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Rare Copy Number Variants in a Population Based Investigation of Hypoplastic Right Heart Syndrome

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Abstract

Background—Hypoplastic right heart syndrome (HRHS) is a rare congenital defect characterized by underdevelopment of the right heart structures commonly accompanied by an atrial septal defect. Familial HRHS reports suggest genetic factor involvement. We examined the role of copy number variants (CNVs) in HRHS.

Methods—We genotyped 32 HRHS cases identified from all New York State live births (1998–2005) using Illumina HumanOmni2.5 microarrays. CNVs were called with PennCNV and prioritized if they were ≥20Kb, contained ≥10 SNPs and had minimal overlap with CNVs from in-house controls, the Database of Genomic Variants, HapMap3 and CHOP database.

Results—We identified 28 CNVs in 17 cases; several encompassed genes important for right heart development. One case had a 2p16–2p23 duplication spanning *LBH*, a limb and heart development transcription factor. *Lbh* mis-expression results in right ventricular hypoplasia and pulmonary valve defects. This duplication also encompassed *SOS1*, a factor associated with pulmonary valve stenosis in Noonan syndrome. *Sos1*$^{-/-}$ mice display thin and poorly trabeculated ventricles. In another case, we identified a 1.5Mb deletion associated with Williams Beuren syndrome, a disorder that includes valvular malformations. A third case had a 24Kb deletion

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upstream of the TGFβ ligand *ITGB8*. Embryos genetically null for *Itgb8*, and its intracellular interactant *Band 4.1B*, display lethal cardiac phenotypes.

Conclusions—To our knowledge, this is the first study of CNVs in HRHS. We identified several rare CNVs that overlap genes related to right ventricular wall and valve development, suggesting that genetics plays a role in HRHS and providing clues for further investigation.

Keywords

hypoplastic right heart; hypoplastic right ventricle; copy number variants; *SOS1*; *LBH*; *ITGB8*; *PRRX2*

INTRODUCTION

Hypoplastic right heart syndrome (HRHS, BPA code: 746.882, ICD10: Q22.6) is a rare cyanotic congenital disorder characterized by underdevelopment of the right ventricle, accompanied by pulmonary valve atresia, hypoplasia of the tricuspid valve or the pulmonary artery, as well as an atrial septal defect (Dib et al., 2012). Patients exhibit a wide spectrum of cardiac abnormalities with the most severe being atresia of the right ventricle outflow tract, severe right ventricle and tricuspid valve hypoplasia as well as coronary artery abnormalities (Tworetzky et al., 2009). On the other end of the spectrum of defects, membranous atresia of the pulmonary valve with a relatively normal sized right ventricle that does not cause severe cyanosis after birth might, occasionally, remain undiagnosed until adulthood (Prasad et al., 1992; Dib et al., 2012).

So far, no specific environmental or demographic factors have been described in relation to HRHS. Familial reports of the syndrome, however, suggest a potential genetic component. Occurrence in siblings has been reported in several cases (Medd et al., 1961; Becker et al., 1971; Eriksen et al., 1989; Chitayat et al., 1992). Isolated right ventricular hypoplasia has also been identified in both siblings and offspring of affected individuals (Sackner et al., 1961; Chessa et al., 2000). Additionally, pulmonary atresia with intact ventricular septum and hypoplastic right ventricle was identified in monozygotic twins (De Stefano et al., 2008). Therefore, some authors suggest that the condition is caused by a single gene or that it has an autosomal dominant pattern of inheritance (Chitayat et al., 1992; De Stefano et al., 2008).

To our knowledge, this is the first population based investigation of the potential genetic cause of HRHS. The objective of our study was to identify novel, potentially causal, copy number variations (CNVs) in HRHS.

METHODS

Cases

Physicians and hospitals in New York State (NYS) are required to report to the state's Congenital Malformation Registry all children born in NYS who were diagnosed with a reportable congenital defect before two years of age. Defects are coded using the expanded British Paediatric Association (BPA) coding system. We identified all cases that included the

code 746.882 (hypoplastic right heart syndrome, hypoplastic right ventricle). We also performed a term search for “hypoplastic right heart” and “hypoplastic right ventricle” to minimize the possibility of missed cases. From all live births occurring in NYS from January 1, 1998 through December 31, 2005, we identified 73 cases with this specific code then excluded those with associated aneuploidy or other chromosomal anomalies, and cases where the diagnosis was equivocal. In total, we identified 34 cases that met our inclusion criteria. Among them, there were nine with the HRHS variant pulmonary atresia with intact ventricular septum and two cases with isolated right ventricular hypoplasia. Archived newborn screening DNA samples were located for 32 cases and none had been marked that the parents had refused use of the specimen for research.

Demographic and medical characteristics of mothers and cases were extracted from NYS vital records and compared to the population of all live births occurring in NYS between 1998 and 2005 (n= 2,023,049). Statistical analyses were performed using t-tests or Fisher’s exact tests, where applicable. Before genotyping and analysis was performed both cases and controls were assigned random identification numbers to ensure that no personal information was accessible. This study was approved by the NYS Department of Health (IRB 07-007) and the NIH Office of Human Subjects Research Protection (OHSRP#3687).

Genotyping

DNA extraction was performed at Wadsworth Center of the NYS Department of Health from two 3mm dried blood spot (DBS) punches using a previously described laboratory method (Saavedra-Matiz et al., 2013). Genotyping was performed at the University of Minnesota using Illumina HumanOmni2.5-8_v1 bead arrays and the Infinium HD assay protocol. Data were analyzed with Illumina GenomeStudio v2011.1 with a genotype no-call threshold set at <0.15. In total, 32 HRHS samples were genotyped (one in duplicate) concurrently with 138 cases with other unrelated phenotypes, 3 in-house controls and one additional HapMap control (also in duplicate). The genotyping analysis was based on Illumina’s Infinium Genotyping Data Analysis Technical Note (Illumina, 2014). A total of 2,278,660 autosomal single-nucleotide polymorphisms (SNPs) were included in the CNV analysis. The average sample SNP call rate ± SD (range) was 99.2% ± 1.3 (range 93.1–99.8%) and the mean log R ratio (LRR) deviation was 0.142 ± 0.035 (0.099–0.231). After cleaning, SNP genotype reproducibility (based on two duplicates included among the 173 samples genotyped) was 100%.

CNV calling and annotation

CNV calling was performed using PennCNV version 2011/05/03 and Illumina’s cnvPartition algorithm version 3.1.6 and calls were compiled and annotated as previously described (Rigler et al., 2014). Potential relevance to HRHS pathogenesis was assessed based on intersecting gene and transcript content (identified using GENCODE Genes track version 19, December 2013, HAVANA and Ensembl Datasets), number of cases and controls with an overlapping CNV and concordance rates between PennCNV and cnvPartition calls. The percent overlap with common variants reported in other CNV databases including the Childrens Hospital of Philadelphia (CHOP) database (Shaikh et al., 2009), HapMap

(International HapMap 3 Consortium, 2010) and Database of Genetic Variants (DGV2) was also calculated.

CNV Selection and Prioritization

Several exclusion criteria were implemented when selecting candidate CNVs for follow up. CNVs were excluded if they were smaller than 20Kb, contained fewer than 10 consecutive SNP probes or had more than 20% overlap with common variants reported in HapMap, CHOP or with any variant that was identified in previous birth defect studies performed in our laboratory (including heterotaxy (Rigler et al., 2014) and posterior urethral valves (Boghossian et al., 2016)). The remaining candidates were examined for overlap with known CNVs reported in DGV (release date – 2014/10/16) using build37/hg19 coordinates. Identified variants with less than 50% overlap or encompassing genes in the non-overlapping region were selected for further investigation.

CNV Validation

CNV validation was performed with real time polymerase chain reaction (qPCR) using two to four TaqMan copy number assays (Applied Biosystems, Carlsbad, CA) per region on either an ABI 7900HT or an ABI QuantStudio (Supplementary Methods). Subsequently, CNVs that were validated were screened against 193 unaffected randomly selected and race-ethnicity matched NYS controls using at least one assay per area of interest (Supplemental information).

RESULTS

The birth prevalence of HRHS in the NYS population was 1 per 60,000 live births. Demographic characteristics from HRHS cases were compared to the total population of live births in NYS from 1998 through 2005 (n=2,023,049) (Table 1). Cases had a significantly lower birth weight than controls (2,956g vs 3298g, p=0.0011), but there was no difference in the gestational age. Additionally, no statistically significant differences in race/ethnicity, maternal pre-pregnancy BMI or infant sex were detected. Table 2 summarizes the clinical manifestations of HRHS in our cases. Cases one through six had a validated CNV of interest.

We successfully genotyped 32 HRHS cases that resulted in 7,860 PennCNV calls in the microarray analysis. After applying the selection criteria previously described, we identified 28 CNVs in 17 cases. We prioritized 10 CNVs in 9 cases for real time PCR confirmation (Supplemental Table 1). Validated CNVs included four duplications and three heterozygous deletions ranging from 25Kb to 27Mb (Table 3) encompassing genes in pathways implicated in cardiac development and specifically in the development of the right heart. A 27Mb duplication spanning *LBH* and *SOS1*, two factors linked to right heart defects, was identified in one case (Case 1, Table 3). In Case 2, we identified a 25Kb heterozygous deletion upstream of *ITGB8*, a TGFβ ligand essential for heart development (Jung et al., 2011). A 1.5Mb heterozygous deletion was identified at 7q11.3 (Case 3, Table 3) spanning *ELN*. Interestingly, this genomic region is associated with Williams Beuren syndrome, which includes valvular malformations as well as peripheral pulmonary stenosis (Collins, 2013).

Case 4 had a 180Kb duplication encompassing *PRRX2*, a transcriptional factor ubiquitously expressed in the developing heart that is also a transcription factor for *HAND2* (Leussink et al., 1995). Three CNVs did not validate.

DISCUSSION

Our study, which is the first population based genomic investigation of HRHS, identified several CNVs that encompassed genes involved in right heart development. The embryonic outflow tract and the right ventricle are derived from a specific population of cells known as the secondary heart field. Signaling pathways hypothesized to control secondary heart field development include Wnt, Fgf, Tgfβ, and Notch (Rochais et al., 2009). Several genes and transcription factors including, *Nkx2.5, Hand2, Tbx5, Isl1*and *Foxh1* seem to have a restricted expression in this area (Black, 2007).

In one case we identified a 27Mb duplication at 2p23.2–2p16.2 encompassing two genes implicated in cardiac development and disease. The first was *LBH*, a transcription factor that is essential for limb and heart development that has been associated with congenital heart defects in partial trisomy 2p syndrome(Briegel et al., 2005). *Lbh* is hypothesized to exert its action by attenuating the signal of two essential WNT pathway molecules that control heart development, *Nkx2-1* and *Tbx5* (Biben et al., 2000; Liberatore et al., 2000). Although in early embryogenesis *Lbh* is ubiquitously expressed in the developing heart, after cardiac tube looping, *Lbh* is specifically expressed in the right ventricle and atrioventricular canal. Transgenic mis-expression of Lbh in murine embryos results in isolated right ventricular hypoplasia, pulmonary stenosis and atresia or both, as well as several other cardiac malformations. (Briegel et al., 2001)

Another gene overlapped by the 27Mb duplication in Case 1 was *SOS1*, a guanine nucleotide exchange factor in the EGFR and Ras/MAPK pathways (Wang et al., 1997; Chen et al., 2010). Mutations in this gene are known to cause Noonan syndrome which is characterized by right heart defects. Interestingly, it has been shown that pulmonic stenosis is more common in Noonan syndrome patients with *SOS1* mutations(Roberts et al., 2007). Additionally, mice with mutant *Sos1* display severe cardiovascular defects including right ventricular hypertrophy and atrial septal defects (Wang et al., 1997; Chen et al., 2010).

In another case we identified a 24Kb deletion 17Kb upstream of *ITGB8*, a member of the integrin family and ligand of the TGFβ pathway. Mice genetically null for *Itgb8* and its intercellular ligand *Band 4.1B* display severe malformations of the cardiac neural crest and the developing outflow tract and die mid-gestation(Jung et al., 2011). *Itgb8* is hypothesized to activate $TGF\beta_1$ and $TGF\beta_3$ signaling (Mu et al., 2002). The TGFβ pathway is essential in cardiac development and specifically in the second heart field (Peng et al., 2014). Abnormal TGFβ signaling results in several right heart defects including double outlet right ventricle and overriding tricuspid valve (Bartram et al., 2001).

A third case had 1.5Mb heterozygous deletion at 7q11.23. Deletions in this genomic region have been associated with Williams Beuren syndrome (WBS), a congenital disorder which includes cardiovascular defects (Kaplan et al., 2001). Cardiac abnormalities are seen in over

80% of the patients; approximately half of these have pulmonary arterial stenosis (Collins Ii et al., 2010; Collins, 2013). Hemizygosity of the *ELN* gene coding elastin has been demonstrated to be responsible for the vascular, and in part the cardiac, pathology in WBS; however, the mechanism is still unclear (Keating, 1995). Additionally, a double chamber right ventricle has been reported in relation to WBS(Mazumdar et al., 2016).

In a fourth case we identified a 180Kb duplication encompassing *PRRX2* (or *PRX2*) a known *HAND2* transcriptional factor that is expressed in the embryonic heart(Leussink et al., 1995; Barbosa et al., 2007). Although it is known that *Prx2* is essential for proper *Hand2* expression in the developing midline mesenchyme, their exact interaction in the developing heart has not been studied (Barbosa et al., 2007). *Prx2* knockout mice display mostly defects of the great arteries, whereas *Hand2* knockout mice exhibit both vascular defects and right ventricular hypoplasia(Bergwerff et al., 2000; McFadden et al., 2005). More research is warranted in order to assess the exact role of *Prx2* in the developing right heart.

Our study has several strengths. This is the first, to the best of our knowledge, population based study of HRHS. The NYS population allowed us to study the demographic characteristics of the cases against >2,000,000 births and identify that HRHS cases have a lower birth weight than controls without having a smaller gestational age indicating compromised intrauterine growth. Our study is also the first genome wide investigation of the syndrome. One limitation of our study is the possibility of missed cases based on incorrect ascertaining or non-reporting. Case ascertainment in the NYS registry, however, has been shown to be excellent (Sekhobo et al., 2001). Additionally, due to the small number of samples our statistical comparisons may have been underpowered. Given that HRHS is a very rare condition (34 cases in over 2 million births) this problem is almost unavoidable. Lastly, as in all CNV studies, potential inaccuracies in mapping breakpoints and bias in genomic databases reduce our ability to confidently assess the clinical significance of the identified CNVs (Duclos et al., 2011).

In conclusion, in this first population based study of HRHS, we identified several rare CNVs spanning genes important for right ventricular wall and valve development, supporting a role for genetic factors in the development of the syndrome. Further investigation of the 2p arm duplication and specifically of *LBH* in the pathogenesis of right heart hypoplasia is warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Selected characteristics of HRHS cases and NYS live birth controls.

Characteristic	HRHS cases (n=34)	NY live births (n=2,023,049)	P value
Maternal age, years (%)			0.36
<20	1 (2.9)	157,085 (7.76)	
20–34	24 (70.6)	1,480,911 (73.2)	
≥35	9 (26.5)	384,744 (19.02)	
Missing	0 (0)	309 (0.02)	
Maternal race/ethnicity, n (%)			0.54
Non-Hispanic white	14 (41.2)	1,051,561 (52)	
African American	8 (23.5)	361,836 (17.9)	
Hispanic	7 (20.6)	437,846 (21.6)	
Asian	4 (11.8)	135,374 (6.69)	
Other	1 (2.9)	31,220 (1.5)	
Missing	0 (0)	5,212 (0.26)	
Maternal education, years (%)			0.64
<12	5 (14.7)	384,781 (19)	
12	9 (26.5)	594,659 (29.3)	
>12	20 (58.8)	1,017,827 (50.3)	
Missing	0 (0)	25,782 (1.27)	
Parity, n (%)			0.6[a]
Nulliparous	16 (47.1)	846,801 (41.86)	
Multiparous	18 (52.9)	1,176,248 (58.14)	
Missing	0 (0)	0 (0)	
Prepregnancy maternal BMI (kg/m^2)			0.72
<18.5	1 (2.9)	40,332 (1.99)	
18.5–24.9	6 (17.6)	523,438 (25.87)	
25–29.9	4 (11.8)	233,251 (11.53)	
≥30	4 (11.8)	182,264 (9.01)	
Missing	19 (55.9)	1,043,764 (51.59)	
Infant sex, n (%)			0.13[a]
Male	22 (64.7)	1,036,825 (51.3)	
Female	12 (35.3)	986,210 (48.8)	
Missing	0 (0)	0 (0)	
Infant gestational age (mean days ± SD)	270.8 ± 21.2	274.6 ± 18.1	0.22
Infant birth weight (mean grams ± SD)	2956 ± 745	3298 ± 610.6	0.0011

Characteristic	HRHS cases (n=34)	NY live births (n=2,023,049)	P value
Maternal Smoking			0.54[a]
No	30 (88.2)	1,842,757 (91.09)	
Yes	4 (11.8)	180,292 (8.91)	
Missing	0 (0)	0 (0)	

[a]Fisher's Exact Test (Two-sided Pr <= P) p value; All p-value calculations exclude missing category.

Table 2

Clinical information on HRHS cases provided by the Congenital Malformations Registry.

Case ID	Cardiac malformations	Extracardiac defects
1	Hypoplastic right heart syndrome Ventricular septal defect	
2	Hypoplastic right heart syndrome Atrial septal defect- ostium secundum Coronary artery anomaly	
3	Hypoplastic right heart syndrome Ventricular septal defect Atrial septal defect Pulmonary valve stenosis Tricuspid atresia	
4	Hypoplastic right heart syndrome Ventricular septal defect Patent foramen ovale Tricuspid atresia	Hydronephrosis
5	Hypoplastic right heart syndrome Patent foramen ovale Coronary artery anomaly	Cleft Lip and palate
6	Hypoplastic right heart syndrome Patent foramen ovale Pulmonary atresia Coronary artery anomaly	
7	Hypoplastic right heart syndrome Coronary artery anomaly	
8	Hypoplastic right heart syndrome Patent foramen ovale Pulmonary atresia	
9	Hypoplastic right heart syndrome	
10	Hypoplastic right heart syndrome Atrial septal defect Pulmonary atresia	
11	Hypoplastic right heart syndrome Pulmonary valve stenosis Tricuspid insufficiency Coronary artery anomaly	

Case ID	Cardiac malformations	Extracardiac defects
12	Hypoplastic right heart syndrome Pulmonary valve stenosis Tricuspid insufficiency	
13	Hypoplastic right heart syndrome peripheral pulmonary stenosis	
14	Hypoplastic right heart syndrome Pulmonary atresia Tricuspid atresia	
15	Hypoplastic right heart syndrome Atrial septal defect Hypoplastic left heart syndrome Peripheral pulmonary stenosis	
16	Hypoplastic right heart syndrome Hypoplastic left heart syndrome Peripheral pulmonary stenosis Pulmonary atresia/pulmonary artery agenesis	Hydronephrosis
17	Hypoplastic right heart syndrome Ventricular septal defect Atrial septal defect- ostium secundum Tricuspid atresia	Brain anomaly (unspecified)
18	Hypoplastic right heart syndrome Atrial septal defect Tricuspid atresia	
19	Hypoplastic right heart syndrome Ventricular septal defect Atrial septal defect- ostium secundum Tricuspid atresia	Brain anomaly (unspecified)
20	Hypoplastic right heart syndrome Ventricular septal defect Atrial septal defect Pulmonary valve stenosis Coronary artery anomaly	
21	Hypoplastic right heart syndrome Pulmonary atresia	
22	Hypoplastic right heart syndrome Tricuspid insuficiency Hypoplastic left heart syndrome	

Case ID	Cardiac malformations	Extracardiac defects
	Peripheral pulmonary stenosis	
23	Hypoplastic right heart syndrome Ventricular septal defect Pulmonary valve stenosis Tricuspid atresia	
24	Hypoplastic right heart syndrome Pulmonary valve stenosis Hypoplastic left heart syndrome Peripheral pulmonary stenosis	
25	Hypoplastic right heart syndrome Ventricular septal defect Pulmonary valve stenosis Tricuspid atresia	
26	Hypoplastic right heart syndrome Tricuspid atresia Aortic insufficiency Right ventricular hypoplasia Pulmonary atresia/pulmonary artery agenesis	
27	Hypoplastic right heart syndrome Hypoplastic left heart syndrome	
28	Hypoplastic right heart syndrome Ventricular septal defect Tricuspid atresia Ectopia cordis	Hypospadias without chordee
29	Hypoplastic right heart syndrome Ventricular septal defect Atrial septal defect Pulmonary valve stenosis Tricuspid atresia Peripheral pulmonary stenosis	
30	Hypoplastic right heart syndrome Pulmonary atresia Tricuspid stenosis or hypoplasia Hypoplastic left heart syndrome	Hydronephrosis Spinal cord anomaly
31	Hypoplastic right heart syndrome Tricuspid atresia Pulmonary atresia/pulmonary artery agenesis Peripheral pulmonary stenosis	

Case ID	Cardiac malformations	Extracardiac defects
32	Hypoplastic right heart syndrome Ventricular septal defect Peripheral pulmonary stenosis	

Table 3

Validated CNVs in HRHS cases.

Locus	Genomic Coordinates (hg19)[1]	Size (Kb)	Type	Case ID	Candidate Genes/Transcripts
2p23.2–2p16.2	28,027,938–54,971,385	27,000	Dupl	1	***LBH***; ***SOS1***; *EPAS1*
7p21.1	20,322,967–20,347,570	24.6	Het Del	2	*AC099342.1*
7q11.23	72,718,278–74,140,708	1.422	Het Del	3	*BAZ1B*; **ELN**; *FZD9; LAT2; LIMK1; MLXIPL; STX1A; TBL2; TRIM50; VPS37D; WBSCR22; WBSCR27; WBSCR28*
9q34.11	132,304,132–132,484,643	180.5	Dupl	4	*ASB6; NTMT1; PRRX2*
19p13.12	15,261,250–15,953,303	692.1	Dupl	4	*BRD4; CYP4F12; CYP4F22; CYP4F3; CYP4F8; EPHX3; NOTCH3; OR10H2; PGLYRP2; RASAL3; UCA1; WIZ*
2q11.2	98,014,115–98,275,354	261.2	Dupl	5	*ACTR1B; COX5B*
10q23.33	96,218,093–96,383,002	164.9	Het Del	6	*HELLS; TBC1D12*

Genes in bold are discussed in the manuscript. Kb=kilobase pairs.

Het Del=heterozygous deletion. Dupl=duplication.

[1]Size and coordinates estimated from array data (pennCNV calls, hg19).