RELATIONSHIP TESTING NEWS

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In This Issue

COVID19/SARS CoV2 and Relationship Testing

While it While it may not appear germane on the surface to the topic of Relationship Testing, laboratories engaged in providing RT service interact in close physical proximity to clients, usually obtaining mouth swab samples. In the present era of Covid-19, it bears reminding that precautions must always be taken to avoid exposure of either the lab staff or the clients to the virus. Dr. Harlan Krumholz, a professor of medicine at Yale, suggests that “we should assume that anyone could be carrying the virus.” Stay safe and be on guard at all times.

Paternity Testing Alleged Fathers of Hydatidiform Moles

Robert E. Wenk MD and John Peterson PhD

Abstract

A hydatidiform mole is a tumorous placenta that may contain embryonic tissue. A mole always inherits one or two paternal genomes so that obligate paternal alleles (OPAs) of independent loci can be used to test the paternity of a mole's alleged father (AF). There are two problems in testing: First, dispermic moles carry loci that can express two different OPAs/locus and second, one visible molar OPA/locus can represent two possible genotypes – either a single OPA if the mole is a familial bi-parental mole or two identical alleles at a homozygous locus in any kind of mole. Each problem has a solution: First, probabilities that a dispermic mole inherits two different OPAs/locus can be calculated using formulas recommended for child chimeras, which also arise by dispermy; and second, paternity indexes (PIs) can be calculated by using one-allele molar phenotypes, the method used for historic ABO serologic tests. These paternity index calculations do not require a cytogenetic examination or classification of the mole.

History and Classification of Moles

In the pre-Elizabethan English language of the 1400s, the word mole meant "a growth in the womb". Midwives had already recognized pregnancies that were partly molar and partly embryonic and other pregnancies that were completely molar. At the turn of the 20th century, mole tissues could be examined microscopically, enabling pathologists to better distinguish "partial" moles, which contained embryonic tissues, from "complete" moles, which didn't. The distinction was important because complete moles were more likely to undergo malignant transformation. In the 1950s, cytogeneticists found that cells of all partial moles were triploid, containing one set of maternal chromosomes (notated M) and two paternal (P) sets. The two paternal homologs appeared to differ in morphology and carried loci that expressed two different alleles. Thus, any two paternal homologous chromosomes were heterozygous (notated P1P2) and triploid moles can be notated cytogenetically MP1P2. Cells of complete moles were diploid, containing two sets of paternal chromosomes but no (0) maternal set. Like triploid moles, 10% of complete moles carried different paternal homologs (P1P2) so that many loci were heterozygous. However, most complete moles (90%) carried paternal homologs whose loci were all homozygous (PP). Around the 2000 millennium, a third kind of mole was described – the diploid familial bi-parental mole, which contained a single set of maternal and a single set of paternal chromosomes (notated MP). A cytogenetic mole classification is shown in Table 1.

Table 1 . Cytogenetic Classification of Hydatidiform Moles

Molar Genotype	Molar Cell Ploidy	Paternal Chromosomal Zygosity	Paternal Alleles per STR phenotype	Usual Description
MP1P2	Triploid	Heterozygous (100%)	2>1	Partial mole
PP	Diploid	Homozygous (90%)	1	Complete mole
P1P2	Diploid	Heterozygous (10%)	2>1	Complete mole
MmPm	Diploid	Not Applicable	1	Familial

				biparental mole

M: maternal genome, P: paternal genome, P1P2: dispermic origin,

PP: endoreplication origin, μ: autosomal mutation

Pathogenesis

Heterozygous diandric triploid moles (MP1P2) and diploid moles (P1P2) both originate by *dispermy* and the fertilization of one ovum by both spermatozoa. Thus, polymorphic loci (e.g., STRs) on any pair of paternal homologous chromosomes often carry different paternal alleles.

Heterozygous diploid moles likely originate as triploid zygotes (MP1P2) whose daughter cells become diploid when they lose their maternal chromosomes in a two-step process. First, one set of paternal chromosomes *endoreplicates*[1] (duplicates to MP1P1P2 or MP1P2P2) and second, the zygote divides by an asymmetric cell division into a diandric cell (P1P2) and a normal bi-parental cell (MP2 or MP2). After many mitoses, the mole is a mosaic that contains two kinds of daughter cells – diandric heterozygous cells (P1P2) and bi-parental diploid cells (MP1 or MP2).[2]

The more common homozygous diploid moles (PP) originate as cytogenetically normal zygotes (MP) that undergo two endoreplications of paternal chromosomes (MPPP) and an asymmetrical cell division. The result is a mosaic mole that contains homozygous diandric cells (PP) and diploid bi-parental cells that express various embryonic phenotypes (MP).

The rare (<1%) familial bi-parental moles inherit a set of chromosomes from each parent (MP) and cellular karyotypes appear normal. However, the mole's parents are carriers of mutations at the same autosomal locus – either NLRP7 at 19q13.42 or KHDC3L at 6q13. Inheriting two mutations/locus inhibits expression of maternal DNA and familial bi-parental moles inherit an autosomal recessive disease. Familial bi-parental moles account for a majority of recurrent moles worldwide.[3]

All Moles Carry Paternal DNA

Embryos are expressions of male and female DNA, but moles are *androgenetic* – expressions of male DNA alone. Diandric diploid moles (PP or P1P2) can express only paternal DNA; diandric triploid moles (MP1P2) overexpress paternal relative to maternal DNA; and familial bi-parental moles (MP) cannot express maternal DNA. Because every mole carries paternal DNA, its alleged father (AF) can be tested for paternity using molecular methods.

To be sure, moles are rarely encountered in paternity test laboratories. There are several reasons why. First, moles occur only once per 1200 pregnancies in the U.S. (Notably, frequencies are more than tenfold greater in some nations of the Far East.) Second, most paternity tests are requested when mothers try to obtain child support from fathers, but molar pregnancies almost never produce viable children. Third, some investigators and lab workers may not realize that moles always contain paternal DNA. The authors have encountered only two moles submitted for paternity tests, both from forensic cases referred after alleged rapes.

Paternity Testing

A mole's biologic father can be identified by the usual typing of microsatellite (STR) alleles of the mole and its alleged father (AF), with or without testing the mother. STR typing at 15-20 loci will reveal presence of heterozygous paternal loci in all triploid moles and in the minority of diandric diploid moles that are heterozygous. While individual loci of heterozygous moles may be homozygous by chance, all loci of homozygous diandric moles exhibit mono-allelic paternal STR

phenotypes. Familial diploid bi-parental moles exhibit both heterozygous and homozygous STR loci but there is only one OPA/locus as there is in a child.

Excluding Paternity

If a mole is diploid and diandric, there is no maternal obligate allele (OMA) per locus. If a mole is triploid (MP1P2) or bi-parental, the OMA/locus is determined from DNA of the mother's (e.g., buccal) cell sample. The OMA at each locus is identified in the mole and the 1 or 2 paternal OPAs per locus are deduced. Evidence of the AF's non-paternity is a failure to carry one (or both) OPAs/locus. **An AF may be excluded when he fails to carry the mole's OPAs at enough STR loci (typically ≥3) to rule out mutations.**

Probable Paternity

A mole's cytogenetic classification, ploidy, and locus zygosity help explain why there can be two OPAs/locus. A mole's class also can suggest maternal DNA contamination of molar DNA when the mole seemingly exhibits two maternal alleles at a locus. Paternity probabilities, however, can be calculated from locus OPAs with no knowledge of a mole's classification.

Locus PI may be determined from a child's locus phenotype (1 or 2 visible OPAs).[1] In fact, the convention of relying on locus phenotype was established at the inception of paternity testing because the ABO locus carried the serologically silent O allele. (Blood type A could represent a homozygous A/A or a heterozygous A/O genotype and blood type B could represent B/B or B/O genotype.) Thus, if a molar locus exhibits a single OPA, the locus PI is calculated in the same way as a paternity case involving a child.

If a molar locus exhibits two different OPAs in the phenotype of a heterozygous locus in a triploid mole (MP1P2) or heterozygous diandric mole (P1P2), a locus' PI is calculated to account for inheritance of both OPAs from the AF and from a RM.[5] Therefore, probability (*p*) of transmitting alleles R and S from an R/S AF is 0.25 because the *p* of transmitting R in one sperm cell is 0.5 and the *p* of transmitting S in a second sperm cell is 0.5 too. The *p* that R and S alleles would be *sequentially* transmitted in dispermy (two independent events) is the product 0.5 x 0.5 = 0.25. The *p* that S and R alleles would be sequentially transmitted in dispermy = 0.25 too and the total *p* that either sequence would occur (mutually exclusive events) is 0.25 + 0.25 = 0.5.

(1) Total *p*(AF transmits both R and S alleles) =0 .5.

A heterozygous R/S RM occurs with a probability of twice the frequency of R × the frequency of S (= 2rs, where r and s are the respective frequencies of alleles R and S if locus alleles are in Hardy-Weinberg Equilibrium). The .5 probability of a molar locus phenotype containing alleles R and S is combined with the independent probability that a heterozygous RM carries alleles R and S. Thus,

(2) p(RM transmits both R and S) = 0.5(2rs) = rs.

PI = p(AF would transmit alleles R & S) / p(RM would transmit alleles R & S) and

(3) PI = 0.5/rs.

Table 2 contains a list of the complex PI formulas for heterozygous diploid and triploid moles that inherit two different paternal alleles per locus. A complete list of algebraic formulas for all possible phenotypes of paternity trios and duos has been tabulated elsewhere.[6]

Table 2. Paternity Indexes of Dispermic Mole Loci Exhibiting Two OPAs/Locus

Maternal Phenotype	Mole Phenotype	Alleged Father's Phenotype	Paternity Index
	Dispermic Triploid Moles		
C	ABC	AB	0.5/ab
AC	ABC	AB	1/(ab + bc)
CD	ABC	AB	0.5/ab
	Dispermic Diploid Moles		
--	AB	AB	0.5/ab

Uppercase: names of alleles per locus.

Lowercase: frequencies of the alleles in uppercase.

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Book Review

"Paternity: The Elusive Quest for The Father", pp. 1-352.

Nara B. Milanich Ph.D., Professor of History, Barnard College.

Harvard University Press, Cambridge MA 2019

Analysis of a paternity "trio" assumes that the child is the offspring of the mother. Once the maternal alleles are identified in the child, the obligate paternal alleles can be deduced. The "maternal assumption" was adopted at the inception of genetic paternity testing, before it was validated by millions of tests worldwide. In contrast, a child's paternity is always open to doubt, denial, dispute, and deception. In the Odyssey, Homer summarized: "Wise is the child that knows its father." In fact, questionable paternity has been the subject of gossip, art, literature, and drama throughout human history.

Nowadays, genetic tests either disprove biologic paternity with near certainty or declare it as a very likely possibility. The biologic truth, however, may not agree with the religious, legal, or social characterizations of the word “paternity”. Accordingly, Nara Milanich, Professor of History at Barnard College, has extensively researched and documented the cultural facets of paternity and their interactions with the biologic reality. She describes in detail the conflicts, attempted resolutions and continuing inconsistencies between the meanings and she presents remarkable case examples drawn from history and newspaper accounts. Her 267-page treatise is engaging and contains a prologue, eight chapters, an epilogue, and an index. The work is illustrated and fully annotated in 63 pages of information taken from source materials. Each succinct chapter demonstrates how Western societies have dealt with differences between the genetic truth (i.e., “modern paternity”) and societal expectations between the 1920s, when ABO blood group serology encompassed human genetics, and the current molecular test (DNA) era.

My own forty years of paternity test experience in the U.S. began in the 1970s when erythrocyte (RBC) serologic tests (for antigens of six loci) were mainstays and RBC agglutination assays were performed mostly in blood bank laboratories. I adopted each new technologic method in order to expand the number of independently assorting test loci and increase the probabilities of exclusion or inclusion. Thus, I learned to interpret test results of serum protein biochemistry and immunochemistry, human leukocyte antigen (HLA) cytotoxicity, and three kinds of DNA tests. None of my learning about valid test methods addressed the historically important pseudoscience and charlatanism that predated and then rivaled blood typing. Fraudsters included laymen, physicians, eugenicists, and a few well-meaning scientists who attempted to apply physiognomy, dermatoglyphics and physical anthropology to paternity testing. As is still true, belief and uncritical acceptance overwhelmed evidence and reason. (Consider the Theranos fraud of our time.) While morphologic methods might have been valid identifiers of individuals and indications of ethnicity, they were invalid paternity tests. They were also slow, complicated, expensive, and required expert interpretation of results. Notably, these so-called paternity tests persisted long after blood group antigen tests had been proven to be Mendelian, fast, inexpensive, accurate, reproducible, and easily interpreted.

In the 1920s, obstetrical deliveries in hospitals began to replace home deliveries. Newborns were separated from mothers and placed in nurseries, producing dramatic “switched-at-birth” errors. Since genetic tests were rudimentary and parentage test results were not yet accepted as court evidence, not all babies were placed with the right families. Newborn identification systems now prevent these errors, but gametes and zygotes are now “switched-*before*-birth”! Plus ca change, plus c’est la meme chose.

Paternity tests have interesting associations with racism and Milanich cites three examples. First, blood group genetics and eugenics were conflated in the 1930s when paternity tests were misused in Germany by the National Socialists to determine the “race” of a child who might have had a non-Aryan parent. Second, after 1945, some married Italian women bore children sired by African American GI’s. Biracial (“negroid”) children caused marital separations, legal disputes about child support and divorce. While paternity tests were unnecessary, religious customs and civil statutes held that a husband is the legal father of his wife’s biracial child. Third, in the early 1950s, the alleged sons of U.S. citizens attempted to emigrate to the U.S. from China, a newly communist country. U.S. racism and xenophobia (the “yellow peril”) led to the paternity testing of thousands of immigrants. Impostors, fraudulent paperwork, and erroneous paternity test results compounded the problem of overwhelming numbers of

tests. Courts found that testing only Chinese petitioners and beneficiaries was discriminatory and preceded more equitable policies.

Clearly, genetic testing cannot replace nor abruptly change traditional, religious, and social concepts of paternity. The embarrassment of a racial disparity between a child and its legal father remain problematic. Genetic testing cannot overcome the discrimination against biracial children or erase the injustice of a husband who must support another man's child. Changes in human thinking and behavior are required to resolve conflicts between an objective genetic truth and prejudices practiced over centuries.

"*Paternity: The Elusive Search for the Father*" contains a few technical errors. (For example, "…If the mother and child were the same [ABO] blood type… no possible father could be excluded.") These aside, this book is historically informative and a pleasure to read. I recommend it to laboratory directors and scientists who deal with kinship testing, forensics, and transfusion.

March 12, 2020
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USCIS UPDATES

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The U. S. Citizenship and Immigration Services (USCIS) has notified AABB that, due to the COVID-19 pandemic, the Department of State has instructed posts to stop scheduling and collecting samples for all visa, citizenship, and USCIS cases until further notice that routine services have resumed.

USCIS Policy Update on Acceptance of DNA Evidence for Sibling Relationships

There have been significant changes to USCIS policies regarding the acceptance of DNA evidence supporting sibling relationships. The news release is available on the USCIS website: https://www.uscis.gov/news/alerts/uscis-updates-policy-dna-evidence-support-sibling-relationships

The complete policy memorandum is available at https://www.uscis.gov/sites/default/files/USCIS/Laws/Memoranda/2018/2018-04-17-PM-DNA-Evidence-of-Sibling-Relationships.pdf

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Do you have an interesting case or question you would like to share through this newsletter?

Repurpose your old talks or presentations
If you have given a talk or presentation in the last 2-3 years on a topic that you think may be of interest to the relationship testing community, share your content as part of AABB's RT Webinar Series. If you decide to submit your content, you can choose to moderate the audio conference or we can assign a speaker for you.

For more information or to submit your content, email us at nikkib@aabb.org

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