Cardiac Defects Are Infrequent Findings in Individuals With 8p23.1 Genomic Duplications Containing GATA4

Shihui Yu, MD, PhD; Xin-Gang Zhou, MD; Stephanie D. Fiedler, MS; Sarah J. Brawner, BS; Julie M. Joyce, BS; Hong-Yu Liu, MD, PhD

Background—The GATA4 gene is critical to regulating myocardial differentiation and function. Haploinsufficiency of GATA4 is strongly associated with congenital heart defects (CHD). However, it is inconclusive whether duplicated GATA4 causes CHD.

Methods and Results—We evaluated 1645 consecutive pediatric patients with various developmental disorders by high-resolution microarray-based comparative genomic hybridization and found 8 probands and 2 relatives with pathogenic genomic imbalances containing GATA4. Four probands contain an ≈4.0-Mb interstitial duplication of 8p23.1 flanked by the 2 olfactory receptor gene clusters REPD and REPP, representing 0.24% (4/1645) of the patients analyzed. None of the 4 patients has CHD or any other heart diseases and 1 mother who transmitted the duplication to her child has a history of aortic stenosis. Two patients who carry multiple genomic abnormalities, including a duplication containing GATA4, have complex CHD. Only 1 of the 3 individuals carrying genomic deletion containing GATA4 has atrial septal and ventricular septal defects.

Conclusions—Cardiac defects are infrequent findings in individuals with 8p23.1 genomic duplications containing GATA4. A 0.24% detection rate of this duplication in this study is significantly higher than previously estimated. Observation in 2 patients with multiple genomic abnormalities and complex CHD is consistent with a 2-hit model that emphasizes accumulative effects of >1 insult to the genome, leading to a visible or more severe clinical manifestation. Haploinsufficient GATA4 may show variable expressivity with a wide spectrum of clinical findings, including CHD.

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Key Words: nucleic acid hybridization ■ GATA4 transcription factor ■ monosomy 8p23.1 ■ duplication 8p ■ heart defects congenital

The GATA4 gene is located on the short arm of human chromosome 8 (8p23.1:11,599,126–11,654,918) containing 7 exons. It encodes member 4 of the GATA-binding proteins, a group of structurally related transcription factors controlling gene expression and differentiation in a variety of cell types.1 This gene is expressed in yolk sac endoderm during fetal development and in adult vertebrate heart, gut epithelium, and gonads. By regulating genes critical for myocardial differentiation and function, including troponin C (TNNC1 and TNNC2), α-myosin heavy chain 6 of cardiac muscle, and brain natriuretic peptide, GATA4 plays an important role in heart formation.1,2 Genetic defects (eg, missense mutation, nonsense mutation, deletion) leading to the haploinsufficiency of GATA4 are strongly associated with conotruncal and septal heart defects.3–9

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With application of molecular cytogenetic techniques, such as florescence in situ hybridization (FISH) and microarray-based comparative genomic hybridization (aCGH), an ≈4.0-Mb genomic duplication of 8p23.1 containing GATA4 has been reported in >10 individuals with a diversity of clinical findings, such as developmental delay, learning disability, mild dysmorphism, and heart defects.10–13 However, there are debates about whether the duplicated GATA4 underlies the congenital genetic defects in these patients.13,16

In the present study, we report on 8 probands and 2 relatives carrying genomic imbalances containing GATA4 identified by genomewide high-resolution aCGH as well as other genetic tools. The possible pathogenic role of GATA4 in the generation of the congenital heart defects was assessed and relevant literature reviewed.

Methods

Specimens
From April 2008 to July 2010, we evaluated 1645 consecutive pediatric patients by aCGH technique in our laboratory. Referral
justification included ≥1 of the following clinical findings: developmental delay, autism, dysmorphic features, seizures, or multiple congenital anomalies. Eight patients were found to carry genomic imbalances containing GATA4 of whom 4 were briefly described in our previous study that explored the genomic mechanisms underlying the formation of chromosomal abnormalities on the short arm of chromosome 8.14 The present study protocol was approved by the Institutional Review Board of Children's Mercy Hospitals and Clinics.

### aCGH Testing, Result Verification, and Parental Follow-up

The aCGH tests were performed using the Agilent Human Genome Microarray Kit 244K or 4×180K platforms following the protocols described (Agilent Technologies; Santa Clara, CA).17 Chromosome karyotyping (G banding) using a standard protocol was performed for all cases. FISH was carried out for cases 5, 6, and 8 as recommended by the manufacturers (Vysis Inc; Downers, IL). The genomic imbalances containing GATA4 identified by aCGH also were verified by quantitative real-time polymerase chain reaction using test primers targeted to GATA4 gene clusters. Recurrent features in the 4 patients include developmental delay (4/4), dysmorphic features (3/4), and neurodevelopmental problems (2/4). Some isolated findings also were observed, such as, abnormal movements, familial congenital hypothyroidism, portal vein thrombosis, and hypospadias. None of the 4 patients has CHD or any other heart diseases.

### Clinical Features in the 12 Patients With Genomic Abnormalities Containing GATA4

The major clinical findings in these patients are summarized in Table 2. Patients 1 to 4 contain an ~4.0-Mb interstitial duplication of 8p23.1 flanked by the REPD and REPP clusters. Recurrent features in the 4 patients include developmental delay (4/4), dysmorphic features (3/4), and neurodevelopmental problems (2/4). Some isolated findings also were observed, such as, abnormal movements, familial congenital hypothyroidism, portal vein thrombosis, and hypospadias. None of the 4 patients has CHD or any other heart diseases. Patient 3 inherited the 8p23.1 duplication from her mother, who has a history of learning disability and aortic stenosis.

In addition to the 8p23.1 duplication, patients 5 and 6 have other genomic abnormalities. Clinically, both patients have complex CHD, with bicuspid aortic valve with aortic dilation, patent ductus arteriosus, and anomalous superior left pulmonary venous return in patient 5 and tetralogy of Fallot, patent ductus arteriosus, and complete atrioventricular canal defect in patient 6. Patient 5 has severe global developmental delay, mood disorder, and behavioral abnormality, whereas patient 6 has had growth delay both prenatally and postnatally.

Different from patients 1 to 6 who carry 8p23.1 duplication, patients 7 and 8 carry 8p23.1 deletion. Clinically, both patients have developmental delays and behavioral problems. Patient 7 has atrial septal defect (ASD) and ventricular septal defect.
(VSD), whereas patient 8 has a normal heart. Although the 8p23.1 deletion in patient 7 was inherited from her father, the father is healthy without detectable problems in his heart and other organs.

**Discussion**

It is controversial whether a duplicated GATA4 may cause cardiac defects.13,16 Barber et al10,13 carried out genotype-phenotype correlation in both prenatal and postnatal cases with 8p23.1 duplication containing GATA4 and >50 other genes. They summarized the clinical data from 11 patients with 8p23.1 duplication in 7 families. CHD was the most common single feature, having been found in 6 of 11 individuals. They proposed that duplication of GATA4 can give rise to a variety of conotruncal or septal heart defects with variable penetrance and expressivity. Basinko et al15 identified a partial trisomy containing ~60 genes, including GATA4, in a patient with CHD, delayed puberty, and learning disabilities. They supported the proposal that the 8p23.1 duplication is associated with ASD, probably because of GATA4 duplication. However, opposite opinions exist. Joziasse et al16 identified a 132.9-kb duplication containing only 1 gene, GATA4, in a boy with developmental delay; dysmorphic features; and multiple congenital anomalies, including CHD. This duplication was inherited from his healthy mother. Three additional family members carrying the GATA4 duplication were identified, and none of them had cardiac symptoms or any observable congenital cardiac defect. Joziasse et al concluded that GATA4 duplication is not sufficient to cause CHD. They further proposed that the CHD in the proband might be caused by a different gene, FAM123B, which also was duplicated in his genome, rather than by the GATA4 duplication. Additional evidence that does not support the involvement of duplicated GATA4 in CHD came from a report by Zogopoulos et al19 that the ~4.0-Mb duplications containing GATA4 occur in the general population at a frequency of 0.6% (7/1190).

In the current study, none of the 4 patients (patients 1–4) who carry an ~4.0-Mb interstitial duplication of 8p23.1...
containing GATA4 has CHD or any other heart diseases. However, the mother of patient 3 who carries the duplication has a history of aortic stenosis. Based on these data, we believe that duplicated GATA4 is less likely to be sufficient to cause CHD. Interestingly, both patients 5 and 6 who carry multiple genomic abnormalities have complex CHD. A possible explanation is that the CHD in these 2 patients was caused by other genomic abnormalities rather by the duplicated GATA4. An alternative explanation is that there are genes in other abnormal regions in concert with the duplicated GATA4 leading to CHD development, which is consistent with a recently described 2-hit model in which 2 insults are necessary during development to result in a visible or more severe clinical manifestation as pediatric disease.20

Different from the CHD observed in only 1 of the 5 individuals (patients 1–4 and the mother of patient 3) who carry the ~4.0-Mb 8p23.1 duplication containing GATA4, developmental delay was present in each of the 4 probands, ranging from mild to severe, and learning disability was present in the mother of patient 3. Dysmorphic features also were found in 3 of the 4 probands. Both developmental delay and dysmorphic features were observed in previously reported patients who carried genomic duplication containing GATA4.10,13,15 However, some isolated findings in the 4 probands, such as portal vein thrombosis and hypospadias, were not previously observed. The finding of 4 probands with 8p23.1 duplication, or a 0.24% detection rate, indicates that the 8p23.1 duplication may be more common than the estimated prevalence of 1 in 64 000 by Barber et al.10,13 However, it remains unknown why the frequency of 0.6% (7/1190) of the /H11015 4.0-Mb duplications containing GATA4 occurred in the general population reported by Zogopoulos et al,19 although they were not examined with regard to neurological and psychiatric phenotypes. It is noteworthy that no examples of the /H11015 4.0-Mb duplication containing GATA4 have been observed in the 1287 healthy individuals21 or documented among the 66 741 copy number variants currently in the Database of Genome Variants (http://projects.tcag.ca/variation).

Table 2. Major Clinical Features in Patients With Genomic Abnormalities Containing the GATA4 Gene

<table>
<thead>
<tr>
<th>Pt</th>
<th>Sex</th>
<th>Development</th>
<th>Behavior</th>
<th>Neurology</th>
<th>Dysmorphic Features</th>
<th>CHD</th>
<th>Other Findings</th>
<th>Inheritance</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>Moderate</td>
<td>GDD</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>Not</td>
<td>Unavailable</td>
<td>.</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td>tremor-related</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>abnormal</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>movements</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>Mild DD</td>
<td>.</td>
<td>Macrocephaly</td>
<td>Prominent forehead</td>
<td>.</td>
<td>Congenital</td>
<td>De novo</td>
<td>The father has</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>hypothyroidism, portal vein</td>
<td></td>
<td>macrocephaly and a history</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>thrombosis, hypospadias</td>
<td></td>
<td>of hypothyroidism</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>Moderate</td>
<td>GDD</td>
<td>.</td>
<td>Seizures and EEG abnormalities</td>
<td>.</td>
<td>.</td>
<td>Maternally inherited</td>
<td>The mother has a history</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Widely spaced deep-set eyes, epicantial folds, short philtrum, upturned nose, downturned mouth</td>
<td>.</td>
<td>.</td>
<td></td>
<td>of aortic stenosis and learning</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>sleep problems, short broad thumbs, dysplastic nails, short stature</td>
<td></td>
<td>disability</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>Mild DD</td>
<td>.</td>
<td>.</td>
<td>Epicanthal folds, broad nasal bridge, smooth philtrum</td>
<td>.</td>
<td>.</td>
<td>De novo</td>
<td>.</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>Severe GDD</td>
<td>Mood disorder and behavioral abnormality</td>
<td>.</td>
<td>Forehead is tall</td>
<td>.</td>
<td>Bicuspid aortic valve with aortic dilation, PDA, anomalous superior left pulmonary venous return</td>
<td>De novo</td>
<td>Family history is remarkable for speech delay in some of siblings and learning difficulty in mother</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sleep problems, short broad thumbs, dysplastic nails, short stature</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>.</td>
<td>.</td>
<td>Mild microcephaly</td>
<td>.</td>
<td>TOF, PDA, complete atrioventricular canal defect</td>
<td>IUGR, small physical size</td>
<td>De novo</td>
<td>.</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>Moderate DD</td>
<td>ADHD and significant behavioral abnormality</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>ASD and VSD</td>
<td>Paternally inherited</td>
<td>The father is healthy</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>GDD</td>
<td>Behavioral abnormality</td>
<td>.</td>
<td>.</td>
<td>Bilateral renal reflux with recurrent UTI</td>
<td>.</td>
<td>Unavailable</td>
<td>Slight limb hypotonia</td>
</tr>
</tbody>
</table>

ADHD indicates attention deficit hyperactivity disorder; ASD, atrial septal defect; CHD, congenital heart defect; DD, developmental delay; GDD, global developmental delay; IUGR, intrauterine growth restriction; EEG, electroencephalogram; PA, pulmonary valve atresia; PDA, patent ductus arteriosus; TOF, tetralogy of Fallot; UTI, urinary tract infection; VSD, ventricular septal defect.
Patient 7 inherited an \( \approx 4.0\)-Mb 8p23.1 deletion from her father, which is reciprocal to the 8p23.1 duplication in patients 1 to 4 (Figure, Table 1). She has ASD and VSD consistent with the clinical findings in previously reported patients carrying the \( \approx 4.0\)-Mb 8p23.1 deletion with a spectrum of cardiac defects that included ASD, VSD, atrioventricular septal defect, pulmonary valve stenosis, tetralogy of Fallot, and combinations of these defects.\(^{22}\) Patient 7 inherited this deletion from her father, who has no observable cardiac defect, reflecting incomplete penetrance of haploinsufficient GATA4 causing CHD. This idea is supported by the observation in patient 8, who is absent of CHD but carries a 15.66-Mb 8p terminal deletion containing GATA4.

In summary, we identified 7 individuals (patients 1–6 and the mother of patient 3) with genomic duplications containing GATA4. Only 1 of the 5 individuals who carry an \( \approx 4.0\)-Mb interstitial duplication of 8p23.1 has a history of aortic stenosis, indicating that cardiac defects are infrequent findings in individuals with this duplication. A 0.24% detection rate of 8p23.1 duplication in this study indicates that the previously calculated prevalence of this duplication was underestimated. Both patients who carried multiple genomic abnormalities, including a duplication containing GATA4, have complex CHD consistent with the 2-hit model emphasizing accumulative effects of >1 insult to the genome, leading to a visible or more severe clinical manifestation. Only 1 of the 3 individuals carrying genomic deletion containing GATA4 has ASD and VSD, reflecting incomplete penetrance of haploinsufficient GATA4 causing CHD.

**Disclosures**

None.

**References**

The GATA4 gene is critical to regulating myocardial differentiation and function. Haploinsufficiency of GATA4 is strongly associated with conotruncal and septal heart defects. However, it is inconclusive whether duplicated GATA4 may cause congenital heart defects (CHD). This study reports on 8 probands and 2 relatives with pathogenic genomic imbalances containing GATA4 through evaluation of 1645 consecutive pediatric patients with various developmental disorders by microarray-based comparative genomic hybridization. Four probands contain an ∼4.0-Mb interstitial duplication of 8p23.1 flanked by the 2 olfactory receptor gene clusters. None of the 4 patients has CHD or any other heart diseases, whereas 1 mother who transmitted the duplication to her child has a history of aortic stenosis. Two patients who carried multiple genomic abnormalities, including a duplication containing GATA4, have complex CHD. Only 1 of the 3 individuals carrying genomic deletion containing GATA4 has atrial and ventricular septal defects. Based on these findings, we have made the following conclusions: (1) Cardiac defects are infrequent findings in individuals with 8p23.1 genomic duplications containing GATA4; (2) a 0.24% detection rate of this duplication in this study is significantly higher than previously estimated; (3) observation in the patients with multiple genomic abnormalities and complex CHD is consistent with a 2-hit model emphasizing accumulative effects of >1 insult to the genome, leading to a visible or more severe clinical manifestation; and (4) haploinsufficient GATA4 may show variable expressivity with a wide spectrum of clinical findings, including CHD.
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**Supplemental Table 1. qPCR information targeting to the GATA4 gene**

<table>
<thead>
<tr>
<th>Primer sets</th>
<th>Primers sequences</th>
<th>Amplified region</th>
<th>Amplicon size (bp)</th>
<th>Target genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target primers</td>
<td>Forward: tcactcaccagaaattgcccaacc</td>
<td>chr8: 11599345—11599469</td>
<td>125</td>
<td>GATA4</td>
</tr>
<tr>
<td></td>
<td>Reverse: acggaaagaatccaaaggcgcttc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference primers</td>
<td>Forward: aggtgttctgctgctgagatggaa</td>
<td>chrX:13693097—13693233</td>
<td>137</td>
<td>OFD1</td>
</tr>
<tr>
<td></td>
<td>Reverse: tccctttgtgcccagatgaagaga</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Supplementary Figure 1.** Patients 1 to 4 contain a ~ 4.0 Mb interstitial duplication of 8p23.1 flanked by the two olfactory receptor gene clusters (REPD and REPP)
Supplementary Figure 2. Patient 5 has three genomic abnormalities, a 6.83 Mb terminal duplication of 8p with the proximal breakpoint residing within the REPD cluster, a 752 kb interstitial duplication of 8p23.1 with distal breakpoint within between REPD-REPP clusters and the proximal breakpoint residing within the REPP cluster, and a 1.62 Mb terminal deletion of 15q26.3. FISH analysis using the DNA probes for the subtelomeres of chromosomes 8p and 15q shows three copies of 8pter and one copy of 15qter. One 8p subtelomere probe is located on the 15q terminus with loss of the 15qter subtelomere probe [ish der(15)t(8;15)(8pter+;15qter-)(D8S504+;D15S369-)]
Supplementary Figure 3. Patient 6 contains two genomic imbalances, a 6.91 Mb terminal deletion with its proximal breakpoint residing within the REPD cluster, and a consecutive 18.35 Mb interstitial duplication. Within the 18.35 Mb interstitial duplication, there is a 563 kb quintuplicated region with its distal breakpoint residing within the REPP cluster.
Supplementary Figure 4. Patient 8 has a 15.66 Mb terminal deletion of 8p and a 27.13 Mb terminal duplication of 15q. G banding and FISH analyses revealed a mosaic unbalanced translocation between chromosome 8 short arm and chromosome 15 long arm with a karyotype of mos 46,XX,der(8)t(8;15)(p22;q24.1)[17/20],ish der(8)t(8;15)(D8S504-,154P1+)[17]46,XX[3], nuc ish(D8S504x2)[3], nuc ish(D8S504x1,VIJyRM2053x2)[184/200]. The unbalanced translocation results in loss of 8p22-8pter and gain of 15q24.1-qter.