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Loss of p57 Expression in Conceptions Other Than Complete Hydatidiform Mole:

A Case Series With Emphasis on the Etiology, Genetics, and Clinical Significance

Deyin Xing, MD, PhD[*,†,‡], **Karin Miller, MD**[*], **Katie Beierl, MS**[*], **Brigitte M. Ronnett, MD**[*,†]

[*]Department of Pathology, The Johns Hopkins Medical Institutions, Baltimore, MD

[†]Department of Gynecology and Obstetrics, The Johns Hopkins Medical Institutions, Baltimore, MD

[‡]Department of Oncology, The Johns Hopkins Medical Institutions, Baltimore, MD

Abstract

Combined p57 immunohistochemistry and DNA genotyping refines classification of products of conception specimens into specific types of hydatidiform moles and various nonmolar entities that can simulate them. p57 expression is highly correlated with genotyping and in practice can reliably be used to identify virtually all complete hydatidiform moles (CHM), but aberrant retained or lost p57 expression in rare CHMs and partial hydatidiform moles (PHM), as well as loss in some nonmolar abortuses, has been reported. Among a series of 2329 products of conceptions, we identified 10 cases for which loss of p57 expression was inconsistent with genotyping results (none purely androgenetic). They displayed a spectrum of generally mild abnormal villous morphology but lacked better developed features of CHMs/early CHMs, although some did suggest subtle forms of the latter. For 5 cases, genotyping (4 cases) and/or ancillary testing (1 case) determined a mechanism for the aberrant p57 results. These included 3 PHMs—2 diandric triploid and 1 triandric tetraploid—and 1 nonmolar specimen with loss of p57 expression attributable to partial or complete loss of the maternal copy of chromosome 11 and 1 nonmolar specimen with Beckwith-Wiedemann syndrome. For 5 cases, including 2 diandric triploid PHMs and 3 biparental nonmolar specimens, genotyping did not identify a mechanism, likely due to other genetic alterations which are below the resolution of or not targeted by genotyping. While overdiagnosis of a PHM as a CHM may cause less harm since appropriate follow-up with serum β-human chorionic gonadotropin levels would take place for both diagnoses, this could cause longer than necessary follow-up due to the expectation of a much greater risk of persistent gestational trophoblastic disease for CHM compared with PHM, which would be unfounded for the correct diagnosis of PHM. Overdiagnosis of a nonmolar abortus with loss of p57 expression as a CHM would lead to unnecessary follow-up and restriction on pregnancy attempts for patients with infertility. Genotyping is valuable for addressing discordance between p57 expression and morphology but cannot elucidate certain mechanisms of lost p57 expression. Future studies are warranted to determine whether chromosomal losses or gains, particularly involving imprinted genes such as p57, might play a role in modifying the risk of persistent gestational trophoblastic

Correspondence: Deyin Xing, MD, PhD, Department of Pathology, The Johns Hopkins Hospital, 401 North Broadway, Weinberg 2242, Baltimore, MD 21231 (dxing2@jhmi.edu).

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disease for PHMs and nonmolar conceptions that are not purely androgenetic but have some abnormal paternal imprinting of the type seen in CHMs.

Keywords

genotyping; hydatidiform mole; p57 immunohistochemistry

Distinction of hydatidiform moles from nonmolar specimens and the subclassification of molar pregnancies as complete hydatidiform mole (CHM) versus partial hydatidiform moles (PHM) are important for both clinical practice and investigational studies.[1,2] The risk of persistent gestational trophoblastic disease (GTD) and clinical management differ for CHMs, PHMs, and non-molar specimens,[3–5] illustrated by the fact that the persistent GTD risk following a CHM is 9% to 20%, whereas the risk following a PHM is 0% to 4%.[5–9] Therefore, accurate classification is critical to ascertaining the actual risk of GTD associated with the various subtypes of hydatidiform moles and determining the appropriate nature and duration of clinical follow-up. Both underdiagnosis and overdiagnosis of hydatidiform moles can result in a faulty estimation of the risk of persistent GTD and improper clinical management.

The most common symptom of a molar pregnancy is vaginal bleeding. On imaging, a CHM is characterized by a vesicular ultrasonographic pattern consisting of multiple echoes/holes with no detected fetus, whereas a PHM typically has focal cystic spaces within the placental tissue and can have a fetus.[10] Although not always, hydatidiform moles are commonly associated with elevated human chorionic gonadotropin (hCG) levels above those of normal pregnancy.[10] Histologically, features raising concern for hydatidiform moles include trophoblastic hyperplasia/proliferation, immature chorionic villi with irregular shapes and enlarged size, the presence of trophoblastic inclusions, and villous stromal change.[2] Genetically, CHMs, which are most often diploid and occasionally tetraploid, are purely androgenetic conceptions (only paternal chromosome complements present).[1,2] Familial biparental hydatidiform mole (FBHM) is a pure maternal-effect recessive disorder that has morphologic features of a CHM/early CHM and demonstrates a biparental rather than androgenetic genotype.[11–13] In contrast, PHMs are almost always characterized by diandric triploidy (2 paternal and 1 maternal chromosome complements) but rare examples exhibiting triandric tetraploidy (3 paternal and 1 maternal chromosome complements) have been described.[1,2] Nonmolar abortus specimens are usually characterized by biparental diploidy (1 paternal and 1 maternal chromosome complements with allelic balance [equal allele ratios at informative loci]), but some can be biparental tetraploid, digynic triploid (2 maternal and 1 paternal chromosome complements), or have other genetic abnormalities in the setting of predominantly biparental diploidy, such as trisomy or monosomy.[1,2]

It has been well demonstrated that combined application of p57 immunohistochemistry and short tandem repeat (STR)-DNA genotyping efficiently overcomes morphology-based suboptimal diagnostic reproducibility and refines classification of products of conception (POC) specimens into specific types of hydatidiform moles and various nonmolar entities that can simulate them.[14–20] p57kip2 (p57) is the protein product of the paternally imprinted,

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maternally expressed gene *CDKN1C,* a cyclin-dependent kinase inhibitor located on chromosome 11p15.5.[21–23] Because of lack of maternal DNA and paternal imprinting of the *p57* gene, CHMs, including early forms, lack p57 expression in the villous cytotrophoblast and villous stromal cells; decidua and intermediate trophoblastic cells are positive, serving as internal positive control for an immunostain that relies on a negative result.[1,2] In contrast, PHMs and nonmolar specimens each contain a maternal genomic component from which expression of p57 occurs in villous cytotrophoblast and villous stromal cells, serving to distinguish these entities as a group from CHMs; however, p57 cannot distinguish between PHMs and nonmolar specimens since they both share p57 expression.[1,2]

Molecular genotyping distinguishes CHMs, PHMs, and nonmolar abortuses on the basis of identification of the parental source of polymorphic alleles and their ratios. While studies have established that immunohistochemical analysis of p57 expression is highly correlated with molecular genotyping results, aberrant retained p57 expression in rare examples of CHMs as well as loss of p57 expression in PHMs and nonmolar abortuses have been reported.[24–28] Genotyping can identify cases in which the p57 immunostain does not accurately reflect the true genetic composition of the tissue because of additional genetic alterations, such as chromosomal gains or losses, point mutations, and epigenetic changes, that can affect the p57 gene on chromosome 11 and generate an aberrant p57 result.[26,27] In a recently published updated series of 2217 cases, we found 5 of the 497 PHMs (1%) and 2 of the 900 nonmolar specimens (0.2%) had loss of p57 expression that was contrary to the expected result for the diagnostic category established by the genotyping result.[15] The lack of p57 expression in cases that do not appear to have any morphologic features of a CHM/early CHM and are not confirmed as androgenetic by genotyping, as well as in rare cases that are biparental and p57-negative but also lack the morphology of CHM/early CHM, is an uncommon but problematic issue because molecular genotyping can address some but not all of the possible mechanisms for loss of p57 expression. For example, genotyping can detect loss of the maternal copy of chromosome 11 but other alterations disrupting the p57 gene or affecting p57 expression, for example, point mutations and epigenetic changes, are below the resolution of or not targeted by the genotyping assay. Here we described a series of cases of p57-negative PHMs and nonmolar abortus specimens with emphasis on the etiology, genetics, and clinical significance.

MATERIALS AND METHODS

Case Selection

Archived product of conception cases encountered on the Gynecologic Pathology Consultation and In-house Services of The Johns Hopkins Hospital, Baltimore, MD, from July 2007 through December 2020, were searched for PHMs or nonmolar abortuses that lost p57 expression or contained very limited scattered p57-postive cells in the villous cytotrophoblast and villous stromal cells, with internal positive control in decidua and/or intermediate trophoblast to assure that the immunostaining technique was optimal. Among a series of 2329 product of conception specimens, 10 cases were identified; 7 of these were briefly described in our recent large series[15] and 3 were encountered on our consultation service since that series was collected.

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Clinicopathologic Features

The patients' age, gestational history, clinical presentations, serum β-human chorionic gonadotropin (β-hCG) levels, and ancillary studies, if any, were retrieved and reviewed. Archived H&E slides for each case were reviewed by 2 pathologists (D.X. and B.M.R.) and the following morphologic features were assessed: hydropic changes, variable villous size (2 villous populations), irregular villous shapes, trophoblastic hyperplasia, trophoblastic inclusions, cisterns, villous stromal hypercellularity, nucleated red blood cells, karyorrhectic nuclear debris, and fetal tissue.

p57 immunohistochemistry.—Immunohistochemical analysis of p57 expression was performed using a mouse monoclonal antibody against p57 protein (predilute, Neomarkers, Fremont, CA), as described previously. Briefly, the presence or absence of nuclear positivity of p57 was assessed in villous stromal cells, cytotrophoblast, intermediate trophoblast, and maternal decidua. The p57 immunostain was interpreted as negative if villous stromal cells and cytotrophoblast were either entirely negative or demonstrated only limited expression (nuclear staining in <10% of these cell types) with satisfactory expression in maternal decidua and/or intermediate trophoblastic cells as internal positive control. The cases for which p57 staining results could not be interpreted due to extensive necrosis/degenerative changes leading to loss of immunoreactivity, technical failure, or a negative preparation lacking internal positive control, were excluded from this study.

Molecular genotyping.—The polymerase chain reaction (PCR)-based STR analysis has been described in detail previously.[14,15,18] Initially (2007 to 2013), PCR amplification of 9 STR loci (AmpFlSTR Profiler kit; Applied Biosystems, Foster City, CA) from 8 different chromosomes (chromosomes 2, 3, 4, 5, 7, 11, 12, 13) and the amelogenin locus (for XY determination) was performed. An expanded analysis with PCR amplification of 15 STR loci from 13 different chromosomes (chromosomes 2, 3, 4, 5, 7, 8, 11, 12, 13, 16, 18, 19, 21) and the amelogenin locus (for XY determination) (AmpFlSTR Identifier kit; Applied Biosystems) replaced the initial 9-marker panel analysis since 2013. To more completely investigate chromosome 11, 7 additional microsatellite (STR) markers mapping to chromosome 11 (spanning 11p15.5 [D11S1984 and THO1] to 11q22.3 [D11S2000]) were analyzed in villous tissue in 2 cases (case 1 and case 7). Interpretation of the molecular genotyping result was described in detail previously.[14,15,18]

Other ancillary tests.—Additional analyses, including single nucleotide polymorphism array (case 2), fluorescence in situ hybridization (FISH, case 3 and case 7), DNA ploidy analysis (case 4), cytogenetics/karyotyping (case 7), quantitative fluorescence-PCR (case 8), and genetic testing (fetal blood) for allele-specific methylation (case 10), were provided in the accompanying pathology reports (consultation cases) or obtained through follow-up with the contributing institution/pathologist and were incorporated into the final interpretation of the cases as appropriate.

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RESULTS

Clinical and molecular features are summarized in Table 1 and histopathologic features are summarized in Table 2. The patients with PHMs (4 diandric triploidy and 1 triandric tetraploidy) that displayed a negative p57 immunostaining ranged in age from 23 to 39 years (mean, 34; median, 37). The 4 nonmolar hydropic abortuses with some degree of abnormal villous morphology and loss of p57 expression were identified in patients ranging in age from 27 to 36 (mean, 33; median, 34). The last case (case 10) was a 30-year-old woman with placental mesenchymal dysplasia (PMD) for which p57 was overwhelmingly negative and the delivered baby had Beckwith-Wiedemann syndrome (BWS).

Loss of p57 Expression in PHMs Due to Partial or Complete Loss of Maternal Chromosome 11

In this series, 3 cases of PHMs lacking p57 expression were due to partial or complete loss of maternal chromosome 11. Case 1 has been reported previously and we re-reviewed the slides retrieved from our consultation archives. Briefly, the patient was a 37-year-old woman (gravidity 2, parity 1) who presented with 10 weeks of amenorrhea and a subsequent serum β-hCG test was 13,028 mIU/mL. A follow-up sonogram demonstrated a “starry sky/snowstorm” heterogenous echogenic pattern characteristic of a molar pregnancy. Case 2 was a 38-year-old woman who presented with clinical suspicion of molar pregnancy. She had 2 previous successful pregnancies with no history of abortion or molar pregnancy. Case 3 was a 23-year-old patient who presented with vaginal bleeding at ~7 weeks of gestation with a serum hCG of 1518 mIU/ML, which was decreased from 2809 mIU/mL.

Histologically, both case 1 and case 2 exhibited some morphologic features suspicious but not fully diagnostic for an early CHM. The morphologic features of case 1 have been described previously.[25] Case 2 displayed features similar to that of case 1: some hydropically enlarged villi had trophoblastic inclusions but lacked trophoblastic hyperplasia (Fig. 1A); some villi displayed bulbous cauliflower-like villi (Fig. 1B), cellular villous stroma (Fig. 1C), and focal trophoblastic hyperplasia (Fig. 1D), and some small villi showed karyorrhectic debris (Fig. 1E). The immature chorionic villi in case 3 exhibited variable villous size, hydropic changes, and karyorrhectic nuclear debris.

Immunohistochemically, the villous cytotrophoblast and villous stromal cells in both case 1 (3 blocks analyzed at our institution) and case 2 (both outside immunostain and repeat immunostain at our institution) were negative for p57 (Fig. 1F). For case 3, immunohistochemical analysis of p57 expression (2 blocks) demonstrated that the villous cytotrophoblast and villous stromal cells were overwhelmingly negative for p57, but very focal expression was identified in these cell types (< 5% of cells). All 3 cases showed positive p57 staining in decidua and/or intermediate trophoblast as internal positive control. The morphology combined with loss of p57 expression was suggestive of subtle forms of early CHMs in these cases. Case 1 was initially interpreted as such, with genotyping pending to confirm that impression based on our early approach to genotyping all cases before using a negative p57 immunohistochemical result alone to finalize a diagnosis of a CHM/early CHM.[16] However, when the genotyping result of diandric triploidy was obtained, further investigation was undertaken to address the unexpected loss of p57

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expression for that genotyping result. The marker on chromosome 11p in the genotyping kit (THO1 locus) and additional markers on chromosome 11 demonstrated loss of the maternal allele as the explanation for the negative p57 result.[25] In case 2, there was concern for an early CHM based on a negative p57 immunostain but some confusion related to an ancillary single nucleotide polymorphism array analysis performed by the outside institution, which was reported as showing "digynic triploidy with disomy for chromosome 11 (68 XXY, −11), with loss of the paternal copy of chromosome 11." However, analysis of DNA polymorphic markers (microsatellites) at our institution demonstrated diandric triploidy, rather than digynic triploidy, with loss of the maternal allele at a locus on chromosome 11p (THO1 locus) providing an explanation for the loss of p57 expression (Fig. 2). Molecular genotyping for case 3 showed the presence of an excess of nonmaternal (obligate paternal) alleles compared with maternal alleles, as well as the presence of contribution from at least 2 sperm (some loci with 2 obligate paternal alleles), with allele patterns and ratios at most informative loci best explained as triandric tetraploidy. However, of note, 1 exception was the locus on chromosome 11p (THO1 locus) where there were only 2 allele peaks shared between villous and decidual tissue with ratios in conjunction with the loss of p57 expression best explained as loss of the maternal allele at that locus (material not available for testing with other markers to confirm this). This assessment of allele ratios as most consistent with tetraploidy was confirmed by FISH analysis which demonstrated 4 signals in cells (XXYY; outside laboratory data not available to show) (see Murphy et al[29] for an example of genotyping data for tetraploid PHM).

Loss of p57 Expression in PHMs Due to Unknown Mechanism

There were 2 PHMs with loss of p57 expression identified, but the underlying mechanisms remained unclear. Case 4 was a 39-year-old G2P1 woman with missed abortion at 7 weeks of gestation with a serum hCG of 72,258 mIU/ML. By outside report, DNA ploidy analysis demonstrated evidence of triploidy (DNA index of 1.47). The submitted immunohistochemical stains for p57 (4 blocks) and repeat p57 stains (2 blocks) at our institution demonstrated that the villous cytotrophoblast and villous stromal cells were negative for p57 with internal positive control in decidua and/or intermediate trophoblast. Case 5 was a 32-year-old woman with missed abortion clinically suspicious for partial molar pregnancy. Similar to case 4, the villous cytotrophoblast and villous stromal cells in case 5 were essentially negative for p57. The tissue in both case 4 and case 5 displayed morphologic alterations suggesting a PHM rather than a CHM. Analysis of DNA polymorphic markers (microsatellites) in both cases demonstrated that the DNA pattern from villous tissue was most consistent with diandric triploidy. Of note, the micro-satellite (STR) marker (THO1) on chromosome 11 revealed a DNA pattern also consistent with diandric triploidy in both cases, thus arguing against loss of the whole maternal copy or (based on the location of this marker) against loss of the entire 11p arm of this chromosome (data not shown). Thus, a mechanism was not identified by genotyping.

Loss of p57 Expression in Nonmolar Abortus Specimen Due to Partial or Complete Loss of Maternal Chromosome 11

Case 6 was a 35-year-old woman with missed abortion. Morphologically, some hydropically enlarged villi displayed irregular shapes (Fig. 3A), trophoblastic inclusion (Fig. 3B), and

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focal trophoblastic hyperplasia (Fig. 3C). Some villi showed karyorrhectic debris (Fig. 3D) and nucleated red blood cells (Fig. 3E). These features can suggest the possibility of a subtle form of early CHM but some non-molar abortuses with abnormal villous morphology due to other (non-molar type) genetic alterations can share these features. The villous cytotrophoblast and villous stromal cells were completely negative for p57 with optimal internal positive control (Fig. 3F). Molecular genotyping demonstrated that the DNA pattern from villous tissue was most consistent with a biparental conception with allelic balance at most but not all loci, indicating a nonmolar abortus. Of note, the allele pattern for the locus on chromosome 11 demonstrated only a nonmaternal allele compatible with paternal monosomy 11 and the allele pattern for the locus on chromosome 4 was compatible with trisomy 4 with an additional paternal chromosome (paternal:maternal allele ratio 2:1) (Fig. 4). Thus, either partial or complete loss of the maternal copy of chromosome 11 was the explanation for the loss of p57 expression.

Loss of p57 Expression in Nonmolar Abortus Specimens Due to Unknown Mechanism

We identified 3 cases of nonmolar abortus specimens lacking p57 expression but the mechanisms related to this observation were unclear. All 3 cases displayed some degree of abnormal villous morphology but not having features of CHM/early CHM. Case 7 was a 27-year-old woman with intrauterine fetal demise and cystic spaces noted in the placenta. The p57 immunostains (multiple blocks, outside institution) and repeat p57 immunostains (3 blocks at our institution) demonstrated loss of p57 expression in the villous cytotrophoblast and villous stromal cells, with adequate internal positive control. By report, conventional cytogenetic analysis demonstrated a 46,XY karyotype and FISH analysis of villous tissue demonstrated a normal male XY pattern without evidence of trisomy of chromosomes 13, 16, 18, and 21. Case 8 was a 32-year-old woman (gravidity 3, parity 1, abortion 2) who presented with missed abortion (suspicious for molar pregnancy) at 5 to 6 weeks of gestation. Similar to case 7, submitted (several blocks) and repeat p57 immunostains (2 blocks at our institution) demonstrated that the villous cytotrophoblast and villous stromal cells lacked p57 expression, with adequate internal positive control. By report, quantitative fluorescence-PCR demonstrated no numerical abnormalities in chromosomes 13, 18, or 21 and STR genotyping by an outside institution demonstrated a biparental genotype. Case 9 was a 36-year-old woman with first trimester ultrasound showing features suspicious for a PHM. Histologically, the villi were hydropically enlarged, and alternating villi showed stromal hypercellularity and hypocellularity without appreciable trophoblastic hyperplasia. Immunohistochemical analysis of p57 expression performed on multiple blocks at our institution revealed loss of p57 expression in the villous cytotrophoblast and villous stromal cells, with adequate internal positive control.

Analysis of DNA polymorphic markers (microsatellites) for case 7 demonstrated that the DNA pattern from both villous tissue and fetal tissue was most consistent with a biparental conception. Of note, the microsatellite (STR) marker (THO1) on chromosome 11p revealed a DNA pattern consistent with diploidy but was not informative as to the parent of origin (both alleles shared between villous and maternal decidual tissue so the parental origin could not be discerned). To more completely investigate chromosome 11, 7 additional microsatellite (STR) markers mapping to chromosome 11 (spanning 11p15.5 to 11q22.3)

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were analyzed in villous tissue. Of these 7 markers, 6 revealed a pattern consistent with diploidy. Of the 6 diploid STRs, 4 were consistent with a biparental pattern while 2 were not informative as to the parent of origin (both alleles shared between villous and decidual tissue), and the most distal 11p15 STR studied (STR D11S1984 which maps ~1.3 Mb distal to p57) was not informative as to ploidy or the parent of origin (data not shown). Thus, the analysis did not identify a potential mechanism for the p57 loss. While for case 7 a familial biparental very early CHM might not be absolutely excluded, particularly since there was no pregnancy history available, there was no morphology to favor that rare entity. Molecular genotyping for both case 8 and case 9 demonstrated biparental conceptions, indicating non-molar gestations. In particular, the microsatellite (STR) marker (THO1) on chromosome 11p revealed a DNA pattern consistent with biparental diploidy without gain or loss in both cases. The pregnancy histories and lack of morphology to favor CHMs/early CHMs argued against the rare possibility of FBHMs.

Loss of p57 Expression in a Placenta With PMD Due to BWS

Case 10 was an unusual case. The patient was a 30-year-old woman who delivered a baby with multiple anomalies at an approximate gestational age of 31 weeks and 2 days. The placenta was characterized by 2 morphologically distinct villous components (Fig. 5A): 1 consisted of small villi appropriate for the reported gestational age (Fig. 5B); the other consisted of markedly enlarged villi with mildly cellular myxoid stroma but lacking evident trophoblastic hyperplasia (Figs. 5C, D). A submitted immunostain for p57 demonstrated that these abnormally enlarged villi had only focal/patchy p57 expression in some cytotrophoblastic cells, with most of these cells and all of the stromal cells being negative. The morphology and this pattern of expression suggested PMD attributable to androgenetic/biparental mosaicism (p57-negative cells are the androgenetic population and p57-positive cells are the biparental population). To further evaluate this case, immunohistochemical analysis of p57 expression was repeated at our institution (3 blocks). The abnormally enlarged villi were confirmed to be overwhelmingly negative for p57, with only focal/patchy p57 expression in some cytotrophoblastic cells and complete loss of expression in stromal cells, with appropriate internal positive control (Fig. 5E). Normal placental tissue displays a similar p57 staining pattern (Fig. 5F).

Analysis of DNA polymorphic markers (microsatellites) in 4 different areas of villous tissue demonstrated 2 patterns in 2 populations of villi: 2 areas with small, normal-appearing villi demonstrated a biparental pattern with allelic balance; 2 areas with enlarged villi demonstrated allele patterns indicating an excess of paternal alleles compared with maternal alleles (paternal: maternal allele ratios > 2:1), with some variability in the 2 different areas analyzed (Fig. 6). These results for the enlarged villi were best explained as a mixture of 2 genetically distinct cell populations, 1 androgenetic and the other biparental. Thus, the combined findings (morphology, p57 expression pattern, and genotyping data) were most consistent with PMD related to androgenetic/biparental mosaicism. Further ancillary analysis using fetal blood at an outside laboratory demonstrated abnormal allele-specific methylation of loci in the 11p15.5 chromosomal region consistent with uniparental disomy or abnormal imprinting affecting these loci, supporting a diagnosis of BWS.

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DISCUSSION

The ready availability of p57 immunohistochemistry and advocacy of using a low threshold to apply this ancillary technique to POC specimens with any morphologic alterations that might suggest the possibility of a hydatidiform mole, and in particular subtle early CHMs since it is the CHMs that are targeted by this assay, have led to increased use of the p57 immunostain in routine practice for a good decade now.[1,2] As a result, practicing pathologists are encountering occasional cases for which there appears to be some concern for addressing the possibility of an early CHM, leading to application of a p57 immunostain, yet some reluctance to accept a negative p57 result as diagnostic in the setting of subtle morphologic alterations, leading to consultation to address this situation. This is evidenced by the cases presented herein, with 6 of 10 cases accompanied by a p57 immunostain that had been performed by the contributing laboratory. While our large prospective analysis demonstrates that the finding of nonmolar POC specimens with loss of p57 expression is rare overall, this is an important subset of cases to recognize.[15] If practicing pathologists perform the p57 immunohistochemical triage step we advocate to evaluate for a possible subtle early CHM and then accept that negative result as diagnostic, there is some chance that an occasional peculiar case of a nonmolar abortus with some abnormal villous morphology and some other genetic mechanism for p57 loss will be interpreted as an early CHM. While rare, overdiagnosis as an early CHM is clinically significant. That diagnosis will lead to unnecessary clinical follow-up with serum HCG levels and contraception, which incur cost and are undesirable for infertility patients, and labels a patient as having an entity with significant risk for persistent GTD, which is erroneous. Despite the rarity, we believe it is useful to draw attention to this subset of cases for the aforementioned reasons. A more general assessment of the approach to using these techniques in a large series of prospectively collected cases, summary analysis of over 2000 cases, and general reviews are beyond the scope of this study and have been provided in our prior publications to which the reader is referred.[1,2,14–16,30]

Our first observation of aberrant loss of p57 expression in a conception that was not a CHM was a unique example of a diandric triploid PHM with lack of p57 expression attributable to loss of the maternal copy of chromosome 11 for which the morphology and p57 expression pattern simulated an early CHM, but molecular genotyping was most consistent with classification as a PHM.[25] Since that report, we have encountered several additional examples of PHMs as well as nonmolar abortus specimens with loss of p57 expression. In the current study, we provide a summary of clinicopathologic and molecular data of 10 cases, with emphasis on assessment of their etiology and genetic mechanisms.

In this series, we identified 4 cases (3 PHMs and 1 nonmolar abortus) for which lack of p57 expression was attributable to loss of at least that portion of the maternal copy of chromosome 11 where the *p57* gene is located and from which p57 expression is derived. It can be speculated that this loss can involve either the entirety or a portion of chromosome 11. In fact, 8 STR markers on chromosome 11, physically located on chromosome 11 from 11p15.5 (D11S1984 and THO1) to 11q22.3 (D11S2000) were assessed in case 1, and molecular genotyping results were overall consistent with 2 paternal alleles, suggesting that most, if not all, of the maternal chromosome 11 was lost in this case.[25] In contrast, only 1

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locus (THO1) on chromosome 11 was assessed in 3 cases (cases 2, 3, and 6); thus whether the negative p57 immunostaining results were due to partial or entire loss of chromosome 11 remains unclear for those cases.

Although not found in our cases, regional or complete paternal uniparental disomy affecting chromosome 11 may account for lost expression of p57 in a subset of cases.[26,27] Uniparental disomy is an abnormal genetic condition defined as 2 copies of a chromosome or part of a chromosome derived from 1 parent and no homologous copy from the other parent. Uniparental disomy arises through meiotic nondisjunction with mitotic correction or gametic complementation when 2 members of a chromosome pair to separate into 2 daughter cells but fail to do so.[31] It has been demonstrated that, as a mechanism for human genetic disease, uniparental disomy is associated with certain genetic syndromes, such as Angelman syndrome, Prader-Willi syndrome, and BWS, when it involves chromosomes that carry imprinted, differentially expressed genes depending on their parental origin.[32,33] Several cases of gestations characterized by abnormal villous morphology mimicking a hydatidiform mole and negative p57 staining of villous cytotrophoblast and stroma were found to contain paternal uniparental disomy at the locus involving p57.[26,27] In theory, paternal uniparental disomy and loss of maternal chromosome 11 lead to loss of p57 expression, however, there are some exceptions. In fact, some cases with this genotype can display retained p57 expression in cytotrophoblastic cells of the small fibrotic villi but loss of expression in enlarged hydropic villi, or even normal p57 expression in all villi.[26] The underlying mechanisms for this phenomenon remain unknown but epigenetic relaxation of imprinted genes including p57 may be a possible explanation. We have observed, on rare occasions, that very limited p57 nuclear staining can be seen in the villous cytotrophoblast and villous stromal cells of CHMs, usually <10% of these cell types, but this degree of expression is allowed and such cases have been confirmed as androgenetic CHMs in our experiences.[14,15] Likewise, although there is no maternal genetic complement for p57 in our case 3 (loss of maternal chromosome 11 in triandric tetraploidy), immunohistochemical analysis of p57 expression for that case still demonstrated very focal expression (< 5%) in the villous cytotrophoblast and villous stromal cells in an overwhelmingly negative background. We suspect epigenetic imprinting relaxation may also explain this observation.

Other alterations, for example, point mutations and epigenetic changes, can also lead to loss of p57 expression in POC specimens. One entity included in our series (case 10) is BWS, a disorder associated with various epigenetic and/or genetic alterations that dysregulate the imprinted genes on chromosome 11p15.[34–36] BWS is characterized by tissue and organ hyperplasia (organomegaly), developmental anomalies (abdominal wall defects, renal malformations, cleft palate) and an increased risk of childhood tumors (Wilms tumor, hepatoblastoma, neuroblastoma and rhabdomyosarcoma).[37] It has been well-established that a subset of BWS has abnormal placentas in the form of PMD.[38] PMD is characterized by placentomegaly and grape-like vesicles that can resemble a molar pregnancy.[39,40] In fact, PMD can be potentially misdiagnosed as a PHM, particularly when a fetus is present, and is also known as “pseudo-partial mole.”[41–43] Case 10 in this series displayed features of PMD characterized by markedly enlarged villi with mildly cellular myxoid stroma but lacking evidence of trophoblastic hyperplasia. However, this morphology also raised concern for a PHM in a twin gestation given that it was a near-term pregnancy comprised of placental

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tissue with 2 discrete populations of villous tissue, namely, hydropic and normal-appearing, with a fetus. It is also possible that when a fetus is not present, as in earlier cases, this could even simulate a CHM in a twin gestation, although the lack of trophoblastic hyperplasia would make that less likely. While the villi in PMD associated with androgenetic/biparental mosaicism usually display a discordant p57 expression pattern, commonly positive staining in cytotrophoblast and negative staining in villous stromal cells,[44] our case 10 showed virtually near total loss of p57 expression, illustrated by only focal/patchy p57 expression in some cytotrophoblastic cells and complete loss of expression in stromal cells. Subsequent molecular genotyping and other ancillary testing for confirmation of a diagnosis of BWS with associated PMD provided a plausible explanation, with the alteration involving the region of the p57 gene likely accounting for loss of p57 expression through a genetic or epigenetic mechanism. We believe it is important to distinguish PMD from molar twin pregnancies because these entities are genetically and biologically distinct.

Several mechanisms including epigenetic and genetic alterations have been linked to BWS, with the most common etiology being epigenetic loss of maternal allele-specific methylation of the more centromeric imprinting center on 11p15.5, occurring in 50% of BWS cases.[38,45] As a result, the methylation status of the maternal allele simulates that of the imprinted paternal allele, leading to loss of p57 expression. An estimated 20% to 25% of BWS cases are due to paternal uniparental disomy of chromosome 11, as we discussed above, resulting in an imbalanced paternal and maternal gene imprinting and decreased or lost p57 expression. In contrast, the genetic causes of BWS, including maternal *CDKN1C/p57* gene mutations or duplications, inversions or translocations within 11p15.5 region, are less frequent than the epigenetic causes.[38,46,47] While some studies demonstrated that the majority of BWS-unrelated PMD have been associated with androgenetic/biparental mosaicism and BWS-related PMD have been associated with paternal uniparental disomy, our case represents an example of androgenetic/biparental mosaicism as well as abnormal allele-specific methylation affecting 11p.15.5 region.

Of 10 cases in this series, the mechanisms for loss of p57 expression could not be determined in 5 cases, including 2 PHMs and 3 nonmolar abortuses. Molecular genotyping for these cases demonstrated a retained maternal copy of chromosome 11 based on the locus on 11p (THO1), indicating other mechanisms than loss of the maternal copy are associated with loss of p57 expression. The other genetic alterations mentioned above, which can affect the *CDKN1C/p57* gene and p57 expression, are not assessed by the genotyping assay used to evaluate these cases. In fact, standardized diagnostic criteria for PMD in early pregnancy specimens are not well studied or established and one could imagine 3 of the nonmolar abortus cases (cases 7, 8, and 9) have some possibility of representing this entity, although this is purely speculative in the absence of other analysis.

p57 expression is finely regulated in a tissue-specific manner. In addition to promoter methylation, various transcription factors (activators and repressors), micro-RNAs, long noncoding RNAs, and RNA modifications have been found to involve regulation of p57 expression. Genetically, 2% to 3% of cases with loss of p57 expression are due to chromosomal rearrangements in this region, including extensive paternal duplications (1%), translocations/inversion (1%), and microdeletions (< 1%).[36,48] It has also been reported that

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pathogenic mutations in the maternal *CDKN1C/p57* allele are identified in 5% to 8% of sporadic, and up to 50% of familial BWS cases.[48] The vast majority of these mutations are either nonsense or insertion/deletion mutations that lead to truncation of the protein or strongly alter the structure of the protein. All of the above-mentioned mechanisms can result in a diminished or negative p57 immunohistochemical staining result but, unfortunately, cannot be assessed by STR-based molecular genotyping. One limitation of our study is that, while these consultation cases were genotyped prospectively, we collected them retrospectively once we had a series of problematic cases with unexpected p57 results and at that point did not have sufficient DNA or paraffin blocks available for additional investigation of other mechanisms for loss of p57 expression.

According to the diagnostic algorithm we proposed previously, p57 immunohistochemistry is used to triage cases for molecular genotyping: all CHMs are diagnosed as such when appropriate morphology is accompanied by a negative p57 result without subsequent genotyping; all p57-positive specimens are subjected to genotyping to correctly diagnose and distinguish PHMs and nonmolar abortuses.[16] In our experiences, p57 immunohistochemistry is highly reliable for diagnosis of CHMs, including the early forms; however, molecular genotyping is required to refine the diagnosis in certain uncommon situations,[14,15] and in particular, those described in this study. In fact, of 5 molecular genotyping-confirmed PHMs with negative p57 expression, 2 cases exhibited some subtle morphology suggestive of early CHMs and 3 cases had features of PHMs. These cases would have all been interpreted as CHMs had genotyping not been pursued. This is a particular pitfall for the rare triandric tetraploid PHM with loss of the maternal copy of chromosome 11, as the finding of tetraploidy and loss of p57 expression in a conception with molar features would usually overwhelmingly favor a CHM over a PHM. While underdiagnosis of a CHM as a PHM is significant in that it would underestimate the potential risk of persistent GTD and might lead to shorter than desirable follow-up, overdiagnosis of a PHM as a CHM/early CHM may cause less harm since appropriate follow-up with serum β-hCG levels would take place for both diagnoses. However, this could cause longer than necessary follow-up and excessive anxiety due to the expectation of a much greater risk of persistent GTD for CHM compared with PHM, which would be unfounded for the correct diagnosis of a PHM. Overdiagnosis of a nonmolar abortus with loss of p57 expression as a CHM/early CHM would also lead to unnecessary follow-up and restriction on pregnancy attempts for patients with infertility.

Loss of p57 expression in cases that do not appear to be a CHM/early CHM and have biparental genotyping results is extremely uncommon but can be problematic for accurate diagnosis of gestational specimens. Loss of p57 expression in a biparental conception can occur in FBHM, but these are rare and are expected to have the morphology of a CHM/early CHM. FBHM is a pure maternal-effect recessive disorder resulting, in most cases, from mutations of *NLRP7* or *C6orf221*.[11–13,49,50] We have previously described 4 patients with molar specimens that were consistent with FBHMs based on morphology, negative p57 immunostains, and biparental DNA patterns per genotyping.[15] These cases highlight the importance of correlating morphology, p57 results, and genotyping data to avoid misinterpretation of FBHM cases as nonmolar conceptions based on the biparental genotyping results, which is important for patient management. Unlike FBHM, the cases

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with loss of p57 expression and biparental origin in this series did not display typical features of CHM or early CHM, although the morphology of the latter can be subtle at times. Moreover, 4 of 5 cases contained nucleated fetal red blood cells in the villous stroma, a finding that usually argues against a CHM except in some rare situations such as mosaic/chimeric conceptions and a twin pregnancy with coexistence of a CHM and a normal conception. Thus, interpretation as non-molar abortuses with some abnormal villous morphology would be favored for these cases, provided that the clinical setting and other evaluation excludes the rare FBHMs which are less likely based on morphologic features and the pregnancy histories in most of these cases. These examples of loss of p57 expression in POC specimens lacking better developed features of a CHM/early CHM are clearly uncommon but emphasize the need to carefully correlate morphology with p57 results and adopt a low threshold for employing molecular genotyping.

Interestingly, 1 case (case 6) showed features equivocal for a subtle form of hydatidiform mole or nonmolar abortus with abnormal villous morphology but immunohistochemically lost p57 expression, thus raising specific concern for a subtle form of early CHM. Molecular genotyping revealed a biparental conception with paternal monosomy 11 (partial or complete loss of maternal chromosome 11) and trisomy 4 of paternal origin (extra paternal copy). Abnormal villous morphology in nonmolar abortus specimens can be associated with other genetic abnormalities, such as trisomy(ies) and monosomy.[15,27,51,52] Our previous study demonstrated that trisomy 4 is a relatively rare genetic alteration in nonmolar specimens, with only 3 such cases identified among 147 cases with trisomy/trisomies.[15] Since the morphologic abnormalities of the villi of hydatidiform moles is influenced by the number of paternal chromosome complements, it seems reasonable to speculate that some degree of abnormal villous morphology that can simulate a hydatidiform mole might be attributable to some imbalance in the maternal:paternal chromosome complements, in this case due to a paternal monosomy and a paternal trisomy (loss of maternal chromosome 11 with associated loss of p57 expression and gain of paternal chromosome 4). We have observed this in a prior case of a triple trisomy of paternal origin that simulated a PHM.[51] Others have described examples with single or multiple trisomies —not all specified or informative regarding parental source—which showed variable p57 expression and displayed abnormal villous morphology simulating hydatidiform moles. Of note among these were 1 case with at least 2 excess paternal chromosomes and uniparental disomy of chromosome 11 and loss of p57 expression and another with single trisomy (undetermined parental source) and uniparental isodisomy of chromosome 11 yet retained p57 expression.[26,27]

As a putative tumor suppressor gene, p57 is thought to play a vital role in regulating trophoblastic proliferation in molar pregnancies.[53] In theory, it can be reasoned that the loss of p57 expression in PHMs and nonmolar abortuses might result in a biological behavior more similar to a CHM that characteristically demonstrates loss of p57 expression in villous cytotrophoblast and stromal cells as a result of their paternal-only genome. In fact, a recent case series reported that a biparental conception with paternal uniparental isodisomy of chromosome 11 developed persistent GTD that required chemotherapy.[26] One limitation of our study is lack of follow-up information since all these cases were collected from our consultation service. Thus, whether loss of p57 expression in these

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cases, either due to partial or complete loss of maternal chromosome 11 or other unknown mechanism, affects their potential for persistent GTD is unknown. The development of hydatidiform moles and their malignant potential appear to be driven by the unbalanced expression of imprinted genes associated with an excess of paternally derived chromosome complements. At the whole-genome level, ~100 to 200 imprinted genes are expressed in a parent-of-origin-specific manner.[54] As such, loss or gain of 1 or only a few chromosomes, including chromosome 11, may not be sufficient to always confer the biological behavior of a molar pregnancy, but as the above mentioned case indicates persistent GTD is possible in this situation. Nevertheless, additional study of this issue surely seems worthwhile, but probably only in the context of more significant patient follow-up and a larger number of cases to truly clarify the clinical significance of this unique phenomenon.

In summary, the current case series demonstrates some known and other yet unknown mechanisms for loss of p57 expression in PHMs and nonmolar gestations, including loss of the entirety or a portion of the maternal copy of chromosome 11 and BWS. The negative p57 results in cases that do not appear to be a CHM/early CHM and have biparental genotyping results is an uncommon but problematic issue because molecular genotyping does not address many/most of the possible mechanisms for these results. This study also illustrates the importance of combined assessment of morphology, p57 immunohistochemistry, and STR genotyping to evaluate all POC specimens for which there is a consideration of a diagnosis of a hydatidiform mole but for which some discordant features between p57 expression and morphology are noted. Future studies are warranted to determine whether chromosomal losses or gains, particularly involving imprinted genes such as p57, might play a role in modifying the risk of persistent GTD for PHMs and nonmolar conceptions that are not purely androgenetic but have some/additional abnormal paternal imprinting of the type seen in CHMs.

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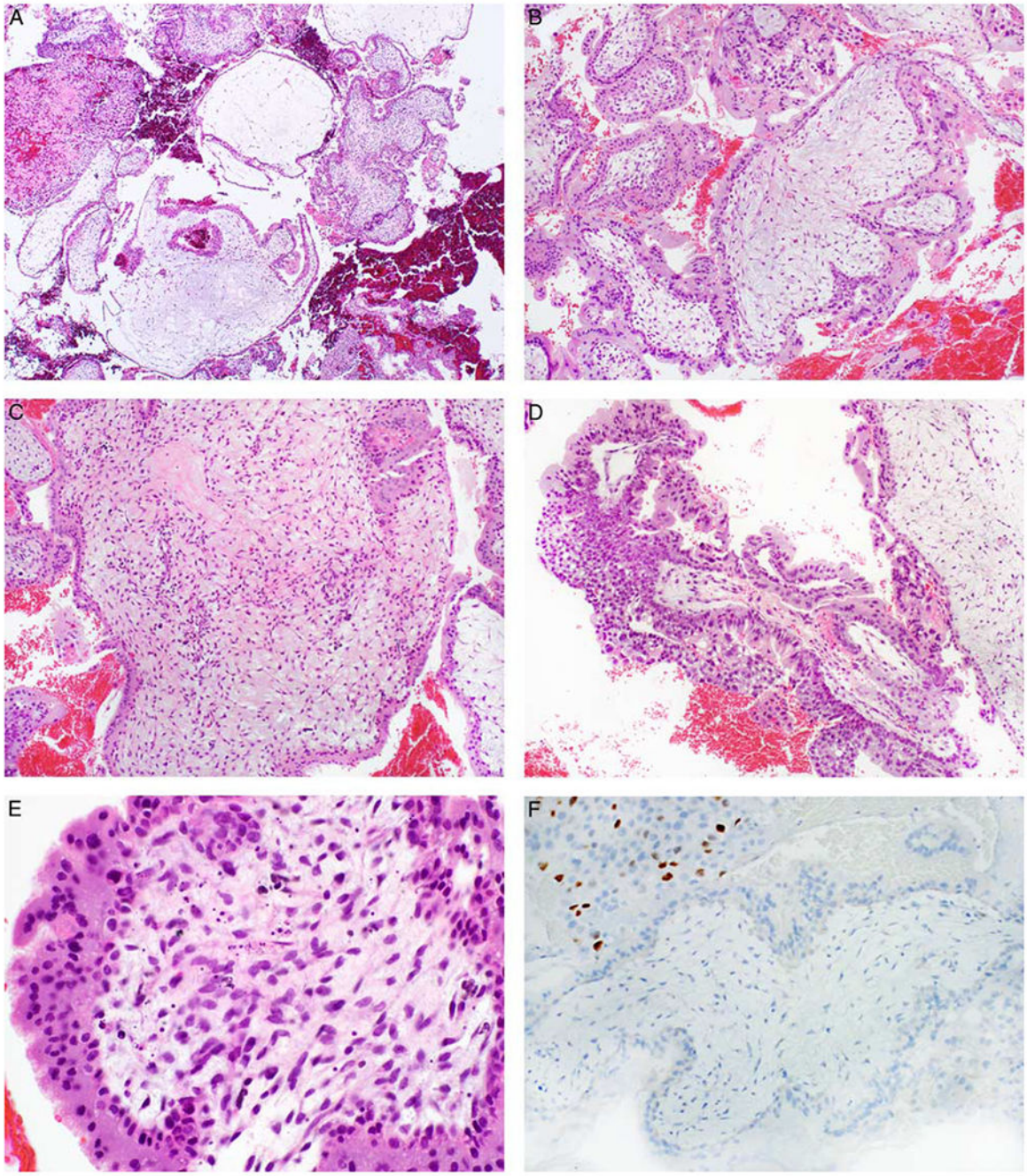

FIGURE 1.

Loss of p57 expression in PHM (case 2). A, Some hydropically enlarged villi have trophoblastic inclusions but lack trophoblastic hyperplasia. B–E, Some villi display bulbous cauliflower-like villi (B), cellular villous stroma (C), trophoblastic hyperplasia (D), and some small villi show karyorrhectic debris (E). F, Loss of p57 expression in villous cytotrophoblast and stromal cells (internal positive control in intermediate trophoblastic cells). The morphology combined with loss of p57 expression simulates a very early CHM; however, genotyping supports interpretation as a PHM (see text and Fig. 2).

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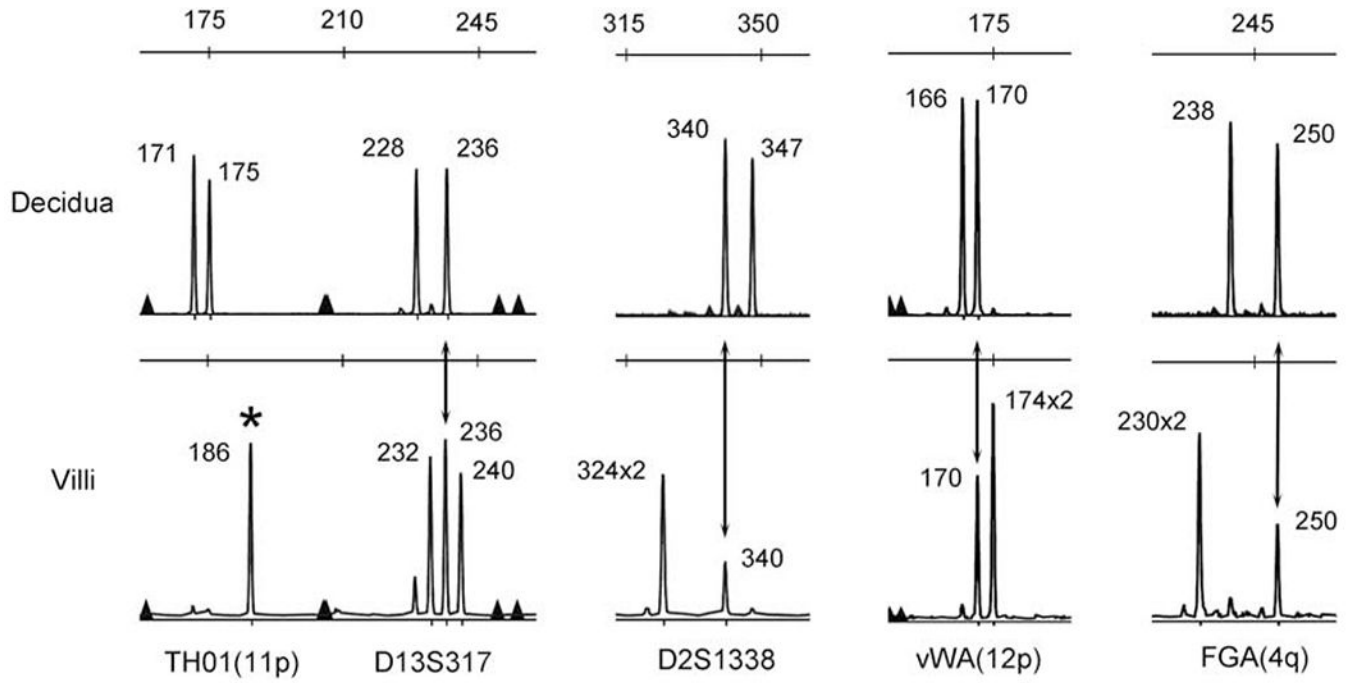

FIGURE 2.

Genotyping result for PHM (case 2) in Figure 1. Analysis of DNA polymorphic markers (microsatellites) demonstrates that the DNA pattern from villous tissue is most consistent with diandric triploidy (1 maternal [PCR product peak with bidirectional arrow] and 2 nonmaternal/obligate paternal [PCR product peak without arrow] chromosome complements) with a paternal: maternal allele ratio 2:1. Of note, the allele pattern for the marker THO1 (11p15.5, the chromosomal location of the p57 gene) is consistent with loss of the maternal allele at this locus (only a nonmaternal/obligate paternal allele is present, star).

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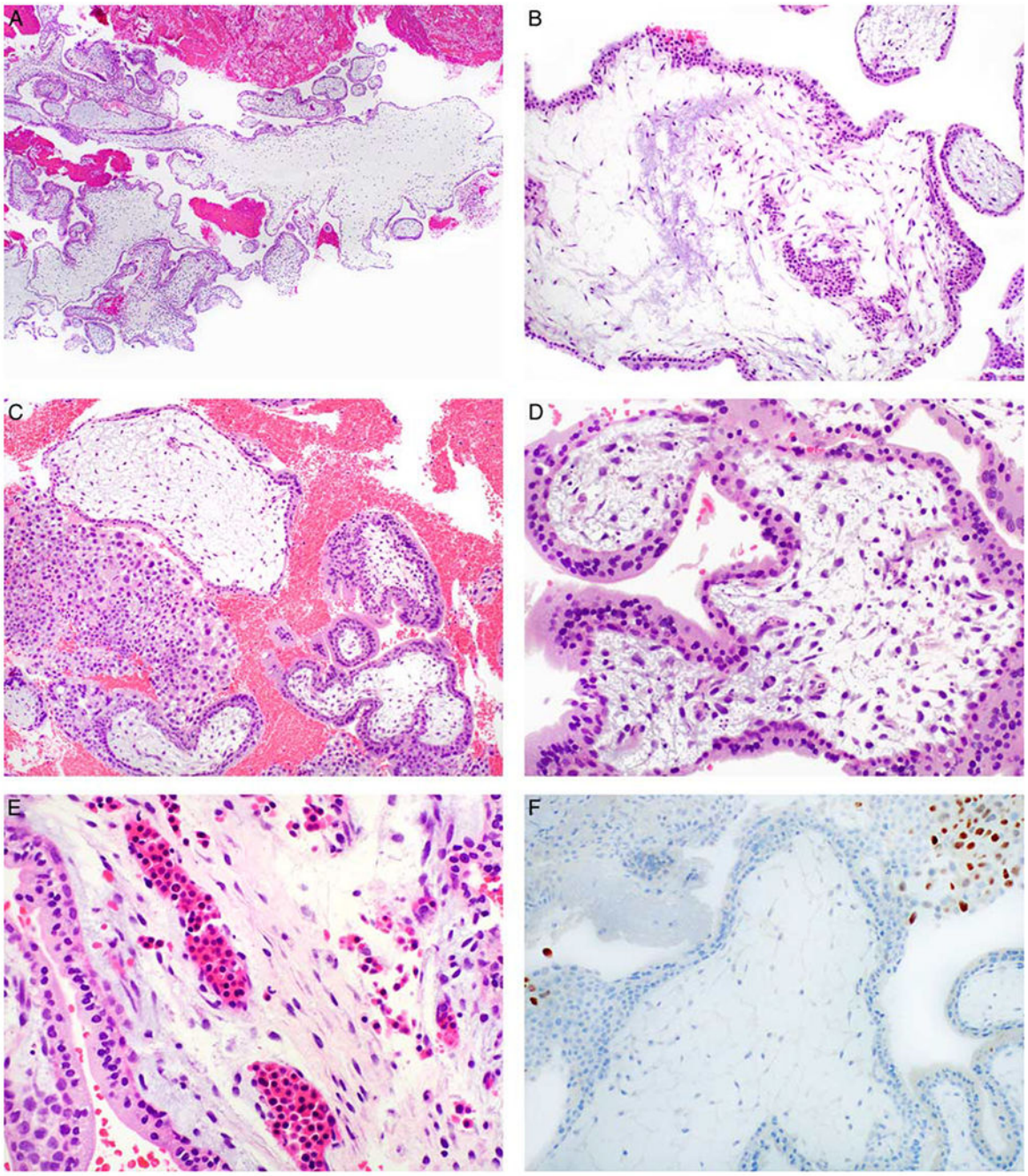

FIGURE 3.

Loss of p57 expression in nonmolar abortus specimen (case 6). A and B, Some hydropically enlarged villi display irregular shapes (A) and trophoblastic inclusion (B). C, Detached trophoblastic proliferation and some small villi with focal trophoblastic hyperplasia. D and E, Some villi show karyorrhectic debris (D) and nucleated red blood cells (E). F, Loss of p57 expression in villous cytotrophoblast and stromal cells (internal positive control in intermediate trophoblastic cells). The morphology combined with loss of p57 expression

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simulates a very early CHM; however, genotyping supports interpretation as a nonmolar abortus (see text and Fig. 4).

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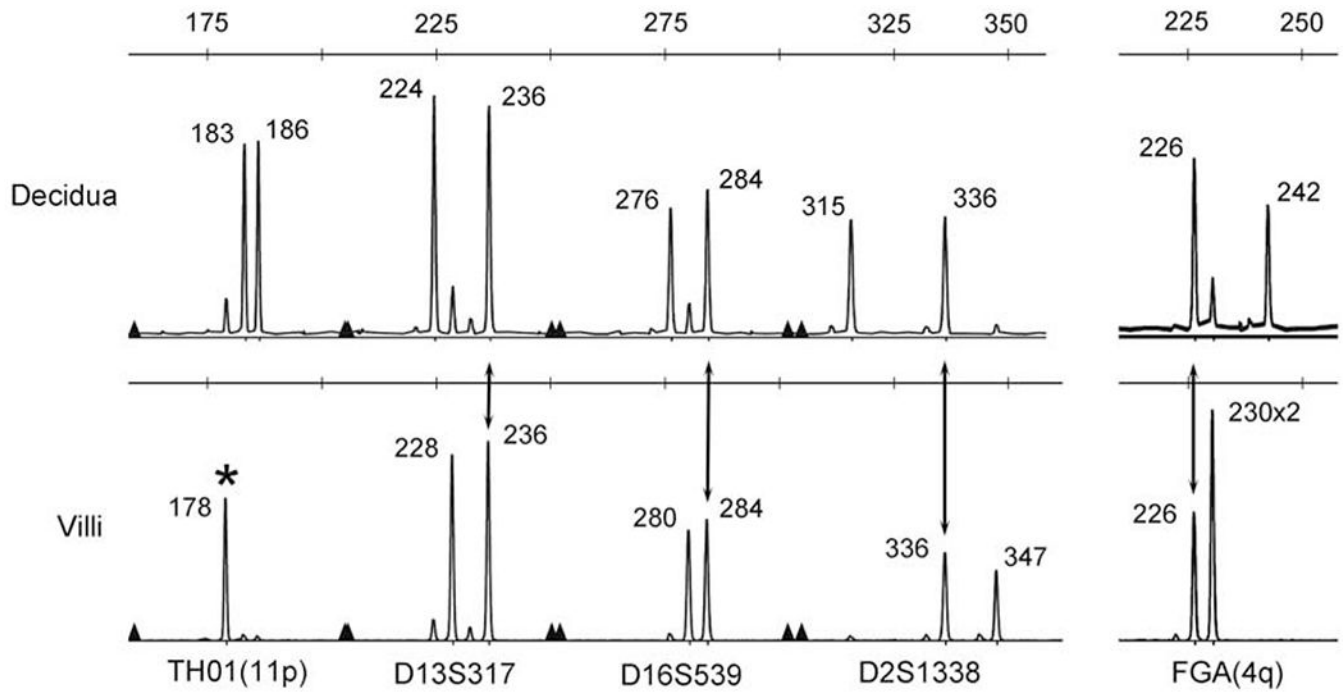

FIGURE 4.

Genotyping result for nonmolar specimen (case 6) in Figure 3. Analysis of DNA polymorphic markers (microsatellites) demonstrates that the DNA pattern from villous tissue is most consistent with a biparental conception with most but not all loci allelic balance (both maternal [PCR product peak with bidirectional arrow] and nonmaternal/obligate paternal [PCR product peak without arrow] chromosome complements present, with equal ratio). Of note, the allele pattern for the marker THO1 (11p15.5, the chromosomal location of the p57 gene) is consistent with loss of the maternal allele at this locus (only a nonmaternal/obligate paternal allele is present, star). In addition, allele pattern for the locus FGA on chromosome 4 is compatible with trisomy 4 (1 extra paternal allele present).

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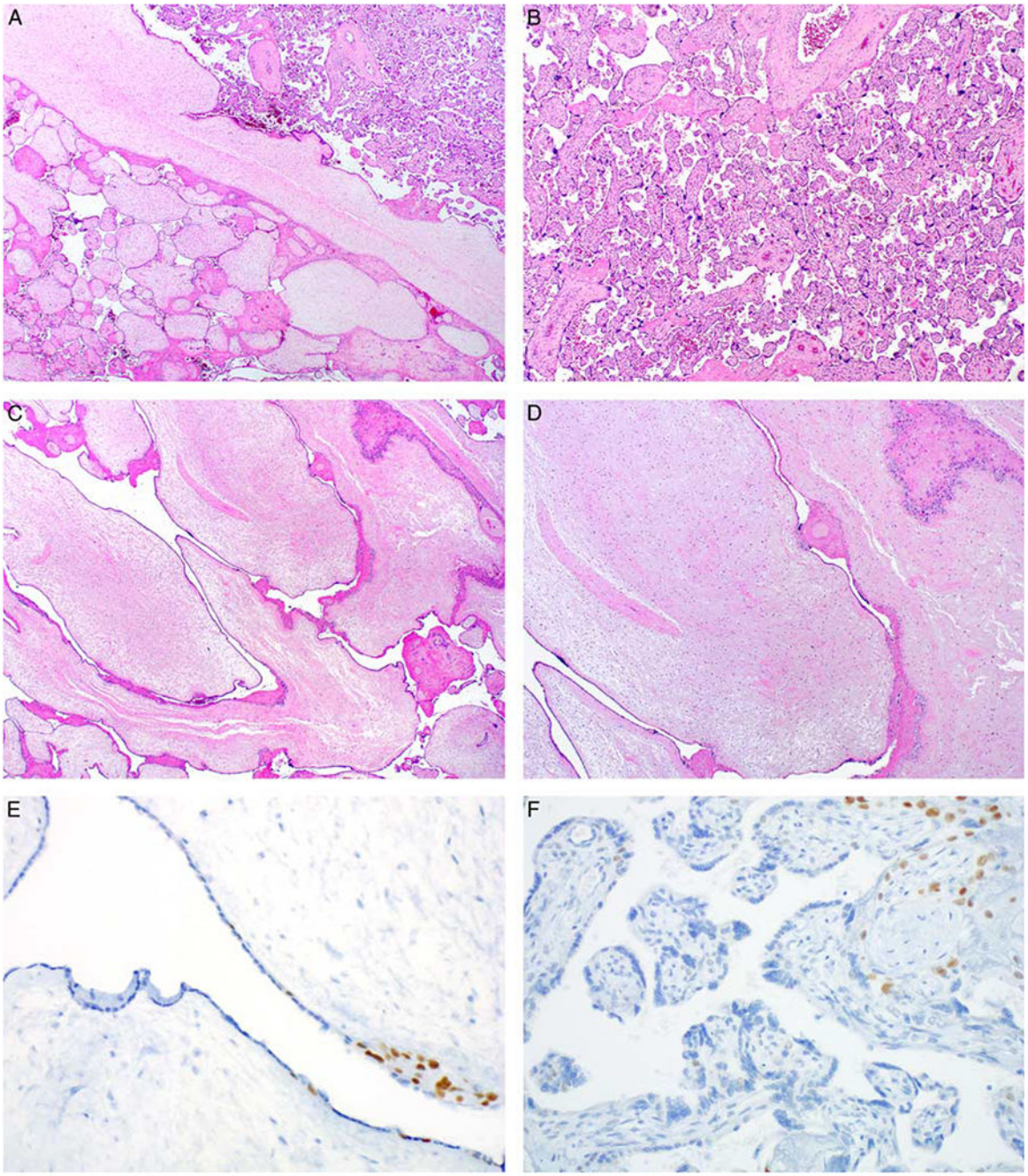

FIGURE 5.

Loss of p57 expression in a placenta with PMD due to BWS (case 10). A, Normal placental tissue with adjacent markedly enlarged villi. B, Normal placental tissue compatible with gestational age (32 wk and 2d). C and D, Markedly enlarged villi with mildly cellular myxoid stroma but essentially lacking trophoblastic hyperplasia. E, The abnormally enlarged villi are overwhelmingly negative for p57, with only focal/patchy p57 expression in some cytotrophoblastic cells and complete loss of expression in stromal cells, with internal positive control. F, Normal placenta displays a similar p57 staining pattern. The terminal

villi and mature intermediate villi are overwhelmingly negative for p57, with only focal/patchy p57 expression in some cytotrophoblastic cells and complete loss of expression in stromal cells, with internal positive control (see text and Fig. 6).

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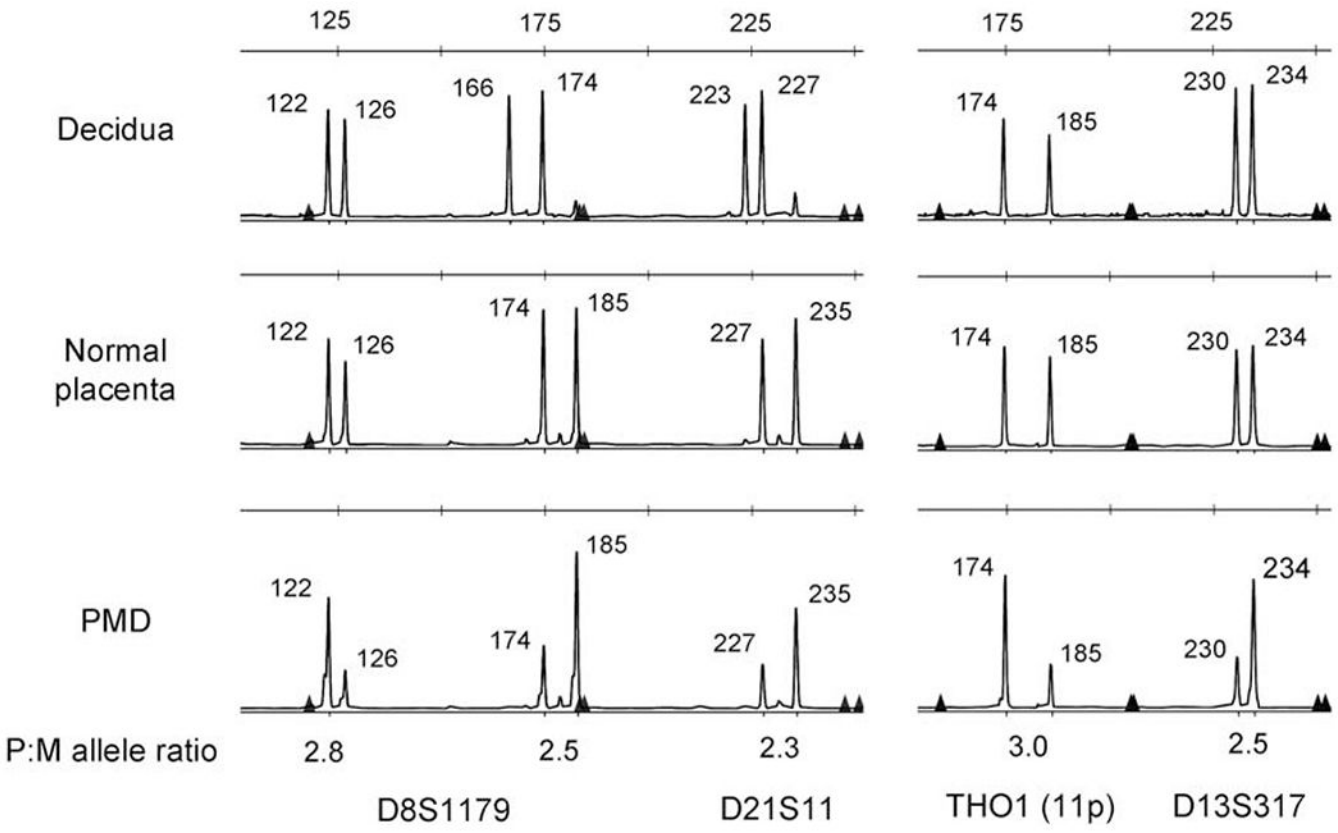

FIGURE 6.

Genotyping result for PMD/BWS (case 10) in Figure 5. Analysis of DNA polymorphic markers (microsatellites) in different areas of villous tissue demonstrate 2 types of patterns in 2 populations of villi: the area with small, normal-appearing villi demonstrated a biparental pattern;the area with enlarged villi demonstrate allele patterns indicating an excess of paternal alleles compared with maternal alleles (paternal:maternal allele ratios > 2:1). These results for the enlarged villi are best explained as a mixture of 2 genetically distinct cell populations, 1 androgenetic and the other biparental. P:M indicates paternal:maternal.

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TABLE 1.

Clinical Features and Molecular Results

Case	Age	Gravidity and Parity	Clinical Presentation	Gestational Age (wk)	Preevacuation β-HCG (mIU/mL)	Molecular Genotyping	Other Ancillary Test	Mechanisms for Loss of p57 Expression	Diagnosis
1*	37	G2P1	Abnormal ultrasound suggesting molar pregnancy	10	13,028	Diandric triploidy	NA	Loss of maternal chromosome 11	PHM
2	38	G2P2002	Molar pregnancy	NA	NA	Diandric triploidy	SNP array (68, XXY, −11)	Loss of maternal chromosome 11	PHM
3	23	NA	Missed abortion	7	2809	Triandric tetraploidy	FISH tetraploidy XXYY	Loss of maternal chromosome 11	PHM
4	39	G2P1	Missed abortion	7	72,258	Diandric triploidy	DNA index 1.47	Unknown	PHM
5	32	NA	Molar pregnancy	NA	NA	Diandric triploidy	NA	Unknown	PHM
6	35	NA	Missed abortion	NA	NA	Biparental diploidy	NA	Loss of maternal chromosome 11	NM
7	27	NA	Fetal demise	NA	NA	Biparental diploidy	Cytogenetics 46,XY; FISH normal pattern of 13, 16, 18, 21, XY	Unknown	NM
8	32	G3P1	Missed abortion, suspicious for molar pregnancy	5-6	> 154,000	Biparental diploidy	QF-PCR normal pattern of 13, 18, 21, XY	Unknown	NM
9	36	G3P3	Ultrasound suspicious for partial mole	First trimester	> 100,000	Biparental diploidy	NA	Unknown	NM
10	30	G3P2	Multiple fetal anomalies	31 and 2/7	NA	Androgenetic/ biparental mosaicism	Genetic testing (fetal blood) shows abnormal allele-specific methylation at 11p15.5	BWS	NM

*DeScipio et al.[25]

NA indicates not available; NM, nonmolar; QF, quantitative fluorescence; SNP, single nucleotide polymorphism.

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TABLE 2.

Histopathologic Features

Case	Hydropic Changes	Variable Villous Sizes	Irregular Villous Shapes	Trophoblastic Hyperplasia	Trophoblastic Inclusions	Cisterns	Villous Stromal Hypercellularity	Nucleated Red Blood Cells	Karyorrhectic Nuclear Debris	Fetal Tissue	Diagnosis
1*	+	+	+	Mild	+	+	Focal/mild	−	+	Not present	PHM
2	+	+	+	Mild	+	+	Focal/mild	−	+	Not present	PHM
3	+	+	+	Rare	−	+	−	−	+	Not present	PHM
4	+	+	+	Focal/mild	+	−	Focal/mild	+	−	Not present	PHM
5	+	+	+	Minimal	+	−	Minimal	−	Rare/minimal	Not present	PHM
6	+	+	+	Focal	+	−	+	+	+	Not present	NM
7	+	++	++	−	+	+	++	+	−	Present	NM
8	+	+	+	−	−	−	−	+	−	Not present	NM
9	+	+	+	−	+	−	−	+	+	Present	NM
10	+++	+	+	−	−	−	−	−	−	Not present	NM

*DeScipio et al.[25]

+ indicates feature present/mild; ++, feature present/moderate; +++, feature present/significant; −, feature absent; NM, nonmolar.