Case Report

Rare Complete Hydatidiform Mole With p57 Expression in Villous Mesenchyme: Case Report and Review of Discordant p57 Expression in Hydatidiform Moles

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Summary: Complete hydatidiform mole (CHM) is a premalignant proliferative disease of the placenta characterized by misexpression of imprinted gene products, most notably p57. The majority of CHM exhibit immunohistochemical absence of p57 protein in villous mesenchyme (VM) and cytotrophoblast (CT) and are thus p57 VM/CT concordant. However, some gestations show loss of p57 in only VM or CT, either in all chorionic villi or a subset thereof (VM/CT discordant). Here, we present a rare case of a p57 VM/CT-discordant CHM with diffuse retention of p57 expression in VM but complete absence in CT. Histologically, the case exhibited typical features of CHM including trophoblast hyperplasia and severe nuclear atypia, but was unusual in the presence of gestational membranes identified ultrasonographically and histologically. Ploidy determination by FISH and genotyping by short tandem repeat analyses showed that this was a diploid gestation with variable allelic ratios and with an androgenetic lineage, similar to previously reported p57 VM/CT-discordant cases. **Key Words:** Gestational trophoblastic disease—Hydatidiform mole—p57—Molecular genotyping—Chimeric gestation.

Complete hydatidiform mole (CHM) is commonly encountered in pathology practice, with an incidence around 1 per 1000 pregnancies. CHM is characterized by absence of embryonic development, edema, cavitation of chorionic villi, and trophoblast hyperplasia/atypia. CHM is associated with persistent gestational trophoblastic disease and malignancies of trophoblast derivation including choriocarcinoma, which occur in ~15% of cases. Partial hydatidiform moles (PHM) share some histopathologic features with CHM but have a much lower risk of neoplastic transformation. Progressive villous enlargement over the course of the pregnancy is characteristic of hydatidiform moles (HM), but such changes can be indiscernible by ultrasound early in pregnancy. CHM can be clinically unsuspected and diagnosed by a pathologist on tissue obtained following a failed early pregnancy (1). Therefore, identification and accurate classification of HM (particularly CHM) by pathologists is essential. The p57 protein is encoded by *CDKN1C*, a strongly imprinted gene expressed from the maternal allele in most cell types, including the placenta. The concordant absence of p57 expression in the villous mesenchyme

(VM) and cytotrophoblast (CT) of CHM (2,3) has made p57 immunohistochemistry (IHC) a useful diagnostic adjunct that is a standard first step in the triage of suspected HM (4).

Genetically, CHM are remarkably diverse. Per comprehensive molecular genotyping studies conducted through short tandem repeat (STR) analysis, the great majority are androgenetic and diploid (AnCHM); 85% of these are monospermic (XX) and 15% dispermic (XX, 3% or XY, 12%) (4). Another rare subtype of HM that mimics CHM histologically and clinically (including risk of malignant transformation) are biparental diploid (BiHM). BiHM have a diploid complement of biparental chromosomes, and are thus indistinguishable from normal pregnancies by STR analysis (5). BiHM result from a Mendelian disorder due to homozygous mutations in *NLRP7* or *KHDC3L*, leading to a syndrome of recurrent HM in the affected mothers (6). BiHM exhibit global imprinting defects similar to AnCHM, with absence of p57 expression in CT and VM in most cases (7). Yet another rare subtype of CHM (<1% of cases) exhibits diffuse expression of p57 in VM and CT due to retention of all or a fragment of the maternal chromosome 11 encoding *CDKN1C*/ p57. These cases represent true p57 "false negatives" that could lead to a missed diagnosis of a CHM, but are identifiable as otherwise androgenetic by STR analysis (8,9).

Whereas all of the above types of CHM exhibit concordant patterns of p57 expression in CT and VM across all chorionic villi, some CHM exhibit discordant patterns of p57 expression in CT and VM (10,11). This is different from twin gestations with a CHM component where one twin is normal and has uniformly p57$^+$ villi and the other is a CHM, with the usual villous pattern of absent p57 in both VM and CT. Here, we present a case report of a very rare p57-discordant HM where p57 was absent in CT but retained in VM, with most histologic features consistent with CHM. Most previously reported cases of p57-discordant gestations have exhibited the inverse pattern (p57 retained in CT but absent in VM). We briefly review the literature of cases of HM with discordant p57 expression in CT and VM, highlighting the need for additional investigations to further illuminate the biological basis of this phenomenon.

CASE REPORT

The patient was a 28 yr old G2P1 at 15 wk 3 d gestation per last menstrual period. Her prior pregnancy (6 yr earlier) was unremarkable. She had not yet established prenatal care, and presented to the emergency room with lower abdominal pain and vaginal spotting. Serum βHCG was positive at 238,000 mIU/mL. An obstetrical ultrasound was performed. Images showed an empty gestational sac, which can represent an anembryonic gestation. There were also areas of cystic spaces, and given the high βHCG level, a partial molar pregnancy was suspected (Fig. 1A). Per size measurements, the gestational sac was estimated at 9 to 10 wk gestational age. The patient underwent uterine dilation and curettage.

Grossly, the pathology specimen consisted of $9 \times 7 \times 2$ cm aggregate of soft tissue and blood. Fluid-filled vesicles consistent with HM were noted, with no fetal tissue identified. The tissue fragments were entirely submitted in three cassettes. The histologic findings were consistent with CHM. Prominent villous edema with villous cavitation was present (Fig. 1B). Many villi had prominent circumferential trophoblast hyperplasia (Fig. 1C) with striking nuclear atypia (Fig. 1D) and mitotic activity. All regions were interpreted as compatible with CHM; however, one unusual finding for CHM was the presence of placental membranes, consistent with the ultrasonographic findings (Fig. 1E). p57 immunostains were performed on all 3 blocks, each of which contained chorionic villi. Maternal decidua showed strong p57 expression, serving as an internal positive control (Fig. 1F, asterisk). All villi showed an identical pattern of discordant p57 expression with CT being p57 negative, as with usual CHM (Figs. 1F, G, red arrows), but with VM showing diffuse positivity in the majority of nuclei in a pattern characteristic of non-CHM villi (Figs. 1E, F, black arrows). This pattern is consistent with a combination of 2 genetically distinct cell lines, namely p57$^-$ androgenetic trophoblast and p57$^+$ biparental VM (see the Discussion section).

To further characterize this unusual CHM, maternal decidua and 3 different regions of chorionic villi were microdissected and genotyped with a standard panel of 16 STR markers (4). The results were complex. Several loci showed 3 alleles (eg, D13S17, Fig. 2B), which could be explained by either triploidy or chimerism. The 3 villous regions demonstrated the same alleles at each locus, but with varying ratios suggestive of mixtures of cells with different genetic constitutions. The results were consistent with a genetic contribution from 2 different sperm and also, with some villous regions being definitively androgenetic. For example, in one villous region, STR marker THO1 showed only an androgenetic component (a single peak not shared
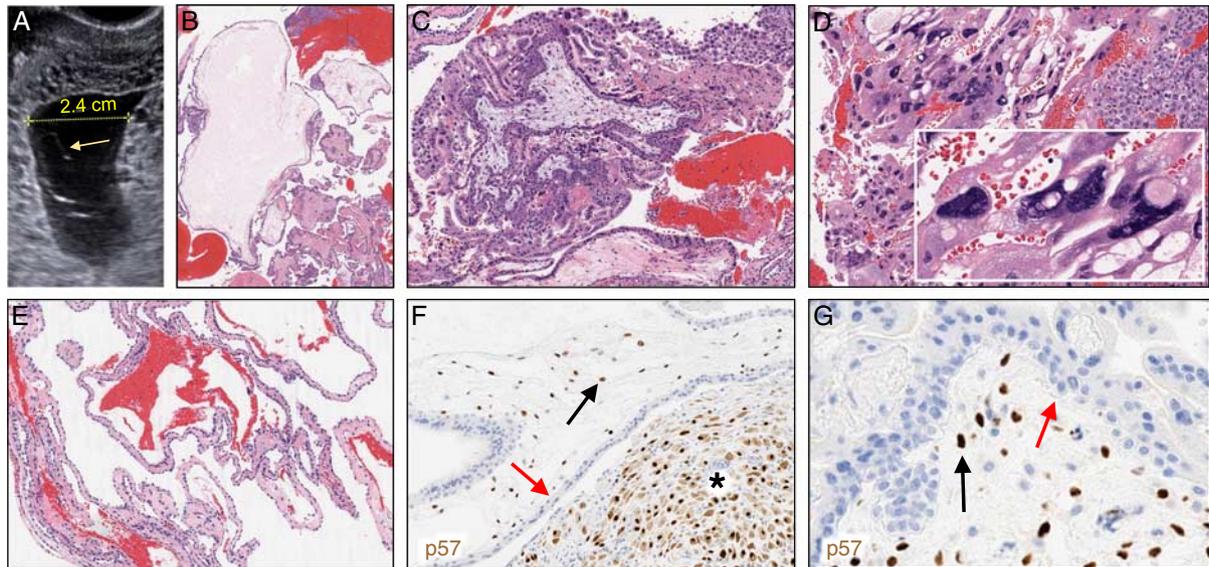
FIG. 1. Ultrasonographic and histopathologic features of case. (A) Obstetrical ultrasound obtained on day of procedure. Plane shows an anembryonic gestation with portions of gestational sac (arrow). (B) Villous edema with definitive cavitation. (C) Prominent circumferential trophoblast hyperplasia. (D) Striking nuclear atypia with highly irregular hyperchromatic nuclei consistent with complete hydatidiform mole (CHM), higher magnification of atypical trophoblast (inset). (E) Gestational membranes, which are not a usual feature of CHM. (F) p57 immunostain. Maternal decidua (*) serves as internal positive control. Cytotrophoblast (CT) shows absence of p57 expression, the typical pattern for CHM (red arrow), but villous mesenchyme (VM) showed strong p57 expression (black arrow) demonstrating CT/VM discordance. (G) Higher magnification of different villus; red arrow = CT, black arrow = VM. All villi in the 3 submitted blocks showed this pattern.

with decidua) in 2 of 3 villous regions sampled (Fig. 2A). FISH with enumeration probes for chromosomes 3, 7, 9, 17 (UroVysion) showed that all villous regions including all VM and CT cells were diploid; that is, no VM or CT cells contained 3 signals for any of the chromosomes (Fig. 2B). One possible explanation for these findings (> 2 alleles and variable allele ratios in the absence of triploidy) is chimerism (ie, the fusion of 2 distinct zygotes), but definitive interpretation is not possible since with this analysis we cannot confidently resolve multiple genotype(s) and we did not specifically genotype isolated CT versus VM. However, the variability of allelic ratios can be readily explained by different ratios of CT versus VM in the regions sampled, with p57$^-$ CT contributing 2 paternal alleles and p57$^+$ VM contributing 1 paternal and 1 maternal allele.

The patient was closely followed with serial serum βHCG measurements every 2 wk. Levels fell gradually and became undetectable (< 2 mIU/mL) 4 mo and 4 d after the procedure. She later became pregnant and was delivered of a healthy infant 1 y and 7 mo after the procedure.

DISCUSSION

CHM are genetically diverse, with misexpression of imprinted genes as their unifying underlying feature.

p57 is a highly sensitive and specific marker of CHM because it is strongly expressed in CT and VM in normal gestations and PHM, but absent in these cell types in the vast majority of CHM. p57 can be expressed in a very small proportion (< 1%) of CT in CHM, and such minor expression should not be misconstrued as positive expression within CT, or as CT/VM discordance (3). In almost all CHM, PHM, and non-HM, p57 expression is concordant in VM and CT: absent (CHM) or present (PHM and non-HM). However, p57 CT/VM discordance is occasionally observed, and we sometimes encounter such cases even in nonconsultation settings, as with this example. Therefore, the phenomenon may be more common than generally appreciated and it is important to recognize such unusual patterns and understand their clinical implications.

Three general patterns of CT/VM discordance have been documented. Cases such as this (VM p57$^+$/CT p57$^-$) are the rarest, with only a few reported cases in the world literature (10,12) (Fig. 3). Gestations with the more common inverse pattern (VM p57$^-$/CT p57$^+$) generally have mesenchymal hyperplasia but no trophoblast hyperplasia, and there is often a fetus; this is a nonmolar entity comprised of p57$^+$ biparental and p57$^-$ androgenetic cell lines and has been termed placental mesenchymal dysplasia in some literature
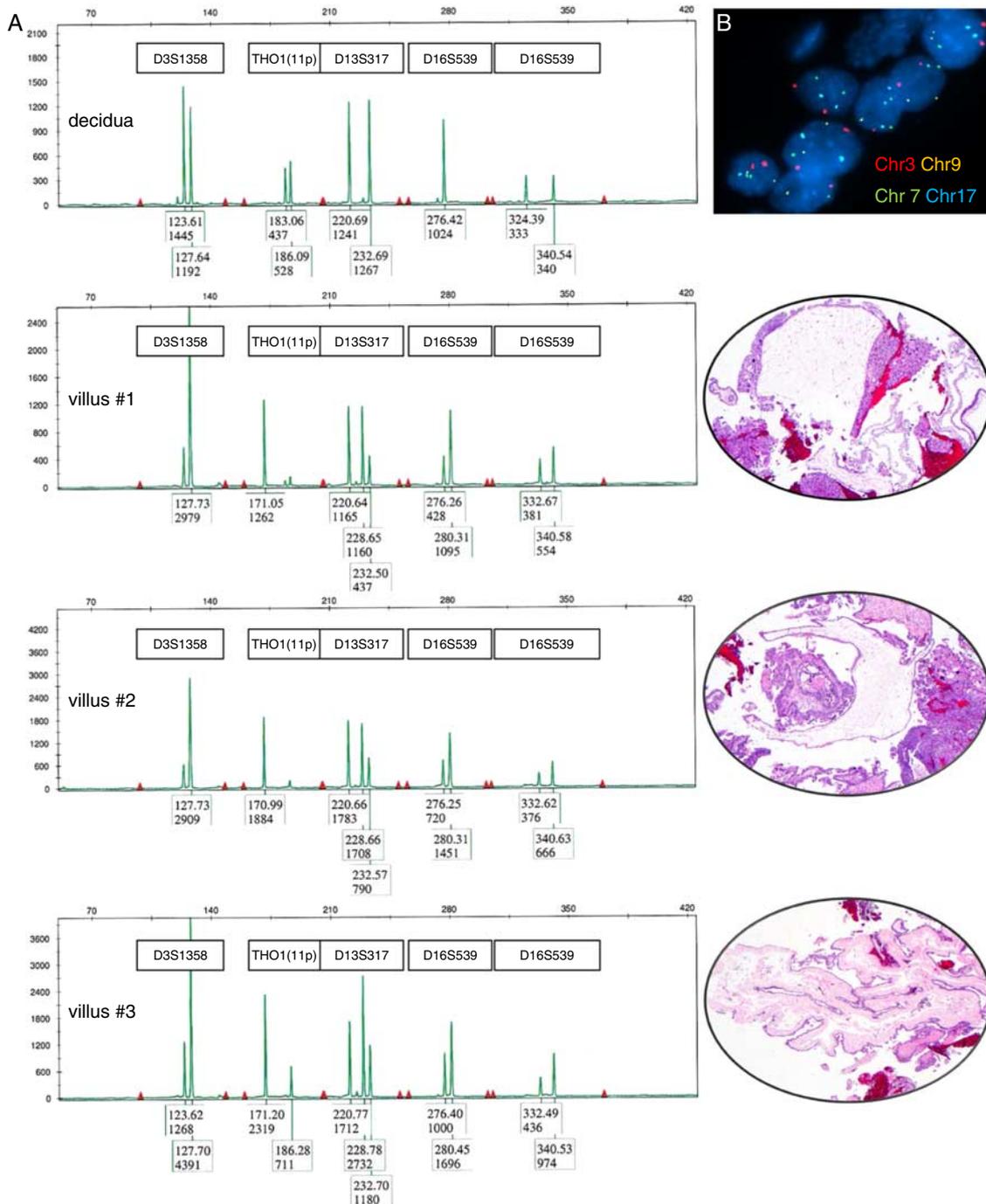
FIG. 2. Molecular and chromosomal analysis. (A) Short tandem repeat-based genotyping. A total of 16 markers were analyzed in maternal decidua and 3 separate villous regions; representative data is shown for 5 markers in decidua and the three villous regions analyzed. Images of the microdissected villus regions are shown on the right. Villus regions #1 and #2 contain a preponderance of hyperplastic trophoblast (the deeply stained cellular areas contain numerous trophoblast nuclei), whereas the edematous villous mesenchyme (VM) has a much lower density of nuclei. Villus region #3 contains a higher proportion of VM and little trophoblast relative to villus #1 and #2. (B) Interphase FISH with enumeration probes for 4 chromosomes (UroVysion assay). The field shows cytotrophoblast (CT); the results were consistent with diploidy in CT and VM in all villi.
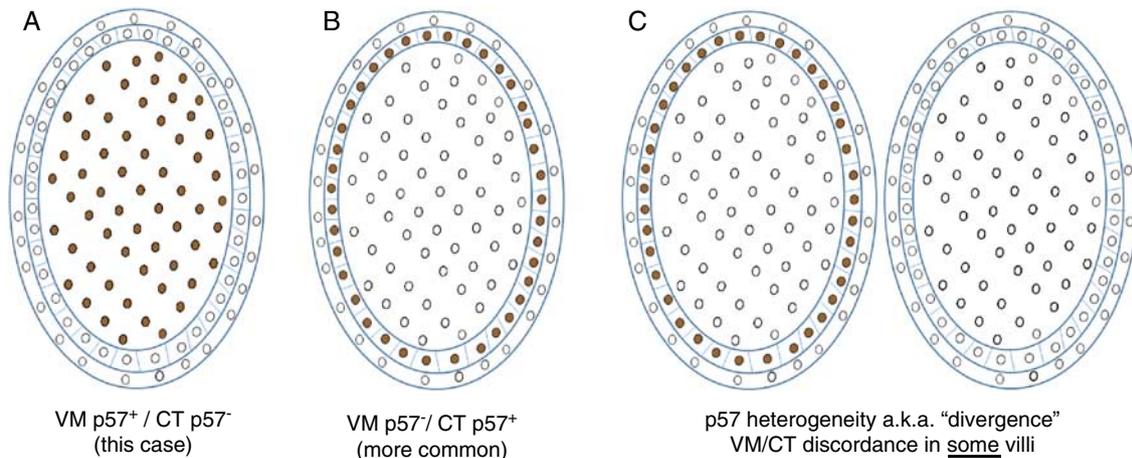
FIG. 3. Schematic showing different patterns of p57 villous mesenchyme (VM)/cytotrophoblast (CT) discordance. The 3 drawings show syncytiotrophoblast (outer layer), CT, and VM illustrating different patterns of p57 discordance. Histologic features of HM such as trophoblast hyperplasia/atypia observed in villi with p57⁻ CT are not illustrated. (A) p57 discordance pattern observed in presented case. (B) More common inverse pattern; such cases in pure form do not exhibit molar features and have been termed placental mesenchymal dysplasia. (C) Other cases show p57 heterogeneity among or within individual villi. In cases where some villi are VM p57⁻/CT p57⁻, such villi typically exhibit histologic features of CHM. Several patterns other than the one illustrated have been documented.

(13). In some cases with this VM p57⁻/CT p57⁺ discordant pattern, there is a second distinct population of VM p57⁻/CT p57⁻ villi with CHM morphology, and such villi are purely androgenetic per genotyping (11). In some cases, p57 VM/CT discordance is heterogeneous among villi, such that the discordance is observed only in a subset of villi, or even within individual villi, and the patterns are admixtures of various proportions of 1 of the first 2 patterns described together with villi with usual p57 expression; this has been termed "divergence" (12). Thus, when VM/CT p57 discordance is observed, we recommend that p57 IHC be performed on several (if not all) blocks.

Malignant progression in CHM is driven by trophoblast, and not VM, suggesting that unusual VM p57⁺/CT p57⁻ CHM such as this have a similar risk of progression or malignant transformation as conventional CHM, whereas gestations with the inverse VM p57⁻/CT p57⁺ pattern are unlikely to undergo malignant transformation (indeed such cases do not exhibit the trophoblast hyperplasia/atypia typical of CHM) (4,10,11,13,14). Accordingly, this case exhibited striking trophoblast hyperplasia and cytologic atypia (Fig. 1) with increased mitotic activity. However, caution is warranted given the potential for heterogeneity among villi. Also, because of the relatively small number of p57-discordant cases so far reported, all p57-discordant cases warrant follow-up to exclude persistent gestational trophoblastic disease or disease progression (4,12).

Lack of p57 expression in CT or VM is indicative of androgenesis of that cell lineage (or less frequently, abnormal imprinting phenocopying androgenesis as in BiHM). p57 IHC is a sensitive indicator of such defects within a specific cell lineage, as evidenced by its ability to detect different types of p57 VM/CT discordance. It is interesting to speculate on the biology of differential p57 expression in VM/CT-discordant cases. Genetic chimerism is a rare but well-documented phenomenon where there are two different sets of DNA originating from the fusion of 2 zygotes (eg, sex-discordant genetic chimerism in individuals with XX and XY cell lineages). This and prior analyses of p57-discordant gestations suggest that some p57-discordant placentae are androgenetic/biparental chimeras consisting of two lineages: one that is androgenetic and p57⁻ and another that is biparental and p57⁺ (4,11,12,14). For example, in this case, the detection of 3 alleles for multiple loci in multiple villous regions in the absence of triploidy could be consistent with chimerism. Deletion of the maternal 11p15.5 chromosomal region encoding *CDKN1C*/p57 (uniparental disomy) is another plausible mechanism for p57 loss in a distinct placental cell lineage (8,9,15). Future studies will benefit from advanced methods such as laser-capture microdissection (12) or single cell sequencing to derive even more detailed molecular profiles of p57-discordant moles and illuminate the origins of their distinct cell lineages. Increased awareness of the phenomenon of p57-discordance and identification of additional cases will aid such investigations.

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