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Heterozygous/dispermic complete mole confers a significantly higher risk for post-molar gestational trophoblastic disease

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Abstract

Hydatidiform moles are classified at the genetic level as androgenetic complete mole and diandric-monogynic partial mole. Conflicting data exist whether heterozygous complete moles are more aggressive clinically than homozygous complete moles. We investigated clinical outcome in a large cohort of hydatidiform moles in Chinese patients with an emphasis on genotypical correlation with post-molar gestational trophoblastic disease. Consecutive products of conceptions undergoing DNA genotyping and p57 immunohistochemistry to rule out molar gestations were included from a 5-year period at Beijing Obstetrics and Gynecology Hospital. Patient demographics and clinical follow-up information were obtained. Post-molar gestational trophoblastic disease or gestational trophoblastic neoplasia was determined by the 2002 WHO/FIGO criteria. A total of 1245 products of conceptions were classified based on genotyping results into 219 complete moles, 250 partial moles, and 776 non-molar gestations. Among 219 complete moles, 186 were homozygous/monospermic and 33 were heterozygous/dispermic. Among 250 partial moles, 246 were triploid dispermic, 2 were triploid monospermic, and 2 were tetraploid heterozygous partial moles. Among 776 non-molar gestations, 644 were diploid without chromosomal aneuploidies detectable by STR genotyping and 132 had various genetic abnormalities including 122 cases of various trisomies, 2 triploid digynic-monoandric non-molar gestations, 7 cases of possible chromosomal monosomy or uniparental disomy. Successful follow-up was achieved in 165 complete moles: post-molar gestational trophoblastic disease developed in 11.6% (16/138 cases) of homozygous complete moles and 37.0% (10/27 cases) of heterozygous complete moles. The difference between the two groups was highly significant ($p = 0.0009$, chi-square). None of the 218 partial moles and 367 non-molar gestations developed post-molar gestational trophoblastic disease. In conclusion, heterozygous/dispermic complete moles are clinically more aggressive with a significantly higher risk for development of post-molar gestational trophoblastic disease compared with homozygous/monospermic complete moles. Therefore, precise genotyping classification of complete moles is important for clinical prognosis and patient management.

Introduction

Hydatidiform moles, complete or partial, are frequently encountered in the histopathological evaluation of products of conception. Accurate subclassification is essential for patient management as 15–20% of complete and 0–5% partial moles eventually develop post-molar gestational trophoblastic disease or post-molar gestational trophoblastic neoplasia (GTN) and require single or multiagent chemotherapy. While advances in reproductive medicine have drastically changed the clinical presentation of molar gestations as most of the lesions are now evacuated in their first trimester, the patient risk for development of post-molar gestational trophoblastic disease/post-molar GTN unfortunately remains unchanged [1, 2].

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According to the WHO, hydatidiform moles are defined at the genetic level, with excessive paternal genomic representation as key etiological requirement in their pathogenesis. The majority of complete moles inherit a diploid diandric genome, devoid of maternal genomic contribution [3–5]. This may arise from the fertilization of an empty egg by one spermatozoon followed by duplication (monospermic 46 XX, 90%) or from simultaneous fertilization of an empty egg by two spermatozoa (dispermic 46 XX or XY, 10%). Most tetraploid moles are triandric-monogynic partial moles [6, 7]. A percentage of complete moles are familial biparental due to germline mutations of NLRP7 or KHDC3L. Partial hydatidiform moles have a triploid dispermic genome arising from the fertilization of a haploid egg by either two spermatozoa (dispermic, 95%) or fertilization by one spermatozoon followed by duplication (monospermic, <5%) [8, 9]. Gestations with triploid digynic and monoandric genome do not represent true partial moles at the pathological and clinical levels. The distinct genetic characteristics of various molar gestations have been exploited for accurate diagnosis in the recent decade, and DNA genotyping is now considered the gold standard for precise classification of hydatidiform moles [10].

While complete moles carry a significantly higher risk than partial moles for the development of post-molar gestational trophoblastic disease, genotypical zygosity of complete moles in correlation with post-molar gestational trophoblastic disease has been a subject of investigations in recent past. A higher frequency of post-molar gestational trophoblastic disease was consistently observed in heterozygous complete moles compared with homozygous complete moles [11–15], and three previous studies found a statistical significance [12, 16, 17]. However, a recent investigation failed to confirm a significant p value [18]. In the current study, we assessed the risk of post-molar gestational trophoblastic disease in correlation with molar genotypes in large cohorts of Chinese patients consecutively diagnosed at Beijing Obstetrics and Gynecology Hospital.

Materials and methods

This study was approved by the institutional review board. Consecutive cases of products of conceptions that underwent DNA genotyping were collected during a 5-year period between 2015 and 2019 at Beijing Obstetrics and Gynecology Hospital. DNA genotyping classification of hydatidiform moles was routinely performed in all cases with a diagnosis of or suspicion for molar gestation based on the initial histological evaluation. The histological features suspicious for early complete mole included enlarged chorionic villi, polypoid villous configurations, cellular

myxoid villous stroma, and abnormal trophoblastic hyperplasia (Fig. 1). Features of fully developed complete mole were seen only in one case presenting in the second trimester and showed marked diffuse villous edema with cistern formation and marked abnormal trophoblastic hyperplasia. Histological features suspicious for partial mole included admixture of enlarged and normal-sized villi, villous stromal edema with cistern formation, irregular (scalloped) villous contours, trophoblast pseudo-inclusions, and abnormal trophoblastic hyperplasia (Fig. 2).

STR genotyping diagnosis of hydatidiform moles

Ten serial sections of 10 μm thick were generated from paraffin-embedded formalin-fixed tissue blocks, with the first one stained with hematoxylin–eosin to verify the distribution of villous and normal maternal tissue, and the remaining nine sections used for the microscopic dissection. Pure chorionic villi and maternal gestational endometrium were scraped separately from the unstained sections using a sterile scalpel into separate microcentrifuge tubes. DNA was then extracted by hydrothermal pressure method of simultaneous deparaffinization and lysis of formalin-fixed paraffin-embedded tissue followed by conventional column purification to obtain high-quality DNA [19]. The concentration of DNA preparation was determined by the absorbance at 260 nm. Tissue genotyping using PowerPlex® 16 System (Promega Corporation, Madison, WI, USA) was performed by multiplex polymerase chain reaction (PCR) at 15 short tandem repeat (STR) loci according to manufacturer instruction. One microliter of the PCR product was mixed with 13 μL of Hi-Di and 0.5 μL sizing marker (GeneScan-500LIZ, Applied Biosystems, Inc.), followed by capillary electrophoresis on an ABI3130 platform. Data collection and analysis were performed using GeneMapper software version 3.7 (Applied Biosystems, Inc., Foster City, California, USA). PCR products were identified by fluorescent color and expected size range. The genotype of paired gestational endometrium was compared with the chorionic villous genotype at each locus to assess if the villous tissue contains unique paternal alleles that are not present in the paired gestational endometrium. Molecular interpretations of the data have been previously described [8, 20]. Briefly, a molecular diagnosis of complete mole was made when the genotyping profiles of the villous tissue demonstrated exclusively paternal alleles of either homozygous/monospermic (Fig. 1) or heterozygous/dispermic patterns. A genotyping diagnosis of partial mole was made when two distinct paternal alleles were found in at least two STR loci along with the presence of two allelic homozygous paternal copies in addition to the presence of maternal allele at the remaining STR loci (Fig. 2). Non-molar hydropic abortions were diagnosed when a balanced biparental
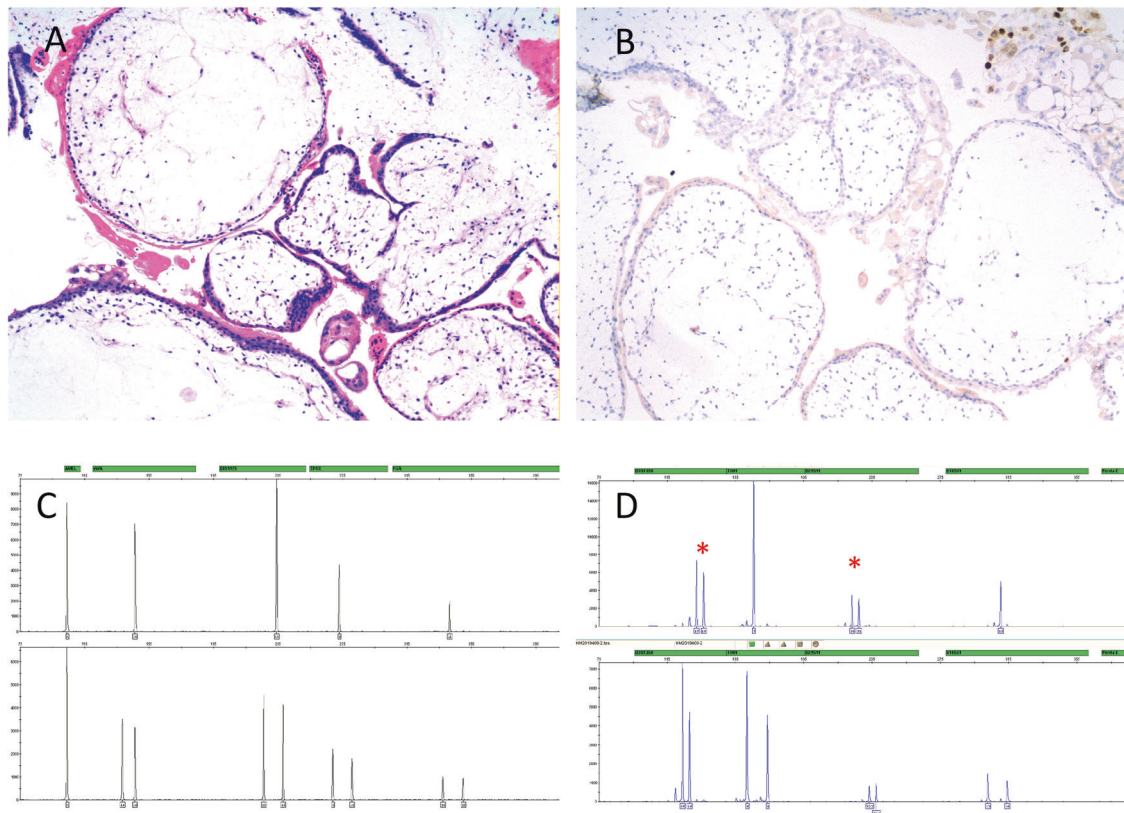
Fig. 1 Complete hydatidiform moles. Morphologic features of early complete moles (**a**) with characteristic bulbous, "cauliflower"-shaped chorionic villi with hypercellular, myxoid stroma, and karyorrhexis. P57 immunohistochemistry in early complete mole (**b**) with loss of nuclear expression in villous cytotrophoblast and stromal cells. STR genotype of homozygous/monospermic complete mole demonstrates a unique paternal allele in duplicate quantity and absence of maternal allele in the chorionic villi at multiple STR loci (**c**): upper panel—chorionic villi; lower panel—paired gestational endometrium. STR genotype of heterozygous/dispermic complete mole shows the presence of two unique paternal alleles (indicated by asterisk) and absence of maternal allele in the chorionic villi at multiple STR loci (**d**): upper panel—chorionic villi; lower panel—paired gestational endometrium.

genetic profile was seen. Various isolated copy changes involving one or two STR loci were interpreted as trisomy (three allelic copies at a particular STR locus, Fig. 3), uniparental disomy (two paternal allelic copies without a matching maternal allele at a particular STR locus), and monosomy (one paternal copy without a matching maternal allele at a particular STR locus).

P57 immunohistochemistry

Immunohistochemical stain was performed in selected cases using p57 antibody (Leica, NCL-p57) at 1:100 dilution by the EnVision+ System from DAKO (Carpinteria, CA) to corroborate the DNA genotyping result and histological findings. Presence of nuclear immunostaining was assessed, and positive staining in chorionic villus stromal cells, cytotrophoblast and intermediate trophoblast were considered a normal expression pattern seen in non-molar gestation and partial mole (Fig. 2). Absence of nuclear staining in villous cytotrophoblast and stromal cells were interpreted as abnormal imprinting loss of p57 expression,

supporting the diagnosis of complete mole (Fig. 1). P57 staining in decidual tissue was used as positive internal control.

Clinical follow-up for post-molar gestational trophoblastic disease

Patient demographics and clinical follow-up data were obtained from the patients' charts, including patient age, gestational history, gestational age at the time of evacuation, pre-evacuation serum hCG, post-molar serum hCG measurements, and pertinent clinical follow-up information. Diagnosis of post-molar gestational trophoblastic disease or post-molar GTN was made following the 2002 WHO/FIGO criteria [21–23], when one of the following findings was observed: (1) a plateau of human chorionic gonadotropin (hCG) (+/−10%) lasted for four measurements over a period of 3 weeks or longer; (2) a rise of hCG over 10% in three consecutive weekly measurements or longer, over at least a period of 2 weeks; (3) the hCG level remained elevated for 6 months or longer; and (4) histological diagnosis
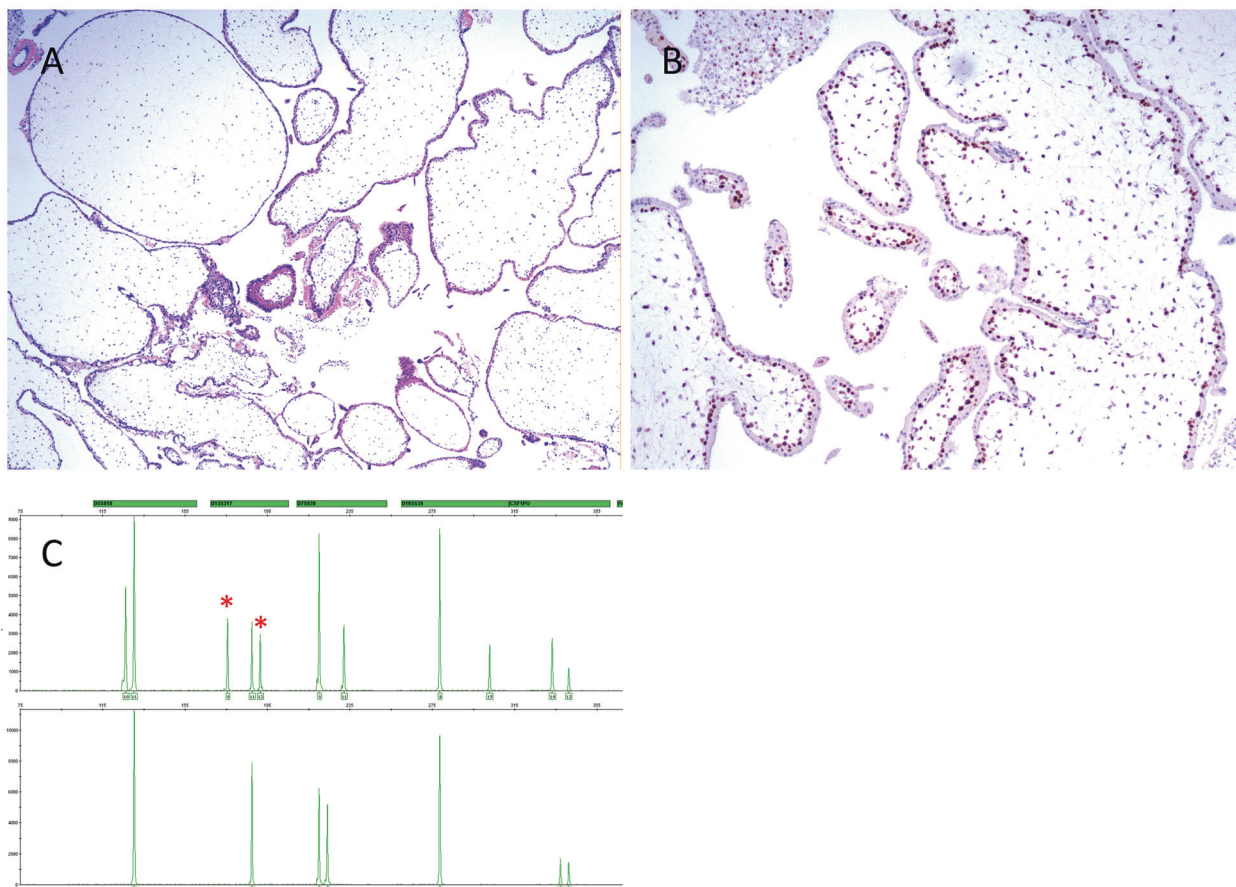
Fig. 2 Heterozygous/dispermic partial hydatidiform mole. Morphological features of an early partial mole with marked villous hydrop, subtle abnormal trophoblastic proliferation, and irregular villous contour (a). P57 immunohistochemistry shows retained nuclear expression in villous cytotrophoblast and stromal cells (b). STR genotype shows the presence of two paternal allelic copies at multiple loci (c): upper panel—chorionic villi, asterisk indicates two distinct paternal alleles at D13S317 locus; lower panel—paired gestational endometrium.
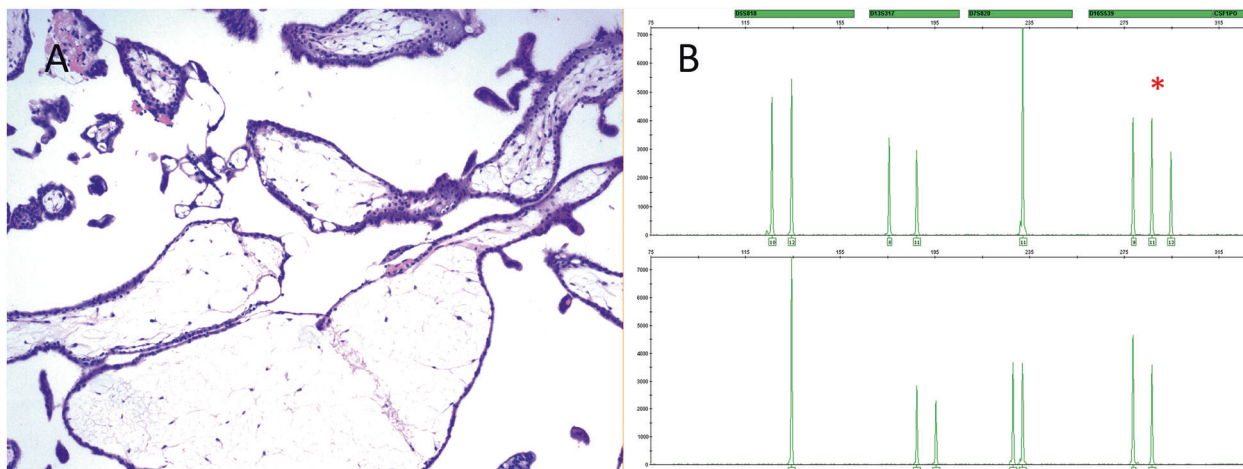

Fig. 3 Product of conception of trisomy 16. Marked villous hydrop is seen in this product of conception of trisomy 16 (a) and STR genotyping shows the presence of three allelic copies (indicated by asterisk) at D16S539 locus (b): upper panel—chorionic villi; lower panel—paired gestational endometrium.

Table 1 Genotypical classification of products of conception ($N = 1245$).

Gestational type			Informative follow-up	Incomplete or loss of follow-up
Hydatidiform moles	CHM (219)	Diploid homozygous CHM	138	48
		Diploid heterozygous CHM[a]	27	6
	PHM (250)	Triploid heterozygous PHM[b]	214	32
		Triploid homozygous PHM	2	0
		Tetraploid heterozygous PHM	2	0
Non-molar Gestations	Diploid HA (644)		305	339
	Trisomies (122)	Trisomy 2	0	1
		Trisomy 3	4	2
		Trisomy 4	0	4
		Trisomy 7	6	6
		Trisomy 7 and 18	1	0
		Trisomy 8	0	4
		Trisomy 11	1	0
		Trisomy 12 and 21	0	1
		Trisomy 13	6	6
		Trisomy 15	0	1
		Trisomy 16	30	31
		Trisomy 16 and 8	0	1
		Trisomy 18	5	6
		Trisomy 21	1	5
	Other abnormal genotypes [10]	Monosomy or uniparental disomy 11	2	0
		Monosomy or uniparental disomy 13	1	0
		Monosomy or uniparental disomy 18	1	0
		Monsomy or uniparental disomy 21[c]	3	0
		Digynic triploid gestation	1	1
		X deletion	0	1
Total	1245		750	495

CHM complete hydatidiform mole, *PHM* partial hydatidiform mole, *HA* hydropic abortus.

[a]Including two cases of diploid heterozygous CHM with trisomy 3 or trisomy 8.

[b]Including two cases of triploid heterozygous PHM with tetrasomy 8.

[c]Including one case with two different alleles at the chromosomal 21 locus.

of gestational choriocarcinoma. Spontaneous remission was defined as a serum hCG level that was within the reference range (<1.0 mIU/mL) for at least 6 months.

Statistical analysis

Chi-square categorical analysis was performed for different genotypes of moles in correlation with the presence of post-molar gestational trophoblastic disease with p value of <0.05 considered statistically significant.

Results

In this single institutional study of Chinese patients during a 5-year period, a total of 1245 consecutive products of

Table 2 Clinicopathological data of patients with successful follow-up ($N = 750$).

Gestational type			# Cases	Patient age in years (range/average/median)	Gestational weeks (range/average/median)	Serum hCG (mIU/ml) at evacuation (range/average/median)
Hydatidiform Moles	CHM	Combined	165	19–50/31.2/30	6–14/8.2/8	5,006–1,240,000/129,954/103,266
		Homozygous CHM	138	19–50/31.6/30	6–12/8.2/8	5,006–862,307/119,246/101,217
		Heterozygous CHM	27	19–40/29.3/29	6–14/8.3/8	7,938–1,240,000/183,100/105,277
	PHM		218	19–48/31.2/31	6–14/9.6/9	4,298–510,000/80,754/58,459
Non-molar gestations	Diploid HA		305	19–45/31.9/31	6–15/8.9/9	1,091–200,000 /45,422/30,624
	Trisomies		54	25–44/32.8/33	7–13/9.1/9	2,476–10,4209 /29,490/20,518
	Other genotypes		8	28–39/32.1/30	6–9/7.3/7	13,013–52,692/32,976/37,359

CHM complete hydatidiform mole, *PHM* partial hydatidiform mole, *HA* hydropic abortus, *hCG* human chorionic gonadotropin.

Table 3 Post-molar gestational trophoblastic disease.

Gestation type	Informative follow-up	Post-molar GTD	% Post-molar GTD
Homozygous CHM	138	16	11.6[a]
Heterozygous CHM[b]	27	10	37.0[a]
Triploid homozygous PHM	2	0	0
Triploid heterozygous PHM[c]	214	0	0
Tetraploid heterozygous PHM	2	0	0
Non-molar gestation	367	0	0
Total	750	26	3.5%

CHM complete hydatidiform mole, *PHM* partial hydatidiform mole, *GTD* gestational trophoblastic disease.

[a]Statistically significant difference between the two groups ($p = 0.0009$).

[b]Including two cases of diploid heterozygous CHM with trisomy 3 or trisomy 8.

[c]Including two cases of triploid heterozygous PHM with tetrasomy 8.

conceptions with various levels of histological suspicion for hydatidiform moles were successfully genotyped (Table 1). There were 219 complete moles, 250 partial moles, and 776 non-molar gestations (Table 1). All hydatidiform moles were evacuated during the first trimester, except one complete mole was evacuated at 14 weeks, and five partial moles were evacuated at 13–14 weeks of gestational age. P57 immunohistochemistry was performed to correlate the histopathological findings and genotyping results in 206 complete moles, 244 partial moles, and 667 non-molar gestations. Abnormal loss of nuclear expression of p57 in cytotrophoblast and villous stromal cells was seen in all complete moles except for one heterozygous complete mole. Normal p57 expression pattern was seen in all partial moles and non-molar gestations. Among 219 complete moles, 186 were homozygous/monospermic (Fig. 2) and 33 were heterozygous/dispermic. Two heterozygous complete moles showed evidence of trisomy 3 or trisomy 8. The

patient age of complete moles ranged from 18 to 50 years with an average of 31.2 years and median of 30 years (Table 2). The gestational age ranged from 6 to 14 weeks with an average of 8.2 weeks and median of 8 weeks. The serum hCG at the initial evacuation ranged from 5,006 to 1,240,000 mIU/ml with an average of 129,954 mIU/ml and median of 103.266 mIU/ml (Table 2). When separately considered, heterozygous complete moles had a higher serum hCG level at the time of evacuation (average of 183,100 mIU/ml, range from 7939 to 1,240,000 and median of 105,277 mIU/ml) than that of the homozygous complete moles (average 119,246 mIU/ml, range from 5,006 to 862,307 mIU/ml and median of 101,217 mIU/ml).

Among 250 partial moles, 248 were heterozygous/dispermic (Fig. 3) and 2 were homozygous/monospermic. Two partial moles were proven tetraploid harboring three haploid paternal chromosome sets. The age of patients with partial moles ranged from 19 to 48 years with an average of 31.2 years and median of 31 years (Table 2). The gestational age ranged from 6 to 14 weeks with an average of 9.6 weeks and median of 9 weeks. Two heterozygous partial moles had four copies at the chromosome 8 locus, suggesting tetrasomy 8. The serum hCG at the initial evacuation ranged from 4298 to 510,000 mIU/ml with an average of 80,754 mIU/ml and median of 58,459 mIU/ml (Table 2).

Among 776 non-molar gestations, 644 cases were diploid without detectable genomic alterations and 132 cases showed various STR abnormalities (Table 1), among which 122 cases were trisomies, including trisomy 16 (61 cases), trisomy 7 (12 cases), trisomy 13 (12 cases), trisomy 18 (11 cases), trisomy 21 (6 cases), trisomy 3 (6 cases), trisomy 8 (4 cases), trisomy 4 (4 cases), trisomy 2 (1 case), trisomy 11 (1 case), combined trisomy 7/18 (1 case), combined trisomy 8/16 (1 case), and combined trisomy 12/21 (1 case). Two cases were proven triploid digynic-monoandric non-molar gestations. One case had loss of one X chromosome complement. In seven cases of non-molar cases, one or possible two copies of identical allele in six cases and two different alleles in one case were found to

derive from the parental source without a matching maternal allele at isolated STR loci involving various chromosomes suggesting the presence of either monosomy or uniparental disomy, including chromosome 11 (two cases), chromosome 13 (one case), chromosome 18 (one case), and chromosome 21 (three cases).

Successful molar surveillance for post-molar gestational trophoblastic disease was achieved in 165/219 complete moles, 218/250 partial moles, and 367/776 non-molar gestations (Table 3). Among 165 complete hydatidiform moles, post-molar gestational trophoblastic disease developed in 11.6% (16/138) of patients with homozygous complete moles and in 37.0% (10/27 cases) of patients with heterozygous complete moles. The difference between the two groups was highly significant (p value of 0.0009, chi-square). None of the 218 partial moles and 367 non-molar gestations—including 305 diploid hydropic abortions, 54 various trisomies, and 8 other non-molar abnormal genotypes—developed GTN in this study.

Discussion

It is clinically important to distinguish hydatidiform moles from non-molar gestations primarily because of the associated risk of developing post-molar gestational trophoblastic disease. Subclassification of molar pregnancies is just as important, as complete moles have a significantly higher risk of post-molar gestational trophoblastic disease (15–20%) in contrast to 0–5% in partial moles. Moreover, 3–5% of complete moles may progress to gestational choriocarcinoma, whereas the risk of choriocarcinoma is <0.2% for partial moles [22]. Although the genetic basis of molar gestations has been established for over three decades, clinical diagnostic applications of this fundamental knowledge only occurred recently [8, 20, 24]. Over the past decade, the clinical sensitivity and specificity of DNA genotyping diagnosis of hydatidiform moles using formalin-fixed paraffin-embedded tissue samples have been confirmed by numerous studies [25–29]. In fact, DNA genotyping is now considered the gold standard for accurate diagnosis and subclassification of hydatidiform moles [10]. Importantly genotyping classification allows more accurate assessment of the clinical behavior of various types of molar gestations. In our current study, post-molar gestational trophoblastic disease was observed only in the two cohorts of complete moles. Among 219 cases of complete moles, successful post-molar follow-up was achieved in 165 cases, including 138 homozygous complete moles and 27 heterozygous complete moles. Post-molar gestational trophoblastic disease developed in 11.6% (16/165 cases) of homozygous complete moles and in 37.0% (10/27 cases) of heterozygous complete moles ($p = 0.0009$).

While it has been well established that complete moles carry a significantly higher risk than partial moles in the development of post-molar gestational trophoblastic disease, the genetic subtypes of complete moles in correlation with post-molar gestational trophoblastic disease have been a subject of many investigations. Although a higher frequency of post-molar gestational trophoblastic disease was consistently observed in heterozygous complete moles compared with homozygous ones, many studies failed to reach a statistical significance, likely due to small study cohorts and technical limitations in the detection of the genetic zygosity [11–15, 30]. Using an identical STR genotyping kit as in our study, one Japanese investigation of 27 patients found a significantly higher risk of post-molar gestational trophoblastic disease in heterozygous complete moles than homozygous ones [16]. Moreover, the most recent study also found a statistical significance in 204 patients with sporadic moles [17]. However, the same Japanese investigators [16] failed to confirm their previous finding in a second, follow-up study of 232 complete moles: post-molar gestational trophoblastic disease was found in 14.2% of homozygous complete moles and 21.4% of heterozygous complete moles (p value of 0.4) [18]. It is worth noting that their second study involved complete moles identified primarily by ultrasonographic findings, supplemented by macroscopic or microscopic evaluation. Moreover, nearly half of their study cases were referral patients with exclusion of those who were referred for persistently high serum hCG or treatment for low-risk GTN after a diagnosis of hydatidiform mole. In contrast, all of our patients were consecutively identified at one major obstetrics and gynecology hospital in China and the selection of study cases was primarily based on histopathological evaluation, therefore minimizing potential bias in the subject recruitment. In our study, patients who developed post-molar GTD/GTN were followed clinically by imaging studies and treated with chemotherapy accordingly. Consistent with the current clinical management guideline, surgical procedures were not given to any patients to obtain a tissue diagnosis of post-molar GTD/GTN. Therefore further histological subclassification of GTD/GTN could not be achieved among the study subjects and a correlation of specific types of post-molar GTD/GTN with the two genetic subtypes of complete moles, particularly the risk of gestational choriocarcinoma, cannot be determined.

The mammalian placenta is enriched with imprinted genes [31–37]. Half of the known 100 or so imprinted genes are functionally related to cellular proliferation [38, 39] and almost all imprinted genes that are specific to the placenta are paternally imprinted and biologically expressed only from the maternal alleles [40, 41]. Based on the "parental conflict hypothesis" [42], the intent of the paternal genome is to maximize resources for the father's own progeny but

the interest of the maternal genome is to distribute resources equally among her offspring, implying that growth-promoting genes are mainly expressed from the paternally inherited genome and are suppressed in the maternally inherited counterparts. Analyses of many imprinted genes in mammals support this theory [43, 44]. Disruption of the normal genomic imprinting in villous trophoblast may result in abnormal trophoblastic proliferation leading to hydatidiform moles. Consistent with the "parental conflict interest" theory, lack of maternal genome combined with abnormal paternal imprinting gene expression is the key molecular event in the pathogenesis of complete moles [45]. It is conceivable that, compared with homozygous complete moles, heterozygous complete moles inherit more diverse alleles from the paternal genome that may lead to additional gain of paternal imprinting gene expression, and therefore results in more aggressive trophoblastic proliferation and increases the risk for post-molar gestational trophoblastic disease.

Our data also confirmed previous observations [16] that heterozygous complete moles had a higher average serum hCG level than homozygous complete moles at the initial evacuation (183,100 vs. 119,246 mIU/ml in our study, Table 2). The maternal age and gestational age at evacuation were comparable between heterozygous and homozygous complete moles (Table 2).

In general, the prevalence of partial mole is higher than that of complete mole [46]. This is consistently observed in our study where among 1245 products of conceptions, 250 cases were partial moles and 219 were complete moles, with a ratio of 1.14. While the patient age and gestational age were comparable between complete and partial moles, patients with partial moles had a lower average and median serum hCG at the time of evacuation than those with complete moles (80,754 and 58,459 mIU vs. 129,954 and 103,266 mIU, respectively). In general, the risk of post-molar gestational trophoblastic disease in partial moles is 0–5% based on prior literature [18, 47, 48]. However, none of the patients with partial moles developed post-molar gestational trophoblastic disease in our study. The reason for this unexpected result is uncertain, but may be due to our study cohort confined to the ethnic Chinese.

In line with the literature [24, 49], trisomies were the most common abnormal genotypes observed among non-molar gestations in our study (Table 1). There were 122 cases of various trisomies with trisomy 16 being the most common one (61 cases), followed by trisomy 7 (12 cases), trisomy 13 (12 cases), trisomy 18 (11 cases), trisomy 21 (6 cases), trisomy 3 (6 cases), trisomy 8 (4 cases), trisomy 4 (4 cases), trisomy 2 (1 case), trisomy 11 (1 case), combined trisomy 7 and 18 (1 case), combined trisomy 8 and 16 (1 case), and combined trisomy 12 and 21 (1 case). Two triploid cases were proven digynic-monoandric non-molar

gestations. One case had loss of one X chromosome complement. Possible monosomy or uniparental disomy was observed in seven cases, involving chromosome 11 (two cases), chromosome 13 (one case), chromosome 18 (one case), and chromosome 21 (three cases). The predominance of trisomy 16 among various trisomy cases is consistent with the published data [49].

One dispermic/heterozygous complete mole was found to have retained p57 expression in villous cytotrophoblast and stromal cells. Careful review of its genotyping data revealed the presence of possible two copies of identical allele at TH01 locus on chromosome 11 in the chorionic villi in contrast to the presence of two distinct TH01 alleles in the corresponding gestational endometrium. P57 is colocalized with TH01 on the short arm of chromosome 11 (11p15). Therefore, a retained maternal chromosome 11 is possible, which may explain the retained expression of p57 expression in this complete mole. However, it cannot be determined with certainty since the two identical TH01 alleles in chorionic villi are shared by one of the two maternal alleles in the corresponding gestational endometrium.

In conclusion, heterozygous/dispermic complete moles are clinically more aggressive with a significantly higher risk for development of post-molar gestational trophoblastic disease than homozygous/monospermic complete moles. None of the partial moles developed post-molar gestational trophoblastic disease in our study cohort of Chinese patients. Genotyping classification of complete moles into precise subtypes based on their genetic zygosity is therefore important for risk assessment for post-molar gestational trophoblastic disease and subsequent patient management. Future studies are needed, particularly at major medical centers with molecular diagnostic capability, to further strengthen the clinical validity of integrating molecular genotyping into the routine diagnostic/risk scoring algorithms for patients with hydatidiform moles.

Compliance with ethical standards

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