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Original Article

STR DNA genotyping of hydatidiform moles in South China

Xing-Zheng Zheng[1], Pei Hui[2], Bin Chang[3], Zhi-Bin Gao[4], Yan Li[1], Bing-Quan Wu[1], Bo Zhang[1]

[1]Department of Pathology, Peking University Health Science Center, Beijing, China; [2]Department of Pathology, Yale University School of Medicine, New Haven, CT, USA; [3]Department of Pathology, Fudan University Shanghai Cancer Center, Shanghai, China; [4]Department of Pathology, Yurao People's Hospital, Zhejiang, China

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Abstract: Objective: To evacuate whether short-tandem-repeat (STR) DNA genotyping is effective for diagnostic measure to precisely classify hydatidiform moles. Methods: 150 cases were selected based on histologic features that were previously diagnosed or suspected molar pregnancy. All sections were stained with hematoxylin as a quality control method, and guided the microscopic dissection. DNA was extracted from dissected chorionic villi and paired maternal endometrial FFPE tissue sections. Then, STR DNA genotyping was performed by AmpFlSTR® Sinofiler™ PCR Amplification system (Applied Biosystems, Inc). Data collection and analysis were carried out using GeneMapper® ID-X version 1.2 (Applied Biosystems, Inc). Results: DNA genotyping was informative in all cases, leading to identification of 129 cases with abnormal genotype, including 95 complete and 34 partial moles, except 4 cases failed in PCR. Among 95 complete moles, 92 cases were monospermic and three were dispermic. Among 34 partial moles, 32 were dispermic and 2 were monospermic. The remaining 17 cases were balanced biallelic gestations. Conclusion: STR DNA genotyping is effective for diagnostic measure to precisely classify hydatidiform moles. And in the absence of laser capture microdissection (LCM), hematoxylin staining plus manual dissection under microscopic guided is a more economic and practical method.

Keywords: Short-tandem-repeat (STR), DNA genotyping, diagnosis, hydatidiform mole, non-molar gestation

Introduction

Hydatidiform mole (HM) is an abnormal pregnancy with nonneoplastic proliferation of trophoblasts [1]. It can be divided into two separate syndromes based on morphologic, genetic and clinical factors [2]. The complete hydatidiform mole (CHM) is a diploid androgenetic conceptus with generalized villous trophoblastic hyperplasia and hydatidiform villous swelling in the absence of an ascertainable fetus. The partial hydatidiform mole (PHM) is a diandric triploid conceptus with focal trophoblastic hyperplasia and focal hydatidiform villous swelling, and with a demonstrable fetus. It is clinically important to distinguish a hydatidiform mole from a non-molar hydropic abortus, primarily because of the associated risk of post-molar gestational trophoblastic neoplasia and subsequent clinical follow-up and management of the patient [3]. Furthermore, because a complete mole has a much higher risk of progression to gestational trophoblastic neoplasia (18-29%) than a partial mole (1.0-5.6%), it is necessary to subclassify for hydatidiform moles [4, 5]. However, histological evaluation of complete hydatidiform mole (especial early CHM), partial hydatidiform mole, digynic gestation and non-molar hydropic abortion is very difficult and easily mistaken [6]. In the past, many ancillary studies, including DNA ploidy analysis, p57 immunohistochemistry, chromosomal enumeration by fluorescent in situ hybridization (FISH), and DNA short-tandem-repeat (STR) genotyping have been developed [7-9]. Most of them were based on the genetic level of hydatidiform moles specific parental chromosomal complements [10].

At the genotype level, most CHMs are androgenetic, containing two sets of paternal chromosomes, with either 46, XX diploid karyotype (monospermic or homozygous, 80%), or 46, XX or XY karyotype (dispermic or heterozygous,

Table 1. Clinicopathologic features and Genotyping Diagosis of 150 cases

Patient	Age (y)	Pathologic Diagnosis	$p57^{kip2}$	Genotyping Diagnosis
1	51	Consistent with HM	POS.	DPM
2	21	Consistent with HM	ND.	MCM
3	27	Consistent with HM	ND.	MCM
4	23	Consistent with HM	NEG.	MCM
5	47	Suggestive of HM	POS.	Non-molar gestation
6	27	Consistent with HM	NEG.	MCM
7	20	Consistent with HM	ND.	MCM
8	27	Suggestive of HM	ND.	MCM
9	24	Consistent with HM	ND.	MCM
10	24	Suggestive of HM	ND.	DPM
11	27	Consistent with HM	ND.	MCM
12	22	Consistent with HM	ND.	MCM
13	30	Consistent with HM	NEG.	MCM
14	22	Consistent with HM	POS.	DPM
15	28	Suggestive of HM	NEG.	MCM
16	28	Consistent with HM	ND.	MCM
17	18	Consistent with HM	NEG.	MCM
18	22	Consistent with HM	POS.	DPM
19	25	Consistent with HM	ND.	MCM
20	30	Consistent with HM	NEG.	MCM
21	19	Consistent with HM	Focal POS.	MCM
22	21	Suggestive of HM	NEG.	MCM
23	41	Consistent with HM	NEG.	MCM
24	29	Suggestive of PHM	ND.	MCM
25	31	Suggestive of HM	NEG.	MCM
26	25	Suggestive of HM	POS.	DPM
27	26	Consistent with HM	NEG.	MCM
28	26	Consistent with HM	ND.	MCM
29	47	Consistent with HM	NEG.	MCM
30	20	Consistent with HM	ND.	MCM
31	28	Consistent with HM	ND.	MCM
32	44	Consistent with HM	NEG.	MCM
33	22	Consistent with HM	NEG.	MCM
34	28	Consistent with HM	ND.	MCM
35	26	Consistent with HM	ND.	Failed detection
36	20	Consistent with HM	ND.	MCM
37	21	Consistent with HM	NEG.	MCM
38	26	Suggestive of HM	NEG.	MCM
39	29	Suggestive of HM	ND.	Failed detection
40	19	Consistent with HM	NEG.	MCM
41	30	Suggestive of HM	NEG.	MCM
42	21	Consistent with HM	ND.	MCM
43	41	Consistent with HM	ND.	MCM
44	41	Suggestive of HM	NEG.	MCM
45	32	Favor HM	ND.	MCM
46	25	Suggestive of HM	NEG.	MCM

20%) [11]. In rare cases, CHMs are diploid, with both a maternal and a paternal chromosome complement (Biparental Complement Hydatidiform Mole, BiCHM) [12]. BiCHM is a recurrent complete mole with strong familial tendency, and some studies reported that NLRP7 gene might be the fundamental genetic of BiCHM [12, 13]. PHMs are triploid, containing one maternal and two paternal sets of chromosomes, with XXX or XXY triploid karyotype with a diandric, monogynic genome arising from fertilization of a haploid egg by either two spermatozoa (dispermic or heterozygous, 90%) or one spermatozoon with duplication (monospermic or homozygous, 10%) [11]. Because of the earlier clinical detection and curettage of abnormal pregnancies, the histopathological features that are often used to distinguish complete moles, partial moles, and non-molar abortions are more subtle and less readily identifiable, leading to increasing difficulties in the proper subclassification of HMs [4, 14]. In daily clinical practice, under-diagnosis of complete mole as partial mole (or non-molar pregnancy), or over-diagnosis of non-molar pregnancy as partial mole (or complete mole) is often encountered [15, 16]. Along with the people aware of that the different subtypes have different clinical

47	34	Favor HM	NEG.	MCM
48	20	Favor HM	NEG.	MCM
49	25	Consistent with HM	ND.	MCM
50	25	Suggestive of HM	ND.	MCM
51	23	Suggestive of HM	NEG.	MCM
52	47	Rule out HM	POS.	Non-molar gestation
53	21	Rule out HM	NEG.	MCM
54	48	Rule out HM	ND.	Failed detection
55	26	Suggestive of HM	NEG.	DCM
56	28	Suggestive of HM	NEG.	MCM
57	43	Suggestive of HM	NEG.	MCM
58	27	Consistent with HM	NEG.	MCM
59	20	Rule out HM	ND.	MCM
60	28	Consistent with HM	NEG.	MCM
61	22	Suggestive of HM	NEG.	MCM
62	22	Suggestive of HM	ND.	MCM
63	46	Consistent with HM	ND.	MCM
64	37	Suggestive of HM	NEG.	MCM
65	33	Rule out HM	POS.	Non-molar gestation
66	28	Rule out HM	POS.	DPM
67	29	Suggestive of CHM	Focal POS.	DPM
68	34	Rule out HM	Focal POS.	DPM
69	26	Suggestive of PHM	POS.	DPM
70	28	Rule out HM	NEG.	MCM
71	26	Suggestive of CHM	Focal POS.	DPM
72	30	Suggestive of PHM	POS.	DPM
73	24	Suggestive of PHM	POS.	Non-molar gestation
74	35	Suggestive of HM	POS.	DPM
75	30	Suggestive of HM	Focal POS.	DPM
76	28	Rule out HM	POS.	Non-molar gestation
77	26	Rule out HM	ND.	Non-molar gestation
78	29	Suggestive of PHM	ND.	Non-molar gestation
79	27	Suggestive of HM	ND.	Non-molar gestation
80	22	Suggestive of HM	ND.	Non-molar gestation
81	34	Consistent with PHM	POS.	DPM
82	26	Suggestive of PHM	POS.	MPM
83	23	Consistent with PHM	POS.	DPM
84	31	Consistent with PHM	POS.	DPM
85	25	Consistent with PHM	POS.	Non-molar gestation
86	34	Suggestive of PHM	ND.	DPM
87	26	Consistent with PHM	POS.	DPM
88	28	Consistent with PHM	POS.	DPM
89	21	Consistent with PHM	NEG.	DPM
90	29	Suggestive of PHM	ND.	DPM
91	38	Consistent with PHM	POS.	DPM
92	26	Rule out CHM	ND.	DPM
93	32	Suggestive of PHM	ND.	DPM
94	39	Rule out HM	NEG.	DPM
95	33	Suggestive of CHM	NEG.	MCM

treatments; the accurate subclassification diagnosis is getting more and more attention in hydatidiform moles. Now, a variety of molecular methods targeting the genetic alterations of hydatidiform moles have been explored to improve diagnostic accuracy [3]. We recently had established a STR analysis platform for DNA genotyping in the diagnosis of molar pregnancy. Our objective was to estimate whether molecular genotyping is effective for diagnostic measure to precisely classify hydatidiform moles.

Materials and methods

Patients and histological evaluation

A total of 150 abortion specimens were selected from February 2009 to March 2014 in department of pathology of YuRao People's Hospital (ZheJiang Province, China). All cases raised the possibility of molar pregnancy based on histology (**Table 1**), with diagnostic terms including "favor molar pregnancy", "consistent with molar pregnancy", "suggestive of or suspicious for molar pregnancy", and "rule out molar pregnancy". All cases had some degree of suspicion for molar gestation by the primary pathologist on the basis of morphologic and/or clinical findings. There were 91 cases that had performed P57 immunohistochemistry men-

96	44	Suggestive of CHM	ND.	Non-molar gestation
97	31	Consistent with CHM	NEG.	MCM
98	22	Consistent with CHM	NEG.	MCM
99	34	Consistent with CHM	NEG.	MCM
100	24	Rule out HM	Focal POS.	DPM
101	29	Consistent with CHM	NEG.	MCM
102	25	Consistent with CHM	ND.	MCM
103	32	Consistent with CHM	ND.	DCM
104	20	Consistent with CHM	ND.	MCM
105	22	Consistent with CHM	NEG.	MCM
106	25	Suggestive of CHM	ND.	DCM
107	22	Consistent with CHM	ND.	MCM
108	34	Consistent with CHM	ND.	MCM
109	32	Consistent with CHM	ND.	MCM
110	28	Suggestive of CHM	ND.	MCM
111	33	Consistent with CHM	NEG.	MCM
112	29	Consistent with PHM	Focal POS.	MPM
113	31	Consistent with CHM	NEG.	MCM
114	27	Suggestive of CHM	ND.	MCM
115	32	Consistent with CHM	ND.	Failed detection
116	26	Suggestive of PHM	POS.	DPM
117	23	Consistent with PHM	POS.	DPM
118	18	Consistent with PHM	POS.	DPM
119	27	Consistent with CHM	POS.	DPM
120	30	Consistent with CHM	NEG.	MCM
121	22	Consistent with CHM	NEG.	MCM
122	24	Consistent with CHM	NEG.	MCM
123	27	Consistent with CHM	NEG.	MCM
124	27	Consistent with CHM	ND.	MCM
125	32	Consistent with CHM	NEG.	MCM
126	20	Consistent with CHM	NEG.	MCM
127	32	Consistent with CHM	ND.	MCM
128	23	Consistent with CHM	NEG.	MCM
129	21	Suspicious for Early CHM	Focal POS.	MCM
130	22	Suggestive of CHM	NEG.	MCM
131	31	Consistent with CHM	ND.	MCM
132	29	Suggestive of CHM	NEG.	MCM
133	32	Consistent with CHM	ND.	MCM
134	32	Consistent with CHM	NEG.	MCM
135	20	Consistent with CHM	NEG.	MCM
136	28	Suggestive of CHM	ND.	MCM
137	38	Consistent with CHM	NEG.	MCM
138	27	Consistent with CHM	ND.	MCM
139	29	Consistent with CHM	NEG.	MCM
140	26	Consistent with CHM	NEG.	MCM
141	24	Suggestive of CHM	POS.	DPM
142	23	Rule out Early CHM	POS.	Non-molar gestation
143	24	Rule out HM	ND.	DPM
144	26	Suggestive of HM	POS.	Non-molar gestation

tioned in retrospective data. This study was approved by the institutional review board (Human Investigation Committee of YuRao People's Hospital).

Referring to Buza N's literature [15], we selected some important parameters to systematically assess, including villous hydrops, maximum size of chorionic villi, villous shape and contour, villous populations, trophoblastic pseudoinclusions, cistern formation, trophoblast hyperplasia, nucleated fetal red blood cells, and other fetal tissues. Each case has been reviewed independently by two gynecologic pathologists. At the same time, we eliminated a few cases which were absence/rare of decidua or rare, finally, 150 cases were selected for STR analyses.

Molecular genotyping detection

Five serial sections 10 micrometers thick were cut from formalin-fixed-paraffin-embedded (FFPE) tissue blocks, the middle section was stained with hematoxylin and eosin to verify the distribution of villous and decidua tissue. In order to isolate pure populations, the remaining four sections were stained with hematoxylin (Dyeing time less than 30 seconds) before the microscopic dissection. Paired tissue samples of chorionic villi and decidua were subjected to DNA extraction by

145	24	Suggestive of HM	NEG.	MCM
146	27	Rule out Early CHM	ND.	Non-molar gestation
147	23	Rule out HM	POS.	Non-molar gestation
148	22	Rule out HM	ND.	MCM
149	26	Suggestive of HM	POS.	Non-molar gestation
150	28	Rule out HM	POS.	Non-molar gestation

HM, hydatidiform mole; CHM, complete hydatidiform mole; PHM, partial hydatidiform mole; MCM, monospermic CHM; DCM, dispermic CHM; DPM, dispermic PHM; MPM, monospermic PHM; POS, positive; NEG, negative; ND, not done.

Hydrothermal Pressure (Pressure Cooking) coupled with chaotropic salt column purification method [17, 18]. DNA was quantified by spectrophotometric absorbance at 260nm using the NanoDrop apparatus (Thermo Scientific Inc.; Wilmington, DE). The quality of the extracted DNA was evaluated by reading the optical density ratio of 260/280. Genotyping was performed with an AmpFlSTR® Sinofiler™ PCR Amplification Kit (Sinofiler kit) (Applied Biosystems, Inc., Foster City, CA). The reaction consists of a short tandem repeat multiplex polymerase chain reaction (PCR) assay that amplifies 15 different autosomal STR loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, D5S818, D13S317, D16S539, D2S1338, D19S433, vWA, D12S391, D18S51, D6S1043, FGA) and the sex-determining marker(Amelogenin) in a single PCR reaction. The producing short amplicons are ranging from 100 to 350 bp. Genomic DNA of 20 to 40 ng was amplified in a 25-microliter reaction containing 10.5 microliters of AmpFlSTR reaction mix, 5.5 microliters of AmpFlSTR® Sinofiler™ primer mix, and 0.5 microliters of AmpliTaq Gold DNA polymerase. The PCR reaction consisted of 11 minutes at 95°C, followed by 28 cycles of 94°C for 1 minute, 59°C for 1 minute, and 72°C for 1 minute, finished by 60°C for 60 minutes. One microliter of the PCR product was mixed with 8.7 microliters of Hi-Di and 0.3-microliter sizing marker (GeneScan-600LIZ; Applied Biosystems, Inc.), followed by capillary electrophoresis on an ABI3500 platform. Data collection and analysis were performed using GeneMapper® ID-X version 1.2 (Applied Biosystems, Inc).

Molecular diagnostic criteria [3]: 1) A molecular diagnosis of complete hydatidiform mole was made when the genotyping profiles of the villous tissue demonstrated exclusively paternal alleles of either monospermic (homozygous paternal alleles) or dispermic (heterozygous paternal alleles) patterns. 2) Dispermic (diandric-monogynic genome) partial hydatidiform mole was diagnosed when the genotyping profiles of the villous tissue showed two distinct paternal alleles in at least two loci but other alleles consist of a duplicate quantity homozygous paternal and one maternal allele. And, monospermic (monospermic duplicate and monogynic genome) partial hydatidiform mole demonstrated homozygous paternal alleles in duplicate quantity, in addition to the presence of one maternal allele in the villous tissue. 3) When the genotyping profiles of the villous tissue showed three alleles in each locus and, two of the three alleles of the villi matched the two maternal alleles of the gestational endometrium, triploid digynic-monoandric gestation was diagnosed. 4) Non-molar gestations, including hydropic abortus, showed balanced biallelic profiles of both paternal and maternal origins in the villous tissue. 5) If the genotyping profiles of the most villous tissue were simiar to nonmolar gestation except only one locus was three alleles or one allele, trisomy or monosomy syndrome diagnosis was made.

Results

DNA genotyping was informative in all cases (**Table 1**), of which 146 cases were succeeded genotype, including 129 hydatidiform moles (95 complete and 34 partial moles) and 17 non-molar gestations (**Figure 1**). Among 95 complete moles, 92 cases were monospermic (**Figure 2**) and three were dispermic. Among 34 partial moles, 32 were dispermic (**Figure 3**) and 2 were monospermic (**Figure 4**). 79 cases with histologic diagnostic terms HMs (including consistent with HM, suggestive of HM, rule out HM) were accurate sub-classified, including 55 monospermic complete moles, 12 dispermic partial moles, one dispermic complete moles and 11 non-molar gestations. 17 cases which diagnosed HMs and PHMs/CHMs by their histologic changes were confirmed non-molar hydropic abortion with DNA genotyping (**Figure 5A-C**). Furthermore, one PHM and 5 CHMs which diagnosed by their histologic changes

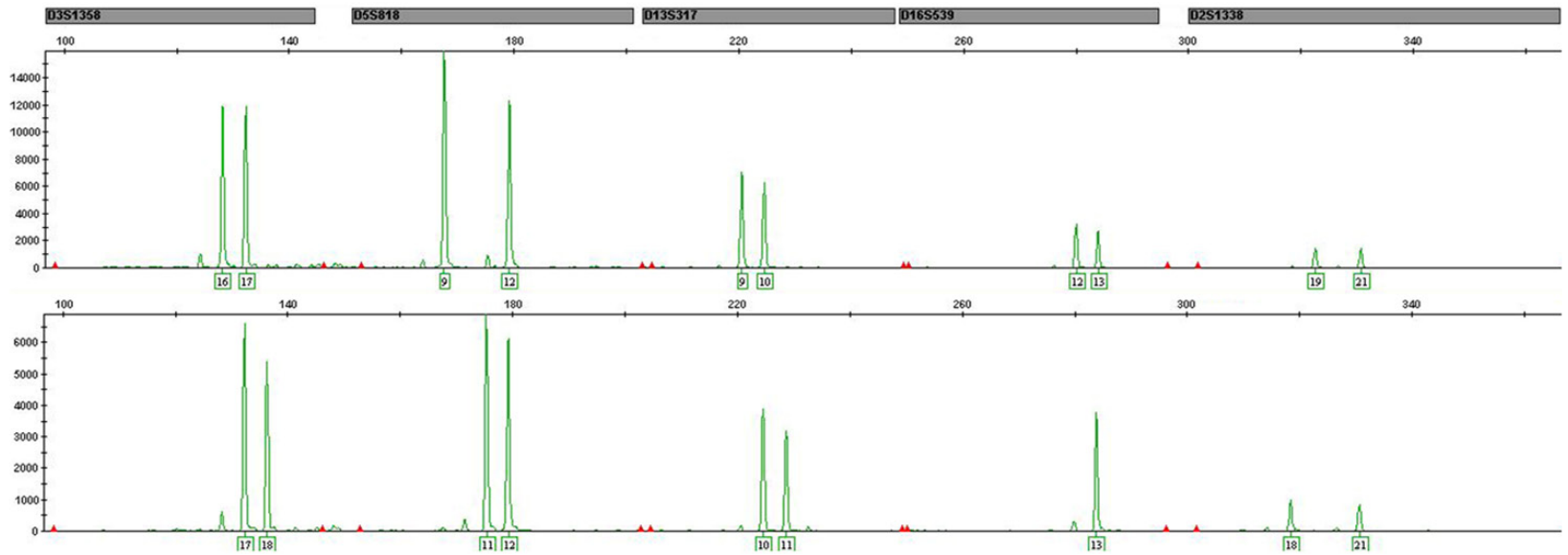

Figure 1. Genetic profiles of a non-molar gestation demonstrating balanced biallelic profiles of both paternal and maternal origins in the villous tissue (top) similar to the maternal endometrium (bottom).

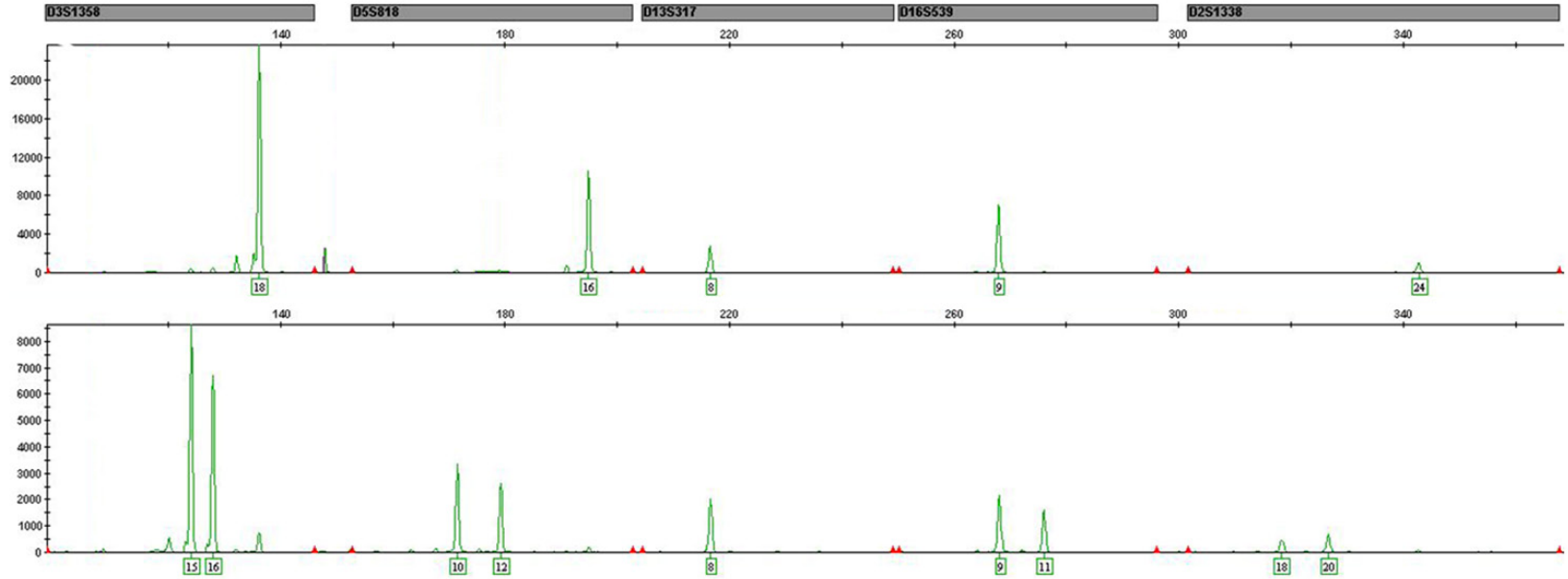

Figure 2. Genetic profiles of a monospermic complete hydatidiform mole. It is demonstrating exclusively paternal alleles in the villous tissue (top). Normal biallelic profiles seen in the maternal endometrium (bottom).

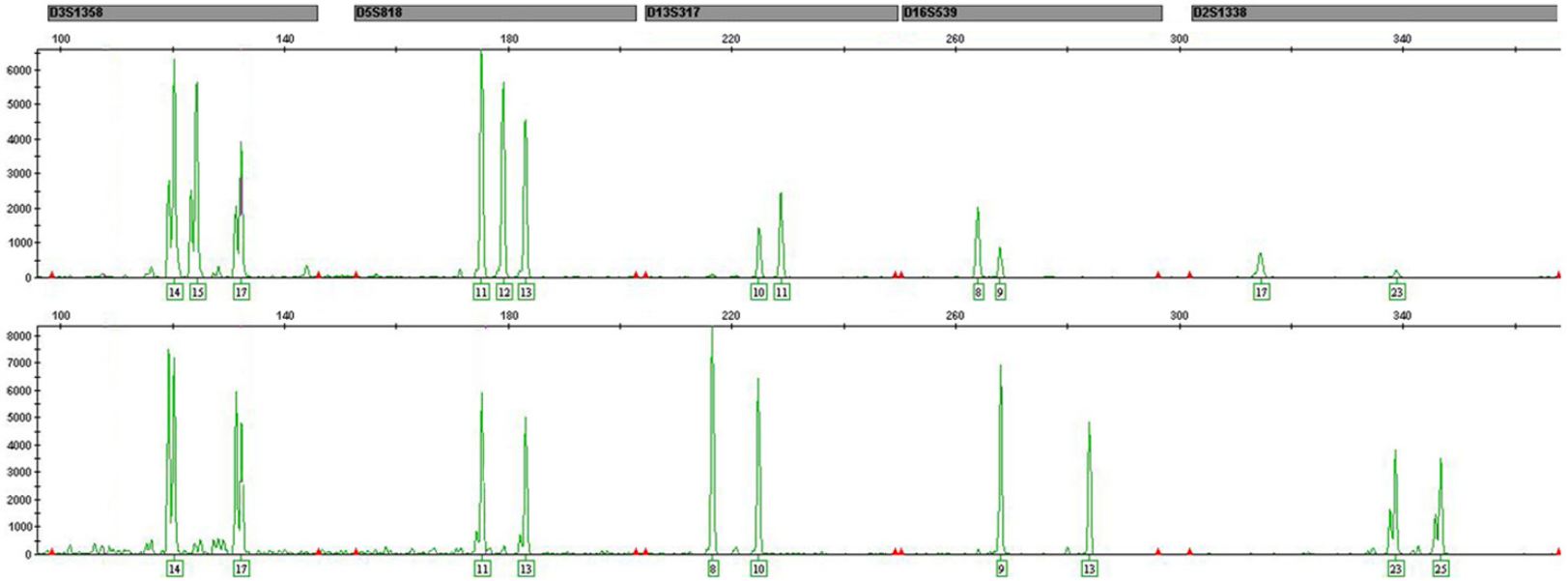

Figure 3. Genetic profiles of a dispermic partial hydatidiform mole. It is showing dispermic paternal alleles (two loci with heterozygous paternal alleles and three loci with homozygous paternal alleles in duplicate quantity) , in addition to the presence of one maternal allele (top). Normal biallelic profiles seen in the maternal endometrium (bottom).

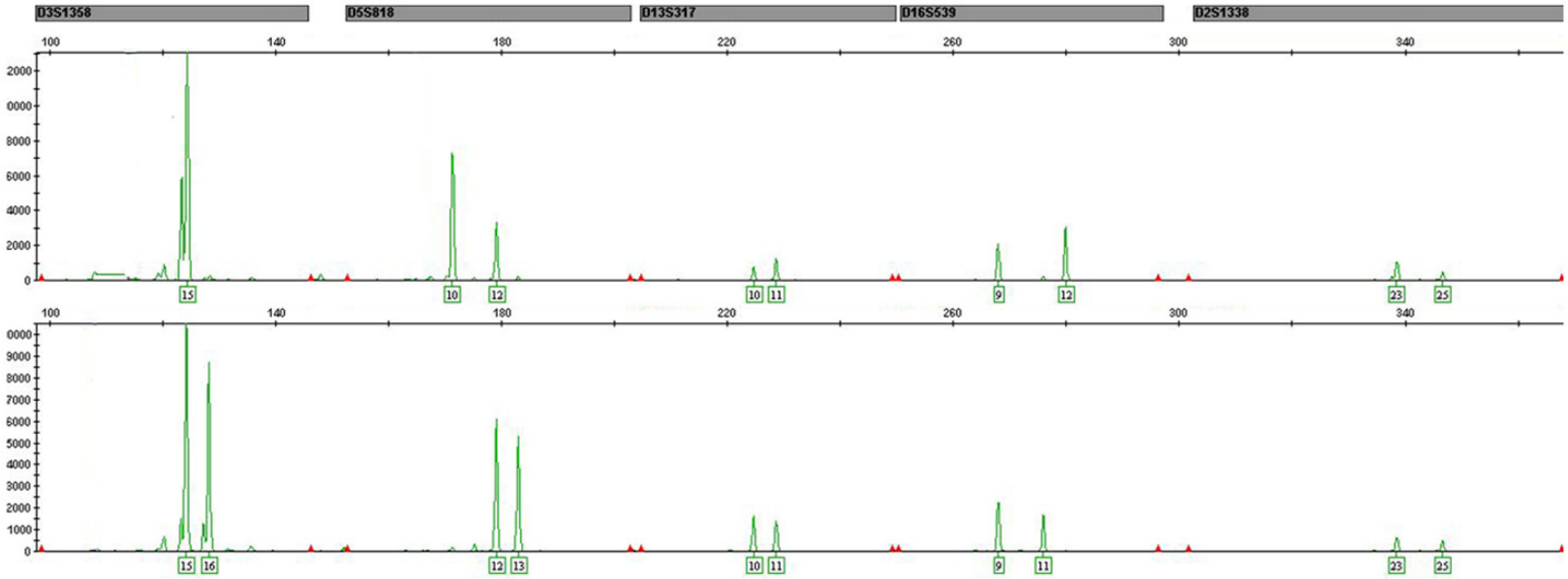

Figure 4. Genetic profiles of a monospermic partial hydatidiform mole showing demonstrated homozygous paternal alleles in duplicate quantity, in addition to the presence of one maternal allele in the villous tissue (top).Normal biallelic profiles seen in the maternal endometrium (bottom).

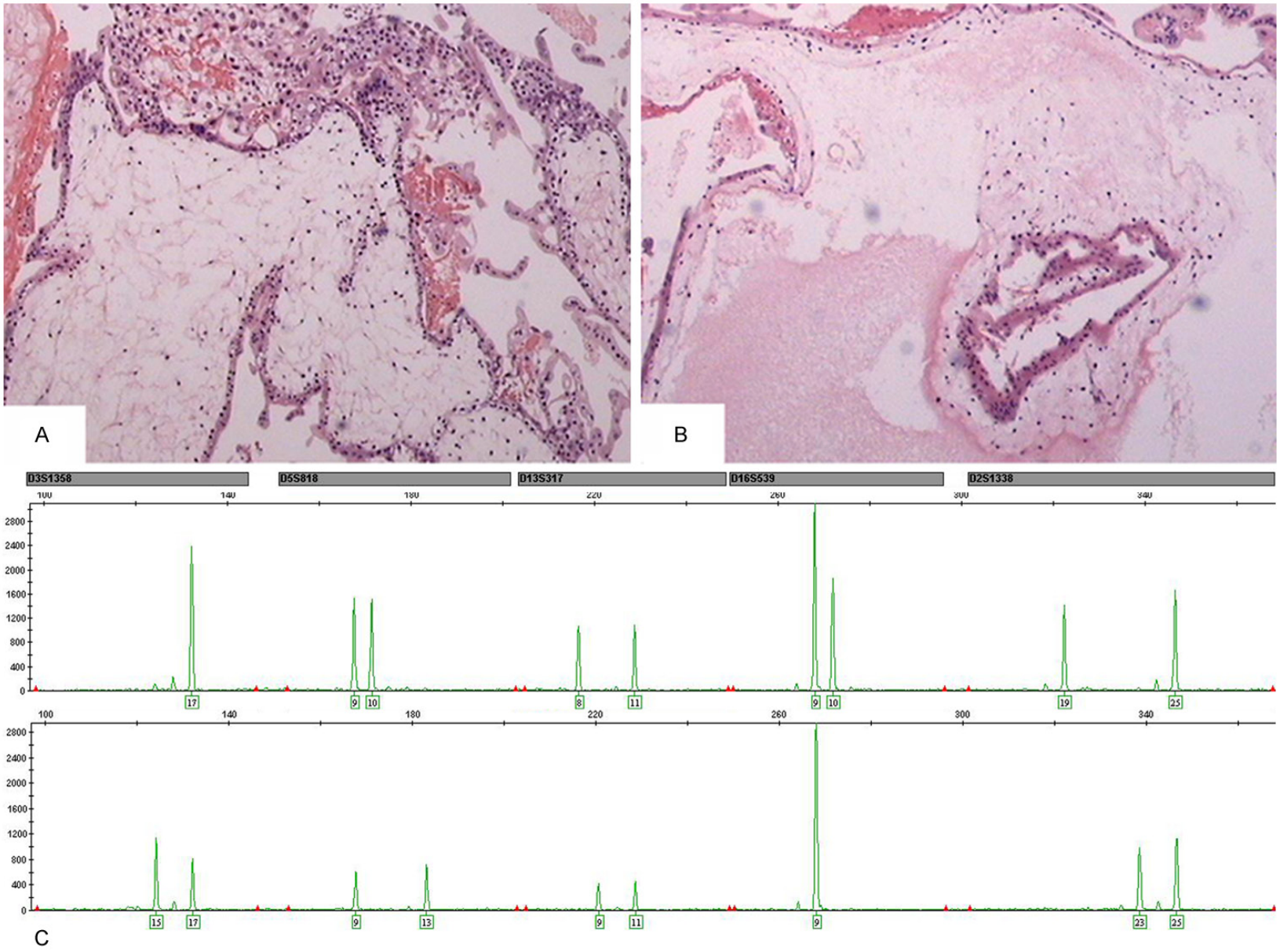
A
B
D3S1358
D5S818
D13S317
D16S539
D2S1338
C

Figure 5. Non-molar gestation. It had been wrongly diagnosed as a PHM by its morphologic features including enlarged admixed with normal sized villi, villous stromal edema with cistern formation, focal trophoblastic hyperplasia (A, B). (C) Genetic profiles of a non-molar gestation demonstrating balanced biallelic profiles of both paternal and maternal origins in the villous tissue (top) similar to the maternal endometrium (bottom).

Table 2. Genotyping diagnosis and $p57^{kip2}$ immunohistochemistry of 95 cases

Genotyping Diagnosis	$p57^{kip2}$ immunohistochemistry	
	-	+
CHM		
MCM	53	2
DCM	1	0
PHM		
DPM	2	24
MPM	0	2
Non-molar gestation	0	11

were precise diagnosed as a monospermic complete mole and 5 dispermic partial moles. We founded 93 cases which had performed $p57^{kip2}$ immunohistochemistry from retrospective study (**Table 1**). 56 cases with $p57^{kip2}$ negative, including 54 cases of CHMs (including 53 MCMs and one DCM) and 2 PHMs. Among 39 cases of $p57^{kip2}$ positive samples, 26 cases were PHMs (including 24 DPMs and two MPMs), 11 cases were non-molar gestation, and 2 cases were CHMs (**Table 2**).

Discussion

Hydatidiform moles are common diagnostic entities in the daily practice of gynecological pathology. It is an abnormal pregnancy with nonneoplastic proliferation of trophoblasts and occurs in about 1 in 1000-1500 pregnancies in Western countries and is somewhat more frequent in Latin America, Southeast Asia and the Middle East [19, 20]. In China, the reported incidences of HMs vary from 1 to 8.83 in every 1000 pregnancies, with the highest incidence being in the province of Zhejiang [21]. Then, Prof. Shi et al reported an incidence of HMs was about 2.5 in every 1000 pregnancies from 143 hospitals in 1990s [22]. As is well-known there are some limitation in HMs pathologic diagnosis, the exact frequency is not known. Although the common form of this disorder is sporadic, 1-6% of patients with a prior mole will have a second mole, and 10-20% will have a second non-molar reproductive wastage, most commonly a spontaneous abortion [23]. It is clinically important to distinguish a hydatidiform mole from a non-molar hydropic abortus, primarily because of the associated risk of post-molar gestational trophoblastic neoplasia and subsequent clinical follow up and management of the patient. Accurate subclassification of hydatidiform moles is also important, as a complete mole has a much higher risk of progression to gestational trophoblastic neoplasia (18-29%) than a partial mole (1.0-5.6%) [4, 5]. Although they occur infrequently, gestational trophoblastic tumors are important to recognize because of their varying clinical behaviors and overlapping histological features with common uterine malignancies.

Histologic changes of early complete molar pregnancy included enlarged chorionic villi with polypoid configurations, cellular myxoid stroma, and mild nonpolar hyperplasia of trophoblasts. Histologic features suspicious for partial molar pregnancy included the presence of fetal parts, enlarged admixed with normal sized villi, villous stromal edema with cistern formation, villi with irregular (scalloping) contours and trophoblast inclusions, and nonpolar hyperplasia of syncytiotrophoblast. In approximately 50% of complete moles and 74% of partial moles, the pathologic diagnoses are incorrectly made in the absence of ancillary studies, even in a gynecologic specialty practice setting [16, 24]. Our trial showed 79 cases with histologic diagnostic terms HM, including “suggestive of or suspicious for molar pregnancy” and “rule out molar pregnancy”, were obtained accurate subclassification by PCR-based short tandem repeat DNA genotyping. And 17 cases which diagnosed HMs and CHMs/PHMs by their histologic changes, were confirmed non-molar hydropic abortion by DNA genotyping. So, in order to improve the accuracy and perform subclassification of HMs, a variety of ancillary techniques can aid in the diagnosis. These include karyotyping, DNA ploidy flow cytometry, chromosomal enumeration by fluorescent in situ hybridization (FISH), and PCR-based short tandem repeat DNA genotyping [7-9].

Although conventional karyotyping is the most accurate chromosomal enumeration method that may be used to confirm the presence of triploidy in a partial mole or diploidy in a complete mole, it cannot specifically ascertain the parental origin of chromosomal contribution to the gestational tissue [25]. DNA ploidy analysis by flow cytometry is frequently used for the separation of a partial mole from a complete mole or a diploid non-molar hydropic abortus by a demonstration of triploidy [26]. However, it is not useful in the distinction between a complete mole and a non-molar hydropic abortus. Furthermore, DNA ploidy analysis cannot distinguish a digynic-monoandric non-molar gestation from a true diandric-monogynic partial mole. In addition, the use of flow cytometry for FFPE material causes not only the problems of tissue contamination, but also cultural artifacts and random or inadequate sampling, so, often increases therefore the specificity additionally by the use of a citrate buffer and RNAse digestion [27, 28]. So, flow cytometry ploidy analysis using FFPE tissue is frequently plagued with technical difficulties and interpretation errors, resulting in significant misclassification of ploidy and misdiagnosis of hydatidiform mole [29]. Interphase FISH can be used for the determination of the number of haploid chromosome sets using both fresh and FFPE tissue samples. But, similar to ploidy analysis, it cannot distinguish a diploid complete mole from a non-molar hydropic abortus and is unable to separate a true diandric-monogynic partial mole from a digynic-monoandric non-molar gestation [8, 30]. Because the above methods have their limitations, in particular, they cannot specifically ascertain the parental origin of chromosomal contribution to the gestational tissue, we need to combine histological morphology and clinical information to analyze.

With IHC markers such as $p57^{kip2}$ used, to some extent, the accuracy rate HMs diagnosis has improved. $P57^{kip2}$ expression has been found to be useful in the distinction of CHMs (including early forms) from PHMs and NMs; however, the latter two entities cannot be distinguished from one another because of shared (retained) $p57^{kip2}$ expression patterns. CHMs, including the early forms, which lack a maternal genetic contribution, have absent (or very limited) $p57^{kip2}$ expression in villous stromal cells and cytotrophoblast, but positive in intervillous intermediate trophoblast, villous endothelial cells, and gestational endometrium [31, 32]. In contrast, both PHMs and NMs (including those with abnormal villous morphology), contain a maternal chromosomal complement and exhibit diffuse $p57^{kip2}$ expression in these cell types, show strong nuclear $p57^{kip2}$ expression in cytotrophoblast, intermediate trophoblast, villous stromal cells, and decidual stromal cells [31, 33]. In our retrospective information, there were about 96.4% (54/56) showed $p57^{kip2}$ immunohistochemistry negative expression in CHMs. A weak nuclear staining was showed in 2 CHMs, probably, that might be in part because of p57 gene incompletely inactive. The cases of $p57^{kip2}$ immunohistochemistry positive expression included 26 PHMs and 11 non-molar gestations. Two DPMs showed $p57^{kip2}$ immunohistochemistry negative expression, the main reason maybe lie in the inadequate of antigen exposure. Overall, $p57^{kip2}$ immunohistochemistry can aid in the diagnosis. But, it cannot differentiate PHM from its mimics that contain maternal genetic material (hydropic abortions, trisomies). A recent study believed that there was different biological behavior between heterozygous and homozygous complete moles, the former have a more aggressive than the latter [34]. Including $p57^{kip2}$ immunohistochemistry and DNA ploidy analysis, these methods cannot distinguish them. Until a few years ago, some studies have demonstrated the value of STR genotyping, for distinguishing HM from non-molar gestations and for subtyping HMs as CHM and PHM [9].

STR genotyping allows for determination of both ploidy and the maternal/ paternal contributions of chromosome complements. Thus, it can distinguish these entities by discerning androgenetic diploidy, diandric triploidy, and biparental diploidy to diagnose CHMs, PHMs, and NMs, respectively. STR is highly prevalent noncoding repetitive DNA sequences of 2 to 7 nucleotides in the human genome and are genetically stable [35]. STR polymorphism denotes that a STR locus differs in the number of repeats between individuals. By identification of the number of STR at specific loci, a genetic profile of an individual or a cell can be ascertained to distinguish one from another. STR polymorphism analysis of gestational tissue in comparison with corresponding maternal tissue offers a determination of parental

genomic contribution and therefore can diagnose and sub-classify hydatidiform moles at the genetic level [9, 11, 36]. In this study, we evaluated 146 products of conception at the genetic level. 129 cases with abnormal genotype were identified, including 95 complete and 34 partial moles. Among 95 complete moles, 92 cases were monospermic and three were dispermic. Among 33 partial moles, 28 were dispermic and 5 were monospermic. It is important to note that 79 cases with histologic diagnostic terms HMs were accurate sub-classified, and 17 cases which diagnosed HMs and PHM/CHM by their histologic changes were confirmed non-molar hydropic abortion with DNA genotyping. 79 cases with histologic diagnostic terms HMs (including consistent with HM, suggestive of HM, rule out HM) were sub-classified into 55 monospermic complete moles, 12 dispermic partial moles, one dispermic complete moles and 11 non-molar gestations. Furthermore, one PHMs and 5 CHMs which diagnosed by their histologic changes were precise diagnosed as a monospermic complete mole and 5 dispermic partial moles. So, STR DNA genotyping is a practical and highly accurate method for the subclassification of hydatidiform moles.

STR genotyping for molar pregnancy assay resembles a conventional diagnostic molecular procedure, including manual tissue dissection, DNA extraction, a STR multiplex PCR reaction, capillary electrophoresis, and data analysis. The first step is to dissect the villous and maternal tissue as far as possible; it is the key to molecular diagnosis of HMs. In most tissue samples of product of conception, well-defined areas of chorionic villi and maternal endometrium are easily recognized in serial tissue sections and can be safely individually dissected into separate test tubes [3]. But, we had been aware of that the position and size of the villous and maternal tissue in each section would be differences, and it was hard to avoid tissue cross-contamination. Therefore, it is not accurate only by one HE section evaluates the distribution of villous and decidua tissue. In order to isolate pure populations, the remaining sections were stained with hematoxylin (Dyeing time less than 30 seconds) before the microscopic dissection. Furthermore, this process does not affect the follow-up experiment (data not shown). Of course, an absolutely pure isolation of villous tissue is generally impossible, as maternal blood and endometrial tissue or cells may be intimately admixed with chorionic villous tissue [3, 11]. In our experiment, we used a novel Hydrothermal Pressure (Pressure Cooking) coupled with chaotropic salt column purification method for DNA extraction. Under the prerequisite of guaranteeing DNA quality, this method does not only short DNA extraction time, but also greatly reduce the cost of reagent. Genotyping was performed with an AmpFlSTR® Sinofiler™ PCR Amplification Kit. The reaction consists of a short tandem repeat multiplex polymerase chain reaction (PCR) assay that amplifies 15 different autosomal STR loci and the sex-determining marker (Amelogenin) in a single PCR reaction. This kit employs the same primer sequences as used in the previous AmpFlSTR® kits with the exception of D6S1043 and D12S391. Degenerate primers for the loci D8S1179, vWA, and D16S539 were added to the AmpFlSTR® Sinofiler™ Primer Set to address mutations in the primer binding sites. The producing short amplicons are ranging from 100 to 350 bp, suitable for FFPE tissue samples. The data were derived and then analyzed by GeneMapper® ID-X version 1.2 after capillary electrophoresis. Interpretation of genotyping data is generally straightforward when the genotyping profile of the pure villous tissue is compared with that of the maternal tissue. The detailed interpretation can refer to “molecular diagnostic criteria” (the part of “Methods”). A few potential pitfalls cannot be ignored in the genotypic diagnosis of a small subset of complete mole of biparental origin, as both the paternal and the maternal genomes are present in the villus and decidua tissue, DNA genotyping is not helpful [3, 13]. In addition, a gestation derived from an egg donor pregnancy is confusing to the genotypic diagnosis. Because a donor egg will present STR alleles that may simulate a dispermic complete mole, DNA genotyping cannot distinguish an egg donor pregnancy from a true dispermic complete mole [3]. And, hydatidiform moles arising from a twin gestation may also potentially complicate analysis [3, 37]. Clinical information (recurrent mole, egg donor recipient, and twin gestation) and careful morphological assessment of the tissue, followed by isolation of pure hydropic villi for genotyping comparison, may resolve such difficult cases [3]. When there is discordance between

the genotyping result and the morphology, $p57^{kip2}$ immunohistochemistry is helpful to identify rare cases of mosaicism, chimerism, or CHM arising from a twin gestation [38, 39]. $P57^{kip2}$ immunohistochemistry and PCR-based STR DNA genotyping are powerful discriminatory markers that can be used to precisely diagnose and subtype both complete and partial hydatidiform moles.

Through this study, we believe that DNA genotyping can be effective for diagnostic measure to precisely classify hydatidiform moles. And in the absence of laser capture microdissection (LCM), hematoxylin staining plus dissection under microscopic guided is a more economic and practical method. In China, the research in hydatidiform moles by DNA genotyping is stills less, not to mention the application for clinical diagnosis. Although our laboratory has performed mature PCR-based STR DNA genotyping platform through a plenty of preclinical validation study, further studies are needed. Combining morphology and $p57^{kip2}$ immunohistochemistry as well as clinical information, integrating DNA genotyping to the routine diagnostic algorithms of hydatidiform moles, precise diagnose and subtype may be beneficial to clinical follow-up and management of the patient.

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Disclosure of conflict of interest

None.

Address correspondence to: Bo Zhang, Department of Pathology, Peking University Health Science Center, Beijing, China. Tel: 86-10-82802627; Fax: 86-10-82805462; E-mail: zhangbo2627@gmail.com

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