnormalities	Renal duplex collecting system Galbladder agenesis Developmental delay	Cryptorchidism Protein losing enteropathy

Note: Clinical description of cardiac, pancreatic, and other phenotypes in Patients 1 and 2.

Abbreviations: AV, atrio-ventricular; CHD, congenital heart disease; DM, diabetes mellitus; PS, pulmonary stenosis; VSD, ventricular septal defect.



**FIGURE 1** Imaging of the heart and pancreas in Patient 1. (a–g) Magnetic resonance imaging showing the anatomy of the heart, at age 21, after surgical correction. (i, j) Computed tomography scan of the abdomen without intravenous contrast at the age 32. (a) Sagittal section showing the left-sided atrio-ventricular concordance and the position of the MV; (b) coronal section showing the central position of the heart in the chest with the apex to the right (dextrocardia). The MV is seen between the LA and LV; (c) Oblique section showing the smaller TV overriding the VSD. Note the superior/inferior relationship of the ventricles; (d) coronal section showing the TV. There is a large surgically created intra-atrial communication and the right atrium is smaller than the LA. Note that the TV and MV are not seen on the same plane; (e) oblique section of the LV outflow tract showing the Ao overriding the VSD; (f) coronal section showing the position of the AoV. Note the FT to the right and posteriorly; (g) sagittal section showing the hypoplastic RV and outflow tract (RVOT). The small PA, seen arising from the RV, has been surgically ligated and ends blindly; (h) Coronal section showing the side-by-side relationship of the great arteries with the Ao to the left (L) of the PA. (i) T1-weighted image, coronal plane. Only the head of the pancreas (dashed white line) is visible, suggestive for dorsal pancreatic agenesis; (j) T2-weighted image, axial plane at the level of the splenic vein (\*) entering the portal vein (#). No pancreatic body or tail could be identified where it would normally be expected (dashed white line). A, anterior; Ao, aorta; AoV, aortic valve; F, foot; FT, Fontan tunnel; H, head; L, left; LA, left atrium; LFP, left foot posterior; LHA, left head anterior; LV, left ventricle; MV, mitral valve; P, posterior; PA, pulmonary artery; R, right; RFP, right foot posterior; RHA, right head anterior; RV, right ventricle; TV, tricuspid valve; VSD, ventricular septal defect [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

Patient 1 was further supported by a high pathogenicity and conservation score (CADD of 35 and GERP of 5.87, respectively; Cooper et al., 2005; Kircher et al., 2014). Taken together, we considered this sufficient evidence to classify the de novo GATA6 variant in this patient as pathogenic. The recessive, compound heterozygous, and X-linked inheritance models did not yield likely candidates (1, 3, and 2 variants, respectively; Table S3).

## 3.2 | Patient 2

### 3.2.1 | Clinical description

We here provided an update on the phenotype of Patient 2, including the description of other abnormalities besides the previously described cardiac and pancreatic abnormalities by De Franco et al. (De Franco et al., 2013; Table 1). One of the most striking abnormalities in this patient was protein losing enteropathy (PLE), which could not be explained by the known and common causes of PLE. This phenotype was described in 1 other GATA6 patient (McMillan, Girgis, & Sellers, 2016) before.

### 3.2.2 | Genetic findings

A GATA6 mutation was identified at amino acid position 479 (c.1435A>G; p.[Arg479Gly], NM\_005257). A de novo state was confirmed with microsatellite analysis of the parent–proband trio (De Franco et al., 2013). This variant was not present in over 130,000 individuals from the Genome Aggregation Database (gnomAD; Lek et al., 2016), had a CADD score of 25.4 and conservation GERP score of 1.29, and was therefore considered pathogenic in this patient.

## 3.3 | Human GATA6 mutations in the literature

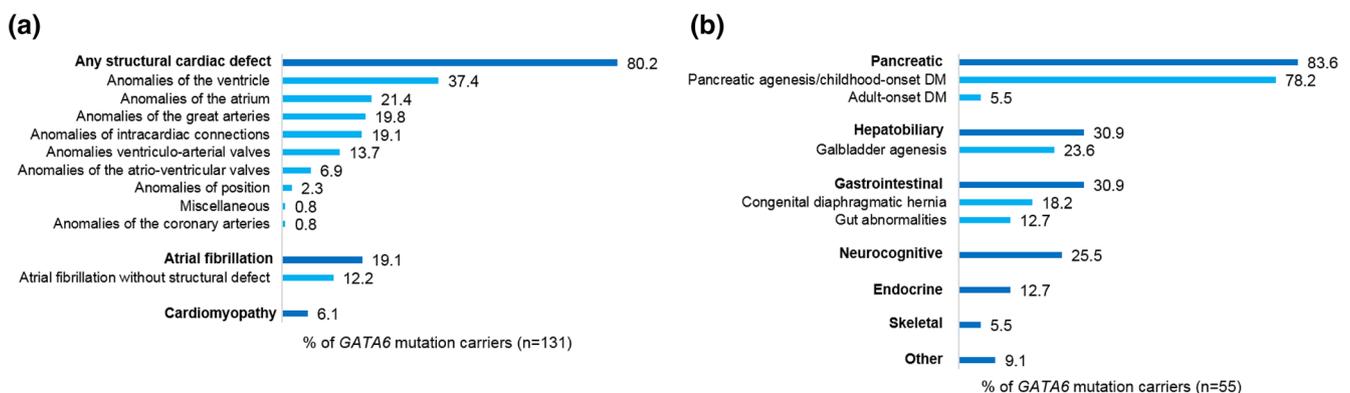
Thirty-eight papers described patients with potentially pathogenic variants in GATA6 (Table S2). Focusing on exonic SNVs, small

insertion/deletions, and splice site variants, there were 68 mutations in 85 independent probands (including the two patients in this manuscript). In 28 probands, the mode of inheritance was (or could) not (be) tested. In the remaining 57 probands, the variant arose de novo in 58% (33 of 57). Interestingly, in three cases, the inherited mutation was found mosaic in the parent (the mother in all cases). Eighty-five percent of these mutations were absent in >130,000 individuals in gnomAD (Lek et al., 2016) and the remaining ones are present at a very low frequency, which fits with the low prevalence of the phenotypes related to this gene.

In total, there were data available on 135 GATA6 mutation carriers (85 probands and 50 family members from 24 families) with available cardiac assessment in 131 (85 probands and 46 family members) of them. Eighty percent ( $n = 105$ ) of the carriers exhibited a structural cardiac phenotype (including cardiomyopathy). Most carriers had a ventricular or atrial anomaly (mainly VSD and atrial septal defect [ASD]; Figure 2). In addition, there were 16 mutation carriers (12%) with atrial fibrillation (AF) in the absence of a structural abnormality.

Data on the presence and/or function of the pancreas were available for 55 GATA6 carriers (43 probands and 12 family members) and were abnormal in 84% (46 of 55). The abnormalities ranged from severe hypoplasia/agenesis of the pancreas with complete pancreatic insufficiency requiring treatment from the neonatal period on (in most cases,  $n = 43$ ) to a milder phenotype such as adult-onset diabetes. The most common other extracardiac features were hepatobiliary (mainly gallbladder agenesis), gastrointestinal (mainly congenital diaphragmatic hernia), and neurocognitive abnormalities (Figure 2).

We assessed the penetrance of the cardiac and pancreatic phenotypes in mutation positive family members of probands with these phenotypes; this largely avoids the selection and assessment bias present in the screened cohorts of probands. Among the family members of probands with a cardiac phenotype, there were data available on cardiac phenotype for 92%. Eighty-seven percent of these family members had a cardiac phenotype as well, representing a high penetrance of cardiac abnormalities. Among the family members of probands with a



**FIGURE 2** Distribution of phenotypes among GATA6 mutation positive individuals. (a) Cardiac features among 131 carriers where cardiac assessment was available. Patients can have cardiac abnormalities in more than one category. (b) Extracardiac features among 55 carriers where other clinical evaluation was available. Patients can have extracardiac abnormalities in more than one category. The main categories are in bold and represented by the dark blue bars, the light blue bars represent the subcategories. DM, diabetes mellitus [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

pancreatic phenotype, there was data available for 91%, and 60% of them had a pancreatic phenotype. There was no statistical difference in the penetrance of these phenotypes between mutation type (missense vs. loss-of-function; LoF).

For 27 mutations, their functional consequence was investigated by measuring the transcriptional activity of GATA6 on several different target genes (e.g., ANF, BNP, and  $\alpha$ MHC) by a luciferase assay in vitro (Table S2). Most of the tested GATA6 mutants (20 of 27, 74%) demonstrated a reduction in transcriptional activity of the GATA6 target genes, compared to wild-type GATA6. For the remaining seven mutations, there was either an increase or no effect observed in transcriptional activity.

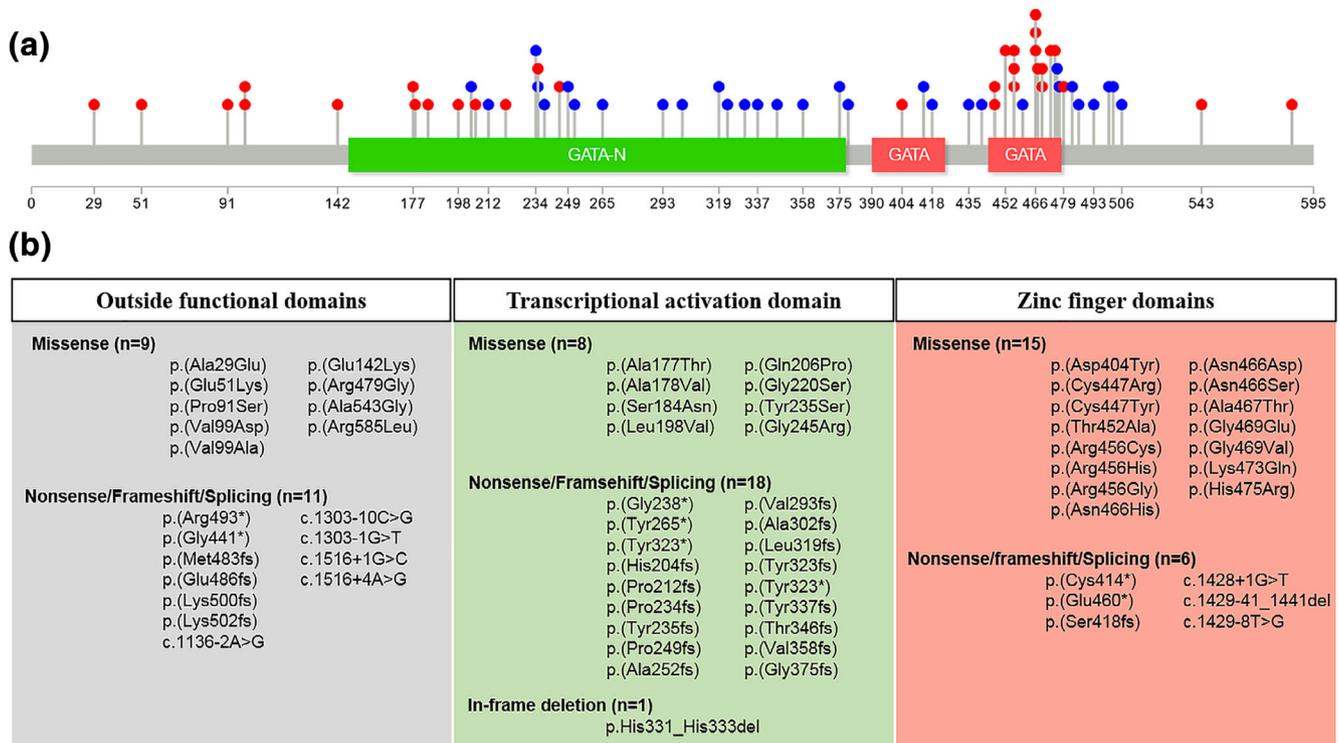
We stratified the patients according to inheritance (de novo vs. inherited) and saw that patients with a de novo mutation more often had an anomaly of intracardiac connections (mainly PTA and transposition of the great arteries [TGA]) and also an anomaly of the great arteries (mainly patent ductus arteriosus), than those with an inherited mutation ( $p = .004$  and  $p = .003$ , respectively).

When stratifying patients according to the type of mutation (missense vs. LoF mutation), we did not observe any statistically significant differences (Table S4). For pancreatic abnormalities and in the different other categories of extracardiac malformations (see Figure 3 for the categories), we did not observe significant differences at the

Bonferroni-corrected  $p$  value threshold, when stratifying patients according to inheritance mode or type of mutation (data not shown). However, endocrine abnormalities (in all cases hypothyroidism) did seem to be present more often in patients with a de novo mutation and in those with a missense mutation ( $p = .03$  and  $p = .01$ , respectively).

Five mutations were found in more than one independent proband (p.(Glu142Lys), p.(Ser184Asn), p.(Arg456Cys), p.(Arg456His) and p.(Lys502fs)) and one of these, p.(Ser184Asn), was even found in 12 independent probands. It is located in the transcriptional activation domain of the protein, essential for transcriptional activity (Brewer & Pizze, 2006). Ten of these 12 probands exhibited outflow tract defects (tetralogy of Fallot [TOF], DORV, and TGA). Unfortunately, information on extracardiac features was not available in any of these 12 patients.

Topologically, the GATA6 protein has at least three functional domains: a transcriptional activation domain essential for its transcriptional activity and two zinc finger domains required for DNA sequence recognition and binding to the consensus motif (Brewer & Pizze, 2006). We plotted all mutations on a schematic diagram of the GATA6 protein (Figure 3). Most mutations were located within the functional domains of GATA6 (48 of 68; 71%). Furthermore, missense mutations were more often located in the zinc finger domains, whereas LoF



**FIGURE 3** Schematic diagram of the GATA6 protein and its protein domains with all described mutations. (a) A schematic presentation of the GATA6 protein with the functional domains “GATA-N” (transcriptional activation domain) and “GATA” (zinc finger) domains. All 68 exonic and splice-site mutations are plotted at their topological location on the GATA6 protein. Red dots represent missense mutations, and blue dots represent loss-of-function mutations (i.e., frameshift, nonsense, and splicing). Splice site mutations are plotted at the closest amino acid position. Bigger dots represent different mutations at the same amino acid position. The figure is created using The Lollipops software (Jay & Brouwer, 2016), which uses the Pfam protein domain database (<http://pfam.xfam.org/>) to retrieve domains. (b) Overview of all 68 exonic and splice-site mutations categorized by position on the protein [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

mutations were more often located in the transcriptional activation domain ( $p = .01$  and  $p = .04$ , respectively). There were no statistically significant differences in presence or type of cardiac or extracardiac phenotypes between mutations located inside and outside of the functional domains (inside vs. outside all functional domains; data not shown).

Besides the 68 exonic and splice site *GATA6* mutations, other genetic variation affecting *GATA6* was described. These involved mutations in the promotor region of *GATA6* ( $n = 4$  probands) or chromosomal aberrations ( $n = 4$  probands; 3 duplications, and 1 deletion). Furthermore, somatic mutations (in right ventricular outflow tract tissue) were described in two TOF patients. The phenotypic characteristics of carriers of these genetic variants can be found in Table S2. In most of these patients, the pancreatic defects were not assessed and the cardiac phenotypes were diverse. Of note, the chromosomal aberrations involved multiple genes and the role of *GATA6* was therefore difficult to assess. Thus, the interpretation of the causality of these categories of genetic variation remains challenging.

## 4 | DISCUSSION

We have presented two cases here with de novo *GATA6* mutations leading to a complex cardiac phenotype, pancreatic malformations, and other extracardiac abnormalities. Both patients add to the broad phenotypic spectrum of *GATA6* mutations and exhibit malformations that distinguish them from the other described patients. Specifically, Patient 1 had a very complex and rare cardiac phenotype, never described before in *GATA6* mutation-positive individuals (superior-inferior ventricles, VSD and PS), and, moreover, the discovery of the *GATA6* mutation has led to identifying the cause of his DM (i.e., pancreatic hypoplasia). Patient 2 had a rare extracardiac abnormality, namely PLE, only described in one other *GATA6* positive patient (McMillan et al., 2016). Although PLE is a well-known complication of the Fontan procedure (Mertens, Hagler, Sauer, Somerville, & Gewillig, 1998), this patient had undergone a Rastelli procedure and moreover, the central venous pressure was low when measured with cardiac catheterization during one of the PLE episodes. Therefore, we did not consider PLE here as secondary to the cardiac surgery but as part of the *GATA6* phenotypic spectrum. We have further provided an overview of all the phenotypes associated with human *GATA6* mutations described in the literature to date.

Human *GATA6* mutations were first described in patients with PTA (Kodo et al., 2009) but have later been shown to give rise to not only a variety of other cardiovascular malformations but also extracardiac abnormalities (mainly pancreatic malformations). There is both clear interfamilial and intrafamilial phenotypic variability, most likely due to differences in genetic background, environmental factors, and epigenetic factors. We found cardiac structural abnormalities and pancreatic abnormalities to be the phenotypes most commonly associated with *GATA6* mutations. The penetrance of the cardiac phenotype was higher than the penetrance of the pancreatic phenotype (87% vs. 60%), however, the absolute numbers are low and the percentage of pancreatic

disease is high enough to warrant screening in all carriers. Other common described extracardiac features included gallbladder agenesis, congenital diaphragmatic hernia, and developmental delay. The mode of inheritance was de novo in 58% of probands, and our stratified analysis showed that patients with a de novo mutation have anomalies of intracardiac connections and the great arteries more often and also exhibit more hypothyroidism than those with an inherited mutation. One possible explanation for this could be that patients with a severe CHD did not reach a reproductive age before the modern surgical era, and thus this group of patients is more likely to carry a de novo mutation. Performing detailed clinical screening in *GATA6* positive patients will help to improve genotype–phenotype correlations in the future.

The mechanism by which mutations in *GATA6* lead to the associated phenotypes is still not completely elucidated. The downstream targets of *GATA6* include the genes encoding atrial natriuretic factor, brain natriuretic peptide,  $\alpha$ -myosin heavy chain, hepatocyte nuclear factor 4 alpha, and other genes (Morrissey et al., 1998; Rémond et al., 2011) required for normal cardiac and pancreatic development. The leading hypothesis is a LoF mechanism due to haploinsufficiency. Seventy-four percent of the *GATA6* mutations described in the literature, where transcriptional activity was investigated, lead to decreased transcriptional activity on target genes. In addition, over 50% of the described *GATA6* mutations fall in the category LoF (i.e., frameshift mutations with a premature stop, nonsense mutation or splice site mutations); whereas in the gnomAD database (with >130,000 individuals), only four such variants could be found (Lek et al., 2016). Although this further supports a haploinsufficiency mechanism, it remains striking that heterozygous *GATA6 null* mice have no phenotype (Morrissey et al., 1998). Moreover, Kodo et al. showed that cotransfecting the same amount of wild-type and p.(Glu486fs) mutant *GATA6* in HeLa cells had a stronger inhibitory transcriptional effect on downstream target genes, compared to half of the wild-type alone, which could suggest a dominant negative mechanism (Kodo et al., 2009).

Among all described *GATA6* positive individuals, there are in total 25 patients from 12 independent families with different mutations reported with AF, the most common arrhythmia in the general population (Anderson et al., 2013). Nine of these patients (36%) have AF accompanied by a structural defect (ASD, VSD, or dilated cardiomyopathy), which might be the substrate for AF in these cases. However, there are also 16 patients with AF in the absence of a structural cardiac defect. This latter category of AF patients is relatively younger compared to the general AF population (mean age 39, range 14–61, vs. 75 years [Feinberg, Blackshear, Laupacis, Kronmal, & Hart, 1995]). The exact pathophysiology remains unclear, and although half of the tested *GATA6* variants associated with AF showed decreased transcriptional activity on downstream target genes, supporting a LoF mechanism, there were also three mutations (p.(Pro91Ser), p.(Ala177Thr), and p.(Arg585Leu)) with increased transcriptional activity, suggesting a gain-of-function mechanism. *GATA6* was not identified in the latest AF genome-wide association study (GWAS) including >1 million people (Nielsen et al., 2018) but interestingly did show up as a candidate gene in a GWAS on resting heart rate (Eppinga et al., 2016), suggestive of a possible role in cardiac electrical function.

The diagnostic yield of screening for *GATA6* strongly depends upon the selected phenotype; in isolated CHD cohorts (Homsy et al., 2015; Jin et al., 2017; Kodo et al., 2009; Kodo et al., 2012; Lin et al., 2010; Maitra, Koenig, Srivastava, & Garg, 2010; J. Wang et al., 2012; X. Wang et al., 2014; Zhang et al., 2018; Zheng et al., 2012), the yield was around ~0.5%, whereas in cohorts selected on pancreatic abnormalities (pancreatic agenesis or neonatal DM; Allen et al., 2011; De Franco et al., 2013; Gong et al., 2013; Sanyoura et al., 2018), the mutation yield was ~11%. Allen et al. showed that 93% of the *GATA6* mutation carriers have a cardiac defect versus none of the *GATA6* mutation-negative patients (Allen et al., 2011). We therefore speculate that the yield will be the highest in patients with a combination of cardiac and pancreatic malformations.

An important limitation in our effort to establish an overview of the full phenotypic spectrum of *GATA6* is that most *GATA6* mutation-positive individuals were identified by screening large cohorts where patients either had CHD and/or pancreatic abnormalities. Detailed clinical evaluation to detect other associated features was missing in those cases. Moreover, most CHD cohorts selected only cases in whom extracardiac anomalies were excluded (i.e., isolated CHD cases), causing bias due to selective inclusion. This might explain the overrepresentation of cases with isolated cardiac phenotypes. As it is impossible to determine how extensive the clinical evaluation was in each patient, when there was no mention of cardiac, pancreatic, or other extracardiac phenotypes (unless specifically stated otherwise), we scored these data as unavailable. For family members we assumed that, if there was a cardiac, pancreatic or other extracardiac phenotype described in the proband, this was also checked in carriers from the same family. In the latter case, if an abnormality was not mentioned in these family members, we scored this data as normal. Nonetheless, as far as we could determine, this is the most comprehensive overview of *GATA6* mutations and related phenotypes to date. It would be interesting to see the yield of *GATA6* testing in a prospective unbiased pediatric cohort with a broad and unselected phenotype, to explore the exact phenotypic spectrum of *GATA6*.

We hope this study will contribute to increased awareness among treating physicians of patients with a combination of cardiac and pancreatic malformations/DM (potentially in the presence of other abnormalities) about the possibility of a *GATA6* mutation. Subsequently, these patients should be referred to the clinical geneticist for diagnostic testing. Conversely, patients in whom a *GATA6* pathogenic mutation has been identified should undergo extensive clinical testing, in order to assess the full disease spectrum in these individuals. This will also hopefully contribute to a better estimation of the mutational yield and to clear genotype-phenotype correlations.

## ACKNOWLEDGEMENTS

We want to thank Dr. S. S. K. S. Phoa (Amsterdam UMC, Department of Radiology and Nuclear Medicine, Amsterdam) for his help with selecting CT images of patient 1. This work is funded by the Dutch Heart Foundation (CVON CONCOR-genes project), the Dekker grant

of F.V. Tjong from the Dutch Heart Foundation, and a research grant from the Children's Heart Foundation.

## ORCID

Doris Škorić-Milosavljević  <https://orcid.org/0000-0001-7089-3703>

## REFERENCES

- Allen, H. L., Flanagan, S. E., Shaw-Smith, C., De Franco, E., Akerman, I., Caswell, R., ... Ellard, S. (2011). *GATA6* haploinsufficiency causes pancreatic agenesis in humans. *Nature Genetics*, 44(1), 20–22. <https://doi.org/10.1038/ng.1035>
- Anderson, J. L., Halperin, J. L., Albert, N. M., Bozkurt, B., Brindis, R. G., Curtis, L. H., ... Wann, S. (2013). Management of Patients with atrial fibrillation (compilation of 2006 ACCF/AHA/ESC and 2011 ACCF/AHA/HRS recommendations). *Journal of the American College of Cardiology*, 61(18), 1935–1944. <https://doi.org/10.1016/j.jacc.2013.02.001>
- Brewer, A., & Pizzey, J. (2006). *GATA* factors in vertebrate heart development and disease. *Expert Reviews in Molecular Medicine*, 8(22), 1–20. <https://doi.org/10.1017/S1462399406000093>
- Cooper, G. M., Stone, E. A., Asimenos, G., NISC Comparative Sequencing Program, Green, E. D., Batzoglou, S., & Sidow, A. (2005). Distribution and intensity of constraint in mammalian genomic sequence. *Genome Research*, 15(7), 901–913. <https://doi.org/10.1101/gr.3577405>
- De Franco, E., Shaw-Smith, C., Flanagan, S. E., Shepherd, M. H., International NDM Consortium, A. T., Hattersley, A. T., & Ellard, S. (2013). *GATA6* mutations cause a broad phenotypic spectrum of diabetes from pancreatic agenesis to adult-onset diabetes without exocrine insufficiency. *Diabetes*, 62(3), 993–997. <https://doi.org/10.2337/db12-0885>
- Decker, K., Goldman, D. C., Grasch, C. L., & Sussel, L. (2006). *Gata6* is an important regulator of mouse pancreas development. *Developmental Biology*, 298(2), 415–429. <https://doi.org/10.1016/j.ydbio.2006.06.046>
- Eppinga, R. N., Hagemeyer, Y., Burgess, S., Hinds, D. A., Stefansson, K., Gudbjartsson, D. F., ... van der Harst, P. (2016). Identification of genomic loci associated with resting heart rate and shared genetic predictors with all-cause mortality. *Nature Genetics*, 48(12), 1557–1563. <https://doi.org/10.1038/ng.3708>
- Fahed, A. C., Gelb, B. D., Seidman, J. G., & Seidman, C. E. (2013). Genetics of congenital heart disease: The glass half empty. *Circulation Research*, 112(4), 707–720. <https://doi.org/10.1161/CIRCRESAHA.112.300853>
- Feinberg, W. M., Blackshear, J. L., Laupacis, A., Kronmal, R., & Hart, R. G. (1995). Prevalence, age distribution, and gender of patients with atrial fibrillation. Analysis and implications. *Archives of Internal Medicine*, 155(5), 469–473. Retrieved from. <http://www.ncbi.nlm.nih.gov/pubmed/7864703>
- Franklin, R. C. G., Béland, M. J., Colan, S. D., Walters, H. L., Aiello, V. D., Anderson, R. H., ... Jacobs, J. P. (2017). Nomenclature for congenital and paediatric cardiac disease: The international paediatric and congenital cardiac code (IPCCC) and the eleventh iteration of the international classification of diseases (ICD-11). *Cardiology in the Young*, 27(10), 1872–1938. <https://doi.org/10.1017/S1047951117002244>
- Gong, M., Simaitte, D., Kühnen, P., Heldmann, M., Spagnoli, F., Blankenstein, O., ... Raile, K. (2013). Two novel *GATA6* mutations cause childhood-onset diabetes mellitus, pancreas malformation and congenital heart disease. *Hormone Research in Paediatrics*, 79(4), 250–256. <https://doi.org/10.1159/000348844>
- Homsy, J., Zaidi, S., Shen, Y., Ware, J. S., Samocha, K. E., Karczewski, K. J., ... Chung, W. K. (2015). De novo mutations in congenital heart disease with neurodevelopmental and other congenital anomalies. *Science*, 350(6265), 1262–1266. <https://doi.org/10.1126/science.aac9396>
- Jay, J. J., & Brouwer, C. (2016). Lollipops in the clinic: Information dense mutation plots for precision medicine. *PLoS One*, 11(8), e0160519. <https://doi.org/10.1371/journal.pone.0160519>

- Jin, S. C., Homsy, J., Zaidi, S., Lu, Q., Morton, S., DePalma, S. R., ... Brueckner, M. (2017). Contribution of rare inherited and de novo variants in 2,871 congenital heart disease probands. *Nature Genetics*, 49(11), 1593–1601. <https://doi.org/10.1038/ng.3970>
- Ketola, I., Otonkoski, T., Pulkkinen, M.-A., Niemi, H., Palgi, J., Jacobsen, C. M., ... Heikinheimo, M. (2004). Transcription factor GATA-6 is expressed in the endocrine and GATA-4 in the exocrine pancreas. *Molecular and Cellular Endocrinology*, 226(1–2), 51–57. <https://doi.org/10.1016/j.mce.2004.06.007>
- Kircher, M., Witten, D. M., Jain, P., O’Roak, B. J., Cooper, G. M., & Shendure, J. (2014). A general framework for estimating the relative pathogenicity of human genetic variants. *Nature Genetics*, 46(3), 310–315. <https://doi.org/10.1038/ng.2892>
- Kodo, K., Nishizawa, T., Furutani, M., Arai, S., Ishihara, K., Oda, M., ... Yamagishi, H. (2012). Genetic analysis of essential cardiac transcription factors in 256 patients with non-syndromic congenital heart defects. *Circulation Journal*, 76(7), 1703–1711. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/22498567>
- Kodo, K., Nishizawa, T., Furutani, M., Arai, S., Yamamura, E., Joo, K., ... Yamagishi, H. (2009). GATA6 mutations cause human cardiac outflow tract defects by disrupting semaphorin-plexin signaling. *Proceedings of the National Academy of Sciences*, 106(33), 13933–13938. <https://doi.org/10.1073/pnas.0904744106>
- Lek, M., Karczewski, K. J., Minikel, E. V., Samocha, K. E., Banks, E., Fennell, T., ... Exome Aggregation Consortium. (2016). Analysis of protein-coding genetic variation in 60,706 humans. *Nature*, 536(7616), 285–291. <https://doi.org/10.1038/nature19057>
- Lepore, J. J., Mericko, P. A., Cheng, L., Lu, M. M., Morrisey, E. E., & Parmacek, M. S. (2006). GATA-6 regulates semaphorin 3C and is required in cardiac neural crest for cardiovascular morphogenesis. *Journal of Clinical Investigation*, 116(4), 929–939. <https://doi.org/10.1172/JCI27363>
- Lin, X., Huo, Z., Liu, X., Zhang, Y., Li, L., Zhao, H., ... Chen, Y.-H. (2010). A novel GATA6 mutation in patients with tetralogy of Fallot or atrial septal defect. *Journal of Human Genetics*, 55(10), 662–667. <https://doi.org/10.1038/jhg.2010.84>
- Maitra, M., Koenig, S. N., Srivastava, D., & Garg, V. (2010). Identification of GATA6 sequence variants in patients with congenital heart defects. *Pediatric Research*, 68(4), 281–285. <https://doi.org/10.1203/PDR.0b013e3181ed17e4>
- McCulley, D. J., & Black, B. L. (2012). Transcription factor pathways and congenital heart disease. *Current Topics in Developmental Biology*, 100, 253–277. <https://doi.org/10.1016/B978-0-12-387786-4.00008-7>
- McMillan, T., Girgis, R., & Sellers, E. A. C. (2016). Neonatal diabetes and protein losing enteropathy: A case report. *BMC Medical Genetics*, 17(1), 32. <https://doi.org/10.1186/s12881-016-0296-0>
- Mertens, L., Hagler, D. J., Sauer, U., Somerville, J., & Gewillig, M. (1998). Protein-losing enteropathy after the Fontan operation: An international multicenter study. PLE study group. *The Journal of Thoracic and Cardiovascular Surgery*, 115(5), 1063–1073. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/9605076>
- Molkentin, J. D. (2000). The zinc finger-containing transcription factors GATA-4, -5, and -6. *Journal of Biological Chemistry*, 275(50), 38949–38952. <https://doi.org/10.1074/jbc.R000029200>
- Morrisey, E. E., Tang, Z., Sigrist, K., Lu, M. M., Jiang, F., Ip, H. S., & Parmacek, M. S. (1998). GATA6 regulates HNF4 and is required for differentiation of visceral endoderm in the mouse embryo. *Genes & Development*, 12(22), 3579–3590. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/9832509>
- Nemer, G., & Nemer, M. (2003). Transcriptional activation of BMP-4 and regulation of mammalian organogenesis by GATA-4 and -6. *Developmental Biology*, 254(1), 131–148. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/12606287>
- Nielsen, J. B., Thorolfsson, R. B., Fritsche, L. G., Zhou, W., Skov, M. W., Graham, S. E., ... Willer, C. J. (2018). Biobank-driven genomic discovery yields new insight into atrial fibrillation biology. *Nature Genetics*, 50, 1234–1239. <https://doi.org/10.1038/s41588-018-0171-3>
- Øyen, N., Poulsen, G., Boyd, H. A., Wohlfahrt, J., Jensen, P. K. A., & Melbye, M. (2009). Recurrence of congenital heart defects in families. *Circulation*, 120(4), 295–301. <https://doi.org/10.1161/CIRCULATIONAHA.109.857987>
- R Core Team. (2017). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Rémond, M. C., Iaffaldano, G., O’Quinn, M. P., Mezentseva, N. V., Garcia, V., Harris, B. S., ... Eisenberg, L. M. (2011). GATA6 reporter gene reveals myocardial phenotypic heterogeneity that is related to variations in gap junction coupling. *American Journal of Physiology-Heart and Circulatory Physiology*, 301(5), H1952–H1964. <https://doi.org/10.1152/ajpheart.00635.2011>
- Sanyoura, M., Jacobsen, L., Carmody, D., Del Gaudio, D., Alkorta-Aranburu, G., Arndt, K., ... Greeley, S. A. W. (2018). Pancreatic histopathology of human monogenic diabetes due to causal variants in KCNJ11, HNF1A, GATA6, and LMNA. *The Journal of Clinical Endocrinology and Metabolism*, 103(1), 35–45. <https://doi.org/10.1210/jc.2017-01159>
- Sifrim, A., Hitz, M.-P., Wilsdon, A., Breckpot, J., Al Turki, S. H., Thienpont, B., ... Hurles, M. E. (2016). Distinct genetic architectures for syndromic and nonsyndromic congenital heart defects identified by exome sequencing. *Nature Genetics*, 48(9), 1060–1065. <https://doi.org/10.1038/ng.3627>
- Tourtellotte, W. G., & Milbrandt, J. (1998). Sensory ataxia and muscle spindle agenesis in mice lacking the transcription factor Egr3. *Nature Genetics*, 20(1), 87–91. <https://doi.org/10.1038/1757>
- van der Linde, D., Konings, E. E. M., Slager, M. A., Witsenburg, M., Helbing, W. A., Takkenberg, J. J. M., & Roos-Hesselink, J. W. (2011). Birth prevalence of congenital heart disease worldwide. *Journal of the American College of Cardiology*, 58(21), 2241–2247. <https://doi.org/10.1016/j.jacc.2011.08.025>
- Wang, J., Luo, X.-J., Xin, Y.-F., Liu, Y., Liu, Z.-M., Wang, Q., ... Yang, Y.-Q. (2012). Novel GATA6 mutations associated with congenital ventricular septal defect or tetralogy of fallot. *DNA and Cell Biology*, 31(11), 1610–1617. <https://doi.org/10.1089/dna.2012.1814>
- Wang, X., Ji, W., Wang, J., Zhao, P., Guo, Y., Xu, R., ... Sun, K. (2014). Identification of two novel GATA6 mutations in patients with nonsyndromic conotruncal heart defects. *Molecular Medicine Reports*, 10(2), 743–748. <https://doi.org/10.3892/mmr.2014.2247>
- Xin, M., Davis, C. A., Molkentin, J. D., Lien, C.-L., Duncan, S. A., Richardson, J. A., & Olson, E. N. (2006). A threshold of GATA4 and GATA6 expression is required for cardiovascular development. *Proceedings of the National Academy of Sciences of the United States of America*, 103(30), 11189–11194. <https://doi.org/10.1073/pnas.0604604103>
- Yu, L., Bennett, J. T., Wynn, J., Carvill, G. L., Cheung, Y. H., Shen, Y., ... Chung, W. K. (2014). Whole exome sequencing identifies de novo mutations in GATA6 associated with congenital diaphragmatic hernia. *Journal of Medical Genetics*, 51(3), 197–202. <https://doi.org/10.1136/jmedgenet-2013-101989>
- Zaidi, S., & Brueckner, M. (2017). Genetics and genomics of congenital heart disease. *Circulation Research*, 120(6), 923–940. <https://doi.org/10.1161/CIRCRESAHA.116.309140>
- Zaidi, S., Choi, M., Wakimoto, H., Ma, L., Jiang, J., Overton, J. D., ... Lifton, R. P. (2013). De novo mutations in histone-modifying genes in congenital heart disease. *Nature*, 498(7453), 220–223. <https://doi.org/10.1038/nature12141>
- Zhang, E., Hong, N., Chen, S., Fu, Q., Li, F., Yu, Y., & Sun, K. (2018). Targeted sequencing identifies novel GATA6 variants in a large cohort of patients with conotruncal heart defects. *Gene*, 641, 341–348. <https://doi.org/10.1016/j.gene.2017.10.083>
- Zheng, G.-F., Wei, D., Zhao, H., Zhou, N., Yang, Y.-Q., & Liu, X.-Y. (2012). A novel GATA6 mutation associated with congenital ventricular septal

defect. *International Journal of Molecular Medicine*, 29(6), 1065–1071.  
<https://doi.org/10.3892/ijmm.2012.930>

#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Škorić-Milosavljević D, Tjong FVY, Barc J, et al. GATA6 mutations: Characterization of two novel patients and a comprehensive overview of the GATA6 genotypic and phenotypic spectrum. *Am J Med Genet Part A*. 2019;179A:1836–1845. <https://doi.org/10.1002/ajmg.a.61294>