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# Distribution of the alleles at loci D16S539, D7S820, and D13S317 in hydatidiform mole genome from Chinese women and its relationship with clinical prognosis

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**Abstract** Using polymerase chain reaction and denaturating polyacrylamide gel electrophoretic techniques, we studied 53 cases of hydatidiform moles. Of these, 41 cases were genetically complete hydatidiform moles (g-CHM) whose genome were totally paternally derived. We investigated the distribution of the alleles in the short tandem repeat sequences at loci D16S539, D7S820, and D13S317 in these cases. In particular, we analyzed the allelic distribution and potential significance in cases with traceable benign and invasive moles (i.e., persistent trophoblastic tumor [PTT]). Among 41 g-CHM cases, there were six alleles at D16S539, five alleles at D7S820 (the frequencies of alleles 9 and 10 were respectively lower and higher than those in Beijing population), and seven alleles at D13S317; the heterozygosity of loci D16S539, D7S820, and D13S317 was 0.0732, 0.0976, and 0.0732, respectively. Among 23 benign cases, there were six alleles at D16S539, four at D7S820, and six at D13S317; among 11 PTT cases, there were five alleles at D7S820 and four alleles each at D16S539 and D13S317. The frequencies of allele 9 at D16S539 and allele 10 at D7S820 were higher than in benign cases ($P < 0.05$). There were significant differences in frequencies of alleles 9 and 10 at D7S820 between the cases and the Beijing population, and heterozygosity at the three loci was lower in the cases than in the population. In addition, invasiveness of hydatidiform mole correlated to the frequency of allele 9 at loci D16S539 and allele 10 at D7S820. 

## 1. Introduction

Complete hydatidiform moles typically result from fertilization of an empty egg by one or (less often) two sperm [1]. Regardless of the number of sperm, genome of complete hydatidiform moles are paternally derived. Pathologic categorization of hydatidiform mole frequently varies from one pathologist to another [2]; categorization using molecular genetic methods promises to be more accurate and consistent. Researchers have long been using DNA polymorphism analysis to study hydatidiform moles. In particular, with progress in the study of short tandem repeat (STR) sequence polymorphism, genetic categorization of hydatidiform moles has become possible, including analysis of fertilization type [3,4]. We used nine STR sequences of high polymorphism to identify hydatidiform moles with genetic material totally paternally derived and then investigated the distribution of the alleles at three STR loci (D16S539, D7S820, and D13S317) and the relationship of this distribution to the mole's invasiveness.

## 2. Materials and methods

Our 53 cases of hydatidiform moles were collected from the Peking University Third Hospital. DNA of moles and peripheral blood from the affected couples was extracted. After molar evacuation, the changes of serum human chorionic gonadotropin (hCG) in the affected women were followed up for 1.5 years: every week for the first 2 months, every 2 weeks for the next 4 months, and then every month for the next year. Persistent trophoblastic tumor (PTT) was diagnosed if (i) the plateau of hCG lasted for four measurements over a period of $\geqslant$3 weeks after evacuation of the mole, or if (ii) there was a rise of hCG for three or more consecutive weekly measurements after evacuation, or if

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(iii) the hCG level remained elevated for 6 months after evacuation or in the presence of metastatic disease except in lung or vagina.

The STR detection kits for nine loci (F13A01, FESFPS, VWA, CSF1P0, TPOX, TH01, D16S539, D7S820, D13S317) were purchased from Promega (Madison, WI). Polymerase chain reaction amplification and silver nitrate staining were performed as previously described [5].

Chi-square testing was performed to evaluate group difference.

## 3. Results

Of 53 cases, 41 had totally paternally derived DNA. Table 1 shows the occurrence of each allele at loci D16S539, D7S820, and D13S317. Chi-square tests showed that the frequency of allele 9 at D7S820 was lower in the cases than in the population ($P < 0.01$), and that the frequency of allele 10 was higher in the cases than in the population ($P < 0.05$); there were no significant differences in the frequencies of other alleles between the cases and the population. Heterozygosity of loci D16S539, D7S820, and D13S317 was significantly lower in the cases than in the population: D16S539 $\chi^2 = 77.45$, D7S820 $\chi^2 = 79.95$, and D13S317 $\chi^2 = 74.37$ (all $P < 0.001$) (Table 2).

Table 3 shows the features of loci D16S539, D7S820, and D13S317 and Table 4 shows the distribution of each allele in benign and invasive cases. Frequencies of allele 9 at D16S539 and of allele 10 at D7S820 were significantly different between benign and invasive cases ($P < 005$); there were no significant differences in distribution of other alleles between the two types of cases.

## 4. Discussion

Statistical analysis revealed significant differences in frequencies of several alleles at D7S820 between the cases and the Beijing population, and the heterozygosity of three loci (D16S539, D7S820, and D13S317) was far lower in the cases than in the population. In addition, allele 9 at D16S539 and allele at D7S820 correlated to the mole's invasiveness.

Hydatidiform moles are of great interest, in part because some cases are invasive [6] (for example, trophoblasts can invade uterine wall and even metastasize to remote sites, such as lung or brain, in which case inappropriate treatment may lead to death of the patient) and also because of their unusual genetic composition. Since the mid-20th century, the development of cellular and molecular genetics has deepened our understanding of hydatidiform mole. Currently, hydatidiform mole is thought to result from altered chromosomal composition due to abnormal fertilization of eggs by sperm [6]. Pathogenesis is thought to follow three different courses.

1. In monospermic g-CHM, an empty egg with no chromosomes is fertilized by a single sperm. The majority of hydatidiform moles fall into this class. Sperm chromosomes are duplicated by themselves and restore diploid DNA, producing a homozygote with all alleles derived paternally.
2. In dispermic g-CHM, an empty egg is fertilized by two sperm. In this class, which accounts for only a minority of moles, a diploid is produced upon fertilization, but the chromosomes are derived from two sperm and alleles are either homozygous or heterozygous. As in the monospermic class, all alleles are paternally derived.
3. In partial hydatidiform mole, a normal egg with chromosomes is fertilized by two sperm. The dispermic fertilization produces a triploid with chromosomes derived both paternally and maternally. In this class, the alleles are of higher heterozygosity than in the empty-egg g-CHM. In the partial mole, three alleles may be present at a single locus in partial mole; this phenomenon is used to define a hydatidiform mole resulting from dispermy.

Table 1
Occurrence of the alleles at loci D16S539, D7S820, and D13S317 in 41 cases of complete hydatidiform moles

| | D16S539 | | | D7S820 | | | D13S317 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Cases | | Pop. | Cases | | Pop. | Cases | | Pop. |
| Allele | Alleles, no. | Allele freq. | Gene freq. | Alleles, no. | Allele freq. | Gene freq. | Alleles, no. | Allele freq. | Gene freq. |
| 8 | 0 | 0 | 0.041 | 13 | 0.159** | 0.203** | 35 | 0.427 | 0.360 |
| 9 | 25 | 0.305 | 0.305 | 3 | 0.037** | 0.172** | 8 | 0.098 | 0.160 |
| 10 | 10 | 0.122 | 0.122 | 22 | 0.268* | 0.172* | 7 | 0.085 | 0.107 |
| 11 | 22 | 0.268 | 0.198 | 31 | 0.378 | 0.241 | 12 | 0.146 | 0.168 |
| 12 | 11 | 0.134 | 0.236 | 13 | 0.159 | 0.185 | 16 | 0.195 | 0.168 |
| 13 | 10 | 0.122 | 0.084 | 0 | 0 | 0.026 | 2 | 0.024 | 0.028 |
| 14 | 4 | 0.049 | 0.015 | 0 | 0 | 0.003 | 2 | 0.024 | 0.008 |
| Total | 82 | | | 82 | | | 82 | | |

*Abbreviations:* freq., frequency; Pop., population (i.e., Han people resident in Beijing).

* $P < 0.05$.

** $P < 0.01$.

Table 2
Heterozygosity of D16S539, D7S820, and D13S317 in cases and in the Beijing population

| Locus | Homozygotic cases, no. | Heterozygotic cases, no. | Heterozygosity | | | |
|---|---|---|---|---|---|---|
| | | | Cases | Pop. | $\chi^2$ | *P* |
| D16S539 | 38 | 3 | 0.0732 | 0.7906 | 77.45 | 0.01 |
| D7S820 | 37 | 4 | 0.0976 | 0.8092 | 79.95 | 0.01 |
| D13S317 | 38 | 3 | 0.0732 | 0.7774 | 74.37 | 0.01 |

Table 3
Features of alleles at loci D16S539, D7S820, and D13S317

| Locus | Chromosome band | GenBank accession no. | Core sequence | Segment length, bp | Allele |
|---|---|---|---|---|---|
| D16S539 | 16q22~q24 | G07925 | (GATA)n | 264–304 | 8–16 |
| D7S820 | 7q11.21~q22 | G08616 | (GATA)n | 256–289 | 6–14 |
| D13S317 | 13q22~q31 | G09017 | (TATC)n | 205–229 | 7–14 |

The empty-egg fertilization mechanism of g-CHMs is a deductive theory based on data from many studies, a theory so far without direct experimental support. Because the majority of hydatidiform moles are genetically complete and result from fertilization by haploid sperm, their genome is haplotypic. Genomic studies frequently deal with haplotype, however (e.g., in the study of single-nucleotide polymorphism, genomic DNA sequence, and gene structures), and g-CHM with its particular haplotype is of value both as model and material for genomic studies [7].

The three loci D16S539, D7S820, and D13S317 examined here localize to human chromosomes 16, 7, and 13, respectively. There are about nine alleles at each of the loci (Table 3). When an empty egg is fertilized, whether by monospermy or dispermy, the genome of such a fertilized egg are partly a paternal copy. If spermiogenesis were regarded as an independent event, frequency of an allele would, in theory, be random among considerable cases of hydatidiform mole—would, in other words, be in accordance with Hardy–Weinberg equilibrium. Accordingly, frequency of an allele in hydatidiform moles should be consistent with that in the population. In the present study, however, we observed statistically significant differences in frequency distribution of a few alleles between the cases and the population (Table 1) [8].

Fertilization of an empty egg by two sperm will give rise to heterozygosity, but such fertilization is rare in hydatidiform moles [9]. Hence, g-CHMs are primarily of homozygotic genotype. Table 2 indicates that there were significant differences in heterozygosity at loci D16S539, D7S820, and D13S317 between the cases and the population. Although the reason is unclear, the frequency of allele 9 at D7S820 was lower in the cases than in the population, and the frequency of allele 10 was higher in the cases than in the population.

Prediction of a mole's invasiveness has long been of great interest; however, whether invasiveness is inborn or acquired is still to be answered. We suggest that invasiveness starts during the occurrence or early stage formation of hydatidiform mole or during its early stages.

Unfortunately, the objective of early prediction of invasiveness has not been satisfactorily achieved. With current progress in the research on molecular genetic markers, hydatidiform moles can be categorized as monospermic or dispermic, and the presence of any maternal genetic materials can be established. By such means, it has been shown that all invasive hydatidiform moles are among those whose genome are totally paternally derived (i.e., g-CHMs), and that invasiveness is not associated with the number of sperm involved [10].

In the present study, we first found that the frequencies of allele 9 at D16S539 and of allele 10 at D7S820 are significantly higher than those of other alleles in invasive hydatidiform moles. The two alleles present in both monospermic and dispermic fertilization cases, and statistically correlate to a mole's invasiveness. Nevertheless, whether there are inherent relationships between these two alleles and invasiveness remains unclear. Further study with more samples is needed. We plan further study of the alleles at more STR loci in hydatidiform mole.

Table 4
Allele distribution at loci D16S539, D7S820, and D13S317 in benign and invasive mole cases

| Allele | Genes at D16S539, no. (freq.) | | Genes at D7S820, no. (freq.) | | Genes at D13S317, no. (freq.) | |
|---|---|---|---|---|---|---|
| | Benign | Invasive | Benign | Invasive | Benign | Invasive |
| 8 | 0 (0) | 0 (0) | 7 (0.152) | 2 (0.091) | 15 (0.326) | 16 (0.727) |
| 9 | 9 (0.196)* | 10 (0.455)* | 0 (0) | 1 (0.045) | 2 (0.043) | 4 (0.182) |
| 10 | 5 (0.109) | 3 (0.136) | 9 (0.196)* | 10 (0.455)* | 6 (0.130) | 1 (0.045) |
| 11 | 14 (0.304) | 6 (0.273) | 18 (0.391) | 8 (0.364) | 10 (0.217) | 0 (0) |
| 12 | 9 (0.196) | 0 (0) | 12 (0.261) | 1 (0.045) | 11 (0.239) | 1 (0.045) |
| 13 | 7 (0.152) | 3 (0.136) | 0 (0) | 0 (0) | 2 (0.043) | 0 (0) |
| 14 | 2 (0.043) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Total | 46 | 22 | 46 22 | | 46 22 | |

*Abbreviation:* freq., frequency.
* $P < 0.05$.

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