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Teaching Case

Androgenetic complete mole with trisomy 13: Report of a case with microsatellite genotyping and review of the literature

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ABSTRACT

Hydatidiform moles are gestational diseases with abnormal development of the villous trophoblast and characterized by an excess of paternal to maternal genetic material. Complete moles are usually diploid and androgenetic, and are thought to develop after the fertilization of an "empty ovum" by either a haploid spermatozoon or two spermatozoa. We report a case of a complete mole in which fluorescence *in situ* hybridization (FISH) incidentally disclosed trisomy 13. Microsatellite genotyping showed a single allele at each of the markers tested on the chorionic villi, and comparison with parental peripheral blood specimens revealed that the markers were all of paternal origin. These results confirmed the paternal origin of all three copies of chromosome 13, and the isodisomy for each chromosome was consistent with duplication of a monospermic fertilization event and subsequent non-disjunction. To the best of our knowledge, this is the only case of an androgenetic complete mole with trisomy 13 described in the scientific literature. We present a review of the literature and hypothesize that the trisomy 13 in our case likely resulted from non-disjunction of chromosome 13.

Introduction

Hydatidiform moles are gestational diseases derived from the villous trophoblast and characterized by hydropic changes, cavitation (cistern formation), and trophoblastic hyperplasia. Hydatidiform moles are characterized by an excess of paternal to maternal genetic material. They are classified as complete and partial moles based on their different pathogenesis and different morphologic and clinical features.

Most partial moles derive from the fertilization of a normal ovum by two spermatozoa. Therefore, partial moles are triploid and have both paternal and maternal contribution to their genetic material in a ratio of 2:1. As for complete moles, most are diploid and, as demonstrated by Kajii in 1977 [7], they are usually androgenetic with their chromosomal content paternally derived and usually no maternal contribution. To explain the mechanism underlying the androgenicity of complete moles, it was hypothesized afterwards by Jacobs and Wake that complete moles arise from the fertilization of an "empty egg" [6] or an ovum whose nucleus has been inactivated or eliminated [15]. Diploidy results from the fertilization of the "empty ovum" by a single haploid spermatozoon which afterwards undergoes duplication (monospermy) [10] or occasionally by fertilization by two spermatozoa (dispermy) [14].

Since these original reports, some studies have shown that complete moles can rarely be of biparental origin [4,8] or infrequently have diverse chromosomal abnormalities [5,13]. Thus, the pathogenesis of complete moles appears to be more complex than previously thought, and further investigation and reporting on the genetic basis of hydatidiform moles is important to improve our knowledge and understanding of this disease.

We have encountered a peculiar case of a complete mole with trisomy 13 in which all three copies of chromosome 13 were paternally derived. To the best of our knowledge, such a genetic abnormality has not been described before in a complete mole.

Materials and methods

Case history and histopathology

The patient is a 48-year-old gravida 8 para 7 whose last full-term pregnancy was 8 years earlier. She presented with second trimester vaginal bleeding, coarse distal tremor, hyperemesis, and increased shortness of breath over the preceding 2 weeks. Her

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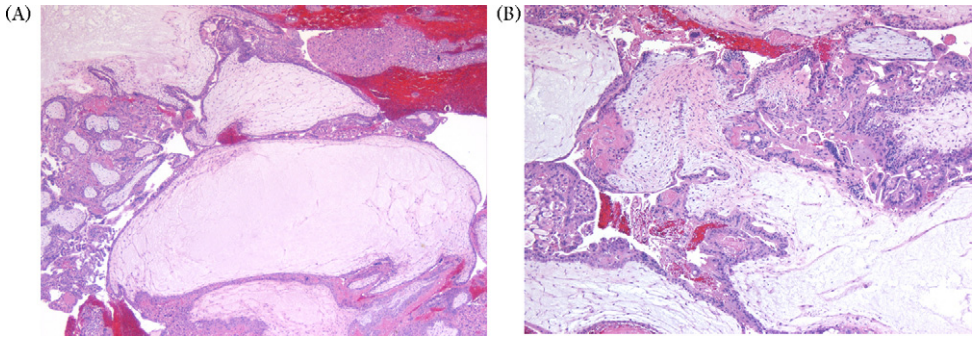

Fig. 1. Histologic sections from the trophoblastic tissue revealed: (A) enlarged edematous chorionic villi with cistern formation (H&E, 20×) and (B) marked trophoblastic hyperplasia (H&E, 40×).

quantitative HCG was over 800,000 mIU/ml, and the ultrasound revealed evidence of a 9 cm intrauterine mass consistent with trophoblastic disease. Physical examination revealed a distended soft uterus reaching the umbilicus and no evidence of vaginal metastasis. The chest X-ray was normal, but the lung CT scan revealed microscopic bilateral pulmonary metastases, the largest measuring 8 mm. No liver metastases were seen. The patient underwent an initial uterine suction curettage. She eventually developed persistent intrauterine disease and was treated with Actinomycin-D. She received 5 cycles of chemotherapy, and she is currently doing well 12 months after her initial diagnosis.

Tissue from the initial uterine curettage showed grossly visible vesicles. Histologic sections revealed morphologic features that were consistent with the diagnosis of complete mole. Chorionic villi were enlarged and edematous with cistern formation. Marked circumferential trophoblastic hyperplasia (Fig. 1) and trophoblastic atypia were also present. No fetal parts were identified. Immunohistochemical study with p57kip2 antibody revealed an ambiguous staining pattern with focal positivity in approximately 20% of the cytotrophoblastic cells. Therefore, to determine the ploidy of the specimen and to confirm the diagnosis of a complete mole, cytogenetics study with fluorescence *in situ* hybridization (FISH) was requested.

Immunohistochemistry

A formalin-fixed, paraffin-embedded tissue section (5 μm) from trophoblastic tissue was stained with the mouse monoclonal antibody directed against p57kip2 protein (clone 57PO6, catalogue #MS-1062-P, Neomarker, Fremont, CA) at a dilution of 1/4000 and incubated for 60 min at room temperature. Pretreatment, heat-induced antigen retrieval was performed at 118 °C using a Biocare pressure cooker and a citrate buffer pH 6.0 for 2 min. The detection system used was the Biocare Mach 3 mouse kit (Biocare Medical, Concord, CA) with diaminobenzidine with copper enhancer as a chromogen. Hematoxylin was used as a counterstain.

Fluorescence in situ hybridization (FISH)

FISH analysis was performed on a 4-μm, formalin-fixed, paraffin-embedded tissue section using the Vysis AneuVysion prenatal test specific for chromosomes X, Y, 13, 18, and 21, as well as a probe specific for chromosome 16 (Abbott Molecular, Des Plaines, IL) using established protocols. A chromosome 13-specific probe set (D13S319, D13S25) was also used to confirm the trisomy 13 result. Briefly, the slides were deparaffinized using three xylene washes at room temperature followed by 100% ethanol dehydration and a water wash. Tissue sections were pre-treated with 0.2N HCl at room temperature for 20 min and 1 M sodium thiocyanate at 80 °C for 30 min, then rinsed in water at room temperature for 2 min, followed by washing in 2× SSC. The slides were dehydrated in an ethanol series (70%, 85% and 100%) at room temperature for 2 min each and air-dried. The Spectrum Aqua-labeled CEP16 probe was added to the LSI 13/21 cocktail of the AneuVysion probe set. The CEP 18/X/Y cocktail was used on a second area of interest. Between 2 and 4 μl of each mixture was added to the slides, and then the slides were cover-slipped. The cells and probes were co-denatured at 73 °C for 6 min and incubated overnight at 37 °C using the HYBrite™ denaturation/hybridization system (Abbott Molecular, Des Plaines, IL). Post-hybridization washing was performed in 1.2× SSC/0.2% NP40 at 73 °C for 2 min, followed by 2× SSC/0.1%NP40 at room temperature for 30 s. The slides were air-dried in the dark and counterstained with DAPI stain. Fifty nuclei within areas marked by the pathologist were scored on an Olympus BX61 fluorescent microscope for each probe cocktail. FISH was repeated with the LSI 13/21 cocktail, where another 25 nuclei were scored. Thirty-three further nuclei were scored with the confirmatory probe set (D13S319, D13S25).

Microsatellite genotyping

Microsatellite genotyping was performed using thick cuts from formalin-fixed, paraffin-embedded trophoblastic tissue and peripheral blood samples from the patient and her spouse. DNA from the tissue section and the peripheral blood samples was extracted using a resin-based procedure (Instagene Matrix, Bio-Rad Laboratories, CA). Quantitative fluorescent PCR (QF-PCR) was performed according to the manufacturer's instructions for each specimen using the Aneufast kit (Molgentix, Barcelona, Spain). The Aneufast kit consists of two multiplex QF-PCR assays that amplify four STRs (short tandem repeats) for each of chromosomes 21 (D21S1411, D21S1414, D21S1435, D21S1446), 18 (D18S386, D18S390, D18S391, D18S535) and 13 (D13S258, D13S305, D13S631, D13S634), 2 pseudoautosomal STRs (DXYS218, X22), 1 X chromosome-specific STR (HPRT), as well as the amelogenin and the SRY genes. The fluorescent QF-PCR products and size standards were analyzed by capillary electrophoresis on the ABI 3130xl Automated Genetic Analyzer using GeneMapper 4.0 software (Applied Biosystems, Foster City, CA).

Results

Due to the ambiguous pattern of staining for p57kip2 in this otherwise morphologically usual complete mole, FISH studies were requested. FISH was performed with probes specific for chromosome X, Y, 13, 16, 18, and 21, and 150 cells were analyzed. The FISH study revealed that this mole was composed of 2 copies each of the X chromosome and chromosomes 16, 18, and 21 (Fig. 2). It also revealed the presence of 3 copies of chromosome 13 (trisomy 13), which was confirmed using another chromosome 13-specific probe