




Comprehensive analysis of 204 sporadic hydatidiform moles: revisiting risk factors and their correlations with the molar genotypes

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Abstract

Hydatidiform mole (HM) is an aberrant human pregnancy characterized by excessive trophoblastic proliferation and abnormal embryonic development. HM has two morphological types, complete (CHM) and partial (PHM), and non-recurrent ones have three genotypic types, androgenetic monospermic, androgenetic dispermic, and triploid dispermic. Most available studies on risk factors predisposing to different types of HM and their malignant transformation mainly suffer from the lack of comprehensive genotypic analysis of large cohorts of molar tissues combined with accurate postmolar hCG follow-up. Moreover, 10–20% of patients with one HM have at least one non-molar miscarriage, which is higher than the frequency of two pregnancy losses in the general population (2–5%), suggesting a common genetic susceptibility to HM and miscarriages. However, the underlying causes of the miscarriages in these patients are unknown. Here, we comprehensively analyzed 204 HM, mostly from patients referred to the Quebec Registry of Trophoblastic Diseases and for which postmolar hCG monitoring is available, and 30 of their non-molar miscarriages. We revisited the risk of maternal age and neoplastic transformation across the different HM genotypic categories and investigated the presence of chromosomal abnormalities in their non-molar miscarriages. We confirm that androgenetic CHM is more prone to gestational trophoblastic neoplasia (GTN) than triploid dispermic PHM, and androgenetic dispermic CHM is more prone to high-risk GTN and choriocarcinoma (CC) than androgenetic monospermic CHM. We also confirm the association between increased maternal age and androgenetic CHM and their malignancies. Most importantly, we demonstrate for the first time that patients with an HM and miscarriages are at higher risk for aneuploid miscarriages [83.3%, 95% confidence interval (CI): 0.653–0.944] than women with sporadic (51.5%, 95% CI: 50.3–52.7%, p value = 0.0003828) or recurrent miscarriages (43.8%, 95% CI: 40.7–47.0%, p value = 0.00002). Our data suggest common genetic female germline defects predisposing to HM and aneuploid non-molar miscarriages in some patients.

Introduction

Hydatidiform mole (HM) is a human pregnancy characterized by abnormal embryonic development, hydropic

degeneration of chorionic villi, and excessive trophoblastic proliferation. In the past, HM used to be divided into two types, complete HM (CHM) and partial HM (PHM), based on morphological and cytogenetic evaluation [1, 2].

Since the original description of the two morphological entities, different methods have been developed to determine the parental contribution to the molar genomes and led to the conclusions that most CHM is diploid androgenetic monospermic, most PHM is triploid dispermic, and several morphologically diagnosed CHM or PHM are diploid biparental [3]. In the last 10 years, the improvement of existing methods, the emergence of more

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informative and efficient genotyping methods by multiplexing several markers, and the combined use of several methods have led to more accurate conclusions about molar genotypes. While androgenetic monospermic, androgenetic dispermic, and triploid dispermic genotypes are now believed to be the only genotypic types of sporadic HM, their frequencies were slightly revised as follows. Of androgenetic CHM, 85% are monospermic and 15% are dispermic [4]. Among triploid PHM, 98% are dispermic and 2% are monospermic [4, 5]. However, most diploid biparental conceptions previously diagnosed as HM are now believed to have been misclassified as HM and are indeed diploid biparental aneuploid conceptions that have some morphological features of moles [5–8]. The only exception to this is recurrent diploid biparental HM from patients with biallelic mutations in *NLRP7* [9–11], *KHDC3L* [12–15], or rarely *PADI6* [16], which may have the morphological features of complete and/or partial HM [9] and are sometimes diagnosed as atypical HM [10, 11]. Other very rare types of conceptions that may morphologically mimic HM and be misdiagnosed as CHM or PHM are tetraploid conceptions and triploid digynic conceptions.

The goal of this study is to comprehensively analyze a large cohort of sporadic moles, mostly from the Quebec Registry of Trophoblastic Diseases, with complete follow-up and postmolar hCG monitoring and re-evaluate some of the risk factors for HM in relation to the accurate molar genotypes. In addition, a history of miscarriages is a well-documented risk factor for HM [17–19]. However, the causes of miscarriages in women with one HM have remained unknown. To answer this question, we investigated the causes in the non-molar miscarriages of patients with CHM or PHM.

Materials and methods

Patients

Patients with an HM were referred to our laboratory between 2006 and 2017; the majority (184 out of 204) were from the Quebec Trophoblastic Disease Registry (Registre des Maladies Trophoblastiques du Québec, RMTQ, <http://www.rmtq.ca/en/>) [20] and some were from other collaborators. All recruited patients provided written consent to participate in our study, agreed to a blood draw for genotyping analysis, and agreed for us to retrieve their molar and non-molar products of conception (POCs) from their various histopathology laboratories for research purposes and to have access to their medical files. Our study population was also combined with that of Banet et al. [4] to test for certain associations. This study was approved by the McGill Institutional Review Board (IRB# A01-M07-98 03A).

Histopathological review

Hematoxylin and eosin-stained tissue sections of the POCs were morphologically evaluated independently by two pathologists (K.R. and J.A.) according to standard criteria [1]. For all molar tissues, the diagnosis was revised to take into consideration the integrated data from various methods. GTN diagnosis and staging were performed according to the International Federation of Gynecology and Obstetrics (FIGO) criteria [21]. Choriocarcinoma diagnosis (by K.R.) was based on histopathology and the presence of biphasic proliferation of mononucleate trophoblast and syncytiotrophoblast cells with the absence of chorionic villi and the presence of hemorrhagic areas associated with significant and variable amounts of necrosis.

Parental contribution to the molar tissues

P57^{KIP2} immunohistochemistry

P57^{KIP2} immunohistochemistry was performed on 4- μ m sections of formalin-fixed paraffin-embedded (FFPE) tissues as previously described [22]. For each POC, the p57^{KIP2} immunostaining result was interpreted as negative when endometrial and/or extravillous trophoblastic cells (EVT), which serve as an internal positive control, exhibited nuclear p57^{KIP2} staining but villous stromal and/or cytotrophoblastic cells did not. The result was interpreted as positive when cytotrophoblast and/or villous stromal cells showed nuclear staining of p57^{KIP2}.

Flow cytometry

Flow cytometry was performed on FFPE tissues following Hedley's protocol [23] with modifications as previously described [24]. Briefly, two 60- μ m sections from each FFPE block were deparaffinized with xylene and gradually rehydrated. The proteins were digested in 1 ml of 5 mg/ml pepsin (Sigma-Aldrich, St. Louis, USA) in 0.9% NaCl (adjusted to pH 1.5 with HCl). Propidium iodide solution (0.1 mg/ μ l, Sigma-Aldrich) and 50 μ l of RNase (1 mg/ml) were added to the cell suspension and then incubated at 37 °C for 30 min. They were then filtered through a 48- μ m mesh nylon filter and analyzed using a BD FACS Canto II at the Immunophenotyping Core Facility of the McGill University Health Centre Research Institute. Data were analyzed using FCSalyzer (Wien, Austria).

Microsatellite DNA genotyping

The FFPE blocks used for analysis were chosen based on the amount of chorionic villi (CV) they contained. Five to twelve serial 10- μ m sections were cut from each block. The

sections were mounted on slides and stained with hematoxylin and eosin (H&E). Under a stereomicroscope, CV were collected from the slides using Kimwipes and forceps and used for DNA extraction using the Qiagen QIAamp DNA FFPE Tissue Kit (Catalog number 56404, Hilden, Germany). Extracted DNA was quantified using a Nanodrop and loaded on a 2% agarose gel for quality evaluation and to determine the required amount for multiplex fluorescent microsatellite genotyping with the PowerPlex 16 HS System (Promega Corporation, Fitchburg, Wisconsin, USA). The reaction consisted of a short tandem repeat (STR) multiplex polymerase chain reaction (PCR) assay that amplifies DNA at 15 different STR loci and a fragment from the X and Y Amelogenin gene. DNA from the POCs and their available parents was amplified and the PCR products were resolved by capillary electrophoresis using an Applied Biosystems 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA) at the Centre for Applied Genomics (<http://www.tcag.ca>). The data were analyzed with PeakScanner, version 1.0 (Applied Biosystems, Foster City, CA, USA) and the POC alleles were compared with the parental alleles to determine their origin.

Fluorescent in situ hybridization

Fluorescent in situ hybridization (FISH) was performed on 4- μ m sections that were hybridized to centromeric probes from chromosomes X, Y, and 18 as previously described [25]. On some tissues, other probes were also used. At least 100 cells for each POC were scored with each probe.

SNP microarray

Single-nucleotide polymorphism (SNP)-based whole-genome chromosomal microarray analysis was performed using the HumanCytoSNP-12 microarray (Illumina, San Diego, CA) at Invitae as previously described [26].

Statistical analysis

We estimated 95% confidence intervals (CI) using exact binomial calculations and tested for differences using Fisher's exact test (two tailed, <http://www.quantitativeskills.com/>).

Results

Strategy of the analysis, main limitations of various methods, and the benefits of their combination

To determine the parental contribution to the POC, we performed comprehensive analyses using three independent

methods, p57 immunohistochemistry, ploidy analysis by flow cytometry, and STR genotyping. These methods were performed systematically for all cases when appropriate materials were available. The results from the three methods, as well as those of the morphological evaluations, were compared and reconciled. Any discrepancies were resolved by repeating whichever methods led to discordant results; in some cases, discrepancies were resolved either by performing FISH on tissue sections or by performing additional simplex genotyping with appropriate markers. The systematic use of different methods along with the comparison and integration of their results allows for an accurate diagnosis and for the identification of errors obtained when relying on a single method. From our experience of genotyping ~350 FFPE molar tissues, the limitations of the various methods and the lessons we have learned can be summarized as follows.

p57^{KIP2} immunohistochemistry

The main limitation of this methodology stems from the quality of the tissue preparation and fixation that may lead to inappropriate p57^{KIP2} reactivity. Such a problem may reveal itself when the EVT and/or endometrial cells, used as an internal control, are not stained. In a subtler example, the EVT and/or endometrial cells may be less than optimally stained, and this may be accompanied by negative staining of the cytotrophoblast nuclei in a tissue that expresses p57^{KIP2} because in normal first-trimester trophoblastic tissues, the expression of p57^{KIP2} in the cytotrophoblast is much lower than that in the EVT. A more in-depth description of problems encountered with p57^{KIP2} immunohistochemistry and their troubleshooting are described on this excellent website (<http://www.nordiqc.org>).

Flow cytometry

The main problem may lie in insufficient amounts of chorionic villi in the FFPE blocks, which can prevent the detection of a triploid peak in a triploid PHM. Furthermore, tetraploid conceptions were not detected by flow cytometry under our experimental parameters because the tetraploid DNA content corresponds to the same DNA content of diploid cells in the G2 phase of the cell cycle.

STR genotyping

While this is an invaluable technique, it has numerous challenges that one needs to be aware of to benefit from this method's full potential. The most critical problems include the following: (1) contamination with maternal tissues in POCs that have CV intermingled with maternal tissues. (2) The poor quality of the DNA extracted from

Table 1 Frequencies of neoplastic transformation among different genotypic types of HM.

	HM genotype			
	Androgenetic monospermic	Androgenetic dispermic	Triploid dispermic	Twin (HM and fetus)/mosaic
Cohort size ($n = 204$)	114 (55.9%)	12 (5.9%)	69 (33.8%)	9 (4.4%)
Number with known follow-up	95	12	62	7
No GTN	<u>49</u>	<u>1</u>	<u>61</u>	4
GTN	<u>46</u> (48.4%)	<u>11</u> (91.7%)	<u>1</u> (1.6%)	3 (43%)
95% CI	0.38–0.59	0.62–1.0	0.0–0.087	n.a.
	57 (53.3%)			
Low risk ≤ 6	39	6	1	1
High risk ≥ 7	5 (10.9%)	3 (27.2%)	0	1
CC	<u>2</u> (1.8%)	<u>3</u> (25%)	<u>0</u>	0
95% CI	0.003–0.074	0.055–0.57	0–0.058	n.a.
Unknown risk	2	2	0	1

GTN gestational trophoblastic neoplasia, CI confidence interval, CC choriocarcinoma, n.a. not applicable. Categories and numbers represented in the histograms of Fig. 1 are underlined

FFPE tissues due to prior fixation and processing or long-term preservation. This may result in the amplification of low amounts of contaminating DNA from various sources that can in turn lead to non-maternal peaks that complicate the interpretation of the genotyping results (e.g., these peaks could be mistaken as paternal alleles in the absence of the paternal genotype). (3) The low quality of the STR genotyping and the amplification of too few markers may not allow the detection of all XX androgenetic dispermic moles. Throughout our analyses, two out of the five XX androgenetic dispermic CHM were initially misdiagnosed as monospermic CHM. After improvements to our protocol, the genotyping analysis was repeated and revealed that the two CHM were in fact androgenetic dispermic. Based on our experience, we believe that many studies underestimate the number of XX androgenetic dispermic CHM, which should theoretically account for one-third of all dispermic androgenetic CHM. Since YY conceptions do not survive early cleavage stages, the remaining androgenetic dispermic CHM (two-thirds) is expected to be XY.

Distribution of the HM genotypes and their neoplastic transformation

The analyses described above allowed us to uncover the genetic mechanisms of origin of a total of 204 sporadic moles. A summary of the genotyping results is provided in Table 1 and the results of all methods are portrayed in Supplementary Table 1. Of the 204 tissues, 114 (55.9%) were found to be androgenetic monospermic, 12 (5.9%) androgenetic dispermic, 69 (33.8%) triploid dispermic, and the remaining 9 cases (4.4%) consisted of twin or mosaic

conceptions detected initially by ultrasound or p57^{KIP2} immunohistochemistry (Table 1).

For the analysis of the association of neoplastic transformation across HM genotypes, we only included HM that were referred to us by the RMTQ and for which complete follow-up and hCG measurements were available that allowed for accurate staging according to the FIGO guidelines [27]. We found that 48.4% (46/95) of androgenetic monospermic moles developed GTN and 1.8% (2/95) developed CC. Of the 12 androgenetic dispermic moles with complete follow-up, 91.7% (11/12) developed GTN and 25% (3/12) gave rise to CC. Among the triploid dispermic moles, 1.6% (1/62) led to a GTN, and none developed a CC. Among the 9 twin/mosaic conceptions, complete follow-up was available for 7 cases, of which 43% (3/7) developed GTN and none gave rise to a CC (Fig. 1 and Table 1). CIs for the rate of GTN do not overlap between the three genotypes, androgenetic monospermic, androgenetic dispermic, and triploid dispermic, supporting significant differences in the propensities of each group to develop GTN (Table 1). The risk of GTN is the highest for androgenetic dispermic HM, and this is significantly different from that of androgenetic monospermic HM (p value = 0.0015) and triploid dispermic HM (p value = 0). Also, the risk of GTN for androgenetic monospermic HM is higher than that of triploid dispermic HM (p value = 0). The propensity to develop CC follows a similar trend but did not reach statistical significance, given the small number of patients that developed CC ($n = 5$).

Among androgenetic CHM that led to GTN, we looked for an association between the severity of the GTN according to the FIGO score, low risk (≤ 6) vs. high risk (> 6) [27], and molar genotypes, monospermic vs. dispermic. High-risk GTN

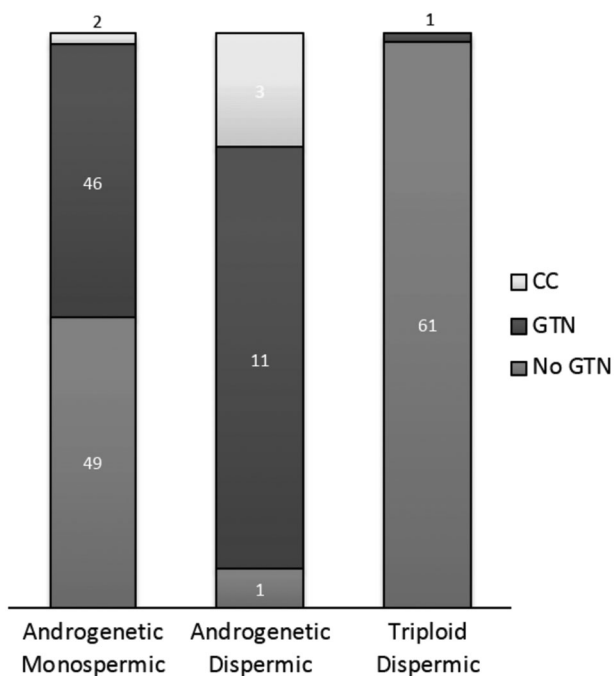


Fig. 1 Frequencies of neoplastic transformation among different genotypic types of HM. GTN stands for gestational trophoblastic neoplasia, and CC for choriocarcinoma.

Table 2 Maternal age and risk for GTN after androgenetic monospermic moles.

	Total	Maternal age	
		>35	>40
Total number of AnMo HM	95	32	20
GTN	46	21	14
% GTN	48.4%	65.6%	70.0%
95% CI	0.38–0.59	0.47–0.81	0.46–0.88

AnMo androgenetic monospermic, GTN gestational trophoblastic neoplasia, CI confidence interval

was more frequent among patients with dispermic CHM, 27.2% (3/11), than among patients with monospermic CHM, 10.9% (5/46) (Table 1), suggesting an association between androgenetic dispermic CHM and high-risk GTN.

Maternal age and risk for GTN

We investigated whether maternal age affects the propensity of a HM to degenerate into a GTN. This was only possible for androgenetic monospermic moles because of the large size of this cohort. Out of our 95 patients with androgenetic monospermic CHM and complete hCG follow-up and accurate staging, 46 (48.4%) went on to develop GTN. If we only consider maternal age older than 35, 21 (65.6%) developed GTN. Out of those whose maternal age was older than 40, 14 (70.0%) developed GTN (Table 2). While CIs of the total

cohort and the advanced maternal age groups do overlap, they are notably different, indicating a possible association between advanced maternal age and the propensity of an androgenetic monospermic HM to degenerate into a GTN (Table 2).

Maternal age and HM genotype

Age was available for all patients, which allowed us to include all 204 HM samples in our analysis of a possible association between maternal age and HM genotype. Out of the 114 patients who had an androgenetic monospermic HM, 6 (5.2%) were ≤20 years old at the time of HM evacuation, 45 (39.4%) were in between 21 and 30, 43 (37.7%) were in between 31 and 40, and 20 (17.5%) were older than 40 years of age. Out of the 12 patients who had an androgenetic dispermic HM, 2 (16.6%) were ≤20 years old, 6 (50%) were in between 21 and 30, 4 (33.3%) were in between 31 and 40, and none were over 40 years of age. Last, out of the 69 patients who had a triploid dispermic HM, 1 (1.6%) was ≤20 years old, 26 (37.6%) were in between 21 and 30, 42 (60.8%) were in between 31 and 40, and none were over 40 years of age (Fig. 2a, b).

We next combined our cohort of sporadic HM with another independent and well-characterized large cohort [4] consisting of a total of 297 HM, 121 CHM (106 androgenetic monospermic and 15 androgenetic dispermic), and 176 triploid dispermic PHM. In this combined cohort, we looked for an association between maternal age and HM genotype. Out of the combined cohort of patients who had an androgenetic monospermic HM, 22 (10%) were ≤20 years old, 93 (42.2%) were in between 21 and 30, 67 (30.4%) were in between 31 and 40, and 38 (17.2%) were over 40 years of age. Out of the patients who had an androgenetic dispermic HM, 2 (7.4%) were ≤20 years old, 12 (44.4%) were in between 21 and 30, 8 (29.6%) were in between 31 and 40, and 5 (18.5%) were over 40 years of age. Last, out of the patients who had a triploid dispermic HM, 24 (9.7%) were ≤20 years old, 106 (43.2%) were in between 21 and 30, 111 (45.3%) were in between 31 and 40, and 4 (1.6%) were over 40 years of age (Fig. 2c, d).

It is notable that in both cohorts (ours alone and the combined cohort), few women after the age of 40 had triploid dispermic PHM (Fig. 2). Statistical analyses of the combined cohort demonstrate that both androgenetic monospermic and dispermic CHM are significantly associated with advanced maternal age (>40) when each is compared with triploid dispermic PHM (*p* value = 0 and 0.00115, respectively).

History of miscarriages and HM

A recapitulation of the number of miscarriages in 106 patients with androgenetic monospermic CHM and 67 patients with

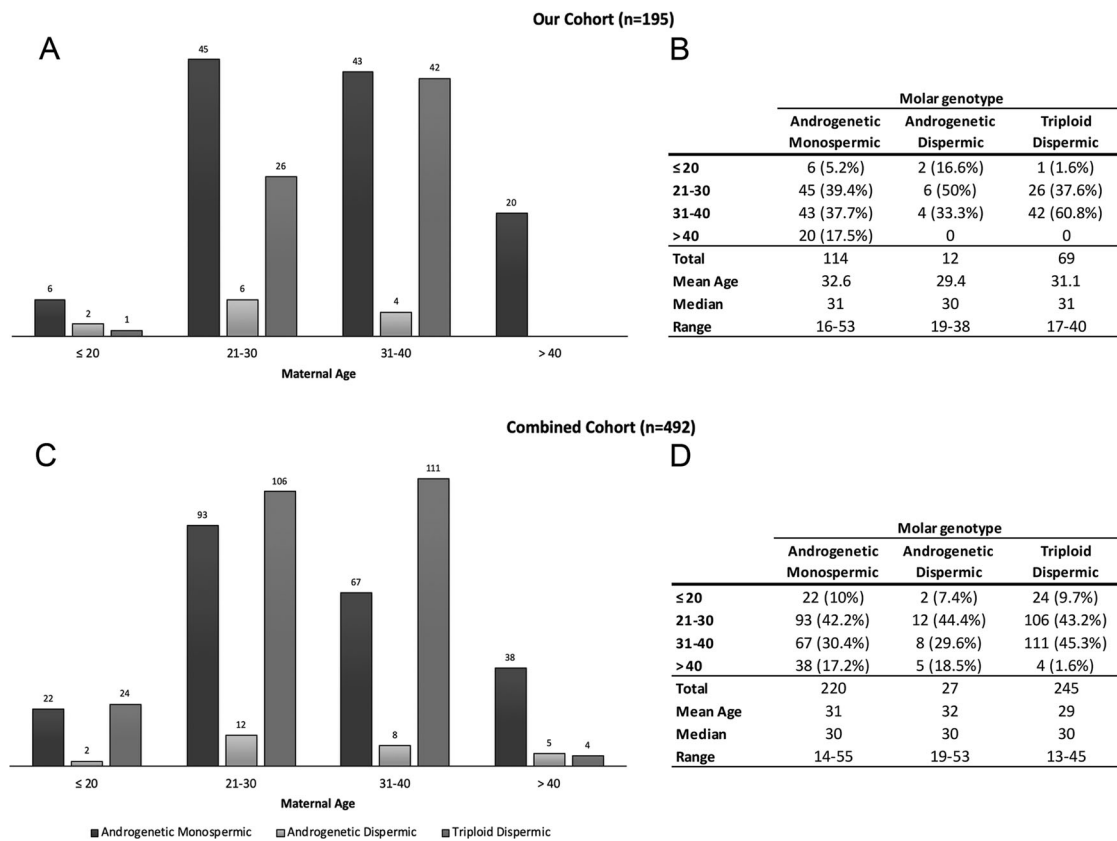


Fig. 2 Age distribution of the different molar genotypes. Age distribution of the different molar genotypes in our cohort of 195 sporadic HM in (a, b), and in the combined cohort of 492 sporadic HM in (c, d), which includes our cohort and that of Banet et al. (2013).

triploid dispermic PHM for whom a full reproductive history was available is shown in Fig. 3. Our data show that 36.8% and 53.7% of our patients with androgenetic monospermic CHM and triploid dispermic PHM, respectively, had at least one miscarriage. This difference was not statistically significant nor was the distribution of the number of miscarriages, whether 1, 2, 3, or >3, among patients with the 2 genotypic types of HM (Fig. 3).

We next asked whether chromosomal abnormalities were at the origin of these miscarriages. To answer this question, we first reviewed the files of our patients and found that five of them had terminations of pregnancies because of fetal ultrasound abnormalities and chromosomal abnormalities identified by karyotype analysis (patients 1160, 1601, 924, 1158, and 1417) (Table 3). Then, we attempted to retrieve FFPE blocks from all available miscarriages of the 173 patients with sporadic HM (114 with androgenetic monospermic CHM and 69 with triploid dispermic PHM). We were able to retrieve 23 POCs with sufficient amounts of CV and performed SNP microarray analysis on them (Table 3 and Supplementary Fig. 1). In total (by karyotype and SNP microarray), 25 out of the 30 analyzed POCs were aneuploid [83.3%, 95% CI: 65.3–94.4%], which is higher than the frequencies of aneuploidies in women with recurrent (436/995 or 43.8%, 95% CI: 40.7–47.0%) or sporadic (3342/6491 or

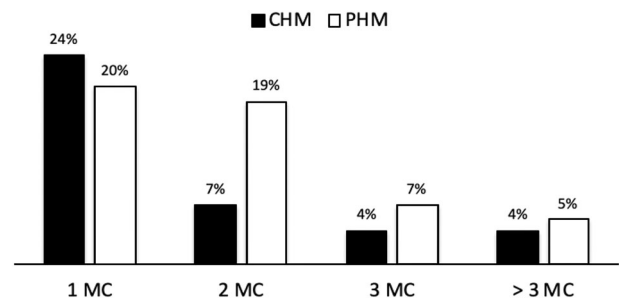


Fig. 3 Distribution of miscarriages across CHM and PHM. MC stands for miscarriage; CHM, complete hydatidiform mole; PHM, partial hydatidiform mole.

51.5%, 95% CI: 50.3–52.7%) miscarriages obtained with the same type of microarray [26] and with other microarray platforms or methods [28–34]. This high frequency of aneuploid miscarriages remained the same (14 out of 17 POC or 82%) even after removing all cases that were referred to us as with recurrent HM and the diagnosis of some of them was revised after genotyping (underlined patient IDs in Table 3).

The ages of the patients at the time of the dilation and curettage of the molar and non-molar miscarriages are recapitulated in Table 3. These data show that the CHM occurred at an older average age (36 years) than their non-molar miscarriages (33 years), while the PHM occurred

Table 3 Summary of chromosomal abnormalities identified in the non-molar miscarriages of patients who had androgenetic monospermic CHM or/and triploid dispermic PHM.

Patient ID	Reproductive history	POC ID	Age at MC	Age at HM	Karyotype or FISH	SNP microarray	Multiplex STR genotyping (Powerplex 16 HS)	Origin
<i>Patients with androgenetic monospermic CHM</i>								
698	4 MC, HM, several failed IVF, MC*, MC, 4 MC	09-09	30	28		Normal male		
	4 MC, HM, several failed IVF, MC, MC*, 4 MC	10-43	30			Normal female		
817	MC, MC*, CHM-GTN (4:7), LB, CC (III:7)	06-11	36	36		Monosomy X	Diploid biparental XX	Paternal X lost
983	LB, eTOP, CHM, MC*, MC, CHM	06-811	39	38		Male, T15	Diploid biparental XY	Maternal MI
			44					
957	MC*, MC, CHM	09-76	34	41		Normal male		
1160	MC, LB, MC, CHM, TOPabn*	12-12	34	33	47,XX,+21			Maternal MI
1198	eTOP, MC*, CHM, IVF-LB	10-04	40	42		45,X		
1601	2 MC, MC*, CHM, TOPabn, CHM revised to MC	11-89	25	27		69,XXX	Triplod digynic	Maternal MII
	2 MC, MC, CHM, TOPabn*, CHM revised to MC	14-41	29		46,XX,t(13)(p11.2q14.2)			
	2 MC, MC, CHM, TOPabn, CHM revised to MC*	16-92	30			69,XXX	Triplod digynic	Maternal MI
			33	36				
<i>Average age</i>								
<i>Patients with androgenetic monospermic CHM or/and triploid dispermic PHM</i>								
899	CHM (CHM and fetus, dizygotic), MC revised to PHM*, 2 LB	11-44	25	23		69,XXY	Triplod Dispermic XXY	
1713	LB, CHM, MC*, CHM revised to PHM	16-37	36	35		Female, T15	Triplod diandric	Paternal 15
	LB, CHM, MC, CHM revised to PHM*	17-76		37				
<i>Patients with triploid dispermic PHM</i>								
808	MC, MC revised to PHM, MC*, IVF-2 LB	09-89	36	36		Monosomy X,+Yq mosaic	Diploid biparental XX	Maternal~
875	PHM, MC, MC*, LB	09	35	34	69,XXX + Triploid by FISH*		Diploid biparental XY	Maternal MII
917	LB, PHM, MC*	10-50	37	37		Male, T22	Diploid biparental XY	Maternal MII
924	PHM, LB, TOPabn, MC, MC*, MC, 2 MC, IVF-MC	09-11	38	34		Female, T16	Triplod digynic XXX	Maternal MI
	PHM, LB, TOPabn, MC, MC, MC*, 2 MC, IVF-MC	09-66	38				Diploid biparental, T7 (mat)	Maternal MI
	PHM, LB, TOPabn, MC, MC, MC, 2 MC, IVF-MC*	11-14	41		47,XX,+7	Male, T16	Diploid biparental XY	Maternal MI
1088	MC, PHM, MC*, LB	12-78	32	32		Female, T7	Diploid biparental XX, T7	Paternal 7
1158	2 eTOP, MC*, PHM, BO, IVF-twin-TOPabn	06-30	37	37		Female, T10	Diploid biparental XX	Maternal MII
	2 eTOP, MC, PHM, BO*, IVF-twin-TOPabn	10-63	41		47,XX,+21;47,XX,+13			n.a.
	2 eTOP, MC, PHM, BO, IVF-twin-TOPabn*	n.a.	42			Normal male	Diploid biparental XY	Post-zygotic
1228	PHM, PHM revised to MC*, LB, PR	14-91	30	29	Tetraploid by FISH*		Diploid biparental XY	n.a.
1257	MC, PHM, 4x F-IVF, MC, PHM revised to MC*	13-72	33	28		Female, T8	Diploid biparental XY	Maternal MII
1306	1st partner: MC*, MC, 2nd partner: PHM	10-59	21	24	92,XXXXY	Normal male	Diploid biparental XY	Maternal MI
1417	eTOP, PHM, TOPabn*	n.a.	24	23	47,XX,+18			
1530	MC*, LB, PHM, LB	12-14	31	34		Female, T8	Diploid biparental XY	Maternal MI
1686	PHM, MC*	17-46	35	36		Normal male	Diploid biparental XY	Maternal MI
1790	PHM, PHM revised to MC*	18-30	31	30		69,XXX	Triplod digynic XXX	Maternal MII
			34	32				
<i>Average age</i>								

This table recapitulates the analyses performed on the non-molar miscarriages of patients who had an androgenetic monospermic CHM or/and a triploid dispermic PHM. The conception analyzed in each row is indicated by an asterisk (*) in the reproductive history; ID, stands for identification number; POC, product of conception; STR, short tandem repeat; SNP, single nucleotide polymorphism; AnMo, androgenetic monospermic; TrDiS, triploid dispermic; MC, miscarriage; HM, hydatidiform mole; CHM, complete hydatidiform mole; PHM, partial hydatidiform mole; CC, choriocarcinoma; MI, meiosis I, MII, meiosis II; T, trisomy; BO, blighted ovum; LB, live birth; eTOP, elective termination of pregnancy; TOPabn, termination of pregnancy because of ultrasound abnormalities; IVF, in vitro fertilization; F-IVF, failed IVF; PR, pregnant; FISH, Fluorescent in situ hybridization; mat, maternal; n.a., not available. ~, indicates that the triploid miscarriage is digynic based on ultrasound abnormalities typical of digynic triploidy at 21 weeks. The following chromosomal probes were used in FISH on POC of patients 875 (X, Y, 13, 18, and 21) and 1257 (X, Y, 10, 11, 17, and 18). The underlined karyotypes correspond to POCs that were not available for further analysis. Underlined patients indicate those that were referred to us for a history of recurrent HM

at a younger average age (32 years) than their non-molar miscarriages (34 years), which is consistent with the known increased risk for CHM with increased maternal age. Of note, 14 out of the 30 (50%) miscarriages occurred at the age of 35 or more. Furthermore, 9 out of the 13 (69%) trisomies are nonviable trisomies, known to be associated with increased maternal age [35] and most of them occurred at an age >35. Definitely, increased maternal age appears to be an important contributing factor to the aneuploid miscarriages in these patients. However, this is not the only cause because the average age of the patients at the time of non-molar miscarriages is 33–34 and the risk of any chromosomal abnormality at this age is much lower, ~1 in 156 pregnancies. Another contributing factor to the increased aneuploidies in these patients appears to be their genetic susceptibility for reproductive loss since some of these patients had few or no live births even when they were young.

Using telomeric and pericentromeric microsatellite markers, we determined the parental and meiotic origin of the aneuploidies. In this analysis, we only investigated the origin of trisomies and triploidies because they mostly originate in the germ lines or around the time of fertilization, while monosomies may also result from later anomalies during postzygotic development, and consequently, their exact origin cannot be determined. Our analysis confirmed all the trisomies revealed by SNP microarrays. In addition, it demonstrated that 9 out of the 11 (81.8%) analyzed trisomies are of maternal origin (Fig. 4), and in 2, the additional chromosomes are of paternal origin (Table 3). The latter may have originated from aneuploid male gametes or from an impaired block of polyspermy by the oocytes, leading to dispermic fertilization followed by postzygotic diploidization and loss of the other paternal chromosomes [36]. Of the six triploidies identified among the 30 POCs, 4 were found to be digynic miscarriages (Fig. 5) and 2 were found dispermic PHM (Table 3). Notably, both anomalies are due to oocyte defects.

Altogether, our data suggest that patients with complete or partial HM and miscarriages have a higher frequency of aneuploid miscarriages than women with one or more miscarriages, and most of these aneuploidies are of maternal meiotic origin.

Discussion

In this study, we used several approaches to comprehensively characterize the genotypes of 204 HM from patients, mostly referred to us by the RMTQ, and 30 of their non-molar miscarriages. We revisited risk factors for HM and GTN across the different HM genotypes and investigated the genetic causes of their non-molar miscarriages.

In our analysis, we found a higher frequency of GTN (53.3%) as compared with other studies from western countries where 14–28% of patients with CHM are reported to develop GTN [37–39]. This difference is clearly due to a referral bias and the fact that patients' enrolment in the RMTQ is made on a voluntary basis by healthcare professionals. Consequently, this may have favored enrolling severe cases from physicians seeking help or a second opinion in the management of their patients and therefore increased the risk of GTN in our patients with CHM.

It is well known that androgenetic, both monospermic and dispermic, CHM is more prone to GTN than triploid dispermic PHM [37–39], and this is replicated in our analysis, in which 53.3% of CHM and 1.6% of PHM lead to GTN. However, among androgenetic CHM, reports about the differences in the propensity of monospermic vs. dispermic genotypes to neoplasia have been less consistent [40–47]. While many studies found higher frequencies of GTN among dispermic vs. monospermic androgenetic CHM, most did not reach statistical significance because of the small number of patients with androgenetic dispermic CHM [40–44]. To date, only Baasanjav et al. [46] reached a significant increase in GTN after dispermic as compared with monospermic CHM in their own samples but not in a meta-analysis after combining all previously described cases. In an attempt to answer this debated question, we evaluated the incidence of GTN across the various genotypes of HM. Our data show that androgenetic dispermic moles have a higher risk for GTN (91.7%) than androgenetic monospermic moles (48.4%) and therefore confirm previous findings [46]. We also demonstrate that GTN after androgenetic dispermic CHM has higher FIGO risk score (score >6) (33 vs. 6%) and is at higher risk for CC (25 vs. 1.8%) than GTN after androgenetic monospermic CHM. Furthermore, our data demonstrate that frequency of GTN in patients with androgenetic monospermic moles increases with increased maternal age, and this finding is in agreement with previous reports [48–51].

Increased maternal age is a well-known risk factor significantly associated with CHM [48, 52, 53]. In studies where the genotypes of the moles were determined, this association was reported with androgenetic CHM [4]. This was also confirmed in our cohort of 204 HM and after combining our cases with 297 HM samples reported by Banet et al. [4]. In addition, analyzing the combined cohort revealed an association between increased maternal age and androgenetic dispermic CHM, which was not seen in our 12 patients with androgenetic dispermic cases and has not been previously reported.

Aside from maternal age, the second highest risk factor for HM that was demonstrated in several studies and populations is a history of miscarriages [17–19]. In one of these studies [19], miscarriages were found associated with

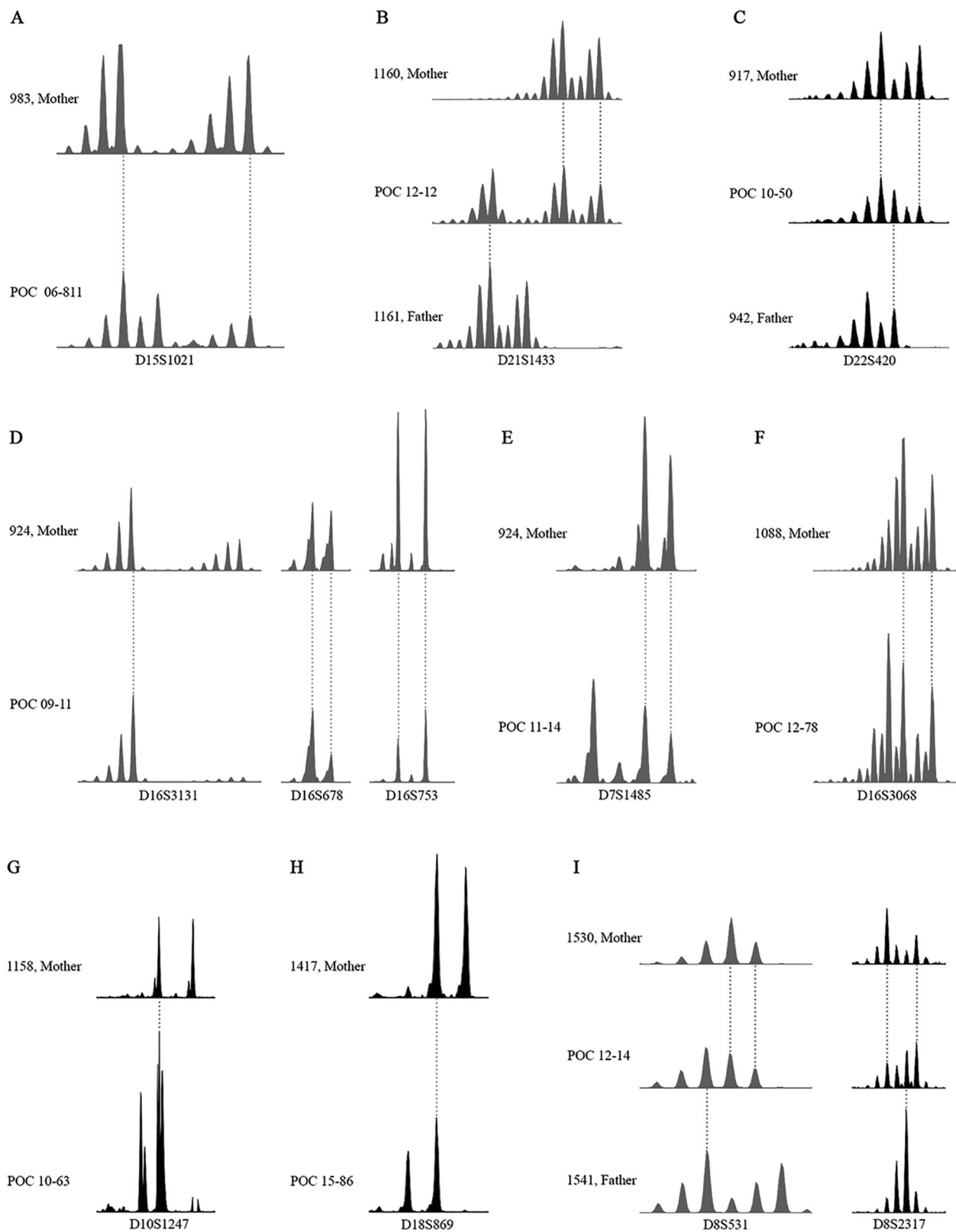


Fig. 4 Genotyping results on the aneuploid miscarriages of maternal origin. The miscarriages in (a, b, c, e, f, i) are due to failure of MI. The miscarriages in (d, g, h) are due to failure of MII. All markers except D16S678 and D16S753 are pericentromeric. The x-axis represents size in basepairs and the y axis represents peak height. Both have been omitted for clarity. POC stands for product of conception.

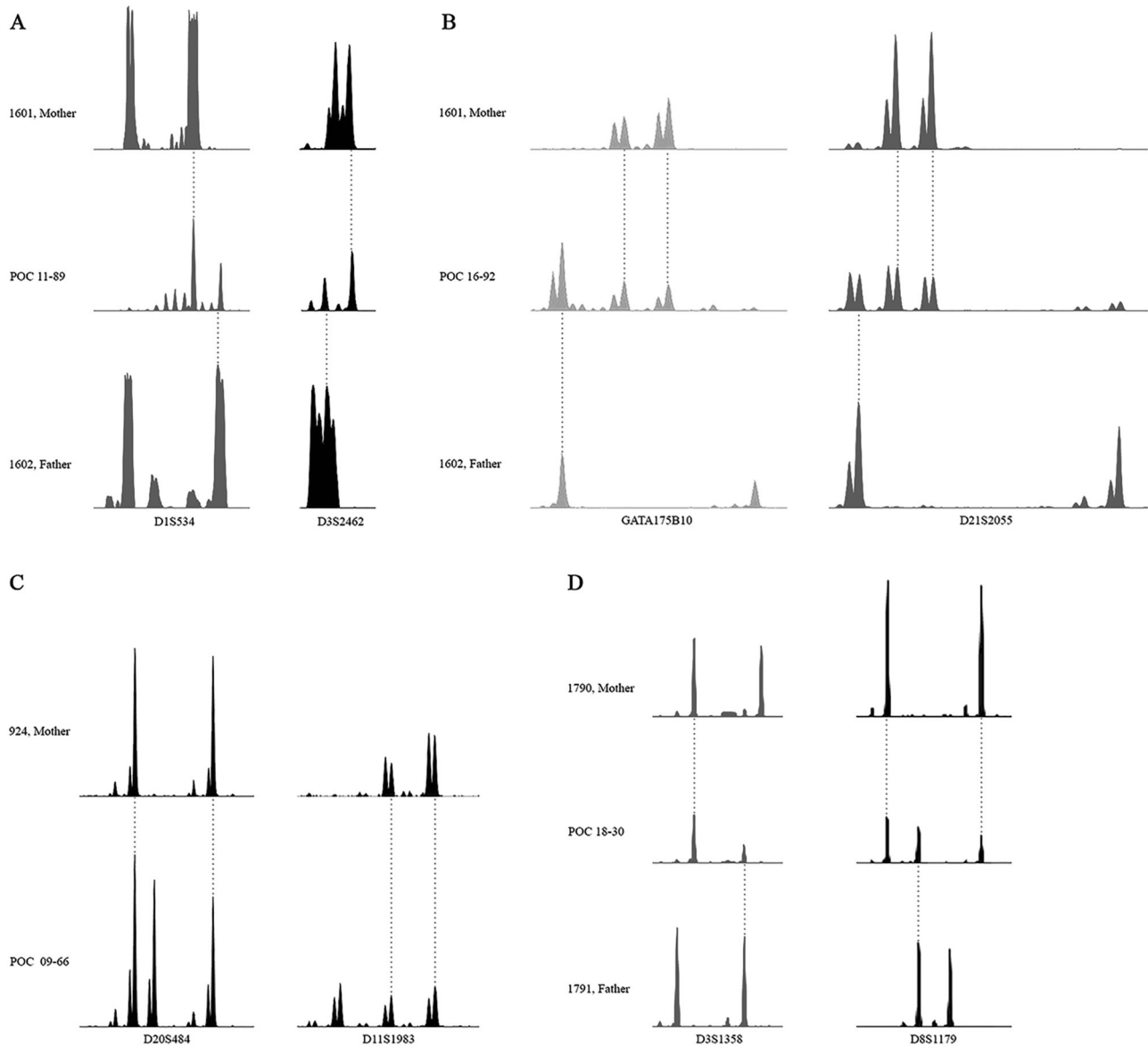


Fig. 5 Genotyping results on the four triploid POCs of maternal origin. The miscarriage in (a) is due to failure of MII and the miscarriage in (c) is due to failure of MI. The markers shown for the miscarriages in (b) and (d) demonstrate the maternal origin of the triploidies. Other markers from the pericentromeric regions were performed on the POCs analyzed in (b) and (d) and demonstrated that they resulted from the failure of MI and MII, respectively (data not shown). The *x* axis represents size in basepairs and the *y* axis represents peak height. Both have been omitted for clarity. POC stands for product of conception.

both histological types of HM. However, the cause of non-molar miscarriages in women with sporadic HM is unknown. From our cohort of patients with sporadic HM, 36.8% of those with androgenetic monospermic CHM and 53.7% of those with triploid dispermic PHM had at least one miscarriage. The rate of miscarriages among our patients is higher than that previously reported and is due, in our judgment, to the following facts: (i) some patients were recruited from the recurrent miscarriage clinic; (ii) others were referred to us with two HM, one of which was found to be a non-molar miscarriage after genotyping; (iii) our follow-up on the reproductive history of some of our

patients continued for several years after the diagnosis of their sporadic HM. Upon analyzing the miscarriages of our patients, 83.3% were found aneuploid, which is higher than the frequency of aneuploidy in women with recurrent (43.8%) or sporadic (53.7%) miscarriages [26, 28–34]. We next determined the parental origin of trisomies and triploidies and demonstrated that 9 out of 13 (69%) of the trisomies and 4 out of 6 (67%) of the triploidies resulted from failure of female meiosis I or II. Representative results are illustrated in Figs. 4, 5. These data suggest that a genetic susceptibility for defects in meiosis I and II may underlie the etiology of HM and aneuploid miscarriages in these

patients. Our findings are in agreement with the fact that increased maternal age (>35) is the most important risk factor for both HM (CHM and PHM) [48, 53, 54] and aneuploid miscarriages [35, 55]. Indeed, age-specific rates of HM and miscarriages follow similar J-shaped curves with a slight increase in teenagers and a steep increase after the age of 35 [56–60]. Furthermore, in a recent study documenting the identification of three novel meiotic genes underlying the etiology of recurrent androgenetic monospermic moles, the patients and their female siblings also had miscarriages, which further support the relationship between meiotic defects, androgenetic CHM, and miscarriages in some patients [61].

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.


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