

Integrated Immunohistochemistry and Genotyping Approach for Diagnosing Recurrent Molar Pregnancies: A Diagnostic Algorithm

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Abstract

We present a case of a couple who had nine pregnancy losses, including five molar pregnancies. Earlier pregnancies often lacked genotyping and immunohistochemistry (IHC), which limited diagnostic accuracy. In the ninth pregnancy, histopathology and IHC suggested a partial mole, but genotyping revealed it to be a biparental complete mole, significantly altering management. This highlights the importance of combining histopathology, IHC, and genotyping to identify parental genomic contributions in recurrent molar pregnancies accurately. Without genotyping, misclassification as a partial mole could have led to inappropriate treatment. Recurrent partial moles may be managed with in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) using the patient's oocytes, while biparental complete moles require donor oocytes due to maternal genomic imprinting defects. The couple was counselled on reproductive options, and an algorithm is proposed to enhance the diagnostic approach for recurrent molar pregnancies.

Keywords: Hydatidiform Moles; Partial Hydatidiform Moles; Complete Hydatidiform Moles; Immunohistochemistry

Introduction

Molar pregnancies arise from abnormal trophoblastic proliferation due to aberrant fertilization processes. Complete hydatidiform mole (CHM) is typically androgenetic, resulting from the fertilization of an empty oocyte by a single sperm, which then duplicates its genetic material, leading to a diploid genome of 46, XX, or 46, XY. In contrast, partial hydatidiform mole (PHM) occurs when an oocyte is fertilized by two sperm, resulting in a triploid zygote with both maternal and paternal contributions, often represented as 69, XXX, or 69, XXY.¹ While most cases of CHM are androgenetic, a subset of it involves a biparental genotype primarily responsible for recurrent molar pregnancies. Maternal-effect genes, such as NLRP7, KHDC3L, and PADI6, are essential for normal embryonic development.²⁻⁴ and mutations in these genes disrupt maternal genomic imprinting, leading to abnormal trophoblast development and recurrent molar pregnancies.⁵

Recurrent molar pregnancies, particularly biparental CHM, are rare and pose significant diagnostic challenges. These cases require careful differentiation from sporadic molar pregnancies and necessitate advanced diagnostic techniques, including immunohistochemistry (IHC) and genotyping, to assess parental contributions. Understanding the genetic mechanisms underlying these pregnancies, particularly the role of genomic imprinting and maternal effects, is vital for reproductive counselling, especially when considering assisted reproductive technologies (ART). Conception using donor oocytes increases the likelihood of a successful pregnancy outcome in these cases.^{6,7} This case report highlights the importance of a comprehensive diagnostic approach to recurrent molar pregnancies, guiding appropriate counselling and treatment to optimize outcomes.

Case Report

A woman in her early 30s, born of a non-consanguineous marriage, presented with her ninth pregnancy at 5 weeks of gestation. Her obstetric history was significant for eight previous pregnancy losses, with no live births. A positive family history was noted, as her sister had experienced three recurrent pregnancy losses. The summary of her prior pregnancies is detailed in Figure 1A. In her second, third, and fifth pregnancies, which were diagnosed before 8 weeks of gestation, histopathology suggested PHM. However, confirmatory IHC and genotyping were not performed at that time. The fourth pregnancy progressed to 16 weeks, at which point ultrasound and histopathology confirmed a CHM. In all instances, serum β -hCG levels normalized within 8 weeks following uterine evacuation.

In the current (ninth) pregnancy, her serum β -hCG was 5,000 IU/L, and transvaginal ultrasound showed a small, empty gestational sac with adjacent cystic changes. A repeat scan after one week demonstrated progression in the cystic changes. Laboratory evaluation revealed: hemoglobin 10.2 g/dL, TSH 2.2 IU/L, β -hCG 24,000 IU/L, and a normal chest X-ray. Given her prior history of suspected PHMs, another PHM was initially considered. She underwent suction and evacuation, and the products of conception showed visible cystic changes. Histopathological examination revealed numerous variably sized villi with hydropic swelling, focal scalloping, cistern formation, and circumferential trophoblastic proliferation (Figure 1B), consistent with a diagnosis of PHM. IHC for p57 demonstrated preserved expression (Figure 1C), which excluded androgenetic CHM and supported the initial diagnosis.

However, genotyping of the conceptus revealed a 46, XY karyotype, which is inconsistent with typical PHM or androgenetic CHM. This prompted a revised diagnosis of biparental CHM, a rare entity distinct from androgenetic forms. Subsequent whole-exome sequencing of the mother did not detect mutations in NLRP7, KHDC3L, or PADI6—genes commonly associated with biparental CHM.

Following the evacuation, the β -hCG levels declined from 24,000 IU/L to 4,000 IU/L at 48 hours, 2,200 IU/L at day 8, 950 IU/L at day 15, and 800 IU/L at day 21. However, by day 28, levels rose to 2,800 IU/L, further increasing to 7,500 IU/L by day 35. Repeat ultrasound at this stage revealed a residual intrauterine mass with cystic features similar to those seen before evacuation.

As the initial histopathology lacked atypical cytotrophoblastic changes, a second suction and evacuation was performed. The histological findings were similar to the initial specimen. Following this intervention, β -hCG dropped sharply to 320 IU/L by day 7, and to 20 IU/L by day 14. Subsequent values were all below 5 IU/L, and monthly monitoring over six months confirmed complete remission.

The couple was provided with comprehensive genetic counselling, and in vitro fertilization (IVF) using donor oocytes was recommended. This approach was advised due to the maternal-effect nature of biparental CHM, where the use of autologous oocytes would not prevent recurrence. Her sister was also referred for genetic counselling, given the familial clustering of gestational trophoblastic disease.

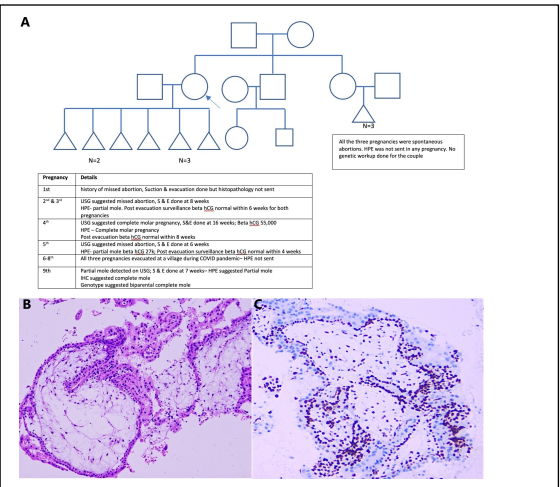


Figure 1: (A) A pedigree chart illustrating the family history of the index case (arrow mark), including her siblings and their reproductive outcomes. The chart details each pregnancy of the index case, with specific information about gestational outcomes provided in the accompanying box below the chart. (USG: Ultrasonography; HPE: Histopathological Examination; hCG: Human Chorionic Gonadotropin); (B) Histopathological image displaying

hydropic changes in the stroma of the villi, lined with cytotrophoblasts. (Hematoxylin and eosin staining, 10x magnification); (C) Immunohistochemistry image showing positive p57 staining in villous trophoblasts and stromal cells, suggesting either a partial hydatidiform mole or a biparental complete mole. (40x magnification)

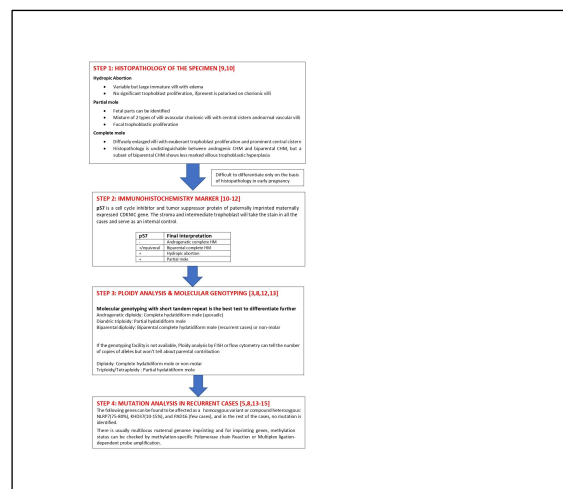


Figure 2: Stepwise algorithm for evaluating recurrent molar pregnancies in early gestation. The approach integrates histopathology, immunohistochemistry, and genotyping to confirm the diagnosis and determine the parental origin of the genome. (CHM: Complete hydatidiform mole; HM: Hydatidiform mole)

Discussion

Recurrent molar pregnancies are defined as the occurrence of at least two molar gestations, separated by a minimum interval of six months between successive pregnancies.^{1,2} The recurrence risk following a CHM is estimated at 1.9–2.3%, and approximately 1.8% following a PHM, representing a nearly 20-fold increase over baseline population risk.^{1–3} This risk significantly escalates to 15–28% after two CHM episodes.⁴ However, the precise prevalence of biparental CHM remains undetermined due to its rarity.

This case exemplifies the diagnostic complexity involved in differentiating early molar pregnancies—particularly between CHM, PHM, and hydropic abortions—where histopathological features may overlap. The initial diagnoses in the earlier pregnancies were based on histopathology alone, which led to misclassification. Features such as pseudo-cistern formation seen in hydropic degeneration and the presence of embryonic vessels, which may appear in PHM or CHM, can be misleading.^{8,9}

In such ambiguous scenarios, IHC with p57 and genotyping are critical adjuncts. The p57 marker is absent in androgenetic CHM but expressed in PHM and also retained in biparental CHM, limiting its utility in isolation. In this case, although p57 expression suggested a PHM, genotyping revealed a 46, XY biparental origin, leading to a revised diagnosis of biparental CHM. This underlines the importance of an integrated, multidisciplinary diagnostic approach that combines histopathology, IHC, and molecular testing.

While histopathology and IHC may guide initial impressions, genotyping remains the gold standard for determining the parental origin of molar tissue and distinguishing androgenetic from biparental CHM. Without molecular confirmation, the current case could have been erroneously managed as a PHM, underscoring the clinical consequences of misdiagnosis.

To facilitate accurate diagnosis in future cases, a working algorithm (Figure 2) can aid in the structured evaluation of early molar pregnancies. The therapeutic implications of a confirmed diagnosis are significant. In cases of recurrent PHM, assisted reproductive technologies such as IVF with intracytoplasmic sperm injection using the patient's own oocytes are often effective. This method minimizes the risk of dispermy. In contrast, biparental CHM arises due to maternal-effect gene mutations, and thus, the use of autologous oocytes is contraindicated, as it does not mitigate recurrence risk.⁶ In such cases, IVF with donor oocytes is the preferred strategy, and appropriate genetic counselling is essential for both the patient and affected family members.

Notably, recurrence of molar pregnancies across different partners, and a frequency exceeding the expected 25% risk in autosomal recessive inheritance, challenges the notion of simple Mendelian transmission.^{1,9} Biparental CHM is now understood as a maternal-effect autosomal recessive disorder linked to multilocus imprinting defects. The majority of such cases are attributed to mutations in NLRP7 (48–80%) and KHDC3L (10–14%), although

other genes like PADI6 may also be implicated.^{10–12} In the present case, whole-exome sequencing failed to detect mutations in known causative genes, suggesting either an undiscovered mutation or the involvement of novel loci.

Previous reports on recurrent molar pregnancies have frequently relied on histopathology or p57 immunohistochemistry alone, without the confirmatory role of genotyping. For instance, studies by Narayan et al. and Koc et al. diagnosed recurrent PHM using histological features alone, while Salima et al. incorporated p57 staining.^{13–15} However, none included genetic profiling, which—as illustrated by the current case—is crucial to distinguish PHM from biparental CHM with diagnostic certainty.

Conclusion

This case illustrates the critical need for an integrated approach combining histopathology, IHC, and genotyping in the evaluation of recurrent molar pregnancies. Accurate diagnosis informs optimal reproductive counselling and ensures the use of appropriate assisted reproductive techniques, particularly in maternal-effect biparental CHM, where donor oocyte IVF is the most viable option to prevent recurrence.

Disclosure

The authors declared no conflicts of interest. Written consent was obtained from the patient.

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