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CASE REPORT

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# Novel 1.3 Mb germline duplication in chromosome 8q21.11 by microarray comparative genomic hybridization plus single nucleotide polymorphism analysis in an adult patient with pancytopenia and urinary bladder complications

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**Key Clinical Message**

We present the case of a 30-year-old woman with a history of perinatal complications as well as bladder and urinary disease through her childhood and adult life. Microarray comparative genomic hybridization (aCGH) analysis revealed a 1.3 megabase duplication at chromosome 8q21.11 encompassing the CASC9 and HNF4G genes.

**KEYWORDS**

HNF4, Howell-Jolly bodies, microarray CGH plus SNP, pancytopenia, urothelial atypia

## 1 | INTRODUCTION

A multitude of inherited and acquired disorders may lead to a presentation of pancytopenia.[1,2] Bone marrow biopsies are instrumental in assessing the status of hematopoiesis in response to stress and disease and the identification of bone marrow failure. However, a hypocellular bone marrow is nonspecific and the differential diagnoses are broad including myelodysplastic syndrome, aplastic anemia, nutritional deficiencies such as inadequate vitamin B12 and folic acid, infectious diseases such as parvovirus B19 and HIV, and inherited bone marrow failure syndromes such as Fanconi anemia and Shwachman-Diamond syndrome.[3] Exogenous insults such as radiation, chemotherapy, and medications may hamper bone marrow production, as well.[4] A thorough clinical history, physical examination, and basic laboratory analyses are often sufficient to elucidate an etiology and guide management. Bone marrow biopsies, flow cytometry, and cytogenetic/genomic analyses are important tools in challenging cases.

## 2 | CASE REPORT

A 30-year-old woman presented with gross hematuria. She was born prematurely and suffered from bilateral retinopathy of prematurity and necrotizing enterocolitis, requiring an ileostomy shortly after birth. Early imaging revealed a duplicated renal collecting system. Later, she was diagnosed with hydrocephalus and asthma. Throughout her life, she has experienced numerous bladder and urinary complications including chronic urinary tract infections, nephrolithiasis, and bladder hemangiomas resected transurethrally at the age of seven. Subsequent multiple bladder biopsies have shown urothelial atypia, but have been negative for malignancy.

On admission at the age of 30, for gross hematuria, laboratory testing revealed marked pancytopenia. During a difficult hospital course that extended over 105 days, she underwent four cystoscopies with clot evacuations. During one of the procedures, numerous hemangiomata were identified and fulgurated. Two additional bladder biopsies again revealed

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urothelial atypia favoring reactive epithelial changes without excluding low-grade or high-grade neoplasia (carcinoma in situ; see Figure S1).

Complete blood counts (CBC) 3 months prior to admission had no significant abnormalities, while mild anemia was noted 74 days prior (see Table S1). A bone marrow aspirate during admission was significantly hypocellular for age at ~10%, with erythroid predominance and notable megaloblastic erythroid maturation with dyspoiesis and pancytopenia (see Figure S2A). The peripheral blood smear showed normocytic normochromic anemia with mild anisopoikilocytosis and markedly decreased lymphocytes, neutrophils, and platelets. The marrow lineages showed decreased granulopoiesis with a left shift and decreased erythropoiesis with a left shift, marked megaloblastic changes and marked dyserythropoiesis including Howell-Jolly bodies, basophilic stippling, irregular nuclear contours, and binucleated forms (see Figure S3). Megakaryopoiesis was markedly decreased. Iron storage was increased; abnormal sideroblasts with increased iron were present, and no ring sideroblasts were seen. The morphologic differential diagnosis was severe aplastic anemia vs hypocellular myelodysplastic syndrome (MDS). Flow cytometry was performed, and no monoclonal B cell or aberrant T-cell populations were identified.

The patient's folate was slightly elevated at 751 ng/mL (normal 180-614), and her B12 was mildly decreased at 196 pg/mL (normal 211-946). Iron studies, after receiving two red blood cell transfusions, were normal except for an elevated ferritin of 323 ng/mL (normal 10-120). She continued to receive multiple transfusions with leukoreduced irradiated platelets and red blood cells throughout her hospitalization. Alkaline hemoglobin electrophoresis showed slightly decreased hemoglobin $A_1$ at 93.3% (normal 96.8%-97.8%) and increased hemoglobin F at 4.3% (normal 0%-1.9%), consistent with a stress hematopoiesis response.[5] Screen for paroxysmal nocturnal hemoglobinuria (PNH) was negative. Telomere length testing and Fanconi anemia chromosome breakage studies were negative. A commercially available next-generation sequencing (NGS) analysis, evaluating >400 cancer-associated somatic mutations/translocations, was deemed negative.

She received B-12 supplements, antithymocyte globulin, cyclosporine, methylprednisolone, and prednisone for severe aplastic anemia/pancytopenia. Her hospitalization continued to be complicated including multiple urinary tract infections treated with antibiotics and transfers to the intensive care unit for respiratory failure after her initial steroid treatment. Imaging showed an extensive infiltrative pulmonary process, although a thorough infectious disease workup revealed no pathogens. She was treated empirically for *Pneumocystis jiroveci* pneumonia and community-acquired pneumonia. The second bone marrow biopsy, performed 19 days after the first, had similar findings. The third bone marrow biopsy, performed 165 days after the first, was hypocellular with trilineage hematopoiesis and interval improvement from the previous in overall cellularity, decrease in the degree of erythroid dysplasia, and an increase in the number of cells in the myeloid lineage (see Figure S2B). A complete blood count revealed persistent pancytopenia with slight improvement (see Table S1). The peripheral blood smear showed macrocytic normochromic anemia with mild anisopoikilocytosis. A flow cytometry MDS panel was run and showed no abnormality in myeloid maturation and no increase in blasts. The patient's pancytopenia is persistent, requiring frequent packed red blood cell and platelet transfusions, although etiology remains elusive with diagnosis of aplastic anemia vs hypocellular MDS and no lasting improvement with antithymocyte globulin and cyclosporine treatment. She is dependent on supplemental oxygen at baseline after her lengthy hospitalization, and her most recent chest CT reveals persistent ground glass opacities in the dependent portions of the lungs, likely edema vs atelectasis. Imaging also reveals bladder wall calcifications, and she continues to have urinary tract complications including intermittent hematuria, infections, renal calculi, bladder spasms, and hydronephrosis necessitating multiple interval hospitalizations. She is awaiting allogeneic stem cell transplant pending improvement in her respiratory function. Possible surgical interventions to alleviate urinary symptoms are pending both improvements in respiratory function and pancytopenia.

## 3 | METHODS

### 3.1 | Chromosome, fluorescence in situ hybridization (FISH), and comparative genomic hybridization (CGH) plus single nucleotide polymorphism (SNP) microarray

Cytogenetic analysis was performed from short-term bone marrow cultures by routine laboratory protocol. For microscopic analysis, metaphase chromosomes were stained with trypsin-Giemsa technique.[6] For chromosome analysis, 20 cells were analyzed; two to five metaphases were karyotyped. For the definition of chromosomal abnormalities, ISCN(2016)[7] was followed. FISH analysis was carried out to confirm the microarray comparative genomic hybridization (aCGH) findings. The bacterial artificial chromosomes' (BACs) probe RP11-846M12 (8q21.11) was obtained from Empire Genomics (Buffalo, NY, USA), and control probe TelVysion 8q was obtained from Abbott Molecular (Abbott Laboratories, Abbott Park, IL, USA). The probes RP11-846M12 and TelVysion 8q were labeled with spectrum green and spectrum orange, respectively, using nick translation kit (Cat # 07J00-001; Abbott Laboratories) according to manufacturer's instructions. Hybridization was performed as per the manufacturer's instructions and standard protocols. The

slides were analyzed using a Nikon (Eclipse 80i) fluorescence microscope attached with a CCD camera; Cytovision FISH software was used for image acquisition and analyses. Ten metaphases and 100 interphase cells were analyzed for confirmation of aCGH findings.

Microarray CGH experiment was carried out on Agilent's SurePrint G3 aCGH-SNP array 4 × 180K, CCMC v2.0 (Agilent Technologies Inc., Santa Clara, CA), custom designed whole genome plus cancer targeted platform containing approximately 120K aCGH probes (60mer) including 20K cancer-specific probes (1 probe/0.5—1Kb; minimum 1 probe/exon, maximum 200 probes/gene) covering over 500 cancer genes and >130 cancer-associated genomic regions and 60K SNP probes which overlaps with Alu1 and Rsa1 sites with minor allele frequency (MAF) >5%. Genotypes on this array are measured using one SNP probe per SNP, providing ~5-10 Mb resolutions for loss of heterozygosity (LOH) detection across the entire genome. Briefly, patient's DNA and control DNA (1.0 µg) were digested with restriction enzymes, Alu1 and Rsa1, and enzymatically labeled with dyes Cyanine-5dUTP and Cyanine-3dUTP, respectively, using Sure Tag DNA labeling kit (Cat # 5190-3399) as per the manufacturer's recommendations. The labeled DNA was hybridized as per the manufacturer's recommendations at 65°C for 40 hours. Data analysis was performed post-washing. The slides were scanned in high-resolution scanner (Model # G2505C; Agilent Technologies Inc.) at 3-µm resolution. The output files were processed and analyzed by CytoGenomics software v2.5 (Agilent Technologies Inc.), using an Aberration Detection Method 2 (ADM-2) algorithm for aberration analysis and visualization, with the threshold set at 6.0. To control small variations appearing in the data analysis and to get the clonal fraction (for mosaicism), we used an extra aberration filter, defining the minimum number

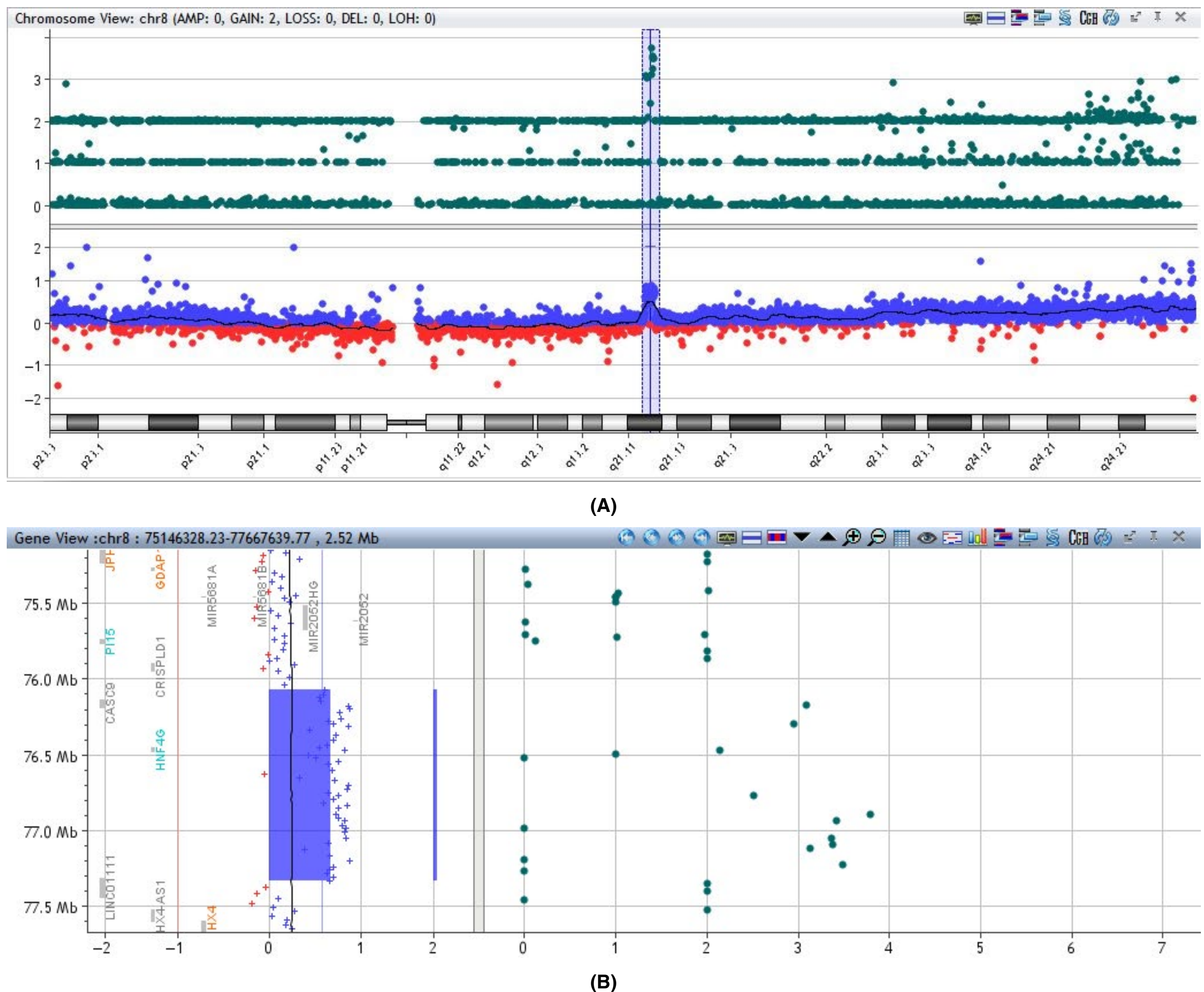


**FIGURE 1** (A) Microarray comparative genomic hybridization (CGH) showing 1.3 Mb mosaic gain at chromosome 8q21.11 segment from 76069471 to 77329144 base pairs. (B), shows the expanded gene view of the 8q21.11 region

of probes that should be present in an aberrant region as five (gain or loss), with the minimum absolute level of average log2 ratio of 0.15. The GC correction, diploid peak centralization, SNP (confidence level 0.95), and LOH (threshold 6.0) parameters were included in the analysis. The cutoff for CNVs was set at 250 kb, and FISH confirmation was performed for confirmation of abnormal CNV.

## 4 | RESULTS

Cytogenetic G-banding karyotype diagnosis at presentation was normal female 46,XX in 19 cells of 20. There was a nonclonal aberration in one cell: 46,XX,del(7)(p13). All follow-up karyotype analysis revealed 46,XX in all 20 cells.

Microarray CGH analysis revealed a 1.3 megabase mosaic gain at 8q21.11 from 76069471 to 77329144 base pairs with an array clonal fraction of approximately 95% (see Figure 1), and SNP array analysis showed a 9.3 Mb copy neutral loss of heterozygosity (cn-LOH) in the long arm terminal region of chromosome 6: arr[GRCh37/hg19] 6q26q27(161,391,546-170,700,775) × 2 hmz (see Figure S4). There were also low-level genomic gains in the short and long arms of all the chromosomes suggestive of genomic instability with unknown clinical significance (see Figure 2A). The telomere noise was confirmed on repeat analysis of initial bone marrow samples. At day 165, a repeat CGH and SNP array analysis did not show the telomere noise nor the 9.3 Mb cn-LOH (see Figure 2B). The duplication was confirmed by FISH with RP11-846M12 in approximately 81% of metaphase cells and approximately 71% of interphase cells analyzed indicating clonal mosaicism (see Figure S5).

To determine the origin of the 8q21.11 duplication, microarray CGH analysis was performed on cultured fibroblast cells from a skin biopsy which revealed 1.3 megabase gain at 8q21.11 indicating germline in origin. Interestingly, the telomere noise in the short and long arms of all the chromosomes observed in the bone marrow samples was not present in the fibroblasts. Also, 9.3 Mb cn-LOH was not observed, supporting an acquired somatic

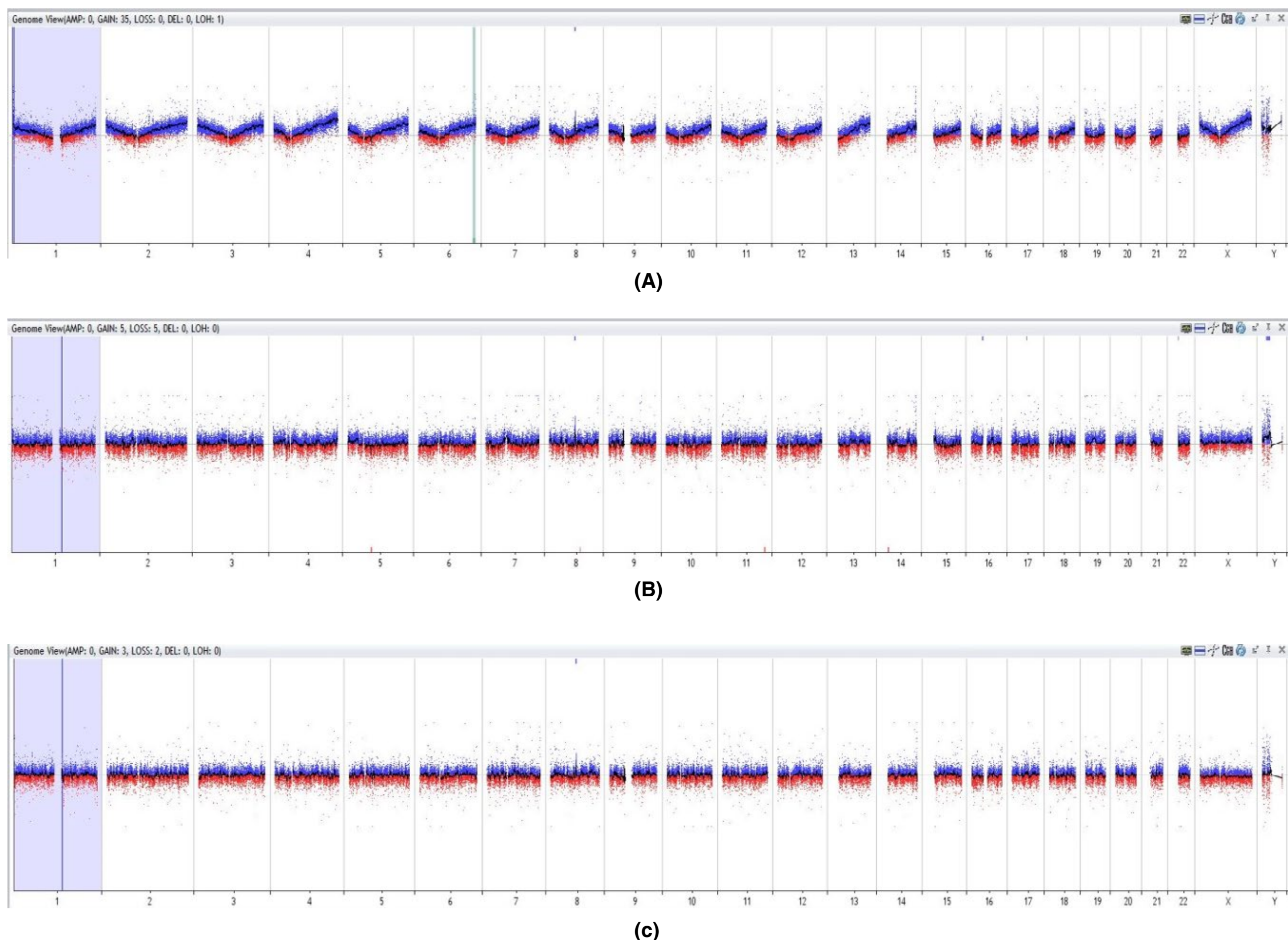


**FIGURE 2** Microarray comparative genomic hybridization (CGH) and SNP array results. (A), initial bone marrow showing the duplication of 8q21.11, low-level genomic gains, short and long arms of all chromosomes, and a 9.3 Mb copy neutral loss of heterozygosity (cn-LOH) in the long arm terminal region of chromosome 6, (B) bone marrow sample after treatment (at 165 d) with only germline duplication of 8q21.11, and (C) cultured skin fibroblast cells with germline duplication of 8q21.11

origin of both these genetic events (see Figure 2C). Additionally, chromosome breakage studies were performed on fibroblast cells which revealed normal findings ruling out Fanconi anemia. A DECIPHER (Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources) database search was conducted for cases with gains/duplications of chromosome eight[8]. (For details see Table S2).

## 5 | DISCUSSION

The duplication of 8q21.11 contains gene CASC9 (cancer susceptibility candidate 9) a nonprotein coding gene and HNF4G (hepatocyte nuclear factor 4, gamma). Nuclear receptors, such as HNF4G, are transcription factors that regulate gene expression. HNF4G is upregulated in certain cancer tissues (eg, bladder urothelial carcinoma), and overexpression in vitro-stimulated cancer cell growth and promoted tumor formation in bladder cancer xenograft models.[9] HNF4G modulation by miR-34a can downregulate its expression, inhibiting bladder cancer cell viability, colony formation, and invasion. The expression levels of this miRNA molecule are significantly reduced in bladder cancer cells, especially muscle invasive sublines.[10] The possibility of an association between the germline mutation 8q21.11 and the patient's urinary bladder pathology and comorbidities is unknown.

The telomere noise observed by microarray CGH caused by low-level genomic gains in the short and long arms of all chromosomes is a rare phenomenon likely associated with Howell-Jolly bodies, small nuclear fragments of DNA material found in erythrocytes under certain conditions. As a red cell matures, it expels its nucleus; however, small fragments of DNA may remain. These fragments are removed from circulating RBCs by a well-functioning spleen. Howell-Jolly bodies are often seen in the peripheral blood smears of patients with hyposplenism, anemia, or MDS. The observation of telomere relative to centromere enrichment throughout all chromosomes has been previously linked to the presence of Howell-Jolly bodies by CGH in an abstract publication[11] for which we found no subsequent research publication. Howell-Jolly bodies in the patient's bone marrow red blood cells concurrent with the telomere genomic gain findings may indicate a source for genomic material at a time of hematopoietic stress. The telomere noise phenomenon was not present in analysis of fibroblast germline cells, nor was it present in microarray analysis of the third bone marrow sample which showed improved hematopoiesis and red cell morphology with no Howell-Jolly bodies.

In conclusion, the patient likely has an acquired bone marrow failure syndrome (aplastic anemia vs MDS), which may be related to an underlying constitutional abnormality. Additional reports of similar patients will help elucidate this unique genetic entity.

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## CONFLICT OF INTEREST

None declared.

## AUTHOR CONTRIBUTION

CRB and AE: involved in hematopathology workup and clinical course. HM: provided urinary bladder pathology analysis. MAI and BZ: involved in cytogenetics, FISH, and microarray CGH analysis. CRB, AE, and MAI: wrote the manuscript. MAI: provided final review and editing.

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## REFERENCES

1. Weinzierl EP, Arber DA. The differential diagnosis and bone marrow evaluation of new-onset pancytopenia. *Am J Clin Pathol*. 2013;139:9-29.
2. West AH, Churpek JE. Old and new tools in the clinical diagnosis of inherited bone marrow failure syndromes. *Hematology Am Soc Hematol Educ Program*. 2017;1:79-87.
3. Steensma DP. Dysplasia has a differential diagnosis: distinguishing genuine myelodysplastic syndromes (MDS) from mimics, imitators, copycats and impostors. *Curr Hematol Malig Rep*. 2012;7:310-320.
4. Cazzola M, Porta MG, Malcovati L. The genetic basis of myelodysplasia and its clinical relevance. *Blood*. 2013;122:4021-4034.
5. Alter BP, Rosenberg PS, Day T, et al. Genetic regulation of fetal haemoglobin in inherited bone marrow failure syndromes. *Br J Haematol*. 2013;162:542-546.
6. Seabright M. A rapid banding technique for human chromosomes. *Lancet*. 1971;2:971-972.
7. ISCN (2016): An International System for Human Cytogenetic Nomenclature, Jean McGowan-Jordan, Annet Simons, Michael Schmid (eds); S. Karger, Basel 2016.
8. Firth HV, Richards SM, Bevan AP. DECIPHER: database of chromosomal imbalance and phenotype in humans using ensembl resources. *Am J Hum Genet*. 2009;84:524-533.

9. Okegawa T, Ushio K, Imai M, Morimoto M, Hara T. Orphan nuclear receptor HNF4G promotes bladder cancer growth and invasion through the regulation of the hyaluronan synthase 2 gene. *Oncogenesis*. 2013;2:e58.
10. Sun H, Tian J, Xian W, Xie T, Yang X. miR-34a inhibits proliferation and invasion of bladder cancer cells by targeting orphan nuclear receptor HNF4G. *Dis Markers*. 2015;2015:879254.
11. Gantt NM, Walker B, Lee T, et al. Genomic composition of Howell-Jolly bodies. *Blood*. 2006;108:1570.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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