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CASE REPORT

Hepatic Malignancy in an Infant with Wolf–Hirschhorn Syndrome

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ABSTRACT

Introduction: Wolf–Hirschhorn syndrome (WHS) is a contiguous gene syndrome involving deletions of the chromosome 4p16 region associated with growth failure, characteristic craniofacial abnormalities, cardiac defects, and seizures. *Case Report*: This report describes a six-month-old girl with WHS with growth failure and typical craniofacial features who died of complex congenital heart disease. Genetic studies revealed a 9.8 Mb chromosome 4p-terminal deletion. At autopsy, the liver was grossly unremarkable. Routine sampling and histologic examination revealed two hepatocellular nodular lesions with expanded cell plates and mild cytologic atypia. Immunohistochemical staining revealed these nodules were positive for glutamine synthetase and glypican 3, with increased Ki-67 signaling and diffuse CD34 expression in sinusoidal endothelium. These findings are consistent with hepatoblastoma or hepatocellular carcinoma. *Conclusions*: A possible association between WHS and hepatic malignancy may be an important consideration in the care and management of WHS patients.

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Liver; malignancy; neonate; Wolf–Hirschhorn; contiguous gene syndrome

Introduction

Wolf–Hirschhorn syndrome (WHS) is a contiguous gene syndrome involving deletions of the chromosome 4p16 region [1, 2]. Features of affected patients include growth failure, characteristic craniofacial abnormalities, cardiac defects, and seizures. This report describes a patient with WHS who died of cardiac disease. At autopsy, two atypical hepatocellular nodules with findings consistent with hepatoblastoma or hepatocellular carcinoma were detected. Two cases of hepatic adenomas have been reported in patients with WHS [3]; the nodules seen in this case, however, were more consistent with a diagnosis of hepatocellular malignancy.

Case report

A 1245-g infant was born at 33 weeks gestation to a 32-year-old mother after a pregnancy complicated by prenatal diagnoses of intrauterine growth restriction and complex congenital heart disease, including tricuspid atresia and hypoplastic right ventricle. Maternal serologies,

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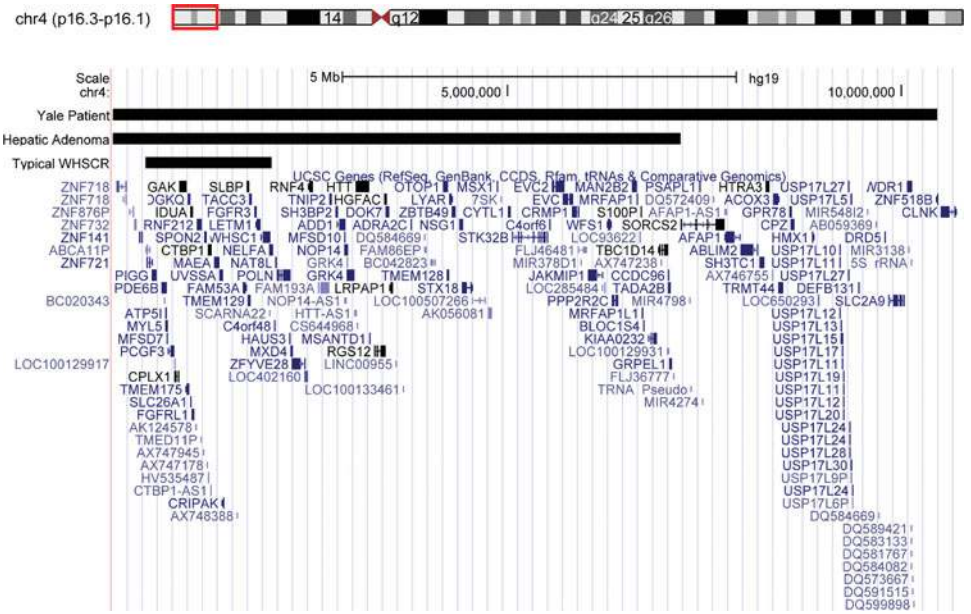

Figure 1. Chromosome 4p region. An integrated genome browser of the 4p region is shown. Top: The red box in the upper panel represents the region typically deleted in patients with WHS. Bottom: Genes in the region of the deletion are shown in conjunction with a bar denoting the extent of the deletions in the patient with WHS reported here, the patient with WHS and a hepatic adenoma previously reported [3], and a typical patient with WHS.

including HIV and hepatitis B titers, were negative. Amniotic fluid karyotype analysis revealed deletion of the short arm of chromosome 4, 46, XX, del(4)(pter-p16.1), in the WHS critical region. Whole genome SNP microarray analysis (Reveal, Integrated Genetics, Affymetrix Cytoscan HD) using amniotic fluid-derived fetal DNA identified a 9.8 Mb terminal deletion of the short arm of chromosome 4, 4p16.3p16.1 (chr4:68, 345-9, 824, 749, CRCh37/hg19 assembly; Fig. 1). This deletion included the WHS-associated genes: *WHSC1*, *WHSC2*, and *LETM1*. The most distal Online Mendelian Inheritance in Man (OMIM) gene deleted was *DRD5*.

At birth, weight, length, and head circumference were less than fifth percentile for gestational age. Craniofacial features were remarkable for a large anterior fontanelle, high forehead, hypertelorism, upward arching eyebrows, downward slanting palpebral fissures, broad nasal bridge, low set ears with simplified folding of the auricles, and micrognathia. The palate was intact. Ophthalmologic examination did not reveal any findings of WHS. Head ultrasound demonstrated slight asymmetry in ventricular size but was otherwise normal. Cardiac ultrasound demonstrated tricuspid and pulmonary valve atresia with right ventricular hypoplasia, absence of pulmonary trunk, atrial septal defect, and bicuspid aortic valve. Prostaglandins were administered to maintain pulmonary blood flow.

The patient's course was complicated by chronic pulmonary edema requiring ventilator support, ventilator-associated pneumonia, and chronic lung disease. She also had intermittent cardiac arrhythmias, early transient indirect hyperbilirubinemia, and transient hyperparathyroidism with hypercalcemia. Since pulmonary blood flow was ductal-dependent, ductal stents were placed during the third month of life and prostaglandin infusion discontinued. An abdominal ultrasound at four months of age, obtained as routine follow-up of early neonatal jaundice, was unremarkable. As she approached six months of age, the patient developed an inter-current febrile illness. Acute cardiac arrest occurred and the patient expired.

At autopsy, the patient was noted to be small for age with both weight and length less than the fifth percentile. Craniofacial features were as described above. Complex congenital heart disease included tricuspid and right ventricular atresia, absent pulmonary trunk, and stenosis of pulmonary veins with near complete occlusion of left pulmonary vein. There was left ventricular hypertrophy, a markedly dilated ascending aorta with a stent present in the proximal aorta, a ductus arteriosus with stent, and a fenestrated foramen ovale bulging into the right atrium. Bilateral pulmonary congestion was present. Other findings included Meckel's diverticulum and multiple ovarian follicular cysts bilaterally.

The liver weighed 172 g (expected weight 170–260 g) and was grossly unremarkable; routine gross photographs were taken at the time of autopsy. Routine sampling and histologic examination revealed two small (~1 mm), ill-defined, hepatocellular nodular lesions (Fig. 2A, E, F, and H). These nodular lesions were non-encapsulated and had expanded cell plates up to five cells thick, confirmed by reticulin staining (Fig. 2G). The cells were mildly pleomorphic and had well-defined cell borders; the nuclei were round to oval with coarse chromatin, and the cytoplasm was eosinophilic and pale. No definite mitoses were identified in the lesions. The background liver parenchyma was largely unremarkable with only mild sinusoidal dilation; trichrome staining showed no fibrosis or disruption of lobular architecture. Immunohistochemical staining revealed the nodular lesions were positive for glutamine synthetase (Fig. 2B) and glypican 3 (Fig. 2C). The signal from Ki-67 staining was estimated at 5–10% (Fig. 2D), compared to <1% in the surrounding normal liver. There was also diffuse CD34 expression in the sinusoidal endothelium. HepPar1 staining was positive in the nodule as well as in the background liver parenchyma. Beta-catenin and alpha fetoprotein (AFP) immunostains were negative. Subsequent re-examination and histologic examination of the remaining liver in its entirety revealed no additional lesions. Taken together, the histologic appearance and immunohistochemical findings are consistent with hepatoblastoma or hepatocellular carcinoma (HCC).

Discussion

The liver is the third most common site for malignant, intra-abdominal tumors in children, with adrenal neuroblastoma and Wilms tumor being the most common. Of malignant hepatic tumors, hepatoblastoma and HCC are the first and the second most common, respectively [4]. HCCs are more common in regions with endemic hepatitis B virus [5, 6].

Hepatic tumors in the neonate are rare [7], with benign tumors, infantile hemangioma, and mesenchymal hamartoma, predominating. The most common malignant tumor in neonates, similar to infants and older children, is hepatoblastoma. HCC is extremely rare in the neonatal period and throughout the first year of life. HCC is primarily described in infants with metabolic disorders, such as tyrosinemia, associated with liver disease that has progressed to cirrhosis [8]. A second group of HCCs found in pediatric patients occurs outside the setting of chronic liver disease. These tumors share many features with hepatoblastoma, including patterns of immunohistochemical staining [9].

The main differential diagnoses for nodular lesions in this case include hepatic adenomas, hepatoblastoma, and well-differentiated HCC. These entities may be histologically ill defined and unencapsulated. Mild cellular atypia may be seen in hepatic adenomas. In hepatoblastoma and HCC, the degree of cellular atypia is variable and cell plate expansion may be present. The immunohistochemical profile of the nodules corresponds with both hepatoblastoma and HCC, with positivity for glutamine synthase, glypican 3, and CD34, and negativity for beta-catenin [9].

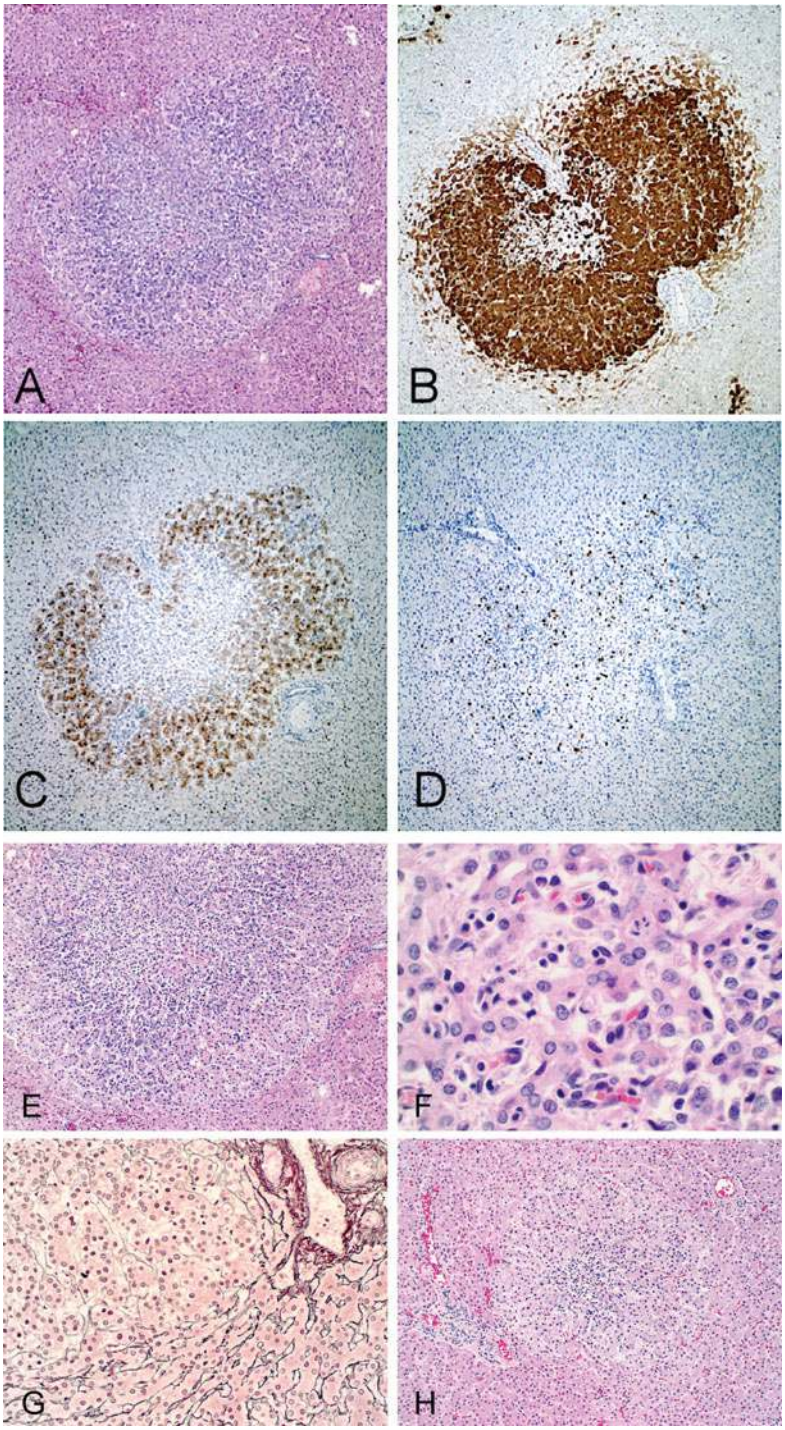

Figure 2. Hepatocellular lesions. Top Panel. (A) A region of cellular proliferation in the liver with expanded cell plates and mild cytologic atypia (H & E, 100×). (B) Immunohistochemical staining with glutamine synthetase (100×). (C) Immunohistochemical staining with glypican 3 (100×). (D) Immunohistochemical staining with Ki-67 (100×). Bottom Panel. (E) Another view of cellular proliferation in the liver with expanded cell plates and mild cytologic atypia (H & E, 100×). (F) Higher power image of the first nodule from the region shown in (E) demonstrating mild cellular atypia (H & E, 400×). (G) Expanded cell plates in the lesion are shown (reticulin staining, 200×). (H) A region of cellular proliferation in the liver with expanded cell plates and mild cytologic atypia in the second lesion is shown (H & E, 100×).

Wolf–Hirschhorn syndrome is a contiguous gene syndrome involving deletions of the chromosome 4p16 region. It is a rare disorder with an estimated frequency of 1 in 50,000–100,000 live births. The core WHS phenotype includes pre- and postnatal growth delay, typical craniofacial features, neurocognitive impairment, and seizures. Craniofacial features, termed as "Greek warrior helmet" include microcephaly, high forehead, prominent glabella, high-arched eyebrows, hypertelorism, protruding eyes, low-set malformed ears, broad or beaked nose, short philtrum, downturned corners of the mouth, and micrognathia [1]. Other findings may include congenital heart disease, ocular colobomas, deafness, cleft lip or palate, renal abnormalities, and antibody deficiencies.

Over half of WHS patients have a deletion of 4p16.3 without other cytogenetic abnormalities, with most occurring *de novo*. The remaining WHS patients have complex cytogenetic abnormalities such as ring 4 chromosome, 4p-mosaicism, or a derivative chromosome 4 resulting from an unbalanced translocation [10, 11]. Phenotype has been loosely correlated with genotype, with disease severity correlated with deletion size and location, i.e., haploinsufficiency of contiguous genes in the 0.4–1.9 Mb terminal 4p16.3 region [11]. Some important genes in this region include *WHSC1* (Nuclear receptor SET Domain containing protein 2 [*NSD2*]), *WHSC2 (NELF-A), LETM1, SLBP, CTBP1, CPLX1, PIGG,* and *FGFRL1*. Our patient had an extended terminal deletion of the 4p region 9.8 Mb in length that included all of these as well as many other genes (Fig. 1).

Some genes deleted in WHS have been implicated in various cancers [12–14]. *WHSC1* is a SET domain-containing protein that participates in chromatin-mediated transcription and cell-cycle progression, and has been implicated in several cancers. A common translocation in multiple myeloma, t(4;14), leads to the simultaneous dysregulated expression of two potential oncogenes, *WHSC1* from chromosome 4p and *FGFR3*, i.e., fibroblast growth factor receptor 3, from chromosome 14. *WHSC1*-encoded *NSD2*, which catalyzes the methylation of histone H3 lysine 36, is overexpressed in multiple myeloma cells. *WHSC2* is a helix-loop-helix domain-containing protein that may function as a transcription repressor or regulator of cell cycle and DNA replication. High-level expression of leucine zipper/EF hand-containing transmembrane-1 (LETM1), a mitochondrial inner membrane protein, has been correlated with multiple human malignancies, including lung and breast cancers, suggesting roles in carcinogenesis and tumor progression.

Allelic deletions with loss of heterozygosity in the 4p16.3 region are often found in breast, colorectal, and bladder cancers. Surprisingly, malignancies have been rarely reported in WHS patients. Two WHS patients developed myelodysplastic syndrome in childhood. One progressed to refractory cytopenia with excess blasts, while the other progressed to acute lymphocytic leukemia [15]. An 11-year-old WHS patient developed a benign inflammatory myofibroblastic tumor of the bladder [16].

Two WHS patients with hepatic adenomas have been described [3]. Information regarding the extent of 14p deletion is available in one of these patients, who, similar to our patient, had an extended deletion (Fig. 1). Since WHS has not otherwise been associated with liver pathology, we identified the genes outside the typical WHS deletion that encompassed the region deleted in both our patient and the reported patient with liver adenoma. We examined the expression of these genes using expression profiles obtained by RNA-seq analysis of mRNA isolated from HepG2 cells, a cell line of hepatic cell origin (UCSC Genome Database). We found a number of genes deleted in both patients that are expressed in hepatic cells (Table 1). These genes encode proteins with a wide variety of cellular functions.

Hepatic lesions with malignant features associated with WHS have not been previously reported. While this WHS patient's liver lesions were discovered incidentally and were not a

Table 1. 4p region genes with hepatic expression deleted in two patients with WHS and liver tumors.

Gene	Gene description	FPKM*
MRFAP1	Morf4 family-associated protein 1	156.3
S100P	S100 calcium binding protein P	152.2
LRPAP1	Low-density lipoprotein receptor-related protein	74.4
HGFAC	HGF activator preproprotein	39.2
MRFAP1L1	Morf4 family-associated protein 1-like 1	38.3
GRPEL1	GrpE-like 1, mitochondrial	32.8
ADD1	Adducin 1 (alpha) isoform a	30.2
ADRA2C	Alpha-2C-adrenergic receptor	26.9
TBC1D14	TBC1 domain family, member 14 isoform a	23.9
WFS1	Wolframin	21.5
MFSD10	Major facilitator superfamily domain containing	18.8
NOP14	Probable nucleolar complex protein 14	17.5
LYAR	Ly1 antibody reactive homolog	15.5
RNF4	Ring finger protein 4	14
MXD4	MAD4	12.2
TNIP2	A20-binding inhibitor of NF-kappaB activation 2	11.3
MSX1	MSH homeobox 1	10.7
HTT	Huntingtin	10.5
TMEM128	Transmembrane protein 128	10.3
BLOC1S4	Biogenesis of lysosomal organelles complex-1, subunit 4	9.5
RGS12	Regulator of G-protein signaling 12 isoform 1	9.1
AC093323.1		7.9
C4orf8		7.4
STX18	Syntaxin 18	6.9
KIAA0232	Hypothetical protein LOC9778	5.8
MAN2B2	Mannosidase, alpha, class 2B member 2	4.9
AC097382.4		4.8
SH3BP2	SH3-domain binding protein 2	4.7
SORCS2	VPS10 domain receptor protein SORCS 2	3.7
HAUS3	Hypothetical protein LOC79441	3.5
EVC	Ellis van Creveld syndrome protein	2.2
PPP2R2C	Gamma isoform of regulatory subunit B55 protein	1.5
CRMP1	Collapsin response mediator protein 1 isoform 1	1.4
ZBTB49	Zinc finger and BTB domain containing 49	1.3
GRK4	G protein-coupled receptor kinase 4 isoform	1.2

*FPKM: mRNA expression determined from RNA-seq of HepG2 cell RNA, expressed as fragments per kilobase of exon per million fragments mapped.

factor in her demise, a possible association between WHS and liver tumors is an important consideration in the care and management of WHS patients, particularly those with large deletions.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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