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De Novo 9q Gain in an Infant with Tetralogy of Fallot with Absent Pulmonary Valve: Patient Report and Review of Congenital Heart Disease in 9q Duplication Syndrome

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Abstract

Genomic disruptions, altered epigenetic mechanisms, and environmental factors contribute to the heterogeneity of congenital heart defects (CHD). In recent years, chromosomal microarray analysis (CMA) has led to the identification of numerous copy number variations (CNV) in patients with CHD. Genes disrupted by and within these CNVs thus represent excellent candidate genes for CHD. Microduplications of 9q (9q+) have been described in patients with CHD, however, the critical gene locus remains undetermined. Here we discuss an infant with tetralogy of Fallot with absent pulmonary valve, fetal hydrops, and a 3.76 Mb de novo contiguous gain of 9q34.2-q34.3 detected by CMA, and confirmed by karyotype and FISH studies. This duplicated interval disrupted *RXRA* (retinoid X receptor alpha; OMIM #180245) at intron 1. We also review CHD findings among previously reported patients with 9q (9q+) duplication syndrome. This is the first report implicating *RXRA* in CHD with 9q duplication, providing additional data in understanding the genetic etiology of tetralogy of Fallot, CHD, and disorders linked to 9q microduplication syndrome. This report also highlights the significance of CMA in the clinical diagnosis and genetic counseling of patients and families with complex CHD.

Keywords

tetralogy of Fallot with absent pulmonary valve; retinoid X receptor alpha; *RXRA*

INTRODUCTION

Congenital heart defects (CHD) are the most common congenital malformations and affect approximately 8 per 1,000 live births [Reller et al., 2008]. The incidence of CHD is

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SUPPORTING INFORMATION

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even higher among fetuses, as many fetuses with complex lesions do not survive to term birth [Hunter and Simpson, 2014]. Tetralogy of Fallot (TOF; OMIM #187500), the most common cyanotic congenital heart defect, is a conotruncal malformation characterized by four abnormalities: pulmonary infundibular stenosis, ventricular septal defect (VSD), overriding aorta, and right ventricular hypertrophy [Ho et al., 2001]. Tetralogy of Fallot with absent pulmonary valve (TOF/APV) is an uncommon CHD accounting for only 3–6% of patients with TOF-spectrum defects [Moon-Grady et al., 2002]. TOF/APV shares the key features of the classical form of TOF as well as an incompetent or absent pulmonary valve.

Genetic factors, epigenetic changes, and environmental influences contribute to the multifactorial nature of CHD. Numerical and structural chromosome anomalies as well as point mutations in over 50 monogenic loci encoding transcription factors, cell signaling proteins, and epigenetic regulators have been identified among infants with isolated or syndromic CHD [Wilson et al., 1985; Kleefstra et al., 1993; Gijsbers et al., 2008; Bittel et al., 2011; Andersen et al., 2014; Hunter and Simpson, 2014]. Murine models of targeted monogenic deletions have identified over 500 genes which when disrupted contribute to the development of CHD [Andersen et al., 2014] (Mouse Genome Informatics; http://www.informatics.jax.org/). For example, homozygous (*Rxra* −/−) and heterozygous (*Rxra* +/−) deletions result in abnormal cardiac structure and function in mice [Kastner et al., 1994; Sucov et al., 1994; Dyson et al., 1995; Gruber et al., 1996], and disruption of *RXRA* may contribute to the pathogenesis of CHD in humans (retinoid X receptor, alpha; OMIM #180245).

Copy number variants have also been identified among individuals with CHD [Hitz et al., 2012; Tomita-Mitchell et al., 2012; Andersen et al., 2014; Bansal et al., 2014; Soemedi et al., 2014]. In humans, microduplications of the long arm of chromosome 9 (9q+) have been associated with CHD as well as low birth weight, failure-to-thrive, craniofacial dysmorphic features (microcephaly, prominent nasal bridge, microstomia, micrognathia), neurologic findings (hypotonia, intellectual disability, developmental delay, and psychomotor delay), skeletal defects (arachnodactyly, joint contractures, abnormally positioned digits), and urogenital malformations [Turleau et al., 1975; Allderdice et al., 1983]. Chromosome microarray analysis (CMA) has identified various overlapping 9q+ breakpoints thereby expanding the clinical spectrum and severity of phenotypic findings. Smaller duplications of 9q34 (9q34+) tend to correlate with milder phenotypes, while larger duplications correlate with more severe malformations and neurologic impairment [Allderdice et al., 1983; Spinner et al., 1993; Papadopoulou et al., 2010]. However, the critical region and candidate genes that impart risk for CHD among patients with 9q microduplications remain to be established [Gelb, 2004; Greenway et al., 2009; Silversides et al., 2012; Glessner et al., 2014; Warburton et al., 2014].

We discuss an infant with TOF/APV, fetal hydrops, and *de novo* contiguous gain in 9q34.2-q34.3, disrupting *RXRA* at intron 1 and duplicating exons 2–10, and review the reported patients with 9q+ duplications. This is the first report of disrupted *RXRA* and complex 9q+ in a patient with TOF or CHD, suggesting that *RXRA* at 9q34.2 may be a critical locus in 9q+-associated CHD. This report highlights the importance of CMA in genomic

and epigenomic characterization, as well as in genetic diagnosis of complex congenital malformations and clinical features associated with 9q microduplication syndrome.

CLINICAL REPORT

A 2,135 g (10th percentile) infant female was born at 35 weeks gestation to a 26-year-old primigravida mother who was rubella immune. The pregnancy was complicated by the second trimester ultrasound finding of a single umbilical artery. Follow-up fetal ultrasound performed at 35 weeks gestation revealed new-onset cardiomegaly, abdominal ascites, and scalp edema consistent with fetal hydrops. Fetal echocardiogram revealed marked cardiomegaly with a cardiothoracic area ratio of 60% (normal <35%). Also noted were a large malaligned VSD with overriding aorta and an enlarged pulmonary outflow tract over three times greater in size than the aortic outflow tract with to-and-fro flow by color Doppler. Rudimentary pulmonary valve leaflets were noted in the annular position and the main and branch pulmonary arteries were markedly dilated. A ductus arteriosus was not present. These findings were consistent with TOF/APV. The left and right ventricles were dilated with moderately decreased and severely decreased systolic function, respectively. Given the poor prognosis of the underlying cardiac defect in the setting of decreased ventricular function and fetal hydrops, the decision was made to provide comfort care upon delivery. The infant was limp and cyanotic at birth with poor respiratory effort. Her physical examination was notable for diminished breath sounds, a distended abdomen, elongated fingers and toes, and loose skin on her legs. Facial exam was notable for triangular facies with a broad forehead, but no other dysmorphic features including hypertelorism, down-slanting palpebral fissures, coarsening of facies, or abnormal helices. Philtrum, nasal bridge, and mouth were normal in appearance. She took a few spontaneous, shallow breaths after delivery and died within minutes of birth. Cord blood was collected at the time of delivery for chromosome microarray and the family agreed to postmortem magnetic resonance imaging (MRI) and genetic testing.

Laboratory/Imaging Studies

Postmortem MRI was notable for marked cardiomegaly with biventricular hypertrophy (Fig. 1). The main pulmonary artery and branch pulmonary arteries were markedly enlarged. Pleural effusion and large volume abdominal ascites were noted as well as small kidneys. No other defects were identified on the MRI.

MATERIALS AND METHODS

Genomic DNA was isolated from cord blood lymphocytes of the infant (proband) for CMA and data output was analyzed using CytoScan HD (Affymterix, Inc., Santa Clara, CA). Lymphocytes from the proband and parental blood samples were propagated in culture and metaphase spreads were obtained for G-banding chromosome analysis and *HIRA* (22q11.21) FISH studies. Post-CMA FISH studies were also performed on interphase nuclei and metaphase chromosomes using the following probes: RP11–644H13 BAC Clone for 9q34.3 locus (185 Kb; [hg19] chr9:140,535,102–140,719,726) and 9q21-specific control probe.

RESULTS

CMA revealed three contiguous copy number gains (triplication-duplication-triplication) at 9q34.2-q34.3 (3.76 Mb; hg19; 137,259,675–141,020,389) (Fig. 2A). This interval includes 2,730 probes and 66 OMIM genes. Karyotype analysis showed extra chromosome material on 9q34 (Figs. 2B and C, arrow). FISH studies revealed an enhanced signal at 9q+ on metaphase chromosomes (Fig. 2D) and approximately five copies (with one enhanced signal) (Fig. 2E) on interphase nuclei, consistent with the copy number gain (>3 copies) detected by CMA. Parental cytogenetic studies were normal and thus the 9q+ finding in the proband was a *de novo* event. The proximal breakpoint revealed disruption at intron 1 and duplication of exons 2–10 of *RXRA* (Figs. 3 and 4B). There was no evidence of deletion of *HIRA* by FISH studies and CMA.

DISCUSSION

TOF/APV, first described by Royer and Wilson [Royer and Wilson, 1908], is a rare variant of TOF characterized by a dysplastic or absent pulmonary valve resulting in free pulmonary regurgitation which produces an increased volume load on the right ventricle and the pulmonary outflow tract resulting in branch pulmonary artery dilation [Lakier et al., 1974]. Dilation of the pulmonary arteries can be massive and compress the tracheobronchial tree, especially the anterior lower trachea and bronchi [Lakier et al., 1974]. Fetal heart failure may ensue due to the ventricular volume overload. Affected infants often develop respiratory failure at birth and, even with ventilatory support and surgery, perinatal morbidity and mortality remain significant. Additional poor prognostic indicators include an abnormal karyotype or identified genetic syndrome [Volpe et al., 2004], hydrops fetalis [Eronen and Heikkila, 2003], left ventricular dysfunction, and increased pulmonary artery valve-to-aortic valve ratio [Szwast et al., 2014].

The CMA for our patient detected copy number gains on distal 9q that disrupted intron 1 and duplicated exons 2–10 of *RXRA* at 9q34.2. RXRA is a subclass of RXR (retinoid X receptors) that bind as homodimers, form heterodimers with RARs (retinoic acid receptors) [Mangelsdorf et al., 1992; Zhang et al., 2014], and together, synergistically bind to DNA response elements to promote transcription of genes eliciting specific signal transduction-mediated pathways [Sucov et al., 1994]. Both receptors are important for retinoic acid signaling pathway during cardiac morphogenesis and formation of the outflow tract [Evans, 1988; Green and Chambon, 1988; Mangelsdorf et al., 1992; Chambon, 1996]. Mice with homozygous deletions of *Rxra* exhibit muscular ventricular septal defects, hypoplasia of the ventricular chamber [Kastner et al., 1994; Sucov et al., 1994; Gruber et al., 1996], and cardiac dysfunction [Dyson et al., 1995]. Heterozygous mice generally have preserved ventricular function, but demonstrate abnormal ventricular chamber structure with abnormal morphology of the trabeculae, myocardium, atrioventricular canal, conotruncal ridges, and papillary muscles [Kastner et al., 1994; Gruber et al., 1996]. These abnormal cardiac findings in mutant *Rxra*-targeted murine models are considered to be phenocopies for human congenital heart defects [Gruber et al., 1996].

The human *RXRA* locus contains multiple regulatory regions (Fig. 4A–D) [Kent et al., 2002; Zhou et al., 2011]. *RXRA* intron 1 (2.2 Kb) contains short interspersed elements or SINEs (MIRs, Alu sequences) that correlate with predicted regions of active regulatory elements (H3K27Ac marks), simple repeats, long interspersed elements (LINEs), and a DNA transposon (MER5B) [Kent et al., 2002; Zhou et al., 2011]. The 5' promoter and other regions within intron 1 of *RXRA* (Fig. 4E, asterisk) are spatiotemporally regulated in fetal and adult hearts as demonstrated by varying peak intensities or patterns of methylation (H3K4, H3K9, H3K27) (Figs. 4E–I). Disruption of this tightly-regulated region, as seen in our patient, may impair *RXRA* expression and disrupt gene-specific signal transduction pathways. Epigenetic studies in patients with TOF demonstrate elevated promoter and LINE-1 methylation resulting in down-regulation of *RXRA* expression [Sheng et al., 2012, 2014; Zhang et al., 2014]. The duplicated interval containing exons 2–10 seen in our patient also contains multiple repeat elements including SINEs, LINEs, and DNA transposons (MLT1D, MER103C, Charlie18a) (Fig. 4D). Duplication of this interval may impair transcript splicing, expression, ligand-RXRA binding, signal transduction, or other mechanisms during fetal heart development. Studies overexpressing *Rxra* in transgenic mice demonstrate abnormal organization of cardiomyocyte contractile elements and dilated cardiomyopathy [Subbarayan et al., 2000]. We speculate that disruption of *RXRA* or additional copies of this gene may have contributed to our patient's cardiac phenotype; however, additional studies are needed to determine how genetic perturbations of *RXRA* contribute to CHD.

Disruption or Duplication of *RXRA* at 9q34.2 and CHD Among 9q+ Patients

In our review of 47 patients with 9q+ microduplication reported in the literature and in the DECIPHER database (Table I, Fig. 3, Supplementary Table SI), duplications of 9q34.2-q34.3 loci were described for 51% (24/47), 9q34.3 locus only for 43% (20/47), and 9q11-q34.1 loci for 6% (3/47). Approximately half (13/25) of the individuals with 9q34.2-q34.3 duplications including our patient had simple or complex CHD. Of the 20 patients with duplication of only the most distal 9q34.3 locus, 95% (19/20) did not have CHD (Fig. 3). Based on these findings, we suggest that *RXRA* at 9q34.2 may be an important locus for CHD among individuals with 9q34+ (Fig. 3). Patient #33 (Fig. 3, box; Table I) has TOF and 9q34.3+ (410 Kb) that does not include duplication of *RXRA* or neighboring *EHMT1,* but has a breakpoint at intron 2 of *NOTCH1* with duplication of exons 3–34. Most described CHD involving *NOTCH1* in humans and mice are attributed to haploinsufficiency, rather than duplication of *NOTCH1* [Timmerman et al., 2004; Garg et al., 2005; McKellar et al., 2007; McBride et al., 2008; Luna-Zurita et al., 2010; Bosse et al., 2013; Wang et al., 2013]. Additionally, microdeletions or mutations in *EHMT1* (9q34.3) result in Kleefstra syndrome as characterized by CHD, developmental delay, intellectual disability, hypotonia, dysmorphic facial features, urogenital defects, and epilepsy [Kleefstra et al., 2006; Willemsen et al., 2012]. Upon careful review of the CMA results from the nineteen other patients with 9q34.3+ (patients 29–48 except #33) one patient's duplication included only *NOTCH1* (#34), three included only *EHMT1* (#40, 41, 44), and three included both genes (#31, 32, 48). None of these seven patients manifested CHD, suggesting that duplication of 9q34.3, *NOTCH1* or *EHMT1* may not contribute to CHD among 9q+ patients. Furthermore, phenotypic variability among patients with cardiac structural defects

may be attributed to incomplete penetrance, epigenetic events, or other mechanisms of disruption of a single gene or combination of *RXRA*, *NOTCH1*, and *EHMT1*. Additional expression studies are needed to determine the role of these genes and the region in the development of CHD.

RXRA and Other Abnormal Findings in 9q+

Patient #27 is the only other individual with duplication of 9q34.2–9q34.3 loci that involves 5' disruption of *RXRA*, but at intron 7 (exons 8–10 duplication) (Fig. 3, box; Fig. 4B), and 3' disruption at intron 12 of *COL5A1* (OMIM #120215) (exons 1–11 duplication). This patient has joint hypermobility and scoliosis, features compatible with *COL5A1*-associated Ehlers-Danlos syndrome, and no documentation of CHD (DECIPHER #284048). The 5' breakpoint in *RXRA* (intron 7) in this patient did not disrupt any CGIs or promoter regions, however, the duplicated interval overlaps with CpG:22 and a few repeat elements including SINEs (Alu, MIR) and LINEs. This interval also exhibits differential regulatory expression and methylation patterns between fetal and adult hearts (Fig. 4B and E–I). Perhaps the disruption or duplication still produces a functional protein or does not affect RXRA binding and signal-mediated pathways in normal heart development; however, without further genomic investigations, this correlation remains speculative. The remaining 32 patients without CHD reveal duplications distributed along the entire 9q (9q11-q34.3) and a phenotypic spectrum consistent with 9q34 microduplication syndrome including hypotonia, dysmorphic facial features, eye defects, arachnodactyly, joint contractures, and urogenital defects (Table I, Supplementary Table I). In 31% (10/32) of these patients, the duplication includes the *RXRA* locus. In addition to cardiac structural defects, RXRA-dependent pathways when disrupted are known to result in CNS anomalies, craniofacial defects, limb dysmorphogenesis and eye defects, suggesting that a common precursor gives rise to different tissue types. and structures via RXRA-mediated pathways. While these clinical features are evident in several of the patients reviewed here [Maden, 1982; Dencker et al., 1987; Wedden et al., 1988; Mascrez et al., 2009], our patient did not have significant craniofacial or extremity defects suggesting others genes, epigenetic changes, or environmental factors modify penetrance.

In summary, this report suggests *RXRA* at 9q34.2 may be a locus for CHD among individuals with 9q microduplications. Although the exact mechanisms remain to be investigated in humans, disruption and/or duplication of this gene may impair the genomic and epigenomic spatiotemporal regulation of *RXRA*-dependent expression, ligand binding, and signaling pathways associated with normal cardiac development and function. This report highlights the importance of CMA and genomics to identify specific breakpoints and affected gene loci among patients with complex congenital malformations including CHD and to inform genetic counseling of families.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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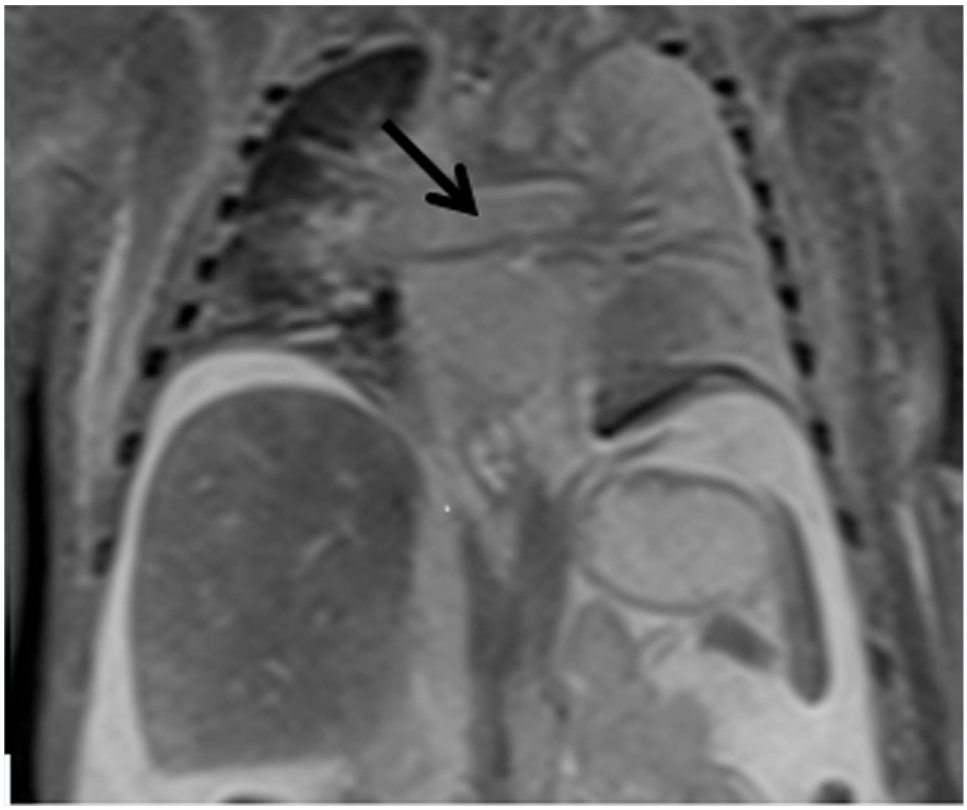

FIG. 1.
Postmortem MRI demonstrating cardiomegaly, enlarged right pulmonary artery (black arrow), atelectasis especially of left lung, and abdominal ascites.

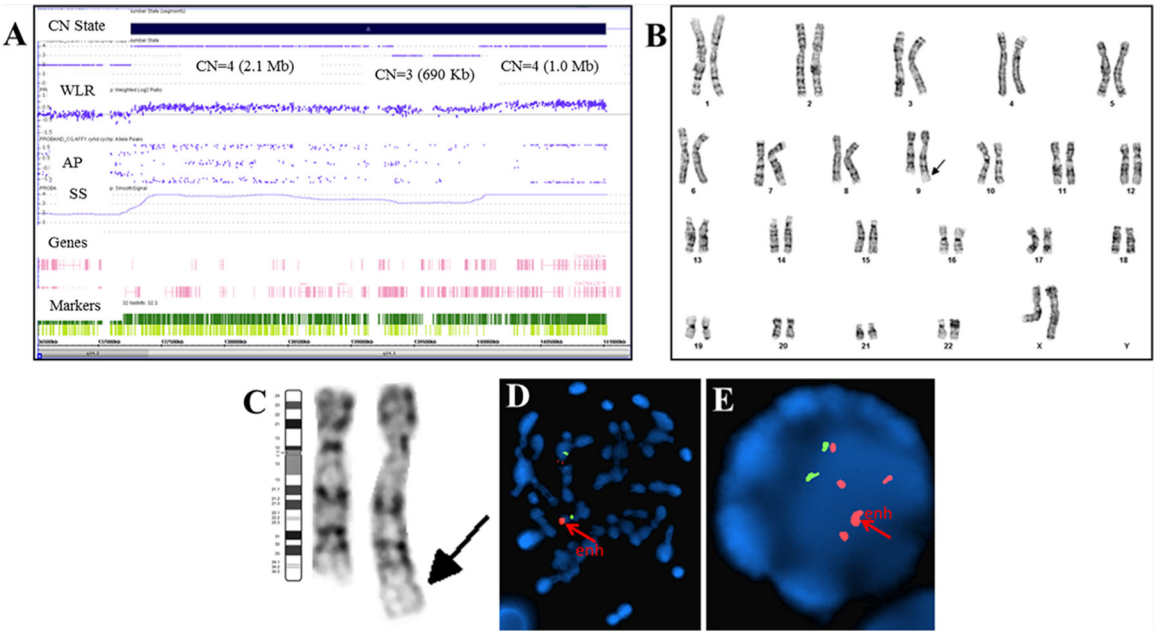

FIG. 2.

Cytogenomic studies in our patient with 9q34.2-q34.3 gains. A: CMA (Affymetrix ChAS Browser) detected contiguous copy number gains; 9q34.2q34.3(137,259,675–139,328,332) x4 (2.1 Mb; 5' breakpoint at intron 1 of RXRA and gains of exons 2–10; 3' breakpoint at intron 3 of *INPP5E* and gains of exons 1–3), 9q34.3(139,328,793–140,018,897) x3 (690 Kb; no gene disruption), 9q34.3(140,018,931–141,020,389) x4 (1.0 Mb; no gene disruption); allele peaks (AP) (0.5, 0, –0.5); weighted log ratio (WLR) (0.5, 0, –0.5); smooth signal (SS) (1–4); oligo and SNP probes (dark and light green). The nearest normal marker is 4.4 Kb from 5' of gain at intron 1 of *RXRA*. B and C: Post-CMA karyotype result supported the copy number gains on 9qter (magnified in C). D and E: FISH studies using RP11–644H13 BAC Clone (185 Kb) for 9q34.3 locus; [hg19] chr9:140,535,102–140,719,726) and 9q21-specific control probe revealed enhanced 9q34.3 signal in metaphase (D) and interphase (E) cells; and at least five 9q34.3 signals in interphase nuclei (E).

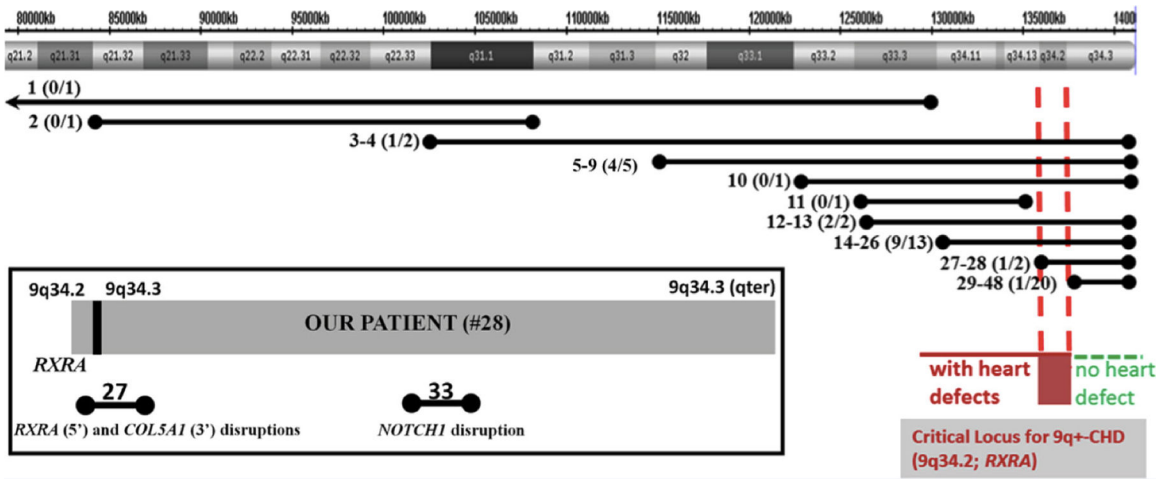

FIG. 3.

Patients #1–48 with 9q+ detected by karyotype analysis or CMA. Each line represents the extent of 9q+ and corresponds to the patient #, and in parentheses, the ratio of the number of patients with CHD of the total number of patients with the same interval. Breakpoints within 9q21.2-qter end in solid circles, and those outside of 9q21.2-qter end in arrows. While 19/20 patients with only 9q34.3+ do not exhibit CHD (green horizontal dotted interval), patients with 9q34.2 have CHD (red horizontal dotted interval). Patients 1, 2, 11 (no CHD) do not overlap with our patient's interval. Based on these findings, we suggest that *RXRA* at 9q34.2 (vertical red dotted lines) may be a critical locus for 9q+-associated CHD. Box: Disruptions in patient #27 (no CHD); and patient #33 at intron 2 of *NOTCH1* (with TOF).

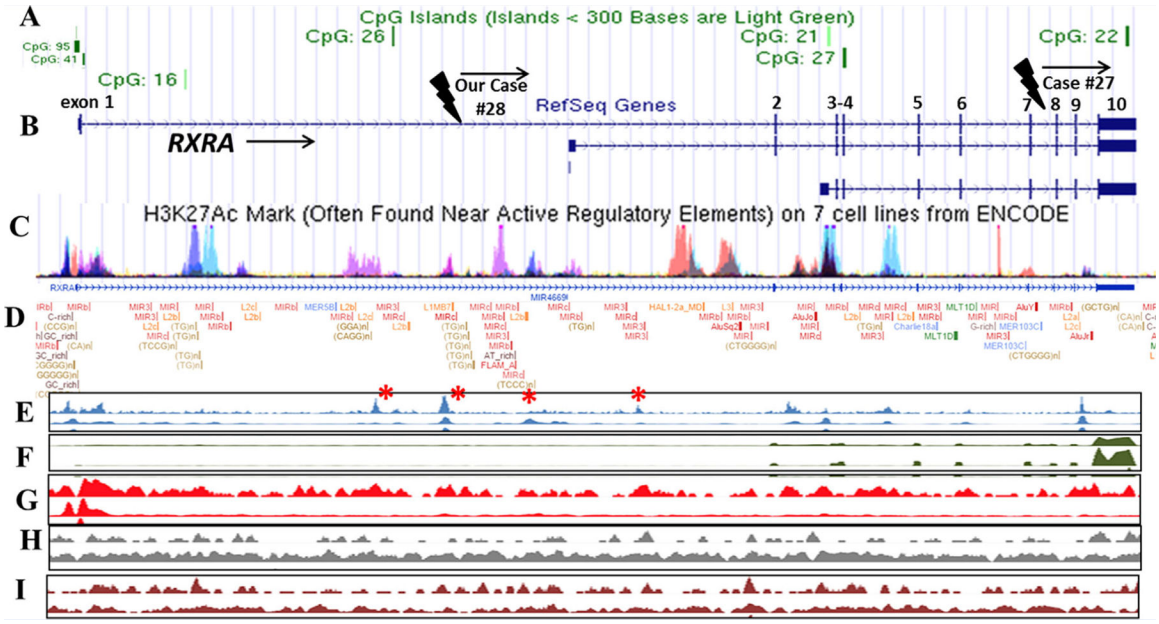

FIG. 4.

The genomics and epigenomics landscape of *RXRA* showing: (A) CpG islands (CGIs) distributed across the gene; (B) the *RXRA* locus including introns and exons 1–10 and the disruptions (lightning bolt) at introns 1 (our patient; #28) and 7 (patient #27); (C) H3K27Ac marks for predicted active regulatory regions; (D) repeat elements including SINEs (Alu, MIR) (red), LINEs (orange), DNA transposons (blue), long terminal repeats (green), simple repeats (brown). E–I: Each square compares fetal (top) and adult left ventricle (bottom): (E) different intensity peaks (asterisk) in DNase hypersensitivity sites in fetal and adult left ventricle; (F) RNAseq profile; (G) H3K4 methylation; (H) H3K9 methylation; (I) H3K27 methylation.

TABLE I.

Summary of Patients with 9q+; Duplications of *RXRA, NOTCH1* and *EHMT1*; Congenital Heart Defects (CHD) and Other Clinical Findings

	9q+ Bands	*RXRA*	*NOTCH1*	*EHMT1*	Test method	Cardiac anomalies	Failure to thrive	Joint contractures	Arachnodactyly	Hypotonia	Craniofacial dysmorphic features	Eye malformations	GI defects	Urogenital malformations	Neurocognitive deficits
#/ N						14	5	9	23	19	34	18	4	13	43
1	q11-q33	N	N	N	K										ID
2	q21.32-q31.1	N	N	N	C				+		+		+		DD, ID, SD
3	q31-q34.3	Y	Y	Y	K			+	+		+	−		−	ID
4	q31-q34.3	Y	Y	Y	K	Congenital heart disease (CHD)		+	+	+	+	+		+	DD
5	q32-q34.3	Y	Y	Y	K	Aortic arch duplication, ventricular septal defect		+	+		+			+	ID
6	q32-q34.3	Y	Y	Y	K			+	+	+	+			+	MD, DD
7	q32-q34.3	Y	Y	Y	K	Complex CHD	+		+	+					DD
8	q32-q34.3	Y	Y	Y	K	Left superior vena cava			+	+	+	+		+	DD
9	q32-q34.3	Y	Y	Y	K	Patent ductus arteriosus	+	+	+	+	+				SD, MD
10	q33.2-q34.3	Y	Y	Y	C								+	+	
11	q33.3-q34.1	N	N	N	C		+		+	+	+			+	MD, SD, mild ID
12	q33.3-q34.3	Y	Y	Y	C	Left superior vena cava, right sided aortic arch, aberrant left	+	+	+	+	+	+	+	+	DD, SD, mild ID

	9q+ Bands	*RXRA*	*NOTCH1*	*EHMT1*	Test method	Cardiac anomalies	Failure to thrive	Joint contractures	Arachnodactyly	Hypotonia	Craniofacial dysmorphic features	Eye malformations	GI defects	Urogenital malformations	Neurocognitive deficits
						subclavian artery									
13	q33.3-q34.3	Y	Y	Y	C	Ventricular septal defect			−		+	+			ID
14	q34.1-qter	Y	Y	Y	K	CHD	+			+					DD
15	q34.1-qter	Y	Y	Y	K	Ventricular septal defect			+	+	+	+		+	ID
16	q34.1-qter	Y	Y	Y	K	Atrial septal defect				+	+	+			MD, DD
17	q34.1-qter	Y	Y	Y	K	Atrial septal defect	−		+	+	+	+	+	+	MD, SD, ID
18	q34.1-qter	Y	Y	Y	C	Ebstein's anomaly	−		−	+	+	+			DD, SD, mild ID
19	q34.1-qter	Y	Y	Y	C	−	−				+	+		−	DD, mild ID
20	q34.1-qter	Y	Y	Y	K				+	+	+	+		+	MD
21	q34.1-qter	Y	Y	Y	K			+	+	+	+	+			SD, MD
22	q34.1-qter	Y	Y	Y	K			+	+		+	−			MD, SD, ID
23	q34.1-qter	Y	Y	Y	K				+	+	+	−		+	MD
24	q34.1-qter	Y	Y	Y	K				+		+	+		+	MD, SD, ID
25	q34.1-qter	Y	Y	Y	K				+		+	+			
26	q34.1-qter	Y	Y	Y	K				+	+	+	+			MD
27	q34.2-q34.3	Y	Y	Y	K			+							
28	q34.2-q34.3	Y	Y	Y	C	Tetralogy of Fallot-Absent Pulmonary Valve			+		+			+	

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	9q+ Bands	*RXRA*	*NOTCH1*	*EHMT1*	Test method	Cardiac anomalies	Failure to thrive	Joint contractures	Arachnodactyly	Hypotonia	Craniofacial dysmorphic features	Eye malformations	GI defects	Urogenital malformations	Neurocognitive deficits
29	q34.3	Y	Y	Y	K										mild ID
30	q34.3	Y	Y	Y	K						+				mild ID
31	q34.3	N	Y	Y	C		−		+	−	+	+			ID, MD, SD
32	q34.3	N	Y	Y	C		−		+	+	+	+			ID, MD
33	q34.3	N	Y	N	C	Tetralogy of Fallot					+				
34	q34.3	N	Y	N	C										ID
35	q34.3	N	N	N	C										ID
36	q34.3	N	N	N	C										seizures
37	q34.3	N	N	N	C					+	+				ID, SD
38	q34.3	N	N	N	C						+				ASD
39	q34.3	N	N	N	C										DD
40	q34.3	N	N	Y	C						+	+			DD
41	q34.3	N	N	Y	C						+				ID, seizures
42	q34.3	N	N	N	C										ASD
43	q34.3	N	N	N	C										ID
44	q34.3	N	N	Y	C										ID
45	q34.3	N	N	N	C						+				ID, SD
46	q34.3	N	N	N	C										seizures
47	q34.3	N	N	N	C						+				ID, MD, SD, ASD, seizures
48	q34.3	N	Y	Y	C				+		+	+			ID

#/N, Patient # (#; vertical, n=48); Total number of patients with congenital heart disease (CHD) and other clinical findings described above (N, horizontal).

Clinical findings: (+) present; (–) absent/normal; () not done/no data available.

Genes within 9q+ interval: *RXRA* (chr9:137,208,944–137,298,240), *NOTCH1* (chr9:139,388,896–139,440,238), *EHMT1* (chr9:140,513,444–140,730,578) (Y)—present by Chromosomal Microarray Analysis (CMA) or Karyotype (N)—not present by CMA or Karyotype.

Test Method: (C)—present by CMA, (K)—present by karyotype analysis.

Exact base pair coordinates for each CMA case are in Supplementary Table I.

Neurocognitive deficits: (ID) Intellectual disability, (DD) developmental delay, (MD) motor delay, (SD) speech delay, (ASD) autism spectrum disorder.

References for published reports and documented patients are in Supplementary Table I.