Characterisation of a rare hydatidiform mole with aberrant p57 expression

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Abstract

Accurate diagnosis and subclassification of hydatidiform mole (HM) are important to stratify the risk of persistent gestational trophoblastic disease (GTD) and gestational trophoblastic neoplasia (GTN). A combination of histomorphology and ancillary studies including p57 immunohistochemistry (IHC) and/or molecular genotyping by short tandem repeat analysis enable subclassification of most HM into partial hydatidiform mole (PHM) or complete hydatidiform mole (CHM). Here we report a rare HM with equivocal morphology and discordant p57 expression within individual villi and divergent p57 expression across villi. Molecular genotyping of DNA extracted from laser capture microdissected (LMD) chorionic villi allowed identification of CHM and rare androgenetic/biparental mosaicism. This study exemplifies a potential diagnostic pitfall and highlights the importance of inter-disciplinary examination such as p57 IHC and molecular genetics in the diagnosis of HMs.

Keywords androgenetic/biparental mosaicism; complete hydatidiform mole; molar pregnancy; molecular genotyping; mosaic; p57 immunohistochemistry; p57-discordant villi; p57DV

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Case report

A patient with a possible hydatidiform mole was referred to the Scottish Hydatidiform Mole Follow-Up Service (SHMFS), one of three regional trophoblastic centres in the UK. The patient was in her mid-30s, with no previous history of hydatidiform mole (HM). Her initial diagnosis was partial hydatidiform mole (PHM). At SHMFS, examination of haematoxylin and eosin slides, immunohistochemistry (IHC) with p57^{Kip2} Ab-6 monoclonal antibody (1:400, Thermo Scientific, Fremont) and ploidy determination by flow cytometry was performed. Molecular genotyping by multiplex short tandem repeat assay of microdissected villous cytotrophoblasts and stromal cells was completed. Laser microdissection (LMD) system was utilized to cut regions of interest on the cresyl violate stained tissue slides, which are then collected in microcentrifuge tubes for DNA extraction. DNA was extracted and PCR amplified using the Promega PowerPlex 16HS system (Promega Corporation, Fitchburg, Wisconsin), followed by analysis on GeneMarker software.

The findings were highly unusual. Flow cytometry indicated diploidy. Three distinct populations of chorionic villi were identified on histology and ancillary studies (Figure 1). The first population had enlarged chorionic villi with irregular scalloped contours and trophoblast pseudoinclusions, appearances that mimic a PHM. p57 IHC showed a differential expression pattern between villous cytotrophoblasts (positive) and stromal cells (negative). LMD villous cytotrophoblasts showed one maternal chromosome complement and one paternal chromosome complement (biparental diploidy) on molecular genotyping. However, villous stromal cells showed two copies of paternal chromosome complement only (paternal isodisomy). Therefore, the findings in this population of chorionic villi were consistent with a mosaic conception in which there is the presence of two or more cell lines within a single conceptus. The first cell line consists of androgenetic (purely paternal) contribution and the second cell line consists of biparental (maternal and paternal) contribution, described as androgenetic/biparental mosaicism (ABM). The second population comprised enlarged oedematous chorionic villi with nonpolar, and in places, circumferential trophoblast hyperplasia and cistern formation. Ancillary studies showed absent expression of p57 in villous stromal cells and cytotrophoblasts, and paternal isodisomy on genotyping. The findings were typical of a complete hydatidiform mole (CHM). The third population were normal appearing chorionic villi, with diffuse positive p57 expression in stromal cells and cytotrophoblasts. Genotyping demonstrated biparental diploidy. Overall, the findings were consistent with a conception, or conceptions, demonstrating biparental genotype, CHM and ABM.

Discussion

HM is the premalignant, abnormal form of pregnancy, which is characterised by hydropic chorionic villi with trophoblast proliferation.[1] Most HMs occur when there is over-representation of

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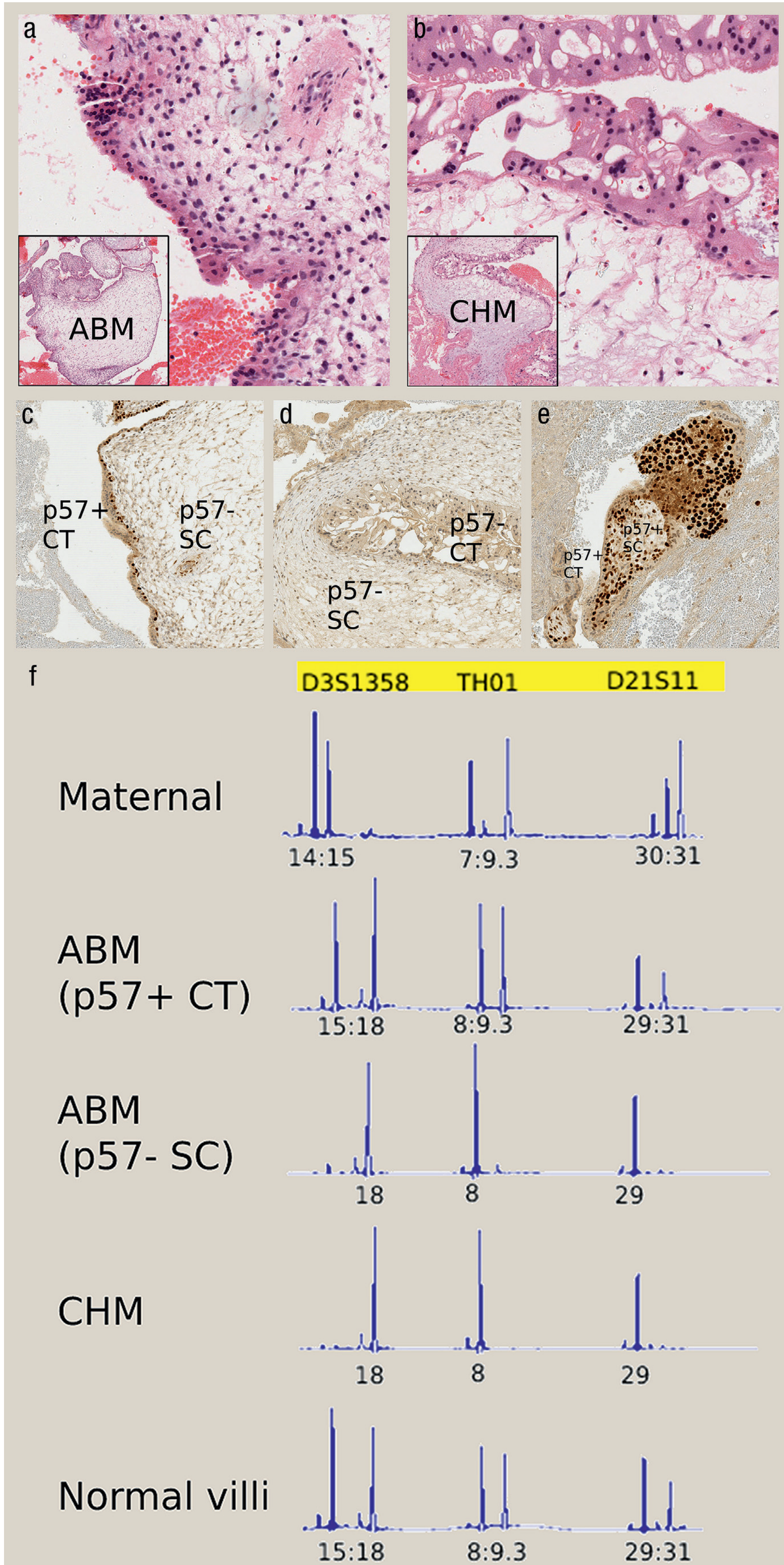
a
ABM
b
CHM
c
p57+
CT
p57-
SC
d
p57-
CT
p57-
SC
e
p57+
SC
p57+
CT
f
D3S1358
TH01
D21S11
Maternal
14:15
7:9.3
30:31
ABM
(p57+ CT)
15:18
8:9.3
29:31
ABM
(p57- SC)
18
8
29
CHM
18
8
29
Normal villi
15:18
8:9.3
29:31

the paternal genome in relation to maternal genome, leading to abnormal imprinting of genes.[1,2] Classical CHM arises when an empty egg is fertilised by a single (monospermic CHM) or two sperms (dispermic CHM)[1,2]; they lack maternal genome and comprise two sets of paternal chromosome complement (abbreviated as PP).[1,2] Most PHMs comprise two sets of paternal and one set of maternal genome (abbreviated as PPM), and therefore, are triploid conceptions.[1] However, rare tetraploid PHMs have been reported, where they comprise three sets of paternal and one set of maternal genome (abbreviated as PPPM).[4] Rarely, recurrent HMs arise from autosomal recessive biallelic maternal mutations in *NLRP7 or KHDC3L*[3]. Pathologic assessment of the products of conceptions (POCs) determines the presence of HM and its subclassification.[4,5] This is crucial for risk stratification since both CHM and PHM carry risk of persistent gestational trophoblastic disease (PGTD) and malignant gestational trophoblastic neoplasia (GTN).[2,4]

Subclassification of HM with morphological features alone can be challenging, particularly subtle PHM and early CHM.[2,4] In addition, hydropic abortus and aneuploid conceptions can also mimic molar features.[2,4] Almost all cases of HM can be resolved by careful examination of the pathological features combined with ploidy determination, and IHC for p57 expression. IHC exploits p57 protein, a cyclin dependent kinase inhibitor encoded by maternally expressed and paternally imprinted *CDKN1C*.[1,4] The p57 expression pattern reflects the genetic makeup of HMs. It is diffusely positive in stromal cells and cytotrophoblasts in PHM due to presence of maternal DNA. It is absent in CHM.[2–5] In cases which have equivocal patterns of p57 expression, indicating mosaicism for an androgenetic component, or where non-molar digynic triploid conception with a single copy of paternal chromosome complement (abbreviated as PMM) is suspected, molecular genotyping may be employed. This will distinguish the HMs from non-molar (with biparental contribution, PM) conceptions and to subclassify HMs (CHM with two sets of paternal chromosome complement – PP or PHM with two sets of paternal chromosome complement and one set of maternal chromosome complement – PPM) which carry an increased risk of GTN compared with non-molar conceptions. Pathologic assessment of potential HMs may require a number of ancillary studies for a definitive diagnosis.

Here we present a rare example of HM with discordant p57 expression within individual villi, referred to as p57-discordant villi (p57DV).[5] p57DV often display overlapping morphological features with CHMs such as stromal karyorrhexis and cistern formation.[5] Differential patterns of p57 expression can exist across villi (p57-divergent), with an associated CHM,[5] as in this case. This supports the usefulness of ancillary molecular studies in proving or refuting the morphological impression, and in identifying subtle early CHMs. Further, molecular genotyping of LMD chorionic villi identified rare ABM. ABMs have been reported to confer risk of PGTD due to the presence of an androgenetic cell line.[6] Patients with ABM, registered with SHMFS, currently follow the CHM clinical follow-up pathway with hCG screening for at least 6 months, regardless of the presence of CHM. Of note, villous trophoblast hyperplasia is striking and often circumferential when the androgenetic cell line is in the cytotrophoblast.[3] In contrast, trophoblast hyperplasia is less apparent or not present when the androgenetic cell line is confined to the villous stroma.[3] Identification and collection of long-term clinical and pathological data of ABM would enable tailored risk stratification and prognostication. The pathologist should be aware of potential diagnostic pitfalls in order to minimise the risk of under-diagnosing CHMs and ABMs. ◆

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Figure 1 H&E, p57^{Kip2} monoclonal antibody (1:400, p57 Ab-6, Thermo Scientific, Fremont) IHC and molecular genotyping by short tandem repeat analysis. a. Low-power (inset) and high-power H&E images of Androgenetic/Biparental Mosaicism (ABM). b. Low-power (inset) and high-power H&E images of Complete Hydatidiform Mole (CHM). c. p57 IHC of ABM. Discordant p57 expression with positive expression in cytotrophoblasts (CT) and absent expression in stromal cells (SC) is appreciated. d. p57 IHC of CHM. There is absent p57 expression in SC and CT. e. p57 IHC of normal chorionic villi. There is positive p57 expression in SC and CT. f. Examples of DNA genotype profiles at microsatellite markers D3S1358, TH01 and D21S11 from maternal decidua and three distinct populations of chorionic villi. Within ABM, p57+ CT show one maternal and one paternal allele at each locus (biparental diploidy) and p57- SC show homozygosity at all alleles with absence of any maternal alleles (paternal isodisomy). CHM demonstrates paternal isodisomy. Normal chorionic villi shows biparental diploidy.

Practice points

- Possible hydatidiform mole (HM) cases are referred to the regional trophoblastic centres in the UK for diagnosis, follow-up and/or treatment.
- Accurate identification of HM is important to stratify the risk of persistent gestational trophoblastic hyperplasia and gestational trophoblastic neoplasia, determining follow-up.
- Multidisciplinary approaches combining morphology with ancillary tests (p57 immunohistochemistry, ploidy analysis and/or genotyping) are important to subclassify HM.
- p57-discordant villi refer to chorionic villi with differential p57 expression in the cytotrophoblasts and villous stromal cells. They can be present with or without complete hydatidiform mole.
- Androgenetic/biparental mosaicism is an example of p57-discordant villi. It is considered to confer a risk of persistent gestational trophoblastic disease due to the presence of an androgenetic cell line.

Self-assessment multiple choice questions

1. Which of the following are features of CHM?

a. Nonpolar circumferential trophoblast hyperplasia
b. Cistern formation
c. Trophoblast pseudoinclusions
d. Nucleated red blood cells
e. a and b

Correct answer: e

2. What is an example of the p57-discordant villi?

a. Coexistence of embryonic material and some enlarged villi with diffusely positive p57 protein expression
b. Enlarged chorionic villi with positive expression of p57 in the cytotrophoblasts and negative expression in the villous stromal cells
c. Variably sized chorionic villi with positive expression of p57 in the stromal cells and positive expression in the cytotrophoblasts
d. Normal sized chorionic villi with negative expression of p57 in cytotrophoblasts and negative expression in the cytotrophoblasts
e. None of the above

Correct answer: b

3. Which of the following confers risk of persistent gestational trophoblastic disease?

a. p57DV with CHM
b. CHM
c. Androgenetic/biparental mosaicism
d. Hydropic abortus
e. a, b and c

Correct answer: e