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ORIGINAL INVESTIGATION

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A familial case of recurrent hydatidiform molar pregnancies with biparental genomic contribution

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Abstract Hydatidiform mole is a benign trophoblastic neoplasia characterized by an abnormal development of the embryo and proliferation of placental villi. Using microsatellite markers amplified by the polymerase chain reaction, we have performed a genetic study on eight independent molar tissues occurring in two sisters. Karyotype and genotype data demonstrate a diploid and biparental constitution in seven of the analyzed moles suggesting a common mechanism underlying the etiology of the various molar pregnancies in this family. The data reported here suggest that complete and partial hydatidiform moles are not always separate entities and that women with familial recurrent hydatidiform moles are homozygous for an autosomal recessive mutation.

Introduction

Hydatidiform mole (HM) is the most commonly occurring form of gestational trophoblastic neoplasia and is characterized by an atypical hyperplastic trophoblast and cavitory hydropic villi. HMs are divided into two types, viz., complete hydatidiform moles (CHMs) and partial hydatidiform moles (PHMs), according to their histology and to ultrasonography examinations. CHMs are characterized by hydropic villi, general trophoblastic proliferation, and the absence of fetal tissues and amniotic membranes (Copeland 1993; Bonilla-Musoles 1993). However, some cases of CHM that are evacuated at an early stage seem to contain embryonic tissues (Zaragova et al. 1997). CHMs are generally diploid and lack a maternal genetic contribution in at least 80% of the cases (Kovacs et al. 1991), a condition known as androgenetic CHM (Kajii and Ohama 1977). PHMs have a mixture of normally appearing villi and hydropic villi, focal trophoblastic hyperplasia, and identifiable embryonic tissues. They are generally triploid with one haploid maternal set of chromosomes and two haploid paternal sets of chromosomes, a condition known as diandric triploidy (Copeland 1993; Bonilla-Musoles 1993). HMs occur in 1 in every 1500 pregnancies in the USA, with partial moles constituting up to 50% of these cases (Lindor et al. 1992). This incidence is 5–15 times higher in Eastern Asia, Japan, Indonesia, and Iran (Lindor et al. 1992).

The exact mechanism responsible for HMs is unknown yet. Most of the recurrent HMs described in the literature are sporadic cases with very few being familial (Ambani et al. 1980; La Vecchia et al. 1982; Parazzini et al. 1984; Kircheisen and Ried 1991). All these familial cases are of the complete hydatidiform type, and none of them has been studied at the molecular level.

Recently, we reported a new familial case of recurrent HMs occurring in three members (two sisters and their cousin) of a Lebanese family (Seoud et al. 1995). Histological analysis of several molar tissues from the two sisters revealed general trophoblastic hyperplasia and the absence of fetal tissues. During the course of our study, we found that a previously published case of four recurrent molar pregnancies belonged to this family (Fig. 1, members IV.8 and IV.9; Vejerslev et al. 1991; Sunde et al. 1993). However, neither Vejerslev et al. (1991) nor Sunde et al. (1993) reported any family history of HM, and the couple was reported as originating from the Middle East. The four molar pregnancies occurring in this couple showed slight focal trophoblastic hyperplasia. Fetal tissues were present in two of them (Vejerslev et al. 1991; Sunde et al. 1993). Therefore, the four molar pregnancies were diagnosed as PHMs and were shown to be diploid with a bi-

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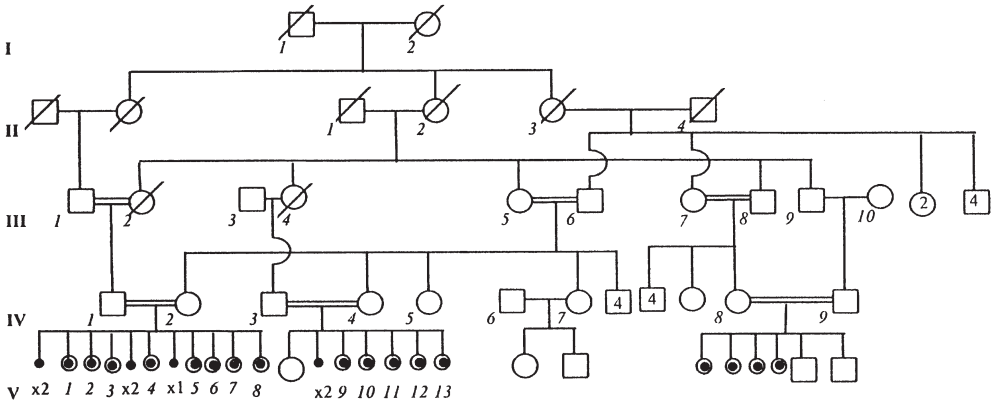

Fig. 1 Partial pedigree of the Lebanese family with recurrent hydatidiform moles. *Encircled black dots* Hydatidiform mole, *black dots* abortion. The sex of the live-born child of IV.5, previously reported as a male (Seoud et al. 1995), is female, whereas the dead child of IV.2 (Seoud et al. 1995) corresponds to a spontaneous abortion of a male fetus

parental contribution (Vejerslev et al. 1991; Sunde et al. 1993).

We describe herein further studies of this Lebanese family. We demonstrate the biparental constitution of seven independent CHMs occurring in the two sisters. Our data suggest that CHM and PHM are not always separate entities and that women with familial recurrent HMs are most probably homozygous for an autosomal recessive mutation.

Materials and methods

Subjects

A partial pedigree of the Lebanese family reported in this study is shown in Fig. 1. No history of HMs in the previous generations was noted by the family members. The clinical and histological examinations of the various molar pregnancies occurring in the two sisters (Fig. 1, members IV.2 and IV.4) were previously described by Seoud et al. (1995). Briefly, the first pregnancy of patient IV.4 ended in a normal newborn and was followed by two first-trimester abortions and five molar pregnancies. Her sister (patient IV.2) had five abortions alternating with eight molar pregnancies. Histological examination of several molar tissues occurring in the two sisters showed general trophoblastic proliferation and the absence of fetal tissues. These moles were therefore diagnosed as CHMs (Seoud et al. 1995). Chromosomal analysis of the two sisters and their spouses revealed normal karyotypes (Seoud et al. 1995). Patient IV.8 had two live-born males after four molar pregnancies (Sunde et al. 1993). The molar pregnancies showed slight focal trophoblastic proliferation. Fetal tissues were present in two of them. The four HMs were therefore diagnosed as PHMs (Vejerslev et al. 1991; Sunde et al. 1993)

Chromosomal analysis

Specimens of villi from three fresh mole tissues were dissected, and the chromosomes were analyzed from direct and/or short-term culture preparations following standard protocols and examined after routine G-banding methods.

DNA extraction and genotyping

Blood and cell samples were obtained with the informed consent of the individuals. Genomic DNA was extracted from parental blood (members IV.1, IV.2, IV.3, and IV.4) according to standard phenol-chloroform protocols. Fresh chorionic villi (from moles V.7, V.8, and V.13) and paraffin-embedded blocks containing chorionic villi cells (from moles V.1, V.2, V.3, V.4, and V.5) were cut into small pieces and then washed once with phosphate-buffered saline. After a brief centrifugation, the cells were lysed, and the DNA was extracted as described for whole blood. Indeed, this protocol gave better results than previously described protocols for extracting DNA from paraffin-embedded tissues. Individuals and molar products were genotyped by the polymerase chain reaction (PCR) by using the published microsatellite markers shown in Tables 1 and 2 (Dib et al. 1996). PCR amplification of

Table 1 Genotype analysis of DNA from parental blood and from three fresh molar tissues (*HM*) genotyped with several microsatellite markers[a]

Locus	IV.1	HM		IV.2	IV.3	HM	IV.4
		V.7	V.8			V.13	
D1S213	ab	bc[b]	bc[b]	ac	ab	aa	aa
D1S479	aa	–	ab[b]	bb	ab	bb	bb
D1S490	ab	ac[b]	ac[b]	bc	aa	ab[b]	bb
ESR (Ch6)	ab	aa	aa	aa	bc	bc	cc
D7S687	aa	–	aa	aa	bc	ca[b]	aa
D7S692	ab	–	ac[b]	cc	ac	cc	cc
D7S496	ab	–	bc[b]	cc	dd	dc[b]	cc
D7S501	ab	–	ab	bb	bb	bb	bb
D7S523	aa	–	ab[b]	bb	aa	ab[b]	bb
D7S525	ab	–	ac[b]	cc	ab	bc[b]	ac
D11S1321	ab	–	bb	bb	bc	cb	bb
D11S916	ab	–	bd[b]	dc	ab	bb	ab
D14S51	ab	aa	ba	aa	ab	ac[b]	cc
D14S250	ab	ba	ba	aa	ac	ab[b]	bc
D21S1257	ab	–	ad[b]	cd	ad	da	ab

[a] The microsatellite markers are from the Généthon map
[b] Hydatidiform mole genotypes with evidence of biparental contribution

Table 2 Genotype analysis of DNA from parental blood and from five paraffin-embedded molar tissues *(HM)* using four microsatellite markers

Locus	IV.1	HM					IV.2
		V.1	V.2	V.3	V.4	V.5	
D6S284	ac	ab[a]	cb	cb	ab[a]	cb	cb
D12S1701	bc	ca	–	ba[a]	ca	cc	ac
D16S3025	ac	ac	cb	ac	ab[a]	ac	bc
D18S1161	ab	ab	ab	ab	ab	ab	bc

[a] Genotype with evidence of biparental contribution

genomic DNA was carried out in a total volume of 25 µl as described previously (Saouda et al. 1998). PCR products were separated on polyacrylamide gels and revealed after transfer and hybridization with a ^{32}P-labeled poly (AC) oligonucleotide (Saouda et al. 1998).

Results

Karyotype analysis

Cytogenetic analysis was performed on three different molar tissues from the two sisters. Analysis of both direct and culture preparations of mole V.7 from patient IV.2 revealed 47,XX,+22 in 50 screened cells (10 on direct chromosome preparations and 40 on culture preparations). Mole V.8 from the same patient (IV.2) was only analyzed on direct preparations and revealed a 46,XY karyotype in 13 analyzed cells. In mole V.13 (from patient IV.4), the quality of chromosomes obtained from direct preparations was too poor to allow a detailed morphological analysis, beyond chromosome counting. However, analysis of the culture preparations of the same mole tissue (V.13) revealed a mosaic karyotype, 46,XX [91]/46,XX,t(9;17)(q34;q21) [6]. The abnormal karyotype with translocation was found in two independent culture tubes at the same frequency (6.1%), reflecting therefore, a true sample mosaicism.

Genotyping

To identify the parental origin of the recurrent HMs in this family (Fig. 1), DNA from parental blood and from three different fresh molar tissues, viz., two from patient IV.2 (moles V.7 and V.8) and one from patient IV.4 (mole V.13), were analyzed at 15 loci from six different chromosomes (Table 1). In mole V.7, a biparental contribution was demonstrated at two informative loci (D1S213 and D1S490) by the presence of one allele from each parental genome. Similarly, a biparental contribution was demonstrated at nine loci in mole V.8 (D1S213, D1S479, D1S490, D7S692, D7S496, D7S523, D7S525, D11S916, and D21S1257) and at seven loci in mole V.13 (D1S490, D7S687, D7S496, D7S523, D7S525, D14S51, and D14S250). These data demonstrate a biparental contribution to the three different moles from the two sisters.

To test whether the same mechanism was responsible for previous molar pregnancies in this family, we extended our analysis to five different paraffin-embedded molar tissues (V.1, V.2, V.3, V.4, and V.5) prepared for histopathological studies. The DNAs extracted from these paraffin-embedded molar tissues were highly degraded and could not be amplified by PCR with the microsatellite markers listed in Table 1. Indeed, most of the PCR-amplified products at these markers ranged from 150–244 bp. However, we were able to amplify DNA segments from the five paraffin-embedded moles by using other microsatellite markers that amplified PCR products between 80–110 bp. Our results also showed a biparental contribution to the five molar tissues (Table 2). Furthermore, these data demonstrated that the eight analyzed moles originated from different independent conceptions and ruled out the possibility of dispermic fertilization.

Discussion

The current study is the first attempt to understand the molecular mechanism responsible for familial CHMs. On the basis of our data from eight independent molar pregnancies derived from two sisters, we demonstrate a biparental contribution to seven of the eight analyzed mole tissues. This result is in agreement with previously reported data from four recurrent moles occurring in a third member of this family (member IV.8; Vejerslev et al. 1991; Sunde et al. 1993). However, in these latter studies, the molar tissues showed slight focal trophoblastic hyperplasia and, consequently, were diagnosed as PHMs. In view of these data, we re-examined several sections of paraffin-embedded molar tissues from our two patients. This analysis confirmed the previous diagnosis and demonstrated that the various HMs from the two sisters had general trophoblastic proliferation and lacked embryonic tissues, hence, fulfilling the criteria of CHMs.

The finding that all HMs in the three family members have a biparental contribution argues in favor of a common genetic factor underlying the etiology of the HMs. However, the differences in the phenotype (partial versus complete) may be explained by a variable expression of the same defect. It should be noted that the occurrence of CHMs and PHMs has previously been observed in the same patient after fertilization by her husband and by a heterologous artificial insemination (Mangili et al. 1993) and in several cases of sporadic recurrent molar pregnancies (Berkowitz et al. 1998). In addition, in our family, the two affected sisters had recurrent abortions; abortions are associated with several cases of familial and sporadic recurrent HMs (Ambani et al. 1980; Parazzini et al. 1985; Mangili et al. 1993) and can also be explained by the variable expression of the same defect.

Genotype-phenotype comparisons between androgenetic diploid CHM and diandric triploid PHM suggest that maternally imprinted genes that are expressed exclusively from the paternal genome are responsible for the trophoblastic proliferation observed in both CHMs and PHMs, whereas paternally imprinted genes that are expressed exclusively from the maternal genome are responsible for the absence of an embryo in sporadic androgenetic CHM (80% of complete moles; Fisher and Newlands 1998). In the remaining sporadic cases of CHM (20% of complete moles) in which a biparental contribution is observed, one possibility is that a paternal disomy of one chromosome or of a paternally imprinted region may prevent normal embryonic development and lead to a phenotype similar to that observed in androgenetic HMs. Such mechanisms have been described in sporadic cases of many inherited diseases associated with imprinted regions, such as the Prader Willi/Angelman syndromes and Beckwith-Wiedemann syndrome (Wilkins-Haug 1993).

However, all described mechanisms leading to uniparental disomy occur de novo and therefore cannot be responsible for familial recurrent moles. Moreover, the inheritance of the defect in our family does not fit with the transmission of a mutation within a paternally or a maternally imprinted gene, since the defect has been transmitted through both maternal and paternal gametes, and no history of HMs or abnormal pregnancies in the previous generations was noted by the family members (Fig. 1).

Familial HM pregnancies are extremely rare. So far, only seven familial cases have been reported (Ambani et al. 1980; La Vecchia et al. 1982; Parazzini et al. 1984; Kircheisen and Ried 1991; Seoud et al. 1995). In all these cases, HMs have never occurred in the mothers or in their daughters. In two of these cases, women with recurrent HMs have sisters who have had normal pregnancies (Parazzini et al. 1984; Seoud et al. 1995). These data are not in favor of a mitochondrial mutation transmitted through the maternal cytoplasm. In addition, all reported familial cases of HMs have included sisters or related women who had more HMs than viable children. Taking into consideration these elements, the pedigree structure, and the high degree of consanguinity of the Lebanese family, we suggest that an autosomal recessive mutation may be responsible for familial HMs. The underlying genetic defect is not in the molar tissue, but in the pregnant women. These women most probably have an autosomal recessive genetic defect. This hypothesis is supported by the finding that one of our patients (member IV.2) has had at least eight molar pregnancies and several abortions with no viable children and by the finding that women with recurrent HMs usually fail to have normal pregnancies.

We hereby suggest that women with recurrent familial HMs are homozygous for an autosomal recessive mutation. The defective gene may be required in the fertilized/unfertilized ovum or in the maternal reproductive tract. It should be noted that the initial development of the mammalian zygote is under the control of maternally inherited proteins and mRNA produced and stored in the oocyte during oogenesis. Moreover, the progression of the fertilized ovum through cleavage, blastocyst formation, and implantation is also dependent on the successful interaction between the pre-implantation embryo and the maternal reproductive tract (Schultz and Heyner 1992). Therefore, a defective maternal gene at any of these levels may deregulate the imprinting process in diploid zygotes and lead to abnormal embryonic development and to a phenotype similar to that observed in androgenetic diploid and diandric triploid conceptuses.

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