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Clinical Features of Three Girls With Mosaic Genome-Wide Paternal Uniparental Isodisomy

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Abstract

Here we describe three subjects with mosaic genome-wide paternal uniparental isodisomy (GWpUPD) each of whom presented initially with overgrowth, hemihyperplasia (HH), and hyperinsulinism (HI). Due to the severity of findings and the presence of additional features, SNP array testing was performed, which demonstrated mosaic GWpUPD. Comparing these individuals

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to 10 other live-born subjects reported in the literature, the predominant phenotype is that of pUPD11 and notable for a very high incidence of tumor development. Our subjects developed non-metastatic tumors of the adrenal gland, kidney, and/or liver. All three subjects had pancreatic hyperplasia resulting in HI. Notably, our subjects to date display minimal features of other diseases associated with paternal UPD loci. Both children who survived the neonatal period have displayed near-normal cognitive development, likely due to a favorable tissue distribution of the mosaicism. To understand the range of UPD mosaicism levels, we studied multiple tissues using SNP array analysis and detected levels of 5–95%, roughly correlating with the extent of tissue involvement. Given the rapidity of tumor growth and the difficulty distinguishing malignant and benign tumors in these GWpUPD subjects, we have utilized increased frequency of ultrasound (US) and alpha-fetoprotein (AFP) screening in the first years of life. Because of a later age of onset of additional tumors, continued tumor surveillance into adolescence may need to be considered in these rare patients.

Keywords

Beckwith–Wiedemann; uniparental disomy; isodisomy; mosaicism; methylation; hyperinsulinism

INTRODUCTION

Genome-wide paternal uniparental isodisomy (GWpUPD) is typically characterized by a mosaic blend of paternal uniparental and biparental cell lineages. Uniparental isodisomy can result in human disease as a result of loss of one parent's allele(s) in an imprinted region or unmasking recessive disease-causing mutations. The clinical consequences depend on the mosaic percentages and the location of the uniparental lineage.

Five paternally imprinted regions have currently been associated with human disease. Paternal uniparental isodisomy for chromosome 11p15 (pUPD11) presents as a spectrum of phenotypes ranging from isolated hemihyperplasia (IH; OMIM 235000) to Beckwith–Wiedemann syndrome (BWS; OMIM 130650), characterized by macroglossia, hemihyperplasia (HH), hypoglycemia, and increased development of embryonal tumors, such as hepatoblastoma and Wilms tumor. Paternal UPD6 (OMIM 601410) manifests as transient neonatal diabetes. Paternal UPD14 (OMIM 608149) presents with facial dysmorphism and skeletal findings including bell-shaped thorax and “coat-hanger” ribs [Sutton and Shaffer, 2000]. Paternal UPD15, Angelman syndrome (OMIM 105830) is characterized by ataxic movements, developmental delay, intellectual disability, and epilepsy [Mabb et al., 2011]. Paternal UPD20 causes pseudohypoparathyroidism (PHP; OMIM 603233) characterized by end-organ resistance to parathyroid hormone (PTH). While no cases of live-born complete UPD have been reported, which suggests incompatibility with life, surprisingly several rare cases of mosaic genome-wide paternal uniparental isodisomy (GWpUPD) have been noted.

Here we describe three individuals who presented with severe features of pUPD11 for whom work-up demonstrated mosaic genome-wide paternal uniparental isodisomy. All presented with prematurity, hyperinsulinism (HI), and hemihyperplasia. In addition, several isolated

unique features included premature thelarche and conjugated hyperbilirubinemia. Multiple tissues from each of these subjects, including tumors from each, were evaluated to determine the levels of GWpUPD mosaicism. These individuals assist in clarifying the phenotypic spectrum of GWpUPD and provide insight into improved medical management of these complex patients.

MATERIALS AND METHODS

Clinical Presentation

Subjects were enrolled into an IRB approved protocol with informed consent at The Children's Hospital of Philadelphia (CHOP) and were evaluated by multiple specialists (genetics, endocrinology, oncology, and gastroenterology). Clinical testing included methylation-sensitive multiplex ligation-dependent probe amplification (MS-MLPA) or quantitative methylation sensitive PCR (MS-PCR) in addition to other laboratory and imaging studies.

Arrays

DNA was purified from peripheral blood, fibroblasts cultured from skin, and tumor tissue. Array analysis was performed using Illumina Quad610 BeadChip SNP arrays as previously described [Conlin et al., 2010]. The subset of probes used for analysis included all intensity-only probes on the Y chromosome and in the pseudoautosomal (XY) region, but excluded these probes elsewhere in the genome, for a total of 594,906 probes. The B-allele frequencies for each sample were examined for imbalance of A and B alleles as indicators of mosaicism. The percent of cells with homozygosity was calculated as previously described [Conlin et al., 2010].

RESULTS

Subject 1 was born via spontaneous vaginal delivery to a 29-year-old G1P0-1 mother and a 39-year-old father. The pregnancy was naturally conceived and there was no known consanguinity. Placental cysts were noted on an early prenatal ultrasound and due to concern for a molar pregnancy, chorionic villi sampling was performed which demonstrated a 46,XX karyotype. Prenatal ultra-sonography at 20 weeks showed hepatomegaly and a two-vessel cord. Ultrasonography at 30 weeks indicated dilated umbilical vessels and possible fetal distress. At 31 $\frac{5}{7}$ weeks gestational age, her weight was at the 85th centile, length at the 40th centile, and head circumference at the 20th centile. Placental pathology demonstrated mesenchymal dysplasia. Following a period of persistent hypoglycemia to 40 mg/dl, she was diagnosed with hyperinsulinism (HI).

Clinical exam revealed her right cheek to be fuller than the left. She also demonstrated telecanthus and slightly cupped ears without ear creases or tags. She had mildly downturned corners of her mouth, no macroglossia, and a small chin. She had a small umbilical hernia, a 0.5 cm raised hemangioma on her left lower chest, and several cutaneous capillary vascular malformations on her face and nape of neck. In addition to her face, she had HH of her left arm, right leg, and the right labia and buttocks. Her postnatal course was complicated by unconjugated hyperbilirubinemia lasting several days treated with phototherapy. She was

noted to have a murmur at birth and echocardiography revealed a fenestrated atrial septum, a small PFO, and a small descending aorta. Repeat echocardiography at 6 months demonstrated resolution of these findings. No cardiomegaly was noted.

Due to features consistent with BWS, 11p15.5 methylation analysis and SNP array testing were performed on peripheral blood. Methylation-sensitive multiplex ligation-dependent probe amplification (MS-MLPA) analysis demonstrated borderline paternal UPD for 11p15.5 and somewhat surprisingly, SNP array analysis demonstrated 30% genome-wide mosaic uniparental isodisomy (Fig. 1A). This was subsequently confirmed to be paternal in origin, as suggested by her clinical features. Since similar SNP array findings can be seen in triploidy, due to genome-wide normalization of the log R ratio, a normal (46,XX) karyotype excluded this condition. To further clarify the findings, DNA from saliva was analyzed by SNP array and demonstrated 10% GWpUPD (Fig. 1B).

Given the increased risk for subjects with pUPD11 to develop Wilms tumor and hepatoblastoma [Shuman et al., 2006], tumor screening was initiated using alpha-fetoprotein (AFP) levels and abdominal ultrasonography (US). The initial AFP at 3 weeks of age was elevated at 98,000 ng/ml and rose to 122,000 ng/ml at 6 weeks of age. Abdominal US followed by MRI demonstrated three discrete lesions; two heterogeneous with complex structures in the right hepatic lobe and a cystic lesion with multiple thin septations in the upper abdomen, thought to be hemangiomas and a hamartoma, respectively. The kidneys demonstrated normal echogenicity. By 6 months of age, the hepatic lesions had enlarged and additional cystic lesions were noted (Fig. 2A–C). Reassuringly, during this period her AFP levels continued to decrease (Fig. 3). At 15 months of age, the dominant intra-abdominal cyst had enlarged to the point of respiratory competition due to abdominal distension. It was subsequently surgically resected, with 2.5 L of fluid removed. Pathology revealed a thick walled hepatic-derived cyst with biliary epithelium containing goblet cells, islands of liver tissue and bile ducts in dense acellular stroma, all consistent with a hamartoma. The lesions with heterogeneous texture were hemangiomas. SNP array analysis of the hamartoma demonstrated 80% GWpUPD and skin fibroblasts demonstrated 30% GWpUPD (Fig. 1B and Table I). Subsequently, imaging by US every 3 months and MRI every 6 months has demonstrated no recurrence of the hamartoma and stability of the hemangiomas. AFP levels normalized by 32 months and are shown in Figure 3.

Her HI was treated initially with diazoxide, which was discontinued by 3 months of age. She developed mild premature thelarche at 10 months of age, prompting testing of thyroid function studies, estradiol, follicle stimulating hormone, luteinizing hormone, bHCG, inhibin A and B, and anti-Müllerian hormone, which were all normal, with no further progression. She has a slightly bell-shaped thorax but does not have “coat-hanger” ribs or other findings consistent with UPD14 (Fig. 4A) [Sutton and Shaffer, 2000]. Since the removal of the hamartoma, her height has been at the 10th centile, weight at the 25th centile, and head circumference at the 10th centile. She is currently 33 months old and has grossly normal cognitive development. She has not had seizures. She had some mild motor delays when corrected for prematurity. She sat at 10 months, pulled to stand at 18 months, walked at 20 months, her first words were at 13 months, and at 30 months she was using two-word phrases.

Subject 2 was born at 30 $\frac{6}{7}$ weeks by emergent cesarean due to decreased fetal heart rate and non-reassuring biophysical profile to a 31-year-old Hispanic G3P2-3 mother and 29-year-old African American father. Echogenic kidneys and bowel were noted on a 20-week prenatal ultrasound. Her birth weight was at the 95th centile, head circumference at the 40th centile, and height at the 40th centile. Placental pathology demonstrated mesenchymal dysplasia. Marked, persistent hypoglycemia at birth (glucose of 20 mg/dl requiring a continuous glucose infusion) resulted in a diagnosis of HI. Clinical exam was remarkable for right facial fullness, macro-glossia, eyelid cutaneous capillary vascular malformations, earlobe creases and pits, and right leg HH (Fig. 5A). Additionally, her skin demonstrated a Blaschkoid mixture of lighter and darker pigmentation (Fig. 5B). MS-MLPA of peripheral blood demonstrated pUPD for 11p15.5. Concurrent SNP array analysis performed on peripheral blood due to the severity of the clinical findings demonstrated 85% GWpUPD (Table I) with a karyotype of 46,XX.

She had persistently elevated AFPs (>700,000 ng/ml) in the neonatal period. Serial abdominal US imaging initiated at 3 weeks of age identified two nodular soft tissue foci in her bladder. At 1 month, bladder polyps were resected and demonstrated 95% GWpUPD. Histology was negative for pleomorphic, atypical cells, or frequent mitoses. At 2 months, a right suprarenal mass and numerous contrast-enhancing liver lesions were noted, all of which continued to enlarge (Fig. 2). Both kidneys contained small medullary cysts with the right kidney 15% larger than the left kidney. Her HI was severe and required a maximum glucose infusion rate (GIR) of 22 mg/kg/min. At 2 months of age she underwent a 95% subtotal pancreatectomy followed by continued management with a GIR of 4.8 mg/kg/min via a gastrostomy tube. During the same surgery, the suprarenal mass and the liver masses were biopsied. Pathology demonstrated hepatic hemangiomas with entrapped bile ducts and hepatocytes with ballooning degeneration. The hepatic parenchyma had canalicular cholestasis, mild steatosis, mild acute inflammation, and persistent extramedullary hematopoiesis. Reticulin staining showed no parenchymal expansion or nodular growth. The pancreas demonstrated marked diffuse proliferation of islet cells, consistent with BWS-associated HI. SNP array analysis of the pancreatic tissue demonstrated 90% GWpUPD. The adrenal gland demonstrated adrenal cortical cytomegaly and mild hemorrhage.

Serial imaging revealed stability of some hepatic and adrenal lesions and increased size in others while her AFP levels continue to trend downward but remain elevated with an AFP of 300 ng/ml at 15 months (Fig. 3). She had conjugated hyperbilirubinemia while on total parental nutrition (TPN), with normal alpha-1-antitrypsin, lactate/pyruvate, coagulation factor, plasma amino acid and urine bile acid studies. Her clinical course was further complicated by peripheral pulmonic stenosis, small bowel obstructions secondary to adhesions from abdominal surgery, deep vein thrombosis associated with an indwelling catheter, a urinary tract infection, pneumonia, and chronic anemia. Her newborn screen suggests that she is homozygous for a *G6PD* mutation carried by her father. She has mild developmental delays when corrected for prematurity. At 10 months of age, she was able to sit. She crawled at 11 months and started to use a walker at 14 months. At 15 months, she said “mama” and “dada” and understood “no.” Her height was at the 5th centile, weight at the 25th centile, and head circumference at the 10th centile.

Subject 3 was born at 24₁₇ weeks due to premature rupture of membranes via spontaneous vaginal delivery to a 25-year-old mother and a 26-year-old father. Her birth weight was 50th centile. After delivery, she developed respiratory distress requiring ventilator support. She also presented with persistent hypoglycemia and was diagnosed with HI. The HI was not responsive to diazoxide or octreotide resulting in a maximum GIR of 38 mg/kg/min. Exam at 1 month of age demonstrated weight at the 75th centile, length at the 15th centile, and head circumference at the 5th centile. She had three fontanelles, hypertelorism, low-set cupped ears, micrognathia, and hypoplastic widely spaced nipples. She had a small umbilical hernia and diastasis recti. She also had mild clitoromegaly. She had normal toes and long tapered fingers. Due to a concern for BWS, AFP and US screening were initiated.

At 1 month, her AFP was 412,100 ng/ml and US revealed a left suprarenal mass. At 2 months of age, US demonstrated increased medullary echogenicity in the right kidney compared to prior studies and new linear calcifications in the liver were noted without biliary dilation or focal parenchyma abnormality. Abdominal CT demonstrated a horseshoe kidney with areas of decreased enhancement suggestive of cystic lesions and the left suprarenal mass was concerning for neuroblastoma (Fig. 2F). She had normal 17-OH progesterone (>25 ng/dl, normal 40–200) and elevated 17-OH pregnenolone (>4,000 ng/dl, normal <935 ng/dl). However, secondary to bacterial sepsis she developed multi-organ system failure and care was withdrawn at 3 months of age. Due to the suprarenal mass and concern for neuroblastoma, a limited autopsy was performed in which massive hepatomegaly was noted. By gross examination, the adrenal mass was initially considered to be a neuroblastoma. However, histologic analysis revealed adrenocortical hyperplasia, disorganized adrenal dysplasia, severe adrenal fetal cortical cytomegaly with atypical polyhedral cells containing eosinophilic granular cytoplasm and large hyperchromatic nuclei with pseudoinclusions. There was no fatty change or migrating neuroblasts. A large paraovarian and paratubal ectopic nodule noted on autopsy was identified by histology as an adrenal nodule of Marchand with disorganized adrenal dysplasia. These findings were consistent with adrenal cortical hyperplasia rather than neuroblastoma. Pathology of the pancreas demonstrated pancreatomegaly of the body and tail and dysplasia, with massive overgrowth of disorganized neuroendocrine tissue consistent with islet cell hyperplasia and a relative paucity of acini and ducts. The neuroendocrine cells stained positive for insulin and p57 and negative for chromograinin similar to control neuroendocrine cells. The detection of p57 in the pancreas suggests there is incomplete imprinting in the pancreas or there is less GWpUPD in the sections of the pancreas that were stained for histology compared to the pancreatic sample tested by SNP array. There was no known examination of the placenta. MS-MLPA analysis of skin demonstrated possible pUPD for 11p15.5. SNP array analysis demonstrated 10% mosaic GWpUPD in skin, 85% in pancreas, and 95% in adrenal mass (Table I).

DISCUSSION

Here we describe clinical and molecular features of three subjects with mosaic GWpUPD. To further understand the clinical features and management, we performed a review of the literature and found 10 previously reported cases of live-born mosaic GWpUPD [Hoban et al., 1995; Bryke et al., 2004; Giurgea et al., 2006; Reed et al., 2008; Wilson et al., 2008;

Romanelli et al., 2011; Yamazawa et al., 2011; Gogiel et al., 2012; Inbar-Feigenberg et al., 2013] (Table II). As one would expect with paternal UPD that includes the sex chromosomes, all patients are 46,XX females. Furthermore, these cases clarify that the predominant phenotype seen in GWpUPD is that of BWS caused by pUPD11 (12/13) with HH (10/13) and HI (11/13). Paternal UPD14 was suggested in three individuals and Subject 1 in our cohort has a bell-shaped thorax [Bryke et al., 2004; Yamazawa et al., 2011; Gogiel et al., 2012]. No other features of UPD14 including coat-hanger ribs, metaphyseal spurs, dysmorphic facial features, hypotonia, polyhydramnios, small size for gestational age, blepharophimosis, small thorax, and joint contractures were seen [Sutton and Shaffer, 2000; Mattes et al., 2007].

Three previously reported individuals had features of paternal UPD15 (seizures and severe developmental delay) [Bryke et al., 2004; Romanelli et al., 2011; Yamazawa et al., 2011], however neither of our surviving subjects have manifested features of paternal UPD15 or Angelman syndrome to date. Only one of the previously reported patients has had features of both paternal UPD6 and UPD20 [Bryke et al., 2004]. Subject 2 in our cohort had conjugated hyperbilirubinemia which has been reported in one individual with paternal UPD6 [Kenny et al., 2009]. However, the hepatic histology reported in that individual included a paucity of bile ducts, while that seen in our Subject 2 had only canalicular cholestasis. Other UPD6 features such as transient neonatal diabetes and low birth weight [Docherty et al., 2010] have not been reported. Subject 1 in our cohort had premature thelarche, which could be a manifestation of paternal UPD20, however, other features of UPD20, characterized by end-organ resistance to parathyroid hormone, were not seen. Paternal UPD7 has been reported in several subjects with both overgrowth and intrauterine growth restriction but no common clinical phenotype aside from the unmasked recessive alleles has been reported [Le Caignec et al., 2007; Hoffmann and Heller, 2011].

Taken together, the cohort of live-born children with GWpUPD now includes 13 subjects. Even within our cohort there is a range of clinical severity of the pUPD11 features with variable HI and macroglossia. Quite notably, tumors of various types are present in a high percentage of these subjects. Fortunately, the percentage with malignant tumors such as Wilms tumor and hepatoblastoma is ~25%, and similar to the percentage seen in BWS patients with paternal UPD11 [Cooper et al., 2005].

Dominance of the Paternal UPD11 Phenotype

Subjects on the severe end of the pUPD11 spectrum typically present with the classic BWS features of macroglossia, hemihyperplasia, hypoglycemia, and embryonal tumors. The phenotype is due to aberrant methylation of the two imprinting regions on chromosome 11 which include *H19*, *IGF2*, and *CDKN1C*. Given the mosaic nature of this disorder, a phenotypic spectrum is expected. However, when the disorder presents with classic clinical features plus additional features as noted in these subjects, further evaluation is warranted.

Additional evidence of the dominant phenotypic features of pUPD11 is seen in subjects with multiple loss of imprinting where pUPD11 is the predominant phenotype compared to other disease-linked pUPD regions [Azzi et al., 2009]. This may be due to the tissue specific effect of imprinted genes and could reflect high-level mosaic pUPD11 in certain tissues that are

largely unaffected by the other disease-causing UPD loci. In contrast, *UBE3A* (UPD15) is only expressed in brain tissue, which would suggest that Angelman syndrome would only be seen in subjects with a certain threshold of GWpUPD in brain. Within our cohort and the GWpUPD patients reported in the literature, BWS was diagnosed in all but one patient [Reed et al., 2008]. The clinical features of BWS present in these patients is summarized in Table II. Given the ability to appreciate subtle clinical features such as hemihyperplasia in even low-level mosaic pUPD11, we would expect that patients with a range of mosaic GWpUPD to demonstrate features of BWS, while they may not demonstrate features of other pUPD regions [Kalish et al., 2013].

Increased Tumor Risk in GWpUPD Subjects

A very large percentage of the GWpUPD subjects developed some type of tissue hyperplasia or mass, which is not surprising as pUPD11 has consistently been associated with increased tumor risk, as well as the dominance of the pUPD11 features in these individuals. In one study, by 5 years of age, 24% of the pUPD11 subjects had neoplasms [Cooper et al., 2005] clearly suggesting that pUPD11 is a major factor in driving tumor risk. The three subjects reported here and the 10 GWpUPD subjects previously reported all had adrenal, renal, and/or hepatic tumors or masses. Adrenal involvement ranged from adrenal cortical hyperplasia (n = 7) to pheochromocytoma (n = 1; Table III). Liver tumors ranged from hamartomas and hemangiomas to hepatoblastomas. While some of these masses are classified as benign from an oncologic perspective, the hamartoma in Subject 1 required surgical resection due to respiratory competition and Subject 2 required a 95% pancreatectomy due to the severity of her hyperinsulinism caused by pancreatic hyperplasia. Although the masses seen in these subjects have all been reported in isolated BWS/pUPD11, their very high incidence underscores the increased risk for tumorigenesis or mass formation. This suggests that the mechanism of over-growth may be augmented in subjects with GWpUPD compared to subjects with pUPD11 alone. This may be due to additional less well-characterized imprinted regions involved in cell growth. Alternatively, GWpUPD may uncover recessive loci in the same tissues being influenced by growth stimulation, which may contribute to the increased incidence of tumors.

Mechanism of GWpUPD Supported by SNP Array Analysis

Several potential mechanisms have been suggested for GWpUPD [Kotzot, 2008]. However, the SNP array analysis used in this work clearly indicates the presence of only one paternal and one maternal haplotype across the entire genome. There is no evidence of crossing over on any chromosome, which would be detected as segments of the genome with multiple paternal haplotypes. This suggests a postzygotic mechanism of GWpUPD resulting from a single sperm and single ovum at fertilization. It also suggests that to see one cell type with two paternal copies, mitotic division of the paternal pronucleus, but not the maternal pronucleus, occurred following fertilization. This may have resulted from endoreduplication of the paternal cell lineage to result in one zygotic cell which contains a paternally derived pronucleus in addition to a normal cell lineage which contains both paternally and maternally derived pronuclei (Fig. 6) [Giurgea et al., 2006; Malan et al., 2006; Kotzot, 2008; Yamazawa et al., 2011]. This mechanism is consistent with other GWpUPD subjects

reported in the literature, which all contain isodisomic and biparental cell lineages in differing ratios in different tissues.

Incidence of GWpUPD

Contrary to our initial impression, GWpUPD does not appear to be lethal and it is clear these individuals are viable with features of pUPD11/BWS. In addition, SNP array analysis has yet to be widely adopted in the assessment of BWS patients. For these reasons, we feel it is likely that GWpUPD may be significantly under-diagnosed. This may be even more relevant for pUPD11/ BWS patients with HI and to an even greater degree in those that are premature. As an example, at our institution, we have evaluated 14 subjects with HI and clinical features of BWS by SNP array. Of these subjects, three were found to have GWpUPD, each also with prematurity. Although our institution has clinical programs for both BWS and HI, reflecting potential ascertainment bias, this 21% incidence of GWpUPD in this cohort of HI/BWS subjects is clearly higher than expected. Although focused methylation testing may detect pUPD of 11p15.5, the utility of additional analysis may be overlooked to assess chromosome- or genome-wide pUPD. Secondly, under-detection may be related to the limitations of methylation assays to detect low-level mosaic differences. If low-level pUPD11 is not noted by this initial testing, additional analysis is less likely to be pursued. Finally, we have noted previously that abnormalities may not be detected in blood and may require testing in a clearly affected tissue [Kalish et al., 2013]. Therefore, we feel a high level of suspicion is key and recommend high sensitivity SNP array analysis in addition to methylation testing in subjects with clinical features of BWS, especially if HI, HH, or prematurity are present.

Management Guidelines

Although the complexity of these patients will likely mandate management on a case-by-case basis, we have adjusted our current management of these subjects to include small modifications to the current BWS/IH recommendations and focus on treatment of HI and tumorigenesis monitoring. The current tumor monitoring recommendations for BWS/IH are AFP levels every 6–12 weeks until age 4 years and abdominal US every 3 months until age 8 years [Tan and Amor, 2006; Clericuzio and Martin, 2009; Weksberg et al., 2010]. Based on the early presentation of tumors in the GWpUPD subjects, and the difficulty in determining their nature by single time point imaging studies, we have found that both surviving cases had findings that led to AFPs every 4 weeks and full abdominal US every 1–2 months during the first year. These were subsequently spaced to standard intervals with AFPs every 6 weeks and ultrasounds every 3 months. In all of these subjects, AFP levels were markedly elevated at birth, even beyond those reported for typical BWS [Everman et al., 2000]. In addition, although they have trended downward, as typically seen, they have taken longer to reach normal levels (Fig. 3). Because of the markedly elevated levels, in some instances, we needed to have the AFP levels measured at a reference lab (ARUP) to enable assessment of the trends. In addition, we have checked AFPs more frequently and used additional imaging modalities (MRI) more readily when US abnormalities were detected in these subjects. We feel that the increased screening program allowed us to follow these patients more closely, provided more time points to evaluate change in their lesions over time, and likely minimized the need for surgeries. Furthermore, the biopsies and

resections coincided with clinically warranted surgeries, for example, Subject 1 had a hamartoma drained when she began having respiratory compromise and Subject 2 had biopsies taken during her pancreatectomy.

Once we had established a baseline and understood the background nature of the lesions, we additionally tailored screening to each subject based on clinical features. For Subject 1, we initiated MRIs every 6 months because it was difficult to differentiate her hamartomas from hepatoblastomas by US. This approach was also supported by prolonged elevated but declining AFPs.

Given the identification of intra-abdominal tumors in several patients in the literature who are older than 8 years of age, we will continue to follow the literature carefully to determine the need for tumor surveillance beyond 8 years of age. This is driven partially by the occurrence of pheochromocytomas and ovarian tumors in subjects older than 8 years [Wilson et al., 2008; Gogiel et al., 2012]. We would consider US imaging every 6–12 months up to 18 years, with assessment of urinary fractionated metanephrines and blood catecholamines and metanephrines in instances of noted adrenal abnormalities. This will need to be balanced with the anxiety and unnecessary procedures that might result from such testing, and information from additional individuals will help to assess these needs.

In addition to tumor screening, management of HI has been challenging and requires endocrinologists familiar with this subject population. While most subjects with HI and BWS have a variable response to medical therapies that can include diazoxide, octreo-tide, enteral dextrose, or a combination of these therapies, Subject 2 had severe HI that required a near-total pancreatectomy. This was not due to recessive mutations of the known HI genes (*ABCC8*, *KCNJ11*, *GCK*, *GLUD1*, *MEN1*, and *UCP2*; data not shown) as is the case for most HI patients who require pancreatectomy. Furthermore, careful evaluation by a developmental pediatrician and/or a neurologist may be beneficial due to concerns for paternal UPD15 as well as effects of HI.

In summary, we describe three subjects with mosaic GWpUPD who display a range of clinical phenotypes, all with features of BWS including HI, HH, prematurity, and tumorigenesis and summarize the existing literature. These subjects highlight the importance of considering GWpUPD in subjects who present with severe BWS and emphasize the necessity of vigilant tumor screening in these subjects.

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FIG. 1.

SNP Array results. A: Genome-wide mosaic uniparental isodisomy. SNP array analysis was performed using IlluminaQuad610 BeadChip. B allele frequency is shown for all chromosomes from lymphocytes in Subject 1. B: SNP analysis results of chromosome 1 for Subject 1 in multiple tissues. For illustration purposes, SNPs for AB/AA mosaicism are pseudocolored in red to demonstrate comparison of these same SNPs between the different tissues. In the cells with more UPD, the frequency of heterozygous SNPs is reduced, and these red SNPs show a shift toward 0%, or an AA genotype. Using this shift, the percentage of cells with loss of heterozygosity was calculated as 30% in lymphocytes, 10% in the buccal cells, 85% in hamartoma, and 20% in skin.

FIG. 2.

Tumor formation in GWpUPD subjects. A: Subject 1 coronal MRI section with contrast demonstrating hamartomas (asterisks). B,C: MRI with fluid sensitive weighting demonstrating fluid-filled hamartomas. D,E: Subject 2 MRI sections showing multiple hepatic hemangiomas (cross) and adrenal cytomegaly (arrow). F: Subject 3 CT scan demonstrating suprarenal mass (arrowhead).

FIG. 3.

AFP levels for normal infants and BWS children compared to GWpUPD subjects. Age in months is corrected for gestational age, where 0 represents 40 weeks gestation. Note Subjects 1 and 2 follow similar trends after the initial newborn period and Subject 1 reached normal values by 32 months of age. Normal and BWS values are from [Blair et al., 1987; Everman et al., 2000], respectively.

FIG. 4. Chest radiographs. A: Subject 1 at 35 weeks corrected gestational age. B: Subject 2 at 35 weeks corrected gestational age. C: Subject 3 at 32 weeks corrected gestational age. Abdominal enlargement and increased density in the right abdomen is suggestive of hepatomegaly. Note a mild bell-shaped thorax in Subject 1 but not in Subjects 2 or 3.

FIG. 5. Subject 2. A: Left leg hemihyperplasia at 14 months. B: Mosaic skin pigmentation. Arrows indicate the darker skin pigmentation.

FIG. 6.

Mechanisms of genome-wide UPD mosaicism. A: Fertilization of an egg by a single sperm, irregular reduplication and division of male pronucleus, followed by cell division and diploid formation from male haploid genome. This results in a normal cell line and a GWpUPD line. B: Fertilization of an egg by a diploid sperm followed by cell division and triploidy formation followed by triploidy rescue. C: Fertilization of an egg by two sperms followed by cell division and triploidy formation followed by triploidy rescue and subsequent haploidy rescue. D: Fertilization of an empty egg and diploid formation from haploid male genome. This would result in two paternal haplotypes that are not present and this would require an additional step of two zygotes fusing.

TABLE I

Percent Mosaicism by Tissue; Values Indicate Paternal UPD Levels

Tissue studied	Subject 1	Subject 2	Subject 3
Blood	30%	85%	—
Saliva	10%	—	—
Skin	30%	<5%	10%
Pancreas	—	85%	85%
Hamartoma/liver	80%	—	—
Bladder	—	95%	—
Adrenal	—	—	95%

TABLE II

Clinical Features of Mosaic GWpUPD Subjects

	Subject 1	Subject 2	Subject 3	Inbar-Feigenberg et al. [2013]	Gogiel et al. [2012]	Yamazawa et al. [2011]	Romanelli et al. [2011]	Wilson et al. [2008]	Reed et al. [2008]	Giurgea et al. [2006], Patient 1	Bryke et al. [2004]	Hoban et al. [1995]
Age at report	32 months	15 months	Deceased at 3 months	14 years	19 years	4 years	26 years	11 years	4 years	11 months	5 years	3 years
LGA	-	+	-	-	-	+	+	+	+	-	NR	NR
Large placenta	+	+	NR	-	+	+	NR	-	+	NR	NR	NR
Polyhydramnios	-	-	-	NR	+	+	NR	+	-	NR	NR	NR
Macroglossia	-	+	-	NR	+	+	NR	+	-	NR	NR	-
Hypoglycemia	+	+	+	+	+	+	+	+	+	+	-	-
Umbilical hernia	+	+	+	+	+	+	NR	+	-	NR	NR	NR
Hepatomegaly	+	+	+	NR	+	+	NR	+	+	NR	+	NR
Hemihyperplasia	+	+	-	+	+	+	+	-	-	+	+	+
Hemangioma	+	+	-	+	NR	+	+	-	-	NR	+	NR
Tumor/mass	+	+	+	+	+	+	+	+	+	+	+	+
Developmental delay	-	-	N/A	+	-	+	+/-	+/-	+/-	NR	+	-

NR, not reported; N/A, not applicable.

TABLE III

Tumor Types and Additional Masses Reported in Individuals with Mosaic GWpUPD

	Age at report	Age at tumor presentation	Tumor type	Other masses or dysplasia
Subject 1	32 months	20 weeks	Hepatic hamartomas and hemangiomas	
Subject 2	15 months	Birth		Pancreatic hyperplasia
		1 month		Bladder polyps
		2 months	Hepatic hemangiomas	Adrenal cortical cytomegaly
Subject 3	Deceased	Birth		Adrenal cortical hyperplasia Pancreatic hyperplasia
Inbar-Feigenberg et al. [2013]	14 years	17 days		Nesidioblastosis
		5 months		Hepatic cysts
		10 months		Choledochal cyst
		4 years		Renal dysplasia
		10 years	Cardiac hamartomas	
Gogiel et al. [2012]	19 years	1 month	Hepatic hamartoma	Nesidioblastosis
		12 years	Ovarian steroid cell tumor	
		18 years	Breast fibroadenoma	
Yamazawa et al. [2011]	4 years	NR		Adrenal mass
Romanelli et al. [2011]	26 years	4 years	Wilms tumor	
		9 years		Pancreatic Hyperplasia
		20 years	Adrenocortical adenoma	
Wilson et al. [2008]	11 years	3 years		Hepatic periportal fibrosis
		8 years	Bilateral pheochromocytomas	
	4 years	6 weeks		Pancreatic hyperplasia
		18 weeks		Cystic adrenomegaly
		5 months	Hemangioendothelioma	
		15 months	Hepatoblastoma	
Reed et al. [2008]	19 months	11 months	Hepatic mesenchymal hamartoma	
Giurgea et al. [2006]	4 years	14 months		Pancreatic hyperplasia
Bryke et al. [2004]	5 years	8 months		Nodular adrenal hyperplasia
		18 months		Nodular hepatic hyperplasia
Hoban et al. [1995]	3 years	11 months	Wilms tumor	Adrenal cytomegaly