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Constitutional 560.49 kb chromosome 2p24.3 duplication including the *MYCN* gene identified by SNP chromosome microarray analysis in a child with multiple congenital anomalies and bilateral Wilms tumor

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ABSTRACT

Fewer than 100 patients with partial chromosome 2p trisomy have been reported. Clinical features are variable and depend on the size of the duplicated segment, but generally include psychomotor delay, facial anomalies, congenital heart defect, and other abnormalities. We report a 560.49 kb duplication of chromosome 2p in a 13 month-old male with hydrocephaly, ventricular septal defect, partial agenesis of the corpus callosum, and bilateral Wilms tumor. After discovery of bilateral renal masses at four months of age, the child underwent neoadjuvant chemotherapy followed by right radical nephrectomy that revealed triphasic Wilms' tumor. A needle core biopsy on one of two lesions on the left kidney also revealed Wilms tumor. A partial left nephrectomy revealed focally positive margins that necessitated left flank radiotherapy. The tumor karyotype was 46,XY,t(7;8)(q36;p11)[8]/46,XY [12] while his constitutional karyotype was 46,XY, suggesting that the t(7;8)(q36;p11) was associated with the malignancy. Single nucleotide polymorphism (SNP) chromosome microarray analysis of peripheral blood identified a maternally-inherited 560.49 kb chromosome 2p24.3 duplication that involved four OMIM genes: *NBAS, DDX1, MYCNOS*, and *MYCN*. SNP array analysis of the tumor revealed the same 2p24.3 duplication. At present, the now 5-year-old boy continues to do well without clinical or radiographic evidence of recurrent disease. This case is instructive because the child's health insurer initially denied authorization for chromosome microarray analysis (CMA), and it took more than one year before such authorization was finally granted. That initial decision to deny coverage could have had untoward health implications for this child, as the identification of constitutional *MYCN* duplication necessitated surveillance imaging for a number of pediatric malignancies associated with *MYCN* overexpression/dysregulation.

1. Introduction

Wilms tumor, or nephroblastoma, is one of the most common pediatric malignancies and is the most common pediatric renal tumor, with an incidence of 1:10,000 live births. Mutations in a number of genes are relatively common in these tumors, including *WT1, CTNNB1, AMER1, TP53, IGF2/H19,* and *MYCN* (Williams et al., 2010; Williams et al., 2015). Specifically, the *MYCN* gene, which is located on chromosome 2p24.3, is a member of the *MYC* family of proto-oncogenes. It functions as a transcription factor, regulates expansive genomic domains, and maintains a widespread genomic structure. As such, it has been shown to play a pivotal role in cell growth and proliferation, in tumorigenesis, and in stem cell biology. (Dang, 2012).

Given the functions of the genes that *MYCN* both directly and indirectly targets, it is not surprising that dysregulation of *MYCN* would be identified in a variety of malignancies. *MYCN* overexpression/dysregulation has been documented in up to 70% of human cancers. Such dysregulation can be an early event in tumorigenesis, but it has also been shown to be involved in tumor maintenance, in establishment of a metastatic phenotype, and to

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cause genomic instability (Dang, 2012).

Somatic amplification of the *MYCN* gene has been documented in a variety of pediatric tumors including neuroblastoma (Brodeur et al., 1984; Seeger et al., 1985), medulloblastoma, (Aldosari et al., 2002), rhabdomyosarcoma (Williamson et al., 2005), and Wilms tumor (Schaub et al., 2007; Williams et al., 2010, 2011; Fievet et al, 2013). Given that the *MYCN* gene is located on chromosome 2p24.3, any constitutional cytogenetic abnormality in this band that results in gain or amplification of *MYCN* would theoretically dysregulate the gene, resulting in an increased susceptibility to malignancies.

In this study, we report a 560.49 kb duplication of chromosome 2p24.3 in a 13 month-old male with hydrocephaly, ventricular septal defect, partial agenesis of the corpus callosum, and bilateral Wilms tumor. The abnormality involved four OMIM genes: *NBAS, DDX1, MYCNOS*, and *MYCN*, and appears to have been inherited from the child's phenotypically normal mother suggesting incomplete penetrance of the mutation. We also describe our experience with the child's health insurer regarding their initial denial of chromosome microarray analysis (CMA) testing, which could have had untoward health implications for this child.

2. Clinical report

The proband is a three-year-old male born to non-consanguineous parents. The pregnancy history was uncomplicated. At two months of age, he was diagnosed with congenital hydrocephalus and a cerebral spinal fluid leak necessitating placement of a left frontal ventriculoperitoneal (VP) shunt. A CT scan performed after VP shunt placement revealed partial agenesis of the corpus callosum. An echocardiogram revealed a patent foramen ovale and a small apical muscular ventricular septal defect. Vision and hearing tests were normal at this time, as was a developmental assessment.

The child presented at the age of four months with bilateral renal masses identified by abdominal CT scan, and thought to represent Wilms tumor. He underwent neoadjuvant chemotherapy with vincristine, actinomycin D, and doxorubicin resulting in interval response in the right kidney. Subsequent right radical nephrectomy and lymphadenectomy of perihilar and periaortic lymph nodes revealed triphasic Wilms tumor with favorable histology and skeletal muscle differentiation (Fig. 1). The surgical margins were negative.

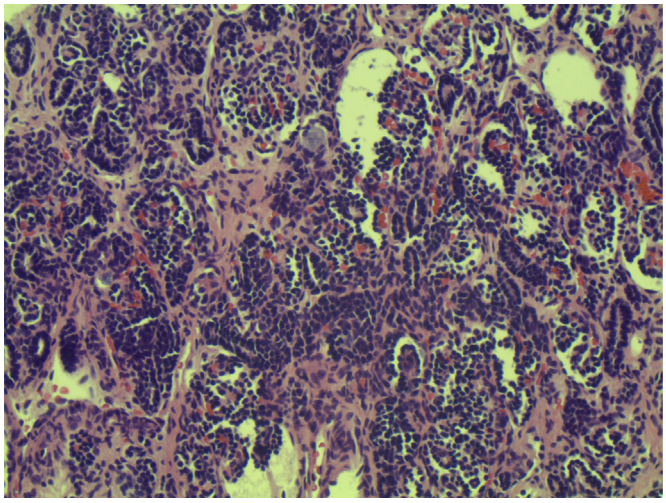

Fig. 1. Right nephrectomy biopsy reveals classic triphasic histology of Wilms tumor with blastema, tubules, and primitive glomerular structures (hematoxylin and eosin stain, original magnification x 200).

Conventional cytogenetic analysis of the tumor from the right kidney revealed a 46,XY,t(7; 8) (q36; p11)[8]/46,XY [12] karyotype (Fig. 2).

A needle core biopsy on one of two lesions on the left kidney revealed Wilms tumor. A left-sided nephron sparing partial nephrectomy was performed after additional chemotherapy failed to elicit a complete response. Wilms tumor with favorable histology and focally positive margins was confirmed in this specimen. Because of the margin status, left flank radiotherapy (total dose of 1080 cGy with 180 cGy per fraction for six fractions) was initiated. Following completion of treatment, MRI and CT scans revealed no evidence of residual disease nor metastatic malignancy.

The child underwent initial genetic evaluation as an inpatient at age 4 months, at which time microarray analysis was recommended but not done. The child returned for genetic follow-up at age 11 months. The examination revealed borderline relative, but not absolute, macrocephaly (Fig. 3). The child's phenotype was not suggestive of any Wilms' tumor-associated genetic syndromes such as, for example, Beckwith-Wiedemann syndrome.

Recommendations for further evaluation included conventional chromosome analysis and CMA, with *WT1* analysis proposed as a second-tier investigation. The constitutional karyotype proved to be 46,XY, suggesting that the t(7;8)(q36;p11) was associated with the malignancy. *WT1* gene analysis yielded normal results. In accordance with requirements of the child's health insurer, a request for prior authorization of CMA was submitted; however, three days later the request was denied, claiming there was "insufficient evidence in the peer-reviewed, published literature to demonstrate the clinical utility of the use of CMA" as of 2012. Eight months after the initial denial of prior authorization for CMA, and after numerous electronic communications and telephone conversations with the health insurer's medical director, an independent review did agree that CMA was of demonstrated clinical utility, and the insurer agreed to alter their coverage policy regarding CMA. Practically, insurer policies caused a delay of thirteen months in the diagnostic workup of our proband.

Chromosome single nucleotide polymorphism (SNP) array analysis was performed on peripheral blood utilizing the CytoScan HD array (Affymetrix, Santa Clara, CA) and identified a 560.49 kb chromosome 2p24.3 duplication copy number variant (CNV) that involved four OMIM genes: *NBAS, DDX1, MYCNOS*, and *MYCN* (Fig. 4). SNP array analysis of the child's mother revealed the same 560 kb duplication of chromosome 2p24.3 involving gain of the same four genes. SNP array analysis of paraffin-embedded tissue from the right nephrectomy specimen revealed the same 2p24.3 duplication. Paraffin FISH analysis of interphase cells from the same biopsy using an *N-MYC* probe (Abbott Molecular, Inc., Downers Grove, IL) confirmed the SNP array impression of a duplication of the *MYCN* gene on chromosome 2p24 (Fig. 5).

The child remains disease free, with the most recent abdominal sonogram, abdomen and pelvis MRI, and chest CT all revealing no evidence of recurrent disease 4.5 years off therapy. He continues to undergo surveillance for other *MYCN*-associated malignancies. Available medical records indicated that the child has been recently diagnosed with hearing loss in his right ear. At his most recent evaluation, his mother reported that he continues to have difficulties with fine motor activities such as writing and cutting, and that he struggles with focus. While his preschool teacher reports that he is improving, he does appear to be somewhat developmentally delayed. His gross motor skills are appropriate for age.

3. Discussion

The child in this study presented with bilateral Wilms tumor with a 560.49 kb chromosome 2p24.3 duplication identified by SNP