# Comprehensive Analysis of 204 Sporadic Hydatidiform Moles: Revisiting Risk Factors and their Correlations with the Molar Genotypes

Yassemine Khawajkie[1], Nawel Mechtouf[2], Ngoc Minh Phuong Nguyen[2], Kurosh Rahimi[3], Magali Breguet[4], Jocelyne Arseneau[5], Brigitte M. Ronnett[6], Lori Hoffner[7], Felicia Lazure[2,8], Marjolaine Arnaud[2], Fabrice Peers[3], Liane Tan[9], Basam Abu Rafea[9], Monica Aguinagua[10], Neil S Horowitz[11], Asangla Ao[12], Seang Lin Tan[13], Richard Brown[14], William Buckett[14], Urvashi Surti[7], Karine Hovanes[15], Trilochan Sahoo[15], Philippe Sauthier[16], Rima Slim[1,2]

[1]Division of Experimental Medicine; [2]Department of Human Genetics, McGill University Health Centre, Montreal Quebec, Canada

[3]Department of Pathology, Hospital network of the University of Montreal (CHUM), Montreal, Quebec, Canada

[4]Department of Obstetrics and Gynecology, Gynecologic Oncology Division, Centre Hospitalier de l'Université de Montréal, Réseau des Maladies Trophoblastiques du Québec, Montréal, Quebec, Canada

[5]Gynecologic Oncology Unit, McGill University Health Centre, Montreal, Quebec, Canada

[6]Department of Pathology, The Johns Hopkins Hospital, Baltimore, Maryland, USA

[7]Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

[8]Lady Davis Institute for Medical Research, Jewish General Hospital, Montréal, Quebec, Canada

[9]The Fertility Clinic, London Health Sciences Centre, London, Ontario, Canada

[10]Genetics and Genomics Department, Instituto Nacional de Perinatologia, Mexico City, Mexico

[11]Division of Gynecologic Oncology, Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; Dana-Farber Cancer Institute, Boston, Massachusetts, USA

[12]MUHC Reproductive Centre, Department of Obstetrics and Gynecology, Department of Human Genetics, McGill University, Montreal, Quebec, Canada

[13]Originelle Fertility Clinic and Women's Health Centre, Montreal, Quebec, Canada

[14]Department of Obstetrics and Gynecology, MUHC Reproductive Centre, McGill University Health Center, McGill University, Montréal, QC, Canada

[15]Invitae, Irvine, CA 92618, USA.

[16]Department of Gynecology Oncology, Hospital network of the University of Montreal (CHUM), Montreal, Quebec, Canada

**Abstract**

Hydatidiform mole (HM) is an aberrant human pregnancy characterized by excessive trophoblastic proliferation and abnormal embryonic development. HM have 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 are 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 are more prone to gestational trophoblastic neoplasia (GTN) than triploid dispermic PHM, and androgenetic dispermic CHM are 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 are diploid androgenetic monospermic, most PHM are triploid dispermic, and severalmorphologically diagnosed CHM or PHM are diploid biparental (3). In the last 10 years, the improvement of existing methods, the emergence of more 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). 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 are 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 of miscarriages 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 (KR and JA) 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 was performed according to the International Federation of Gynecology and Obstetrics (FIGO) criteria (21). Choriocarcinoma diagnosis (by KR) 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-μm 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 μm 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/μl, Sigma-Aldrich) and 50 μl 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 µm 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 µm 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 (Catalogue 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 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 to the parental alleles to determine their origin.

*Fluorescent in situ hybridization.* Fluorescent in situ hybridization (FISH) was performed on 4 µm 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*. SNP-based whole-genome chromosomal microarray analysis (CMA) 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 products of conception, 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 approximately 350 FFPE molar tissues, the limitations of the various methods and the lessons we have learned can be summarized as follow.

*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 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 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) are 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 into 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). Confidence intervals (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 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) versus high risk (>6) (27), and molar

genotypes, monospermic versus dispermic. High-risk GTN were 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 an 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 confidence intervals 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. Lastly, 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 and 2B).

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. Lastly, 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 and 2D).

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 to 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 triploid dispermic PHM for whom a full reproductive history was available is shown in Figure 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 two 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, 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% confidence interval (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 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 recurrent HM and whose diagnosis was revised after genotyping (underlined patient IDs in Table 3).

The ages of the patients at the time of the dilatation 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 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, nine out of the 13 (69%) trisomies are non-viable 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, approximately 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 birth 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 germlines 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 two, 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, four were found to be digynic miscarriages (Fig. 5) and two 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 risks 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 to 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' enrollment in the RMTQ is made on a voluntary basis by health care 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 are 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 versus dispermic genotypes to neoplasia have been less consistent (40-47). While many studies found higher frequencies of GTN among dispermic versus 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. reached a significant increase of GTN after dispermic as compared to monospermic CHM in their own samples but not in a meta-analysis after combining all previously described cases (46). 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 have higher FIGO risk score (score >6) (33% vs. 6%) and are at higher risk for CC (25% vs. 1.8%) than GTN after androgenetic monospermic CHM. Furthermore, our data demonstrate that the 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 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 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; and 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 Figures 4 and 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).


## ACKNOWLEDGEMENTS

The authors would like to thank Dr. Olga Basso for her help with the statistical analysis. This study was support by the Canadian Institutes of Health Research to R.S. (MOP-130364, MOP 102469, and MOP 86546). We also express our gratitude to the Pathology Departments of the following health centres for providing archived products of conception for analyses: McGill University Health Centre, Centre Hospitalier de l'Université de Montréal, Centre Hospitalier Universitaire de Sainte-Justine, Centre de la Santé de Laval, Hôpital Pierre Le Gardeur, Centre Hospitalier Régional de Rimouski, Centre de Santé et de Services Sociaux de Trois Rivières, London Health Science Centre, Hôpital Pierre Boucher, Hôpital Maisonneuve Rosemont, Hôpital Sacré-Coeur, Hôpital Anna-Laberge, Hôpital Général du Lakeshore, Jewish General Hospital, Hôpital Honoré Mercier, Credit Valley Hospital, Hôpital Saint Jean sur Richelieu, Hôpital Regional Saint Jerome, Hôpital Sainte-Mary, and Windsor Regional Hospital.


## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## REFERENCES


1. Szulman AE, Surti U. The syndromes of hydatidiform mole. II. Morphologic evolution of the complete and partial mole. Am J Obstet Gynecol 1978;132:20-7.

2. Vassilakos P, Kajii T. Letter: Hydatidiform mole: two entities. Lancet 1976;1:259.

3. Kovacs BW, Shahbahrami B, Tast DE, Curtin JP. Molecular genetic analysis of complete hydatidiform moles. Cancer Genet Cytogenet 1991;54:143-52.

4. Banet N, DeScipio C, Murphy KM*, et al.* Characteristics of hydatidiform moles: analysis of a prospective series with p57 immunohistochemistry and molecular genotyping. Mod Pathol 2014;27:238-54.

5. Lipata F, Parkash V, Talmor M*, et al.* Precise DNA genotyping diagnosis of hydatidiform mole. Obstet Gynecol 2010;115:784-94.

6. Vang R, Gupta M, Wu LS*, et al.* Diagnostic reproducibility of hydatidiform moles: ancillary techniques (p57 immunohistochemistry and molecular genotyping) improve morphologic diagnosis. Am J Surg Pathol 2012;36:443-53.

7. Colgan TJ, Chang MC, Nanji S, Kolomietz E. DNA Genotyping of Suspected Partial Hydatidiform Moles Detects Clinically Significant Aneuploidy. Int J Gynecol Pathol 2017;36:217-21.

8. Furtado LV, Paxton CN, Jama MA*, et al.* Diagnostic utility of microsatellite genotyping for molar pregnancy testing. Arch Pathol Lab Med 2013;137:55-63.

9. Nguyen NM, Zhang L, Reddy R*, et al.* Comprehensive genotype-phenotype correlations between NLRP7 mutations and the balance between embryonic tissue differentiation and trophoblastic proliferation. J Med Genet 2014;51:623-34.

10. Brown L, Mount S, Reddy R*, et al.* Recurrent pregnancy loss in a woman with NLRP7 mutation: not all molar pregnancies can be easily classified as either "partial" or "complete" hydatidiform moles. Int J Gynecol Pathol 2013;32:399-405.

11. Sebire NJ, Savage PM, Seckl MJ, Fisher RA. Histopathological features of biparental complete hydatidiform moles in women with NLRP7 mutations. Placenta 2013;34:50-6.

12. Hayward BE, De Vos M, Talati N*, et al.* Genetic and epigenetic analysis of recurrent hydatidiform mole. Hum Mutat 2009;30:E629-39.

13. Judson H, Hayward BE, Sheridan E, Bonthron DT. A global disorder of imprinting in the human female germ line. Nature 2002;416:539-42.

14. Reddy R, Akoury E, Phuong Nguyen NM*, et al.* Report of four new patients with protein-truncating mutations in C6orf221/KHDC3L and colocalization with NLRP7. Eur J Hum Genet 2013;21:957-64.

15. Parry DA, Logan CV, Hayward BE*, et al.* Mutations causing familial biparental hydatidiform mole implicate c6orf221 as a possible regulator of genomic imprinting in the human oocyte. Am J Hum Genet 2011;89:451-8.

16. Qian J, Nguyen NMP, Rezaei M*, et al.* Biallelic PADI6 variants linking infertility, miscarriages, and hydatidiform moles. Eur J Hum Genet 2018;26:1007-13.

17. Messerli ML, Lilienfeld AM, Parmley T, Woodruff JD, Rosenshein NB. Risk factors for gestational trophoblastic neoplasia. Am J Obstet Gynecol 1985;153:294-300.

18. Parazzini F, La Vecchia C, Pampallona S, Franceschi S. Reproductive patterns and the risk of gestational trophoblastic disease. Am J Obstet Gynecol 1985;152:866-70.

19. Parazzini F, Mangili G, La Vecchia C*, et al.* Risk factors for gestational trophoblastic disease: a separate analysis of complete and partial hydatidiform moles. Obstet Gynecol 1991;78:1039-45.

20. Sauthier P, Breguet M, Rozenholc A, Sauthier M. Quebec Trophoblastic Disease Registry: how to make an easy-to-use dynamic database. Int J Gynecol Cancer 2015;25:729-33.

21. Ngan HY, Bender H, Benedet JL*, et al.* Gestational trophoblastic neoplasia, FIGO 2000 staging and classification. Int J Gynaecol Obstet 2003;83 Suppl 1:175-7.

22. Castrillon DH, Sun D, Weremowicz S*, et al.* Discrimination of complete hydatidiform mole from its mimics by immunohistochemistry of the paternally imprinted gene product p57KIP2. Am J Surg Pathol 2001;25:1225-30.

23. Hedley DW. Flow cytometry using paraffin-embedded tissue: five years on. Cytometry 1989;10:229-41.

24. Khawajkie Y, Buckett W, Nguyen NMP*, et al.* Recurrent triploid digynic conceptions and mature ovarian teratomas: Are they different manifestations of the same genetic defect? Genes Chromosomes Cancer 2017;56:832-40.

25. Surti U, Hoffner L, Kolthoff M*, et al.* Persistent gestational trophoblastic disease after an androgenetic/biparental fetal chimera: a case report and review. Int J Gynecol Pathol 2006;25:366-72.

26. Sahoo T, Dzidic N, Strecker MN*, et al.* Comprehensive genetic analysis of pregnancy loss by chromosomal microarrays: outcomes, benefits, and challenges. Genet Med 2017;19:83-9.

27. Ngan HY, Seckl MJ, Berkowitz RS*, et al.* Update on the diagnosis and management of gestational trophoblastic disease. Int J Gynaecol Obstet 2015;131 Suppl 2:S123-6.

28. Maslow BS, Budinetz T, Sueldo C*, et al.* Single-Nucleotide Polymorphism-Microarray Ploidy Analysis of Paraffin-Embedded Products of Conception in Recurrent Pregnancy Loss Evaluations. Obstet Gynecol 2015;126:175-81.

29. Hassold T, Chen N, Funkhouser J*, et al.* A cytogenetic study of 1000 spontaneous abortions. Ann Hum Genet 1980;44:151-78.

30. Eiben B, Bartels I, Bahr-Porsch S*, et al.* Cytogenetic analysis of 750 spontaneous abortions with the direct-preparation method of chorionic villi and its implications for studying genetic causes of pregnancy wastage. Am J Hum Genet 1990;47:656-63.

31. Stephenson MD, Awartani KA, Robinson WP. Cytogenetic analysis of miscarriages from couples with recurrent miscarriage: a case-control study. Hum Reprod 2002;17:446-51.

32. Shearer BM, Thorland EC, Carlson AW, Jalal SM, Ketterling RP. Reflex fluorescent in situ hybridization testing for unsuccessful product of conception cultures: a retrospective analysis of 5555 samples attempted by conventional cytogenetics and fluorescent in situ hybridization. Genet Med 2011;13:545-52.

33. Menten B, Swerts K, Delle Chiaie B*, et al.* Array comparative genomic hybridization and flow cytometry analysis of spontaneous abortions and mors in utero samples. BMC Med Genet 2009;10:89.

34. Robberecht C, Schuddinck V, Fryns JP, Vermeesch JR. Diagnosis of miscarriages by molecular karyotyping: benefits and pitfalls. Genet Med 2009;11:646-54.

35. Grande M, Borrell A, Garcia-Posada R*, et al.* The effect of maternal age on chromosomal anomaly rate and spectrum in recurrent miscarriage. Hum Reprod 2012;27:3109-17.

36. Golubovsky MD. Postzygotic diploidization of triploids as a source of unusual cases of mosaicism, chimerism and twinning. Hum Reprod 2003;18:236-42.

37. Garner EI, Goldstein DP, Feltmate CM, Berkowitz RS. Gestational trophoblastic disease. Clin Obstet Gynecol 2007;50:112-22.

38. Golfier F, Raudrant D, Frappart L*, et al.* First epidemiological data from the French Trophoblastic Disease Reference Center. Am J Obstet Gynecol 2007;196:172 e1-5.

39. Sebire NJ, Lindsay I. Current issues in the histopathology of gestational trophoblastic tumors. Fetal Pediatr Pathol 2010;29:30-44.

40. Wake N, Seki T, Fujita H*, et al.* Malignant potential of homozygous and heterozygous complete moles. Cancer Res 1984;44:1226-30.

41. Wake N, Fujino T, Hoshi S*, et al.* The propensity to malignancy of dispermic heterozygous moles. Placenta 1987;8:319-26.

42. Lawler SD, Fisher RA. Genetic studies in hydatidiform mole with clinical correlations. Placenta 1987;8:77-88.

43. Lawler SD, Fisher RA, Dent J. A prospective genetic study of complete and partial hydatidiform moles. Am J Obstet Gynecol 1991;164:1270-7.

44. Cho S, Kim SJ. Genetic study of hydatidiform moles by restriction fragment length polymorphisms (RFLPs) analysis. J Korean Med Sci 1993;8:446-52.

45. Cheung AN, Sit AS, Chung LP*, et al.* Detection of heterozygous XY complete hydatidiform mole by chromosome in situ hybridization. Gynecol Oncol 1994;55:386-92.

46. Baasanjav B, Usui H, Kihara M*, et al.* The risk of post-molar gestational trophoblastic neoplasia is higher in heterozygous than in homozygous complete hydatidiform moles. Hum Reprod 2010;25:1183-91.

47. Kaneki E, Kobayashi H, Hirakawa T*, et al.* Incidence of postmolar gestational trophoblastic disease in androgenetic moles and the morphological features associated with low risk postmolar gestational trophoblastic disease. Cancer Sci 2010;101:1717-21.

48. Savage PM, Sita-Lumsden A, Dickson S*, et al.* The relationship of maternal age to molar pregnancy incidence, risks for chemotherapy and subsequent pregnancy outcome. J Obstet Gynaecol 2013;33:406-11.

49. Xia ZF, Song HZ, Tang MY. Risk of malignancy and prognosis using a provisional scoring system in hydatidiform mole. Chin Med J (Engl) 1980;93:605-12.

50. Tow WS. The influence of the primary treatment of hydatidiform mole on its subsequent course. J Obstet Gynaecol Br Commonw 1966;73:544-52.

51. Tsukamoto N, Iwasaka T, Kashimura Y*, et al.* Gestational trophoblastic disease in women aged 50 or more. Gynecol Oncol 1985;20:53-61.

52. Graham IH, Fajardo AM, Richards RL. Epidemiological study of complete and partial hydatidiform mole in Abu Dhabi: influence age and ethnic group. J Clin Pathol 1990;43:661-4.

53. Sebire NJ, Foskett M, Fisher RA*, et al.* Risk of partial and complete hydatidiform molar pregnancy in relation to maternal age. BJOG 2002;109:99-102.

54. Yen S, MacMahon B. Epidemiologic features of trophoblastic disease. Am J Obstet Gynecol 1968;101:126-32.

55. Choi TY, Lee HM, Park WK, Jeong SY, Moon HS. Spontaneous abortion and recurrent miscarriage: A comparison of cytogenetic diagnosis in 250 cases. Obstet Gynecol Sci 2014;57:518-25.

56. Magnus MC, Wilcox AJ, Morken NH, Weinberg CR, Haberg SE. Role of maternal age and pregnancy history in risk of miscarriage: prospective register based study. BMJ 2019;364:l869.

57. Matalon M, Modan B. Epidemiologic aspects of hydatidiform mole in Israel. Am J Obstet Gynecol 1972;112:107-12.

58. Grimes DA. Epidemiology of gestational trophoblastic disease. Am J Obstet Gynecol 1984;150:309-18.

59. George L, Granath F, Johansson AL, Olander B, Cnattingius S. Risks of repeated miscarriage. Paediatr Perinat Epidemiol 2006;20:119-26.

60. Nybo Andersen AM, Wohlfahrt J, Christens P, Olsen J, Melbye M. Maternal age and fetal loss: population based register linkage study. BMJ 2000;320:1708-12.

61. Nguyen NMP, Ge ZJ, Reddy R*, et al.* Causative Mutations and Mechanism of Androgenetic Hydatidiform Moles. Am J Hum Genet 2018;103:740-51.

## FIGURE LEGENDS

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

**Figure 2.** Age distribution of the different molar genotypes in our cohort of 195 sporadic HM in A and B, and in the combined cohort of 492 sporadic HM in C and D, which includes our cohort and that of Banet et al., 2013.

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

**Figure 4.** Genotyping results on the aneuploid miscarriages of maternal origin. The miscarriages in A, B, C, E, F, and I are due to failure of MI. The miscarriages in D, G, and 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.

**Figure 5.** Genotyping results on the four triploid POCs of maternal origin. Miscarriages in B and C are due to failure of MI and the miscarriages in A and D are due to failure of MII. 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.

**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** | 49 | 1 | 61 | 4 |
| **GTN** | 46 (48.4%) | 11 (91.7%) | 1 (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** | 2 (1.8%) | 3 (25%) | 0 | 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.

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

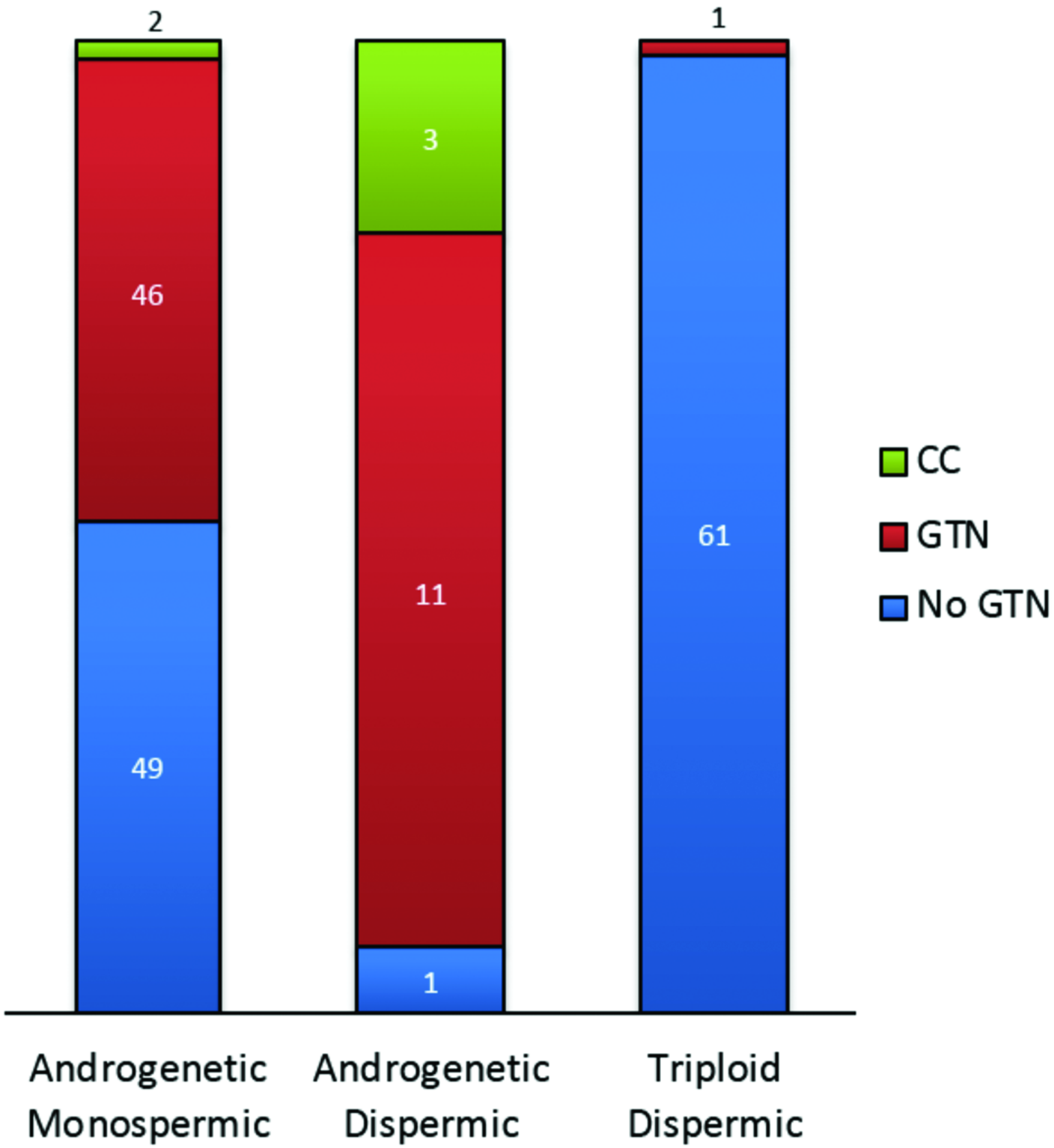
| | | Maternal Age | |
|---|---|---|---|
| | **Total** | **> 35** | **> 40** |
| **Total number of AnMo HM** | 95 | 32 | 20 |
| **GTN** | 46 | 21 | 14 |
| **% GTN** | 48.4% | 65.6% | 70.0% |
| **95% CI** | 0.38-0.59 | 0.47-0.81 | 0.46-0.88 |

AnMo stands for androgenetic monospermic; GTN, gestational trophoblastic neoplasia; CI, confidence interval

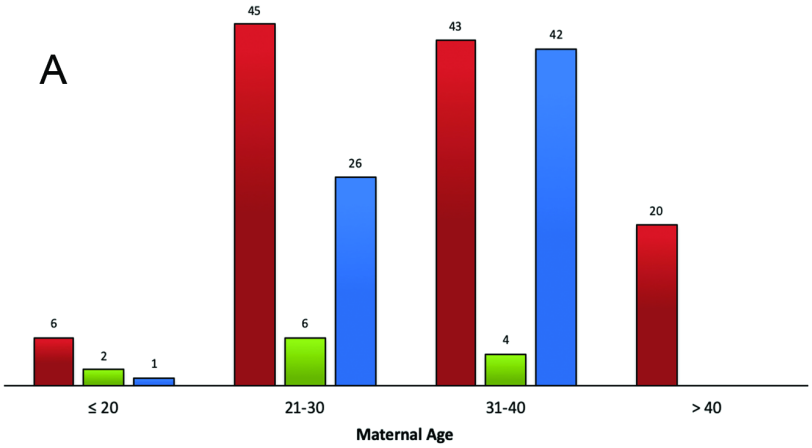
**Table 3. Summary of chromosomal abnormalities identified in the non-molar miscarriages of patients who had androgenetic monospermic CHM or/and triploid dispermic PHM**

| Category | Patient ID | Reproductive history | POC ID | Age at MC | Age at HM | Karyotype or FISH | SNP Microarray | Multiplex STR genotyping (Powerplex 16 HS) | Origin |
|---|---|---|---|---|---|---|---|---|---|
| Patients with Androgenetic Monospermic | 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 (I: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 | Triploid digynic | Maternal MII |
| | | 2 MC, MC, CHM,TOPabn*, CHM revised to MC | 14-41 | 29 | | 46,XY,r(13)(p11.2q14.2) | | | |
| | | 2 MC, MC, CHM, TOPabn, CHM revised to MC* | 16-92 | 30 | | | 69,XXX | Triploid digynic | Maternal MI |
| | **Average age** | | | **33** | **36** | | | | |
| Patients with the AnMo & TriDis | 899 | CHM (CHM and fetus, dizygotic), MC revised to PHM*, 2 LB | 11-44 | 25 | 23 | | 69,XXY | Triploid Dispermic XXY | |
| | 1713 | LB, CHM, MC*, CHM revised to PHM | 16-37 | 36 | 35 | | Female, T15 | | Paternal 15 |
| | | LB, CHM, MC, CHM revised to PHM* | 17-76 | | 37 | | | Triploid diandric | |
| Patients with a Triploid Dispermic | 808 | MC, MC revised to PHM, MC*, IVF-2 LB | 09-89 | 36 | 36 | | Monosomy X,+Yq mosaic | Diploid biparental XX | |
| | 875 | PHM, MC, MC*, LB | 09 | 35 | 34 | 69,XXX + Triploid by FISH[#] | | | Maternal~ |
| | 917 | LB, PHM, MC* | 10-50 | 37 | 37 | | Male, T22 | Diploid biparental XY | Maternal MI |
| | 924 | PHM, LB, TOPabn, MC, MC*, MC, 2 MC, IVF-MC | 09-11 | 38 | 34 | | Female, T16 | | Maternal MII |
| | | PHM, LB, TOPabn, MC, MC, MC*, 2 MC, IVF-MC | 09-66 | 38 | | | | Triploid digynic XXX | Maternal MI |
| | | PHM, LB, TOPabn, MC, MC, MC, 2 MC, IVF-MC* | 11-14 | 41 | | 47,XX,+7 | | Diploid biparental, T7 (mat) | Maternal MI |
| | 1088 | MC, PHM, MC*, LB | 12-78 | 32 | 32 | | Male, T16 | Diploid biparental XY | Maternal MI |
| | 1158 | 2 eTOP, MC*, PHM, BO, IVF-twin-TOPabn | 06-30 | 37 | 37 | | Female, T7 | Diploid biparental XX, T7 | Paternal 7 |
| | | 2 eTOP, MC, PHM, BO*, IVF-twin-TOPabn | 10-63 | 41 | | | Female, T10 | Diploid biparental XX | Maternal MII |
| | | 2 eTOP, MC, PHM, BO, IVF-twin-TOPabn* | n.a | 42 | | 47,XX,+21; 47,XX,+13 | | | n.a. |
| | 1228 | PHM, PHM revised to MC*, LB, PR | 14-91 | 30 | 29 | | Normal male | Diploid biparental XY | |
| | 1257 | MC, PHM, 4x F-IVF, MC, PHM revised to MC* | 13-72 | 33 | 28 | Tetraploid by FISH[#] | | Diploid biparental XY | Post-zygotic |
| | 1306 | 1st partner: MC*, MC; 2nd partner: PHM | 10-59 | 21 | 24 | 92,XXXY | | | n.a. |
| | 1417 | eTOP, PHM, TOPabn* | n.a. | 24 | 23 | 47,XX,+18 | | | Maternal MII |
| | 1530 | MC*, LB, PHM, LB | 12-14 | 31 | 34 | | Female, T8 | | Maternal MI |
| | 1686 | PHM, MC* | 17-46 | 35 | 36 | | Normal male | Diploid biparental XY | |
| | 1790 | PHM, PHM revised to MC* | 18-30 | 31 | 30 | | 69,XXX | Triploid digynic XXX | Maternal MII |
| | **Average age** | | | **34** | **32** | | | | |

This table recpatiluates the analyses performed on the non-molar miscarriages of patients who had an androgenetic monospermic CHM or a triploid dispermic PHM. The conception analyzed in each raw is indicated by an asterisk (*) in the reproduction history; ID, stands for identification number; POC, product of conception; STR, short tandem repeat; SNP, single nucleotide polymorphism; AnMo, androgenetic monospermic; TriDis, triploid dispermic; MC, miscarriage; HM, hydatidiform mole; CHM, complete hydatidiform mole; PHM, partial hydatidiform mole; CC, choricarcinoma; 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 undelined karyotypes correspond to POCs that were not available for further analysis. Underlined patients


2
46
49
3
11
1
1
61
CC
GTN
No GTN
Androgenetic Monospermic
Androgenetic Dispermic
Triploid Dispermic

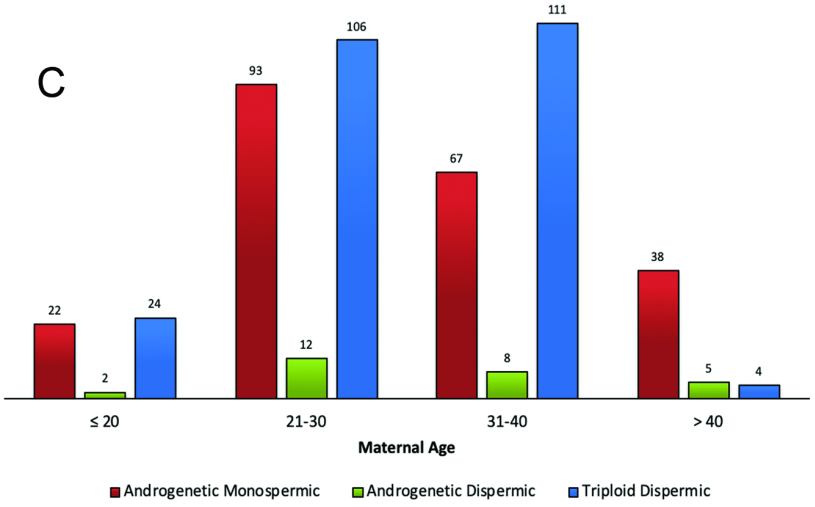
A




B

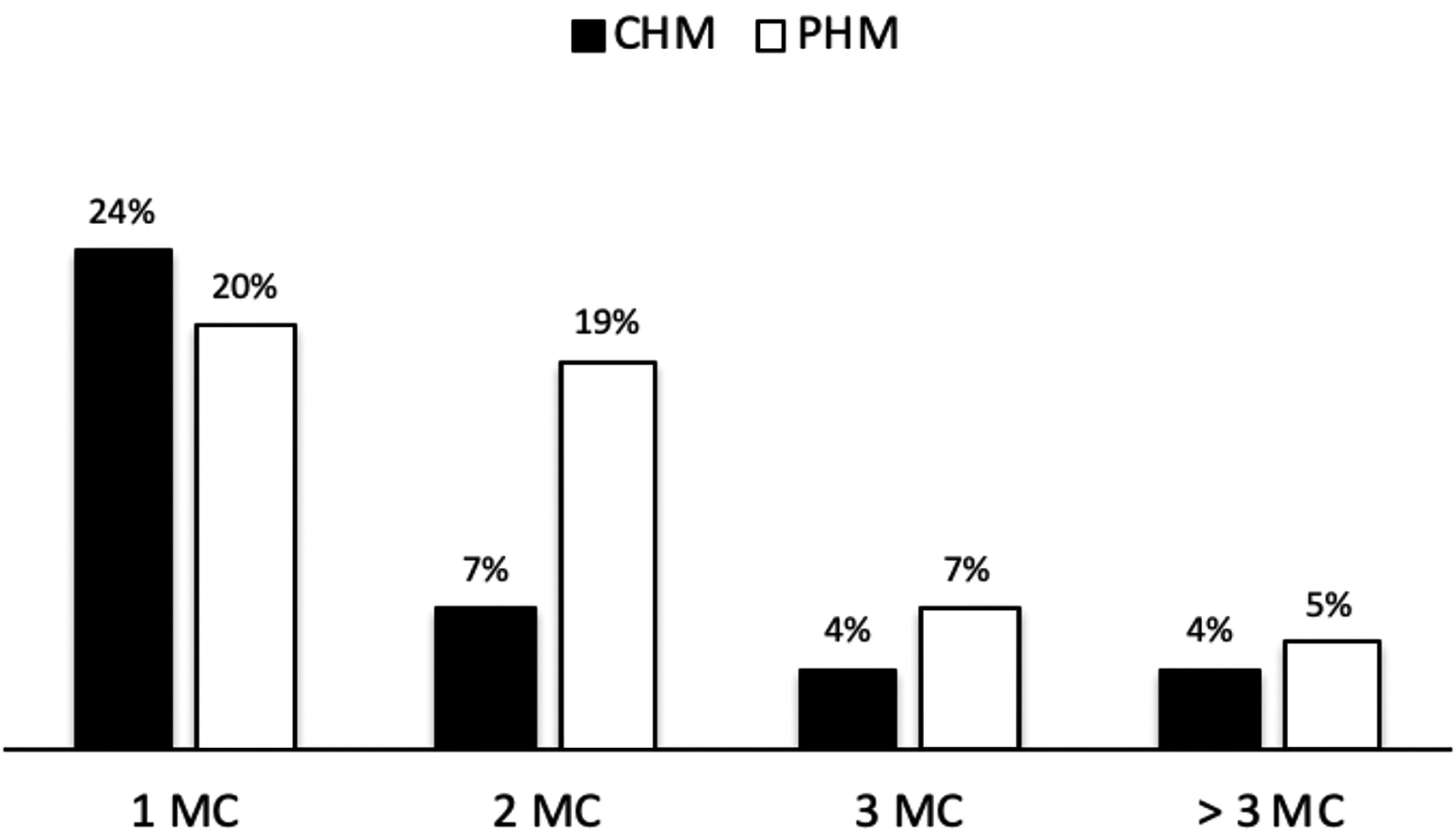
| | Molar genotype | | |
|---|---|---|---|
| | Androgenetic Monospermic | Androgenetic Dispermic | Triploid Dispermic |
| **≤20** | 6 (5.2%) | 2 (16.6%) | 1 (1.6%) |
| **21-30** | 45 (39.4%) | 6 (50%) | 26 (37.6%) |
| **31-40** | 43 (37.7%) | 4 (33.3%) | 42 (60.8%) |
| **>40** | 20 (17.5%) | 0 | 0 |
| **Total** | 114 | 12 | 69 |
| **Mean Age** | 32.6 | 29.4 | 31.1 |
| **Median** | 31 | 30 | 31 |
| **Range** | 16-53 | 19-38 | 17-40 |

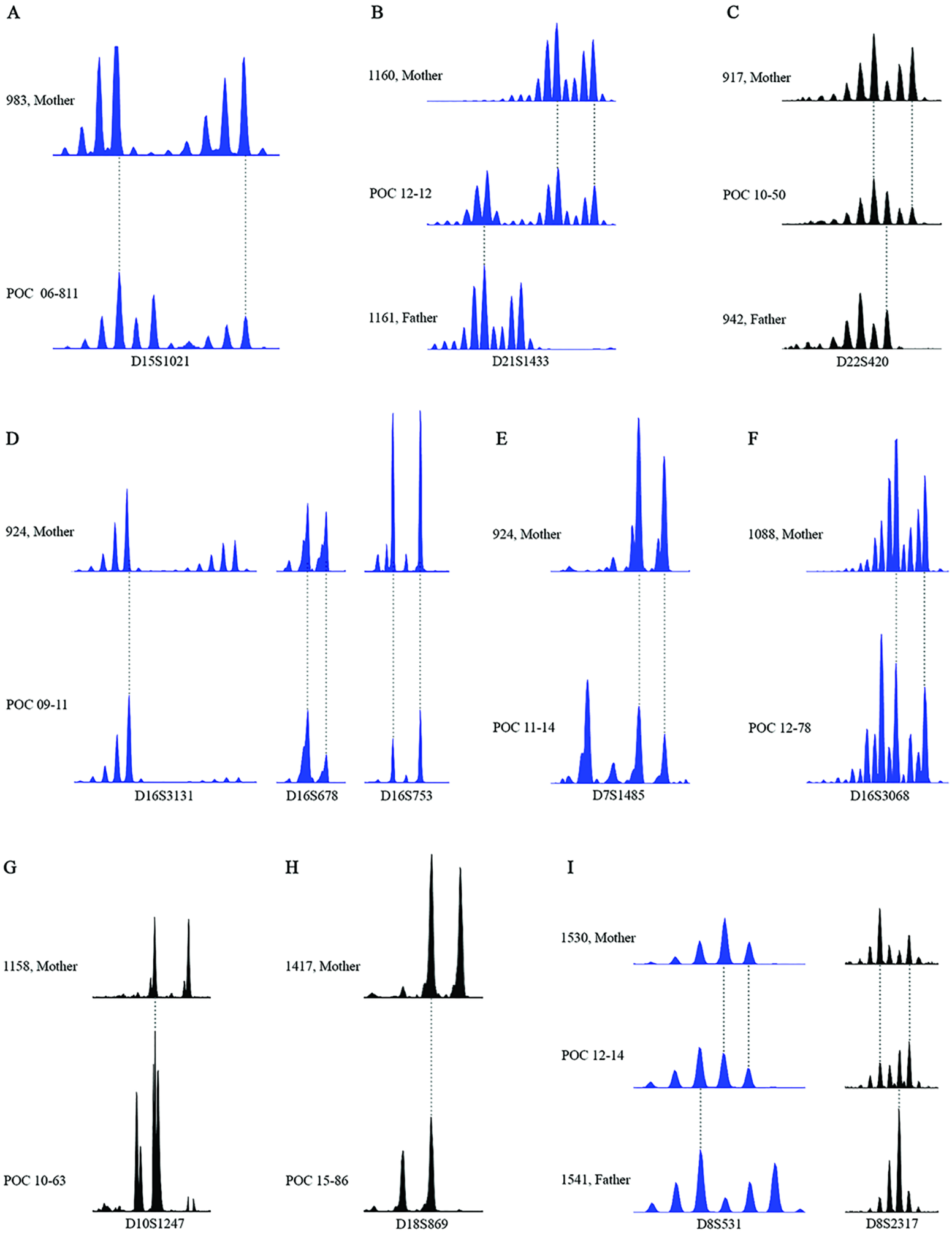
C

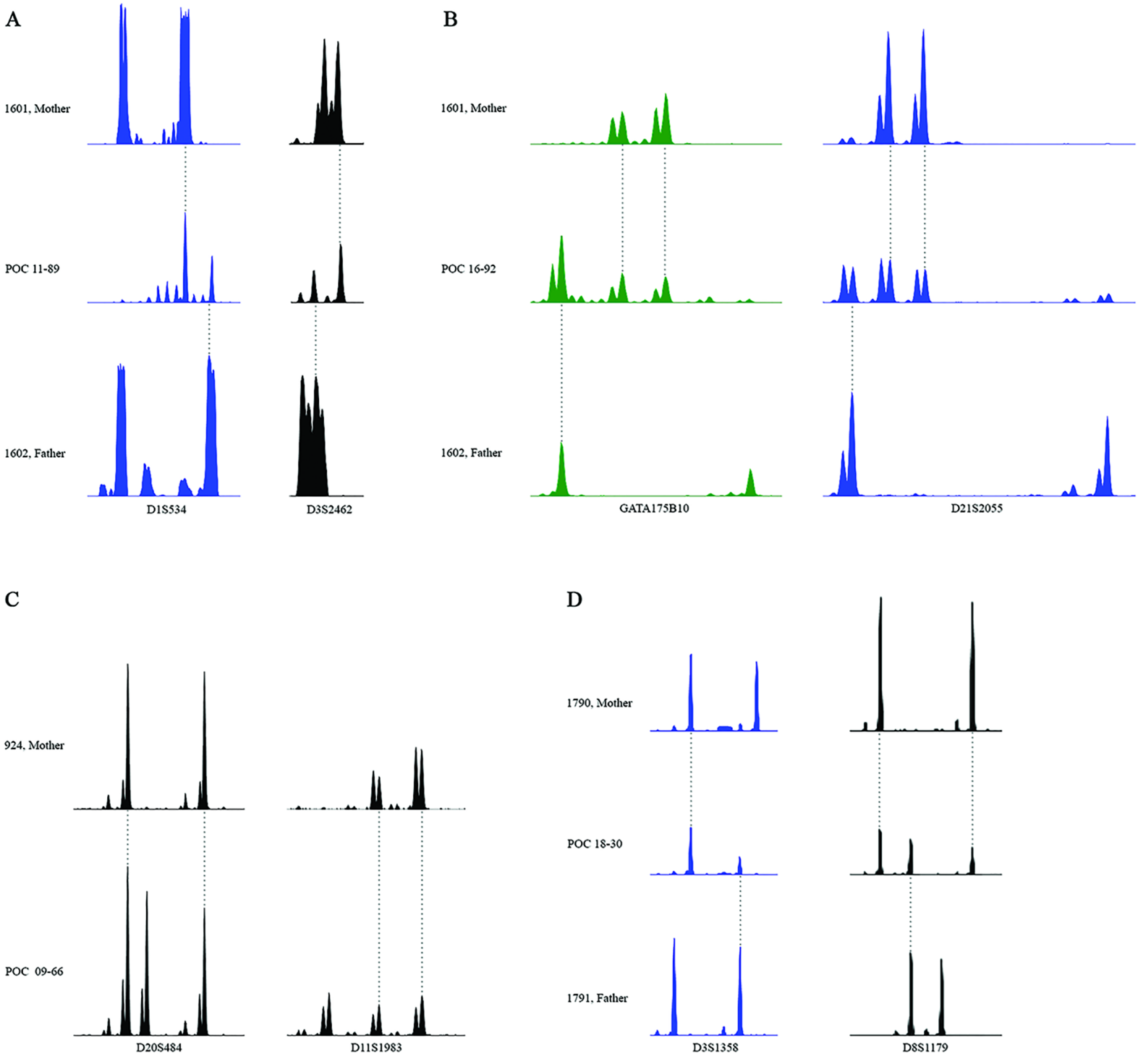



D

| | Molar genotype | | |
|---|---|---|---|
| | Androgenetic Monospermic | Androgenetic Dispermic | Triploid Dispermic |
| **≤20** | 22 (10%) | 2 (7.4%) | 24 (9.7%) |
| **21-30** | 93 (42.2%) | 12 (44.4%) | 106 (43.2%) |
| **31-40** | 67 (30.4%) | 8 (29.6%) | 111 (45.3%) |
| **>40** | 38 (17.2%) | 5 (18.5%) | 4 (1.6%) |
| **Total** | 220 | 27 | 245 |
| **Mean Age** | 31 | 32 | 29 |
| **Median** | 30 | 30 | 30 |
| **Range** | 14-55 | 19-53 | 13-45 |


CHM
PHM
24%
20%
7%
19%
4%
7%
4%
5%
1 MC
2 MC
3 MC
> 3 MC


A
983, Mother
POC 06-811
D15S1021
B
1160, Mother
POC 12-12
1161, Father
D21S1433
C
917, Mother
POC 10-50
942, Father
D22S420
D
924, Mother
POC 09-11
D16S3131
D16S678
D16S753
E
924, Mother
POC 11-14
D7S1485
F
1088, Mother
POC 12-78
D16S3068
G
1158, Mother
POC 10-63
D10S1247
H
1417, Mother
POC 15-86
D18S869
I
1530, Mother
POC 12-14
1541, Father
D8S531
D8S2317


A
1601, Mother
POC 11-89
1602, Father
D1S534
D3S2462
B
1601, Mother
POC 16-92
1602, Father
GATA175B10
D21S2055
C
924, Mother
POC 09-66
D20S484
D11S1983
D
1790, Mother
POC 18-30
1791, Father
D3S1358
D8S1179