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Congenital Anomalies and Rhabdoid Tumor Associated with 22q11 Germline Deletion and Somatic Inactivation of the *SMARCB1* Tumor Suppressor

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Abstract

The most common microdeletion in humans involves the 22q11 region. Congenital anomalies associated with 22q11 loss include cardiac and facial defects. Less frequent is the co-presentation of malignant rhabdoid tumors that are highly aggressive childhood malignancies typically found in renal or extra-renal soft tissues and central nervous system. A newborn patient presented with multiple congenital anomalies consistent with 22q11 deletion syndrome including cleft lip and palate, ear tags and ventricular septal defects co-presenting with an axillary rhabdoid tumor. Comparative genomic hybridization revealed a 2.8 Mb germline deletion in the 22q11.2 region containing genes required for normal fetal development and the *SMARCB1* tumor suppressor gene. Analysis of tumor DNA revealed a somatic deletion of exon 7 in the second allele of *SMARCB1*. Expression of *SMARCB1* was absent, while tumor markers including *MYC*, *GFAP* and *CLAUDIN-6* were upregulated. The presence of tandem oriented *BCRL* modules located within interspersed low copy repeat elements throughout the 22q11 distal region may predispose this area for microdeletions through nonallelic homologous recombination.

Introduction

Genomic deletions within the 22q11.2 region are among the most common microdeletion in the human population (estimated at ~1/4000 live births) and the second most frequent deletion associated with congenital cardiac defects (Goodship et al., 1998; Scambler, 2000; Kobrynski and Sullivan, 2007). Patients with 22q11 deletions manifest malformation phenotypes similar to animal models of neural crest disruption (Scambler, 2000). The 22q11.2 locus is a hotspot for de novo microdeletion, likely due to the presence of a series of 8 low copy repeat (LCR) elements interspersed throughout a 9Mb region that may allow for nonallelic homologous recombination (Shaikh et al., 2007; Stankiewicz and Lupski, 2002). The most common of these microdeletions is a characteristic 3Mb deletion of a locus termed the “Typically Deleted Region,” or TDR (Shaikh et al., 2000). Deletion of the TDR that spans the proximal LCRs 2-4 (also referred to as LCR B-D) is associated with a broad

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spectrum of congenital anomalies ranging from cardiac defects, cleft lip and palate, thymic hypoplasia, parathyroid dysfunction, conotruncal anomalies and facial defects (Shprintzen et al., 1978), as well as psychotic features (Kook et al., 2010) and immunodeficiencies (Zemle et al., 2010). These phenotypes have been clinically referred to as DiGeorge syndrome (DGS; MIM 188400) or velo-cardio-facial syndrome (VCFS; MIM 192430). Both syndromes arise from similar deletions in 22q11, suggesting a similar etiology (Scambler et al., 1992). Deletion of the *TBX1* gene has been proposed to account for many of the phenotypic features of DGS (Lindsay et al., 2001; Merscher et al., 2001). While *TBX1* is a transcription factor that is required throughout all of development for the normal formation of the structures derived from the branchial arches (Xu et al., 2005), there are likely other genetic modifiers that contribute to the DGS phenotype (Aggarwal and Morrow, 2008). Distal deletions encompassing LCRs 4-6 (LCR D-F) have also been reported to be associated with developmental anomalies similar to DGS/VCFS (Mikhail et al., 2007), and have been referred to as 22q11.2 distal deletion syndrome (22q11.2DDS; MIM 611867) (Ben-Shachar et al., 2008; Lafay-Cousin et al., 2009). Germline deletions within this region predispose patients to a variable range of anomalies including cardiac defects and facial abnormalities (Ben-Shachar et al., 2008; Fernandez et al., 2009) that display limited phenotypic overlap with both DGS/VCFS and Goldenhar Syndrome/Oculo-Auriculo-Vertebral Spectrum (OAVS) (MIM 164210) (Derbent et al., 2003; Lafay-Cousin et al., 2009), suggesting the likelihood of many genetic modifiers of these phenotypes associated with defects in branchial arch development. 22q11 distal deletions removing genes residing between LCR6 and LCR7 (F-G) are associated with familial schwannomatosis (MIM 162091) (Hulsebos et al., 2007; Boyd et al., 2008) as well as rhabdoid predisposition syndrome (RPS; MIM 601607) (Sevenet et al., 1999; Roberts and Biegel, 2009) due to the loss of the *SMARCB1* (*IN11/SNF5*) tumor suppressor gene.

A rhabdoid tumor is a highly aggressive and metastatic form of pediatric cancer that typically arises during infancy or early childhood, with time of initial diagnosis being made between 6 months and 5 years of age (median onset of 20 months) (Roberts and Biegel, 2009). Rhabdoid tumors are typically located within the kidneys or the CNS, but less commonly found in bone marrow and bladder. In the CNS, including the brain and spinal cord, they are classified as atypical teratoid/rhabdoid tumors (AT/RTs) (Rorke et al., 1995; Rorke et al., 1996; Pfister et al., 2010); whereas, in soft tissues including the kidney, liver and spleen they are designated malignant rhabdoid tumors (MRTs). Clinical presentation of the tumor depends in large part on the location of the tumor, but often includes a palpable mass, back/neck pain, loss of appetite, nausea/vomiting, and/or hematuria (Madigan et al., 2007). At the time of diagnosis, the majority of these tumors are already metastatic, rendering most therapies ineffective with overall survival of 23% and average survival of 16 months from the time of diagnosis (Tomlinson et al., 2005). These tumors arise from biallelic inactivation of *SMARCB1* (*IN11/hSNF5*) that maps to 22q11.2 between LCRs 6-7 (F-G) and encodes a crucial subunit of the highly conserved SWI/SNF chromatin remodeling complex. The majority of rhabdoid tumors arise from somatic loss of both copies of *SMARCB1*, with 15-30% associated with germline mutation or deletion (Biegel et al., 1999; Biegel et al., 2002; Biegel, 2006). Many of the germline mutations are acquired de novo and individuals with these mutations appear to have a worse prognosis, often associated with earlier presentation, widespread and therapy-resistant metastases (Savla et al., 2000; Roberts and Biegel, 2009). There are only three published reports of four patients with congenital anomalies consistent with 22q11.2 deletion and co-presentation with aggressive rhabdoid tumors (Wieser et al., 2005; Jackson et al., 2007; Lafay-Cousin et al., 2009). In each of these reported cases, the age of rhabdoid tumor diagnosis ranged from 6 months to 5 years old. Our patient presented with phenotypic features consistent with either DGS/VCFS Syndrome or 22q11DDS with subsequent determination of a rhabdoid tumor at

birth, suggesting a possible common germline deletion mediated by chromosome features that are a hallmark of the 22q11.2 region.

Materials and Methods

Immunohistochemistry

Tumor tissues were fixed in 10% buffered formalin and embedded in paraffin. Tissue sections (5-micron) were mounted and immunohistochemistry (IHC) was performed as described (Tang et al., 2008). Citric acid buffer (10mmol/L citrate buffer, pH 6.0) or Tris-EDTA Buffer (pH 9.0) was used for antigen retrieval. Antibodies and working concentrations for IHC detection included SMARCB1/INI1 (mab clone 3E10, 1:1000; Sigma-Aldrich, St. Louis, MO), C-MYC (sc-788, 1:500; Santa Cruz Biotech, Santa Cruz, CA), p53 (sc-4246, 1:500; Santa Cruz Biotech), GFAP (AB5804, 1:500; Millipore Inc., Billerica, MA) and CLAUDIN-6/CLDN6 (01-8865, 1:66; American Research Products, Belmont, MA). Detection was performed using the LSAB + System-HRP kit (K0679, Dako Corp., Carpinteria, CA) according to manufacturer protocols followed by light hematoxylin counterstaining.

Comparative Genome Hybridization (CGH) Array

A peripheral blood sample was used to carry out genomic microarray and FISH analyses by ARUP laboratories (Salt Lake City, UT). Hybridization was performed using the Human Genome CGH Microarray 44K (hg18 assembly) from Agilent Technologies (Santa Clara, CA, USA) with a custom design previously described (Baldwin et al., 2008). The data was analyzed using DNA Analytics 4.0.76 software from Agilent Technologies with the ADM-1 algorithm. FISH analysis (probe RP11-8007) was performed to confirm to array results.

DNA Isolation and PCR Analyses

Tumor DNA was obtained from de-paraffinized 10-micron tissue sections using an Arcturus XT laser capture microdissection instrument (Arcturus Biosciences, Mountain View, CA) as described (Yadav et al., 2010). The DNA was prepared using the Arcturus PicoPure[®] DNA extraction kit (Applied Biosystems, Carlsbad, CA) following the manufacturer's instructions. Standard PCR reactions were carried out using GoTaq[®] Green polymerase (Promega, Madison, WI) and specific primers (see supplementary materials).

Results and Discussion

Clinical Presentation

Our patient was a full term male infant born at 37 weeks via cesarean section to a healthy 26 year old G4 P2 mother. The pregnancy was complicated by gestational diabetes and mild polyhydramnios. Prenatal ultrasound revealed bilateral cleft lip and palate which prompted an amniocentesis that indicated a normal male karyotype (46XY). There was no extended family history of congenital anomalies or malignancies. At birth, physical examination revealed multiple congenital anomalies including bilateral cleft lip and palate, a 2 cm well-demarcated vascular-appearing growth on his left postero-lateral shoulder, bilateral ear tags, a 1cm poorly defined violaceous nodule just medial to the left anterior axillary line and bilateral clubbed feet. An echocardiogram revealed 2 small mid/apical VSDs (Table 1). Shortly after birth, a head CT and MRI revealed cystic encephalomalacia and possible migration disorder of the left frontal lobe, but not other lesions.

At 3 weeks of age, the vascular-appearing growth on the shoulder began to ulcerate and bleed (Fig. 1A). Over the following two weeks the mass significantly increased in size and the arm became edematous. An ultrasound of the left chest revealed the presence of 2

separate lobular, septated masses—a posterior mass measuring $3.0 \times 2.5 \times 1.8$ cm and anterior mass of $2.7 \times 1.5 \times 3.1$ cm. Two subsequent abdominal ultrasounds performed 1 week apart revealed an increasing number of hypoechoic lesions in the liver, demonstrating rapid progression of the tumor to metastatic growth. At 4 weeks of age, the infant developed a pleural effusion in which analysis of the pleural effusion fluid revealed many mitotic figures consistent with a large cell epitheloid malignant neoplasm. A chest/abdomen/pelvis CT scan revealed a heterogenous, multiloculated cystic lesion with rim enhancement in the left neck, shoulder, axilla, and thoracic chest wall with extension to the superior mediastinum with multiple hypodense nodules in the liver, subcutaneous tissue and retroperitoneum. MRI of the axilla done at the same time revealed the same large left-sided mass with associated axillary and supraclavicular lymphadenopathy (Fig. 1B). Following surgical debulking of the tumor the patient developed sepsis with vancomycin-resistant *Enterococcus*, his condition worsened, and the parents made the decision to withdraw care 8 days post-operatively at 7 weeks of age. At autopsy, the infant was found to have bilateral axillary malignant rhabdoid tumors ($5.5 \times 3.5 \times 3$ cm on the right and $6 \times 4 \times 4.5$ cm on the left) with extensive invasion of the thoracic, upper abdominal and cervical organs including pericardium, diaphragm, thymus, thyroid, pleura, paraspinal region, retroperitoneum, mesentery, esophagus, liver, pancreas, and cervical/celiac/retroperitoneal lymph nodes.

Immunohistochemical and Molecular Analyses

Paraffin-embedded tumor tissue removed during surgery was cut and examined by staining with H&E to reveal the presence of cells of rhabdoid morphology (Fig. 1C), with large sections of the tumor displaying large polygonal cells with distinct borders, prominent nucleoli, eccentric basophilic nuclei, eosinophilic inclusions, and an abundant cytoplasm. There were also areas of the tumor that appeared to be highly necrotic (data not shown). As the axillary vascular growth was initially diagnosed as a possible hemangioma, we sought to verify that the tumor was an MRT by testing for the presence of several MRT-associated markers and confirmed that the tumor cells were positive for GFAP (glial fibrillary acid protein), vimentin, pan-keratin (AE1/AE3), EMA (epithelial membrane antigen), CD99 and smooth muscle actin (Fig. S1).

The overwhelming majority of malignant rhabdoid tumors result from inactivation or deletion of the *SMARCB1/INI1* gene. Therefore, we performed an immunohistochemical (IHC) analysis for SMARCB1 and found the protein to be missing in cells displaying rhabdoid morphology (Fig. 2C). Some SMARCB1 positive staining cells were observed within the tumor tissue, likely due to infiltrating non-tumor epithelial and vascular cells.

Given the unusually early presentation of the MRT at birth and the extremely aggressive nature, we next sought to determine whether the rhabdoid tumor was positive for several tumor markers, including MYC and CLAUDIN-6/CLDN6. SMARCB1/INI1, a component of SWI/SNF chromatin remodeling complexes, interacts directly with the MYC promoter to decrease its levels in normal cells (Nagl et al., 2006). Recent analyses of rhabdoid tumor gene expression profiles associated with loss of *SMARCB1* revealed that MYC transcription was indeed upregulated more than 2-fold (Gadd et al., 2010). Our MYC staining results revealed that the protein was strongly expressed in the rhabdoid morphology cells (Fig. 2E). MYC oncoproteins are a collection of transcription factors with a massive number of target genes (Lee and Dang, 2006; Keller et al., 2010) including those essential for coordinating angiogenic and anti-angiogenic factors in cancer and development. The aberrant regulation of angiogenic activity due to the large upregulation of MYC might contribute to the rapid tumor formation and metastasis of RTs (Albihn et al., 2010). MYC may also contribute to cancer formation through its role in proliferation control as repression of MYC is necessary for cells to differentiate (Eilers and Eisenman, 2008).

CLAUDIN-6 (CLDN6) is a component of tight junctions in renal podocytes where it is involved in the regulation of paracellular NaCl transport (Abuazza et al., 2006). CLDN6 displays elevated levels in early development with drastically reduced levels in adult mouse kidneys (Zhao et al., 2008). CLDN6 has been shown to be upregulated in the cell membranes of brain and spinal cord atypical teratoid/rhabdoid tumors (AT/RT) associated with loss of SMARCB1 (Birks et al., 2010). Although CLDN6 was recently found to be a marker for both rhabdoid glioblastomas (Kleinschmidt-DeMasters et al., 2010) and atypical teratoid/rhabdoid tumors (Birks et al., 2010), its explicit role in rhabdoid tumor pathology is currently unknown. We found strong but variable upregulation of CLDN6 in the membranes of the MRT cells (Fig. 2F), and to our knowledge, this is the first time that CLDN6 upregulation has been demonstrated in MRT. Rhabdoid tumor cells are similar to neural crest cells because each cell type possesses the ability to show divergent differentiation, can migrate over considerable distances, and can be present in both CNS and extra-CNS tissue (Gadd et al., 2010). RT cells may therefore arise from cells destined to become neural crest cells and thus retain the characteristics that the RT cells share with the neural crest cells. Elevated CLDN6 expression in rhabdoid cells may therefore be indicative of a developmentally primitive tumor and correlate with malignancy.

Germline deletion of *SMARCB1* followed by a somatic mutation in the second allele is often associated with early onset MRT (<1 yr), aggressive behavior, poor prognosis and is found in ~15-30% of patients with rhabdoid tumors (Roberts and Biegel, 2009). The co-presentation of congenital abnormalities consistent with 22q11 deletion syndromes (preauricular ear tags, cleft lip and palate, cardiac defects) with the MRT suggested that our patient harbored a large germline deletion of chromosome 22q in a region containing genes required for normal fetal development along with the *SMARCB1* gene. Comparative genomic hybridization (CGH) was performed using Agilent arrays (version hg18) along with FISH analyses to determine if the patient harbored a deletion in the 22q11 region (Rickert and Paulus, 2004). These data revealed a 2.8Mb germline deletion (hg18 build; base pairs 20,138,750 – 22,973,264) that resulted in partial monosomy for the 22q11.2 region (Fig. 2A). As expected, the deleted material included the region implicated in 22q11 distal deletion syndrome and the *SMARCB1* gene, as well as *BCR*, *MAPK1* and other clinically important genes that reside between the low copy repeat LCR4 and LCR7 elements. The 22q11.2 proximal region implicated in DGS/VCFS was not deleted. In order to confirm the biallelic inactivation of *SMARCB1*, we isolated genomic DNA from the rhabdoid tumor cells using laser capture microdissection (Yadav et al., 2010). PCR amplification of all nine exons (Fig. 2B) revealed that 8/9 exons appeared to be present in equal amounts; however, the amplification product for exon 7 appeared to be greatly reduced compared to control, suggesting that exon 7 was deleted in the tumor. PCR amplification using additional flanking primer sets (Fig. 2C) confirmed that the exon was completely deleted in the tumor. The extent of the deletion removing exon 7 is not known as both exons 6 and 8 appear to be intact and the distance between these exons is approximately 16.6 kilobases. Predictions of protein coding potential with exon 7 removed indicate a frameshift would follow translation of exon 6 with a subsequent in-frame termination codon, effectively truncating SMARCB1 (Fig. 2D). Since there was no SMARCB1 IHC signal detected in the rhabdoid cells, we conclude that the tumor is null for SMARCB1 protein, most likely due to nonsense mediated decay of the mRNA.

The similarity in clinical phenotype between our patient and others (Wieser et al., 2005; Jackson et al., 2007; Lafay-Cousin et al., 2009) with nearly identical deletions and/or deletion endpoints suggests that the distal 22q11 region may be a hotspot for nonallelic homologous recombination, likely due to the presence of tandem *BCRL* modules within the LCRs (Fig. 3). These LCRs can become improperly aligned fairly easily during meiotic recombination and result in the deletion of all genetic material between the two

recombination sites (Edelmann et al., 1999a; Edelmann et al., 1999b; Shaikh et al., 2001; Shaikh et al., 2007; Ben-Shachar et al., 2008). Deleting the genetic material between LCR1/A and LCR4/D has been implicated in DGS/VCFS while *SMARCB1* lies between LCR6/F and LCR7/G (Jackson et al., 2007). An examination of these published case reports reveals three interesting observations (Table 1 and Fig. 3). First, the appearance of a rhabdoid tumor at birth in our patient indicates that the somatic loss of the second *SMARCB1* allele also occurred in utero during fetal development and that the presence of a large deletion did not influence the time of tumor onset as has been suggested (Jackson et al., 2007; Lafay-Cousin et al., 2009). Second, the presence of tandem *BCRL* modules may predispose patients for specific microdeletions, such that the distal oriented *BCRL* module embedded within LCR4 may be juxtaposed with the similar module on LCR7, while the proximal oriented *BCRL* module in LCR4 may juxtapose with the modules in LCR5 and LCR6. The presence of reported deletions between LCR5 or LCR6 and a site distal to LCR7 in three reported cases suggests that a cryptic distal-oriented *BCRL* module may be present between LCR7 and LCR8. Third, the cardio-facial phenotypes of the patients with similar 22q11.2 distal deletions (LCR4/7) are likely due to the removal of genes important for normal cardiac and facial development between LCR4 and LCR6. These genes are non-redundant with those associated with DGS/VCFS proximal deletions that are phenotypically similar. This similar phenotypic presentation has led to some confusion regarding diagnoses, with resolution dependent on molecular characterization.

Summary

Patients with confirmed MRT have a grave prognosis in which survival is inversely related to age at diagnosis. Infants diagnosed at less than 6 months old have a median survival of approximately 2-4 months from the time of disease progression (Tomlinson et al., 2005). Germline deletions predispose patients for somatic mutations at any earlier age, possibly leading to more aggressive disease (Roberts and Biegel, 2009). Due to the presence of widespread metastases and the extensive invasion of neighboring vessels and organs, the ability to fully resect these tumors is greatly impaired (Madigan et al., 2007). While aggressive therapy (both surgical debulking and chemotherapy) has not been significantly effective in the treatment of rhabdoid tumors, there is some indication that early correct diagnosis may provide some opportunity to initiate therapy sooner and possibly extend survival of RT patients (Katsumi et al., 2008; Nicolaides et al., 2010). Infants diagnosed with congenital anomalies consistent with deletions in 22q11 may benefit from additional tests to determine whether the *SMARCB1* gene had been inactivated. Moreover, patients with distal 22q11.2 deletions that extend beyond the *SMARCB1* gene may benefit from continual monitoring by ultrasound or periodic head CT for signs of tumor development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Rhabdoid tumor analysis

(A) Photograph showing the vascular mass on the posterior aspect of the infant's left shoulder, shortly before excision. The mass had continued to grow in size and has some Vaseline gauze in place over the medial surface due to ulceration and bleeding. (B) T2-weighted MRI image showing a 2.7cm by 1.9cm septated subcutaneous mass in the left axilla. The associated high-signal of the surrounding tissues is consistent with edema. (C-F) Paraffin-embedded tumor sections were used for histologic examination of cellular morphology and immunohistochemical analyses. (C) Tumor section stained with hematoxylin and eosin (H&E). Note the characteristic malignant rhabdoid tumor phenotype, including the large off-center eccentric basophilic nucleus, eosinophilic cytoplasm and prominent nucleolus. (D-F) Immunohistochemical staining of rhabdoid tumor tissue sections. (D) SMARCB1/INI1. Note the absence of staining with the exception of a small population of infiltrating cells. (E) C-MYC and (F) CLDN6/CLAUDIN-6 were upregulated in the tumor cells compared to the non-rhabdoid surrounding cells.

Figure 2. Germline mutation detection and molecular analysis of the rhabdoid tumor
 (A) A peripheral blood sample was used for comparative genomic hybridization (CGH) using the hg18 build on a U-array Cyto6000 platform. A ~2.8Mb deletion resulted in partial monosomy (-1) in the chromosome 22q11.2 region, indicated by the green dots. A magnified view of the region shown on the right indicates loss of genetic material between the low copy repeat regions 4 and 7 (blue *BCR* and *SMARCB1/hSNF5*). (B) PCR analysis of the tumor sample. Chromosomal DNA was isolated following laser capture microdissection of rhabdoid tumor cells and used for PCR analysis. Each of the nine exons of *SMARCB1* was individually amplified using both control and tumor cell DNA as templates. The *GAPDH* gene was used as a control for DNA concentration. Note the strongly reduced PCR product for exon 7. (C) PCR amplification of an extended region around exon 7 was used to verify that the entire exon was deleted in the tumor. The regions indicated by the grey arrows were used for amplification detection. (D) Schematic view of the deletion product that results in a frameshift and premature termination following exon 6. The SMARCB1 protein is shown (top), including the imperfect repeat regions (R1 and R2), as well as the C-terminal coiled-coil (CC). The deletion of exon 7 (lower diagram) results in the complete removal of residues within the R2 and CC regions are required for heterotypic protein interactions and assembly of SMARCB1 into SWI/SNF complexes.

Figure 3. Germline deletions in 22q11.2 predispose to a variety of developmental defects and cancer

A schematic of the chromosome 22q11.2 region reveals the presence of a ~9Mb segment implicated in several congenital birth defect and cancer predisposition syndromes. Interspersed within this region are 8 copies of low copy repeat sequences (LCR, blue ovals) that contain BCRL modules (red arrowheads, direction is related to orientation) that are likely the sites of non-allelic homologous recombination (NAHR). A potential undiscovered BCRL module may reside distal to LCR7 (gold arrow). Recombination between these BCRL modules may result in deletions of intervening chromosomal DNA. Frequent deletion of the region between LCR2 and LCR4 is associated with DGS/VCFS syndrome; while deletion of DNA between LCR4 and LCR6 is associated with 22q distal deletion syndrome. Clinical features of patients with deletions of either region are generally overlapping with several distinguishing features. Select genes located within the deletion syndrome regions are indicated. Molecularly defined germline deletion data obtained from several patients with 22q11 distal deletion syndrome reported in this study or others are indicated. Similar deletions from more than one patient in a given report are indicated by parentheses. Facial defects are frequently linked to loss of genes within the LCR4/6 region, while malignant rhabdoid tumors/rhabdoid predisposition syndrome (MRT/RPS) is associated with loss of genetic material between LCR6/7 (shaded boxes).

Table 1
Clinical and molecular features of patients diagnosed with co-presenting MRT and/or 22q11.2 distal deletion syndrome

Clinical feature	Patient 1 ^a	Patient 2 ^b	Patient 3 ^c	Patient 4 ^d	Patient 5 ^d	Patient 6 ^d	22q11 distal ^e
Deletion (LCR)	2.8Mb (4/7)	2.8Mb (4/7)	2.8Mb (4/7)	2.8Mb (4/7)	2.8Mb (4/7)	1.5Mb (5/7+)	1.4-2.1Mb (4/6)
Malignant rhabdoid tumor, AT/RT	+	+	+	+	+	+	0/6
Location of RT	axilla/MRT	MRT	AT/RT	MRT	AT/RT	axilla/MRT	NA
Age at RT diagnosis	birth	6mos	1yr	5yr	1yr	5weeks	NA
Fetal bilateral cleft lip and/or palate	+	-	-	+	+	NR	2/6
Ear tags	+	+	+	-	-	NR	NR
Simple/folded ears	-	+	+	+	-	NR	NR
Skeletal defects (clubbed feet, spine deformity)	+	-	NR	NR	NR	NR	3/6
Cardiac defects (ASD, VSD)	+	+	NR	+	+	NR	2/6
Mongolian spots	+	-	NR	NR	+	NR	NR
Upslanting palpebral fissures	-	+	+	+	+	NR	NR
Smooth philtrum	-	+	+	NR	NR	NR	6/6
Developmental delay	NA	+	+	+	NR	NA	4/6
Facial dysmorphia/asymmetry	-	+	+	+	+	NR	NR
Downturned mouth	-	+	+	-	+	NR	NR
Gastroesophageal reflux	NR	NR	+	+	+	NR	NR
Hearing loss	NR	+	NR	+	NR	NR	NR

^aThis study;

^bWieser et al. (2005);

^cLafay-Cousin et al. (2009);

^dJackson et al. (2007);

^eBen-Shachar et al. (2008).

NA, not applicable; NR, not reported; MRT, malignant rhabdoid tumor; AT/RT, atypical teratoid/rhabdoid tumor; LCR, low copy repeat