Article type : Case Report

Parentage of Hydatidiform Moles

Robert E. Wenk,[1] M.D., M.S.; Michael Baird,[2] Ph.D.; John Peterson,[3] Ph.D.; Debra Davis,[4] Ph.D.; Richard Lieberman,[5] M.D.; Janice M. Maly[6]; Lindsey J. Campbell,[7] B.S.; and Kathy K. Fox,[7] B.S. Kristin A. Schelling,[8] M.S.

[1]111 Hamlet Hill Road, Unit 205, Baltimore, MD 21210.

[2]Chief Science Officer, DNA Diagnostic Center, One DDC Way, Fairfield OH 45014

[3]Director Forensic Laboratory, DNA Diagnostic Center, One DDC Way, Fairfield OH 45014.

[4]Director Parentage Laboratory, DNA Diagnostic Center, One DDC Way, Fairfield OH 45014.

[5]Associate Professor of Obstetrics and Gynecology and Pathology, University of Michigan Health System, Pathology, 2800 Plymouth Rd. Bldg. 35, Room 36-1221-03, Ann Arbor MI 48109.

[6]Forensic Scientist, Wisconsin State Crime Laboratory, Milwaukee, 1578 South 11th Street, Milwaukee, WI 53705.

[7]Forensic Scientist, Michigan State Police, Forensic Science Division, 7320 North Canal Road, Lansing, MI 48913.

[8]Biology Technical Leader, Michigan State Police, Forensic Science Division, 7320 North Canal Road, Lansing, MI 48913.

Corresponding author: Robert E. Wenk, M.D., M.S. E-mail: rwenk1@verizon.net

Running head: Mole Paternity

ABSTRACT: We were presented with the STR (short tandem repeat) profiles from two separate paternity trios. Each trio consisted of a mother, an alleged father and products of conception (POC) that contained a hydatidiform mole but no visible fetus. In both cases, antecedent pregnancies had followed alleged sexual assaults.

Mole classification and pathogenesis are described in order to explain the analyses and statistical reasoning used in each case. One mole exhibited several loci with two different paternal alleles, indicating it was a dispermic (heterozygous) mole. Maternal decidua contaminated the POC, preventing the identification of paternal obligate alleles (POAs) at some loci. The other mole exhibited only one paternal allele/locus at all loci and no maternal alleles, indicating it was a diandric and diploid (homozygous) mole.

In each case, traditional calculations were used to determine paternity indices (PIs) at loci that exhibited one paternal allele/locus. PIs at mole loci with two different paternal alleles/locus were calculated from formulas first used for child chimeras that are always dispermic. Combined paternity indices in both mole cases strongly supported the paternity of each suspect.

KEYWORDS: genetic kinship, paternity test, DNA testing, hydatidiform mole, mole classification, dispermy

Hydatidiform moles are placental growths with visibly cystic villi. Moles occur about once in 1,200 pregnancies. Moles are encountered infrequently in laboratories that test for paternity because most tests are requested in civil lawsuits brought by mothers seeking

financial support of their children and molar pregnancies very rarely produce a viable child. In the authors' experience, the question of a mole's paternity has only arisen in two criminal investigations.

This report describes mole genesis and classification in order to analyze a mole's paternity in the absence of a fetus or child. The aim is to develop and present paternity index (PI) formulas for mole loci that may exhibit either one or two paternal obligate alleles (POAs) per locus. To our knowledge, there are no previous accounts of how to determine the paternity of moles.

Mole Classifications

In a pathology laboratory, the microscopic absence of fetal tissue characterizes a *complete* mole whereas presence of fetal tissue characterizes a *partial* mole. Both kinds of moles carry a risk of transforming into gestational trophoblastic neoplasms (GTNs). A GTN occurs in up to 20% of complete moles, including a 1% risk of choriocarcinoma. Partial moles carry a 5% risk of a GTN but are not at risk for choriocarcinoma. Some partial and complete moles are difficult to distinguish by routine histopathologic examinations and require immunohistochemical study. Because cells of complete moles lack a maternal genome, their cells lack the $p57^{KIP2}$ antigen encoded on chromosome 11p15.4. (1) The antigen is an expression of only maternal DNA, which is absent from complete moles. Partial mole cells demonstrate this antigen because they contain an active maternal genome.

While pathologic classification of moles remains useful for predicting risks of malignant transformation, a genetic classification is based on cell ploidy and the contributions of parental chromosomes to molar cells. Ploidy and parental contribution help to interpret DNA-based paternity test results. All partial moles are triploid and carry a set of 23 maternal chromosomes in addition to two paternal sets. About 90% of complete moles are diploid and carry only two identical sets of (*homozygous*) paternal chromosomes. (2) Another 10% of complete moles are diploid but carry two different (*heterozygous*) sets of paternal chromosomes. Rare complete moles (<1%) carry one set of each parent's chromosomes – they are called *familial biparental moles*. (3) Regardless of ploidy or karyotype, however, every mole carries one or two sets of paternal chromosomes. Therefore, it is possible to evaluate paternity of the alleged father of any mole by ordinary identification of short tandem repeat (STR) alleles.

Mole Pathogenesis

All triploid moles and the 10% minority of diploid moles carry two different sets of paternal chromosomes. These moles arise after fertilization of an ovum by two sperm cells (dispermy) and are designated *heterozygous* because the two paternal chromosomal homologs differ and many STR loci exhibit two different alleles. (4) Zygotes carry two different (heterozygous) sets of paternal chromosomes (notated P_1P_2). The triploid moles also carry one set of maternal chromosomes (P_1P_2M).

The heterozygous *diploid* moles are believed to arise from dispermic triploid zygotes (P_1P_2M) that undergo *diploidization* (P_1P_2). Diploidization does not result from a simple expulsion of maternal chromosomes from the zygote. Rather, there is duplication (*endoreplication*) of one set of paternal chromosomes ($P_1P_1P_2M$ or $P_1P_2P_2M$) and asymmetric cell division of the momentarily tetraploid cell into two diploid ones. (5) One diploid cell carries a normal set of parental chromosomes (P_1M or P_2M) and the other cell carries two different paternal homologs (P_1P_2). The diandric cell (P_1P_2) develops into a *heterozygous* diploid mole. If the bi-parental diploid cell (P_1M or P_2M) proliferates, it generates various embryonic tissue phenotypes.

The majority (90%) of diploid moles carries two *identical* sets of paternal chromosomes (PP) and are designated *homozygous* because the two paternal homologs carry identical alleles at every locus. Homozygous moles are thought to arise after *endoreplication* of the paternal chromosomes in a normal diploid and bi-parental zygote (PM) to form a triploid zygote (PPM). (5) Any two paternal homologous chromosomes carry identical alleles and are homozygous at every locus. A second endoreplication of paternal chromosomes and an asymmetric cell division produce a diandric diploid homozygote (PP) and a two-parent diploid cell (PM). Proliferation of the diandric cell (PP) produces a diploid *homozygous* mole. If both diploid cells proliferate, a mole may become a mosaic of diandric cells (PP) and bi-parental cells (PM). Very rarely, a bi-parental diploid cell (PM) develops into a complete embryo. Note that embryonic tissue phenotypes require the expression of maternal DNA and mole phenotypes require an *overexpression* of paternal DNA: All moles are "*androgenetic*".

Familial bi-parental moles are also diploid, but they inherit one set of each parent's chromosomes. Each parent carries an autosomal mutation at the same locus and female children who inherit the mutation from both parents experience recurrent molar

pregnancies. Familial bi-parental moles ($P_{\mu}M_{\mu}$ in Table 1) are *functionally* androgenetic because maternal DNA is silenced by mutations of the autosomal NLRP7 locus (19q13.42) or the KHDC3L locus (6q13). The mutations produce an autosomal recessive mole disease that recurs in pregnancies of an affected woman. (Parents of women producing familial biparental moles are often consanguineous.) The mutations alter oocyte-imprinted (methylated) maternal DNA that otherwise would be expressed. (6) Other infrequent genomic events can produce familial biparental moles, as well.[1]

Table 1 summarizes the features of the various mole classes.

Paternity Testing

DNA Sampling

Ideally, fresh products of conception (POC) tissue should be submitted so that the mole can be dissected free of maternal decidua and blood. Then, the mole can be examined without maternal DNA contamination in a paternity-testing laboratory. Parentage studies benefit from using purely molar DNA unmixed with DNA of maternal decidua and blood. If maternal and molar cells remain admixed in POC, extracted DNA will exhibit contaminant maternal alleles as well as inherited ones. Even with contamination, however, some of the mole's paternal alleles can be deduced because molar paternal alleles in the POC are absent from the maternal genomic (e.g., buccal) sample. Paternal obligate alleles (POAs) may not be identifiable at loci where the mother shares them and multi-locus test power is reduced.

Paternity Exclusion

Evidence of exclusion from paternity at a locus consists of finding: A) one visible paternal allele/mole locus that is absent from the alleged father (AF) or B) two visible paternal alleles/mole locus**,** either one of which is absent from the AF. As is true in ordinary paternity cases, one may conclude that the AF is not the mole's biological father (BF) when exclusionary genetic evidence is observed at several STR loci. As usual, exclusionary evidence at several STRs is necessary because of possible mutations.

Paternity Inclusion

STR paternity testing of any mole is possible because every mole carries the DNA of one or two sets of paternal chromosomes. STR phenotype interpretation in molar parentage analysis partly depends on an understanding of the cytogenetic mole classification – i.e., cell

ploidy and the zygosity of paternal chromosomes. Whether diploid or triploid, *homozygous moles* exhibit one visible paternal allele/locus and every locus exhibits a mono-allelic paternal DNA 'phenotype'. Although duplicate paternal alleles/locus increase peak height on an electropherogram, peak height is not a reliable determinant of allele copy number. Furthermore, a molar allele carried by both parents may be ambiguous with respect to its parent of origin. Nevertheless, identifying even one POA per locus provides the same evidence of paternity as it does in an ordinary paternity case.

Heterozygous moles, whether diploid or triploid, are dispermic in origin and a tested STR locus may exhibit two different paternal alleles. If two different paternal alleles/locus are observed, one biologic father (BF) contributed both. Statistical logic, described in the next 2 paragraphs, is identical to that described for paternity testing the AF of a child chimera. (7) Chimeras and heterozygous moles both arise after dispermy: A mole results if a single ovum is fertilized; a chimeric child results if two ova are fertilized and the resulting dizygotic twin embryos fuse or exchange cells.

A mole must inherit two distinct alleles/locus from a heterozygous father who carries those two alleles. If the AF is heterozygous (A/B) and he is the mole's BF, the probability that he would sequentially transmit alleles A and B in two sperm cells is the product of their individual probabilities: 0.5 × 0.5 = 0.25 (by the Product Rule for independent events). The probability that the AF would sequentially transmit alleles B and A is 0.25 too. The total probability that a mole locus would be heterozygous if AF is its BF and AF is heterozygous is 0.25 + 0.25 = 0.5 (by the Addition Rule for mutually exclusive events).

Alternately, if a random man (RM) were the child's BF, he would transmit alleles A and B with probability 0.5 because he must be genotype A/B. A RM who is genotype A/B occurs with a population frequency of 2ab, if a and b are the respective frequencies of alleles A and B in AF's population and if locus genotypes meet Hardy-Weinberg expectations. The joint probability that an A/B heterozygous RM would transmit both A & B alleles is: 0.5 × 2ab = ab (by the Product Rule). Note that the probability with which a RM transmits both A & B alleles to his child (= ab) is less than the probability (= a or b) that a RM transmits one paternal allele. Thus, the paternity index (likelihood ratio) is greater when a molar locus exhibits two different paternal alleles and the AF carries both.

If only one paternal allele/locus is visible in a mole, the statistical logic is identical to that of an ordinary child because, by historical convention, paternity index calculations compare

the probabilities that the AF or a RM contributed to the offspring's locus *phenotype* and not to its presumed (homozygous) genotype. (8)

Table 3 presents formulas for calculating PIs of hypothetical DNA types that might be seen with STR profiles from molar paternity trios and "motherless" duos.

Two Mole Cases

STR test results (electropherograms) of two paternity trios (mothers, moles and AFs) were referred for parentage testing from different crime laboratories. No clinical, pathologic or cytogenetic information was obtained in either case from the crime laboratories or from the mothers' obstetricians. The referring labs had used commercial multiplexed tests of independently assorting STR loci but allele frequencies were those determined by the paternity testing laboratory.

Case 1

Three DNA samples taken from the POC all appeared to be mixtures with identical STR phenotypes. All maternal STR alleles were present in the POC samples, but POC STRs also contained 1 additional allele per locus at 16 loci and 2 additional alleles at 6 loci. All the non-maternal POC alleles, with the exception of one SE33 allele, were found in the STR profile of the AF's buccal DNA. The exceptional SE33 allele appeared to be 1 'repeat' longer than an allele present in both the mother and alleged father so that a mutation could have occurred in either parent. (Among commonly used STR loci, SE33 is known to have a relatively high mutation rate.)

Presence of every maternal allele in the POC is most simply explained by contamination of molar tissue by decidua. Other explanations are either highly improbable or impossible: Tetraploid moles do occur but are infrequent (9); triploid and familial bi-parental moles would exhibit no loci with two maternal alleles/locus; and diploid homozygous moles would exhibit no maternal alleles/locus. Therefore, all maternal alleles in the POC were ignored and a locus POA was identified as any allele that was absent from the mother's (buccal) DNA. STR alleles of the trio from Case 1 are represented in Table 2.

The AF carried the 1 or 2 alleles/locus that were absent from maternal buccal DNA. Two different paternal alleles/locus indicated a *heterozygous* (dispermic) mole that could not be further classified as either diploid or triploid. Assuming that all maternal alleles in the POC

were artifacts of maternal DNA contamination, the paternity index/locus (PI) was calculated from POC alleles absent from the mother's buccal cells. The combined multi-locus PI (CPI) was 1.4×10^{15}:1. Given a 50% prior probability of AF's paternity, the combined posterior probability of paternity (CPI) was >99.9999%.

Case 2

The mole's STR electropherogram exhibited a single allele at every tested locus and that allele was absent from the mother's profile. Findings indicated both an absence of maternal tissue contamination and an absence of inherited maternal alleles. The mole exhibited a single paternal allele/locus at all 23 test loci, a molecular phenotype that suggested endoreplication of paternal chromosomes and a presumably diploid but diandric *homozygous mole*. (See Table 2, Case 2.) Every mole locus exhibited an allele found in the AF. The CPI was 3.5×10^{10}:1 and the percentage posterior probability of paternity was >99.9999%, given a prior probability of 50%.

Conclusions

An alleged father can be tested for the paternity of any hydatidiform mole by using ordinary STR tests. At molar loci with one evident POA, the statistical logic used to determine locus PIs and posterior probabilities of paternity follows the logic of cases involving an ordinary child. At molar loci with two different paternal alleles/locus, we propose using the logic first used in paternity cases involving child chimeras because both chimeric children and heterozygous moles arise from dispermic fertilizations.

References

1. Genest DR, Dorfman DM, Castrillon DH. Ploidy and imprinting in hydatidiform moles. Complementary use of flow cytometry and immunohistochemistry of the imprinted gene product p57KIP2 to assist molar classification. J Reprod Med 2002;47(5):342–6.
2. Candelier JJ. The hydatidiform mole. Cell Adh Migr 2016;10(1-2):226–35. doi: 10.1080/19336918.2015 1093275.
3. Nguyen NMP, Khawajkie Y, Mechtouf N, Rezaei M, Breguet M, Kurvinen E et al. The genetics of recurrent hydatidiform moles: lessons from a comprehensive analysis of 113 patients. Mod Pathol 2018 Jul;31(7):1116–30. doi: 10.1038/s41379-018-0031-9.
4. Scholz NB, Bolund L, Nyegaard M, Faaborg L, Jorgensen MW, Lund H et al. Triploidy – observations in 154 diandric cases. PLoS ONE 2015;110(11):e01425. doi: 10.1371/journal.pone.0142545.
5. Sunde L, Niemann I, Hansen ES, Hindkjaer J, Degn B, Birk U et al. Mosaics and moles. Eur J Hum Genet 2011;19(10):1026–31. doi: 10.1038/ejhg.2011.93.
6. Monk D, Sanchez-Delgado M, Fisher R. NLRPs, the subcortical maternal complex and genomic imprinting. Reproduction 2017;154(6):R161–70. doi: 10.1530/REP-17-0465.
7. Wenk RE, Davis D. Paternity probabilities when a child is a congenital chimera. Transfusion 2017;57(11):2814–5. doi: 10.1111/trf.14317.
8. Morris JW. Probability of paternity: logic 1. In: Probability of inclusion in paternity testing. Silver H, editor. Arlington, VA: American Association of Blood Banks, 1982;46.
9. Banet N, DeScipio C, Murphy KM, Beierl K, Adams E, Vang R et al. Characteristics of hydatidiform moles: analysis of a prospective series with p57 immunohistochemistry and molecular genotyping. Mod Pathol 2014;27(2):238–54. doi: 10.1038/modpathol.2013.143.

TABLE 1—*Hydatidiform mole classifications and characteristics.*

Genotype	Ploidy	Paternal Homologs	Etiology	Usual Pathology	No. Visible* POAs/STR
		Diandric Moles			
P_1P_2M	Triploid	Heterozygous (100%)	Dispermy	Partial mole $p57^{KIP2}$ Ag(+)	1 or 2
P_1P_2	Diploid	Heterozygous (10%)	Dispermy	Complete mole $p57^{KIP2}$ Ag(−)	1 or 2
PP	Diploid	Homozygous (90%)	Endo-replication	Complete mole $p57^{KIP2}$ Ag(−)	1

		Familial Biparental Moles			

$P_{\mu}M_{\mu}$	Diploid	Not applicable	Autosomal mutations	Complete mole $p57^{KIP2}$ Ag(−)	1

*A paternal obligate allele (POA) is *visible* if it is not a duplicate allele.

GLOSSARY - P: a set of 23 paternal chromosomes; M: a set of 23 maternal chromosomes; $P_{\mu}M_{\mu}$: 2-parent autosomal mutations of NLRP7 or KHDC3L; $p57^{KIP2}$ Ag (+): immunochemical stains histologically positive for p57 antigen.

TABLE 2—*Generic locus phenotypes in two paternity cases.*

Case 1: Heterozygous Triploid? (Partial) Mole

Mother	POC*	AF	Loci Matching Generic Phenotypes	Paternity Index†

X	X, Y	X, Y	Amelogenin	NA
A, B	A, B, C, D	C, D	CSF1PO, D1S1656, D12S391, D16S539	.5/cd
A, B	A, B, C	A, C	D2S441, D2S1338, D7S820, D8S1179, D19S433	.5/c
A, B	A, B, C	C, D	D13S317, D21S11, D22S1045, FGA, Penta E	.5/c
A, B	A, B, C	C	D18S51	1/c
A, B	A, B	A	D5S818	1/a‡
A	A, B	B, C	D10S1248, TPOX	.5/b
A	A, B	A, B	D3S1358, Penta D, TH01	.5/b
A	A	A, B	VWA	.5/a‡
A, B	A, B, C	A	SE33	mutation

Case 2: Homozygous Diploid (Complete) Mole

Mother	Mole	AF	Loci Matching Generic Phenotypes	Paternity Index
X	X	X, Y	Amelogenin	NA
A, B	A	A, C	D2S441, D10S1248, D13S317, Penta D, D21S11, D8S1179, D19S433	.5/a
A, B	C	C, D	D1S1656, Penta E, D2S1338, D7S820, FGA	.5/c

A, B	A	A, B	D5S818, D3S1358, D18S51	.5/a
AB	A	A	CSF1PO, D12S391	1/a
AB	C	C	D16S539, TPOX	1/c
A	B	B	D22S1045	1/b
A	B	B, C	TH01	.5/b

Uppercase letters substitute for numbered alleles at each listed locus because of privacy concerns of the submitting forensic laboratories. (STR profiles identify individuals.)

*POC: Products of Conception (Mole + Decidua + Maternal blood)

† Paternity Index = Likelihood Ratio formulas take into account maternal contamination and may differ from the formulas in Table 3.

‡ Because alleles from maternal contamination overlapped the paternal contribution, LRs were not included in final CPI calculations.

TABLE 3—*Phenotypes and likelihood ratios in molar paternity cases.*

Mother Mole	Father	Alleged Paternity Index (PI)
All Heterozygous Triploid (Partial) Moles (100%)		
A	A	AB	.5/a
A	AB	AB	.5/b
C	AC	AB	.5/a
C	ABC	AB	.5/ab*
AB	A	AB	.5/a
AB	AB	AB	1/(a + b)
AC	A	AB	.5/a
AC	AB	AB	.5/b
AC	AC	AB	.5/(a + c)
AC	BC	AB	.5/b
AC	ABC	AB	1/(ab + bc)*
CD	AC	AB	.5/a
CD	ABC	AB	.5/ab*
A	A	A	1/a
B	AB	A	1/a
AB	AB	A	1/(a + b)
AB	A	A	1/a
BC	AB	A	1/a
Homozygous Diploid (Complete) Moles (90%)		
–	A	A	1/a
–	A	AB	.5/a
Heterozygous Diploid (Complete) Moles (10%)		

–	A	A	1/a
–	A	AB	.5/a
–	AB	AB	.5/ab*

Upper case letters indicate alleles and lower case letters indicate allele frequencies.
* PIs of loci with two inherited paternal alleles are calculated in the way that a chimeric child is, (7) whereas PIs of a mole locus with only one visible paternal allele are calculated in the way that an ordinary child is. (8)