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Duplication of *HEY2* in Cardiac and Neurologic Development

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Abstract

HEY2 is a basic helix-loop-helix (bHLH) transcription factor that plays an important role in the developing mammalian heart and brain. In humans, nonsynonymous mutations in *HEY2* have been described in patients with atrial ventricular septal defects, and a subset of individuals with chromosomal deletions involving *HEY2* have cardiac defects and cognitive impairment. Less is known about the potential effects of HEY2 overexpression. Here, we describe a female child with tetralogy of Fallot who developed severe right ventricular outflow tract obstruction due to a combination of infundibular and valvular pulmonary stenosis. She was also noted to have hypotonia, lower extremity weakness, fine motor delay and speech delay. A copy number variation (CNV) detection analysis followed by real-time quantitative PCR analysis revealed a single gene duplication of *HEY2*. This is the only duplication involving *HEY2* identified in our database of over 70,000 individuals referred for CNV analysis. In the developing heart, overexpression of HEY2 is predicted to cause decreased expression of the cardiac transcription factor GATA4 which, in turn, has been shown to cause tetralogy of Fallot. In mice, misexpression of *Hey2* in the developing brain leads to inhibition of neurogenesis and promotion of gliogenesis. Hence, duplication of *HEY2* may be a contributing factor to both the congenital heart defects and the neurodevelopmental problems evident in our patient. These results suggest that individuals with *HEY2* duplications should be screened for congenital heart defects and monitored closely for evidence of developmental delay and/or cognitive impairment.

Keywords

HEY2; *GATA4*; chromosomal duplication; tetralogy of Fallot; developmental delay; cognitive deficits

INTRODUCTION

Hairy/enhancer-of-split related with YRPW motif protein 2 (HEY2) is a member of the HEY family of basic helix-loop-helix (bHLH) transcription factors which also includes HEY1 and HEYL [Weber et al., 2014]. HEY proteins function by forming homo- and

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SUPPORTING INFORMATION

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hetero-dimers that transcriptionally repress other genes. HEY genes are dynamically expressed throughout the body and help regulate differentiation and specification of cells during development [Kokubo et al., 1999; Leimeister et al., 1999; Nakagawa et al., 1999; Chin et al., 2000; Leimeister et al., 2000].

HEY2 plays a critical role in cardiac development. *Hey2*-null mice develop a variety of congenital heart defects including atrial and ventricular septal defects, tricuspid and pulmonary valve stenosis, abnormal mitral valve morphology, overriding aortic valve and tetralogy of Fallot [Donovan et al., 2002; Gessler et al., 2002; Sakata et al., 2002, 2006; Fischer et al., 2004]. In humans, non-synonymous sequence changes affecting the second helix of *HEY2* have been identified in two patients with atrioventricular septal defects who also carried binding domain mutations in other cardiac-specific transcription factors [Reamon-Buettner and Borlak 2006]. In a recent review of cases from clinical and public databases, Thorsson et al. [2014] demonstrated that 15% (2/ 13) of individuals with deletions involving *HEY2* had congenital heart defects.

HEY2 is also thought to play a role in development of the nervous system. In mice, *Hey2* is expressed in the matrix and ventricular zones of the cerebral cortex, the spinal nerves and various cranial ganglia and the sympathetic trunks and their associated ganglia [Leimeister et al., 1999; Sakamoto et al., 2003]. To date, no neurologic deficits have been described in *Hey2*-null mice. However, misexpression of *Hey2* leads to inhibition of neurogenesis and promotion of gliogenesis [Sakamoto et al., 2003]. This suggests that overexpression of HEY2 may have detrimental effects on brain development.

Here, we describe a female child with tetralogy of Fallot, infundibular and valvular pulmonary stenosis, hypotonia, lower extremity weakness, fine motor delay and speech delay. A copy number variant (CNV) detection analysis revealed a single gene duplication of *HEY2* that may have contributed to these phenotypes.

CLINICAL REPORT

Our patient is a 3-year-old Hispanic female who was conceived naturally by nonconsanguineous parents. Pregnancy was complicated by an unspecified infection treated with antibiotics and maternal Bell's palsy which was treated with medications. Our patient was delivered vaginally at term and weighed 3.57 kg (~80th centile) and was 49.5 cm in length (~60th centile). A nuchal cord was present at delivery and was manually reduced. After birth, she was noted to have a heart murmur. An echocardiogram revealed tetralogy of Fallot associated with a large conoventricular septal defect. A subsequent echocardiogram performed at one month of age revealed hypoplasia of the pulmonary annulus (Z score = −3.29) and thickened pulmonary valve leaflets that appeared tethered with systolic doming. Severe right ventricular outflow tract obstruction was demonstrated with a peak velocity of ~5.5 m per second and a mean gradient of ~77 mmHg. This obstruction was due to a combination of infundibular and valvular pulmonary stenosis.

Her outflow tract obstruction worsened over time, and she underwent surgical repair at approximately 3 months of age. This repair included patch closure of the ventricular septal defect, patch augmentation of the main pulmonary artery with some extension into the

pulmonary valve annulus and resection of muscle bundles from the right ventricular outflow tract. Intra-operatively, the left anterior descending artery was found to arise from the right main coronary artery and course across the right ventricular outflow tract.

Our patient's language development was significantly delayed. She said her first word at 17 months of age, and she was using only three purposeful words at 19 months of age. A hearing screen was normal. A brain MRI obtained at 19 months of age revealed a Chiari I malformation and a complex right frontal developmental venous anomaly not associated with further areas of abnormal enhancement. Speech therapy was initiated at that time. Our patient was speaking in short phrases at 27 months of age, but at 3 years and 2 months of age she was still unable to speak in complete sentences.

Although our patient walked at 11 months of age, she was noted to be hypotonic, and there was concern that she had lower extremity weakness. She began receiving physical therapy at 12 months of age and was prescribed braces. At 21 months of age she was examined by a neurologist and a neurosurgeon who both considered her to have normal strength in all extremities. By 27 months of age physical therapy had been discontinued, and she could walk and run well without braces. Her fine motor development was delayed. She fed herself using her hands at approximately 1 year of age but started using a spoon to feed herself at 23 months of age.

Her most recent physical examination was at 3 years, 2 months of age. At the time she weighed 11.7 kg (3rd centile), had a height of 89.9 cm (8th centile), and had a head circumference of 47 cm (~2 standard deviations below the mean). No dysmorphic features were noted.

Our patient has a maternal half-brother and half-sister and a paternal half-brother. The maternal half-brother had hypokalemic seizures around the time of birth. All of these half-siblings are in good health and are developmentally appropriate. There is no family history of heart defects, but our patient's mother has an asymptomatic heart murmur. Her father has no contact with the family but is reportedly in good health.

METHODS AND RESULTS

DNA from our patient was screened for copy number variations (CNVs) using a chromosomal microarray analysis (CMA)-HR+SNP version 8.2 obtained on a clinical basis through the Medical Genetics Laboratories at Baylor College of Medicine (Supplemental Figure S1). This study revealed a copy number gain on chromosome 6q22.31 that spanned between 12 and 84 kb (minimum interval chr6:125,749,186–125,761,299; maximum interval chr6:125,713,307–125,797,245; hg38). The *HEY2* gene is completely encompassed within the minimum interval (Fig. 1). The maximum interval defined by CNV analysis included *LOC643623* which encodes an uncharacterized long non-coding RNA. A portion of the nuclear receptor coactivator 7 gene (*NCOA7*; OMIM #609752) was also located in the maximum interval.

To determine if these genes were affected by the duplication, we performed real-time quantitative PCR analyses as described previously [Scott et al., 2007] using primer pairs

designed within and between the *LOC643623*, *HEY2*, and *NCOA7* genes (Supplemental Table SI). These analyses revealed a normal copy number ratio between the patient and her mother (patient:maternal) for probes within and 3′ of *LOC643623* and within and 5′ of *NCAO7* (Supplemental Figure S2). In contrast all probes within *HEY2* showed an increased patient:maternal copy number ratio. This suggests that the patient carries a single gene duplication of *HEY2* that does not include *LOC643623* or *NCAO7* (Fig. 1; refined maximal interval =chr6:125,721,953–125,780,731; hg38).

Copy number variation analysis also revealed a loss on chromosome 2p16.3 that spanned between 20 and 40 kb (minimum interval chr2:50,832,013–50,852,148; maximum interval chr2:50,822,062–50,862,325; hg38). The maximum interval of this deletion is contained entirely within a large intron of the neurexin 1 gene (*NRXN1*; OMIM #600565). Compound heterozygous mutations and deletions involving *NRXN1* have been described as a cause of Pitt-Hopkins-like syndrome-2 (OMIM #614325) and severe developmental delay with early onset epilepsy [Zweier et al., 2009; Harrison et al., 2011]. Heterozygous deletions involving exons of *NRXN1* have also been shown to predispose individuals to a wide spectrum of developmental disorders [Schaaf et al., 2012; Dabell et al., 2013]. However, the phenotypic consequences of the small, heterozygous intronic deletion seen in our patient are unclear. We note that heterozygous deletions encompassing the maximal interval of our patient's deletion have been described previously in several studies of control individuals catalogued in the Database of Genomic Variants (http://dgv.tcag.ca/) including those by Itsara et al. (1/1557), Shaikh et al. (1/2026) and Xu et al. (3/6533) [Itsara et al., 2009; Shaikh et al., 2009; Xu et al., 2011]. This suggests that this change is likely to be benign.

DNA from our patient's mother was screened for the *HEY2* duplication and the *NRXN1* intronic deletion. Her studies revealed a normal copy number at both of these locations. Our patient's father has no contact with other family members. Hence, his DNA could not be obtained for study.

DISCUSSION

We present a 3-year-old Hispanic female with tetralogy of Fallot, infundibular and valvular pulmonary stenosis, hypotonia, lower extremity weakness, fine motor delay and speech delay. She was found to have a single gene duplication of *HEY2*. This is the smallest duplication involving *HEY2* reported to date. Although her mother was found not to carry this duplication, her father could not be tested. Since it is unclear whether this change is *de novo* or was inherited from a putatively unaffected father, it is possible that phenotypes associated with *HEY2* duplication are incompletely penetrant and that other genetic, environmental and stochastic factors play a role in determining whether an individual develops cardiovascular and/or neurological phenotypes.

No other duplications involving *HEY2* were seen in over 70,000 individuals referred to the Medical Genetics Laboratories at Baylor College of Medicine. Similarly only one individual with a duplication involving *HEY2* is reported in the Database of Genomic Variants (http://dgv.tcag.ca/dgv/app/home). This duplication was reported in one of 90 Yoruban individuals from Ibadan Nigeria by Matsuzaki et al. [2009]. However, this change was not identified by

McCarroll et al. [2008] who analyzed the same cohort. A search of the Decipher database (https://decipher.sanger.ac.uk/) did not reveal individuals with *HEY2* duplications with the exception of Patient # 266259 who has trisomy 6 (possibly mosaic with a mean $\log_2$ ratio of 0.377). This individual's only reported phenotypes were arrhythmia and depressed nasal bridge. Taken together, these data suggest that duplications of *HEY2* are uncommon in the general population.

Only two other cases of *HEY2* duplications with molecular cytogenetic descriptions have been previously reported in the literature [Thorsson et al., 2014]. Although these cases may provide some additional evidence that *HEY2* duplications may contribute to the development of clinical phenotypes, their usefulness is limited by the large sizes of the chromosome 6q duplications involved, the presence of large or clinically relevant deletions on other chromosomes in the same individual and a paucity of clinical information. In addition, there are several case reports of cytogenetically identified isolated 6q duplications that may involve *HEY2*, some of which are associated with structural heart defects (2/6, 33%) and neurological deficits (5/6, 83%). Clinical and cytogenetic summaries of these cases are provided in Supplemental Table SII.

A combination of *in vitro* and *in vivo* studies suggests that HEY2 overexpression can adversely affect cardiac development through dysregulation of *GATA4* which encodes a cardiac-expressed transcription factor. Specifically, forced expression of HEY2 in several cell lines strongly represses expression from the *GATA4* promoter [Fischer et al., 2005]. In addition, HEY2 represses the expression of some of GATA4's cardiac target genes by directly binding to GATA4, blocking its transcriptional activity. Inversely, the expression levels of these GATA4 target genes are elevated in HEY2-deficient mice [Fischer et al., 2005]. The repression of GATA4 activity by HEY2 is particularly germane since heterozygous loss-of-function mutations in *GATA4* have been shown to cause isolated and familial tetralogy of Fallot [Nemer et al., 2006; Yang et al., 2013]. Based on these observations, we conclude that duplication of *HEY2* may be a contributing factor to the development of congenital heart defects in our patient.

Our patient was also found to have hypotonia, lower extremity weakness, fine motor delay and language delay. Her Chiari I malformation may have contributed to some of these phenotypes—such as the lower extremity weakness—but cannot explain others, particularly her language delay. The consequences of HEY2 overexpression during brain development have been studied in mice. Specifically, misexpression of *Hey2* by electroporation in the developing mouse brain at embryonic day (E) 13.5 leads to transient maintenance of neural precursor cells and thereby increases late-born neurons located in the superficial layers of the cortex [Sakamoto et al., 2003]. The same treatment performed at E15.5 inhibits neurogenesis and promotes generation of astroglial cells [Sakamoto et al., 2003]. Hence, an overabundance of HEY2 causes impairment of neurogenesis and promotion of gliogenesis in mice. This suggests that duplication of *HEY2* in humans may have detrimental effects on brain development.

We conclude that duplication of *HEY2* may be a contributing factor to the congenital heart defects and the neurodevelopmental problems evident in our patient. These results suggest

that individuals with *HEY2* duplications should be screened for congenital heart defects and monitored closely for evidence of developmental delay and/or cognitive impairment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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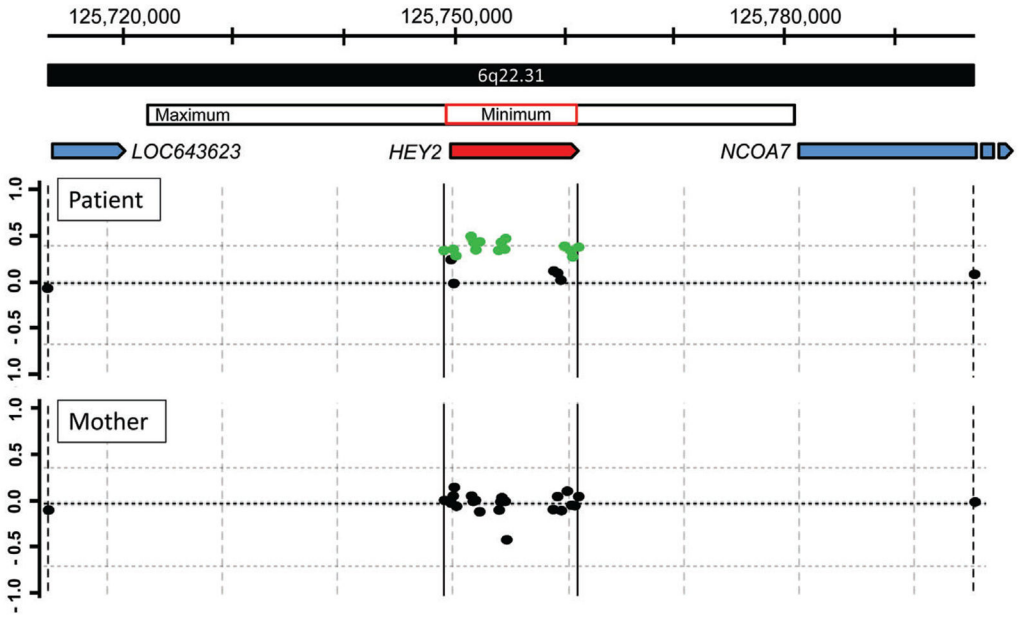

FIG. 1.

Duplication of *HEY2* in a child with congenital heart defects and neurologic phenotypes. An array-based copy number variation (CNV) detection assay revealed a *HEY2* duplication in our patient which was not seen in her mother. Data from these studies, plotted on a $\log_2$ scale, are shown (hg38). The entire *HEY2* gene (red arrow) is located in the minimum duplication interval (red box). Although *LOC643623* and a part of the *NCOA7* gene (blue arrows) were located in the maximum interval identified by CNV analysis, subsequent real-time quantitative PCR (RT-qPCR) analyses revealed that they were not affected by the duplication. The approximate location of the refined maximal interval defined by RT-qPCR is shown as a black box. Arrows representing genes point in the direction of transcription. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/journal/10.1002/ (ISSN) 1552-4833.