European Journal of Human Genetics (2000) 8, 641–644

www.nature.com/ejhg

SHORT REPORT

Mole maker phenotype: possible narrowing of the candidate region

A Sensi[1], F Gualandi[1], MC Pittalis[2], O Calabrese[1], F Falciano[1], I Maestri[3], L Bovicelli[2] and E Calzolari[1]

[1]*Dipartimento Medicina Sperimentale e Diagnostica, Sezione Genetica Medica, Università di Ferrara, Italy;* [2]*Fisiopatologia Prenatale-Università di Bologna-Policlinico, S Orsola, Italy;* [3]*Sezione Anatomia Patologica, Università di Ferrara, Arcispedale S Anna, Italy*

Recent data has suggested that familial recurrent hydatidiform mole is a rare autosomal recessive trait in women experiencing this gestational disease (MIM 231090). Here we provide molecular data on an additional family confirming that recurrent familial hydatidiform moles are diploid, biparental and arise from independent conceptions. A narrowing of the gene interval on chromosome 19q13.3–13.4 is suggested by haplotype analysis in two sisters. *European Journal of Human Genetics* (2000) **8**, 641–644.

Keywords: hydatidiform mole; familial; recurrent; biparental; genetics

Introduction

Hydatidiform mole (HD) can be defined simply as a human conceptus displaying macroscopically visible vesicular villi and trophoblastic hyperplasia.[1] Recurrence of molecular pregnancies is rare (1–5%),[2–5] but after two episodes the risk rises to 11–30%.[3–5] Familial HM (MIM 231090) is exceedingly rare, with only seven families having been reported so far.[6–11] Consanguinity was often noted between the partners, suggesting an autosomal recessive etiology in the conceptus genotype.[10] Recently Helwani *et al*[12] reported a family in which consanguinity was also noted between the parents of the women. This finding together with biparentality of the moles in this family prompted that group to consider an autosomal recessive genotype in the woman. To map the hydatidiform mole locus, Moglabey *et al*[1] performed a genome-wide scan, using a combination of linkage search and homozygosity analysis, on a very complex Lebanese family, with extensive consanguinity, reported by Helwani *et al*[12] and on a German family.[9] A maternal gene was mapped to 19q13.3–q13.4 in a 15.2-cM interval flanked by D19S924 and D19S890. Here we report the molecular results of a study on a second family with recurrent hydatidiform molar pregnancies.

Correspondence: Dott. Alberto Sensi, Dipartimento Medicina Sperimentale e Diagnostica, Sezione Genetica Medica, via L Borsari 46, 44100 Ferrara, Italy. Tel: +39 0532 291380; Fax: +39 0532 291380; E-mail: sna@ifeuniv.unife.it
Received 16 December 1999; revised 15 March 2000; accepted 4 April 2000

Materials and methods

Subjects and materials

A couple from a mountain region of Southern Italy sought genetic counselling for familial recurrence of hydatidiform moles (Figure 1). The proband and her partner are first cousins, while the proband's sister and partner were second cousins. Consanguinity between the proband's parents is not reported, although both come from the same village. The proband experienced eight reproductive failures (from the age of 28 to 37), including six complete moles (with histopathologic documentation) and two miscarriages in the first gestational trimester. The seventh pregnancy (HM V:11) was attempted by ovum donation, but STS analysis, and HLA molecular typing of the molar conceptus established that it was originated by the fertilisation of a maternal ovum (HLA data are not shown). This mole was persistent and treated with methotrexate. The proband's sister reported the recurrence of three molar pregnancies (from the age of 25 to 32).

Ploidy assessment of molar tissues

One molar sample (HM V:11) was studied by standard karyotype and flow cytometry, while a previous molar

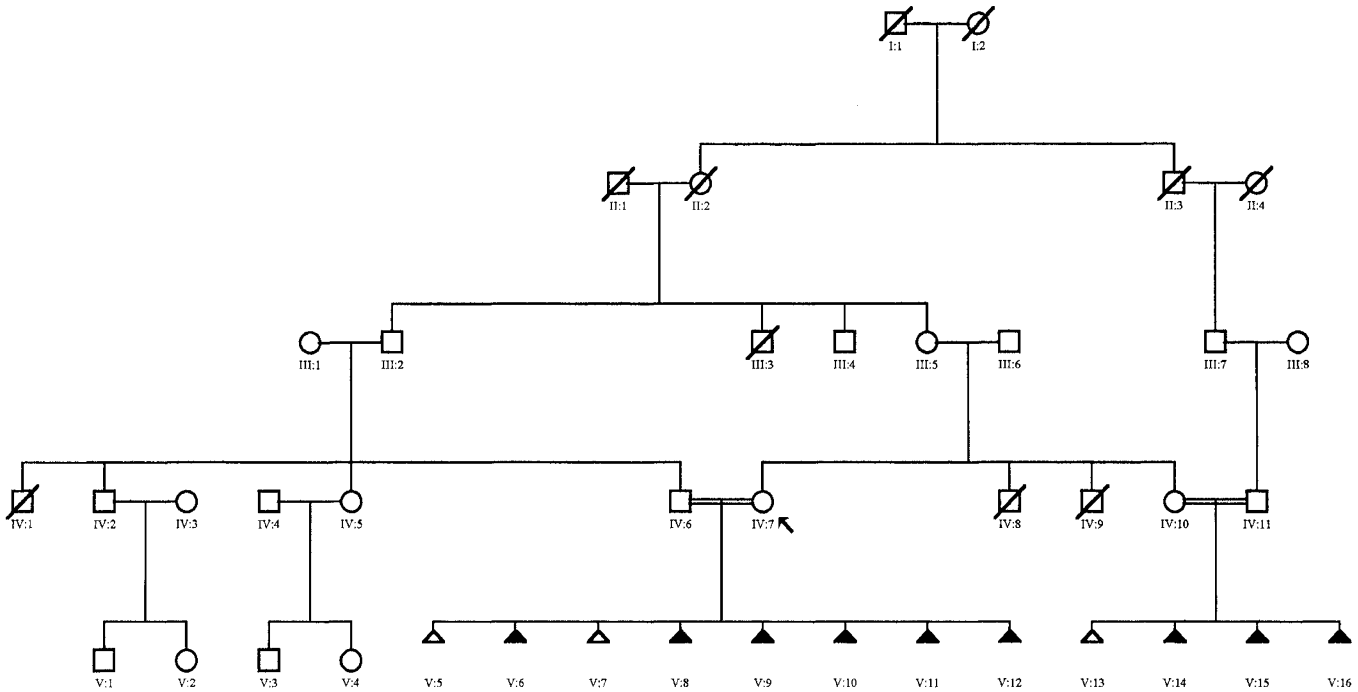

Figure 1 Pedigree of the family with recurrent molar pregnancies. Filled black triangles: molar pregnancy; unfilled triangles: spontaneous abortion.

conceptus (HM V:10), was recovered as a paraffin embedded sample and studied only by flow cytometry.

Molecular analysis

Parental origin of two molar conceptuses was assessed by STS analysis, PCR amplification, PAGE and silver staining detection. A total of 17 microsatellite markers (D7S481, D7S507, D7S636, D7S523, D7S500, D7S550, D11S911, D14S283, D14S77, D14S267, D15S97, D15S211, D15S126, D15S11, GABRB3, D15S113, D15S210), obtained from the Genome Database, were tested to assess parental origin of HM V:11. Eight of the informative markers were used to analyse HM V:10. The chromosome 19 haplotypes, corresponding to the region where the locus responsible for familial HM was mapped, were also assessed in the two sisters by the same methods (Figure 2). HLA data are not shown, but biparentality of HM V:11 was confirmed.

Results

Karyotype of trophoblastic tissue from HM V:11 showed diploidy. The cytofluorimetric analysis of both the molar tissues available confirmed diploidy for HM V:11 and assessed diploidy for HM V:10.

The results of the informative STS polymorphism analysis in two molar conceptuses of the proband are shown in Table 1. Both samples showed biparentality for all the tested chromosomes. It was established that the two molar tissues examined originated from independent conceptions.

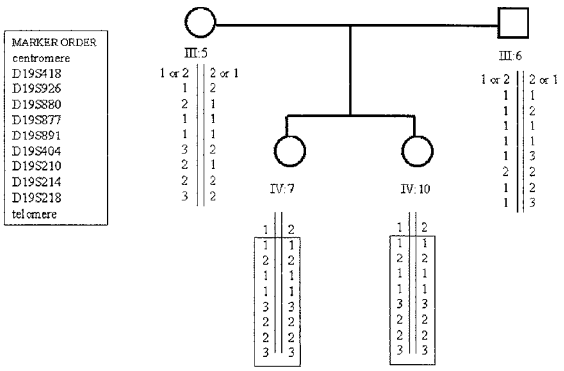

Figure 2 19q13.3–13.4 markers segregation in the family. Closed boxes show the homozygous region shared between the proband and sister.

The two sisters with recurrent molar pregnancies shared both haplotypes in the investigated region. The inherited paternal and maternal haplotypes were identical, except for the locus D19S418 where both sisters were heterozygous (Figure 2).

Discussion

The etiology of sporadic and familial biparental diploid moles is completely unknown. A careful revision of the families reported[6–12] suggests an autosomal recessive genotype of women experiencing recurrent familial molar pregnancies, as proposed by Helwani *et al*.[12] Only one family has

Table 1 STS analysis in two molar conceptuses from the proband

Polymorphism	*Proband IV:7*	*Partner IV:6*	*HM V:10*	*HM V:11*
D7S507	1,2	1,1	/	1,2
D7S523	2,2	1,2	/	1,2
D7S500	1,2	1,3	2,3	1,2
D7S550	2,2	1,2	1,2	1,2
D11S911	1,2	3,4	2,3	2,3
D14S283	1,3	2,3	/	2,3
D14S77	1,4	2,3	2,4	3,4
D14S267	1,2	1,3	1,2	1,1
D15S97	2,3	1,3	/	1,2
D15S211	1,3	2,4	/	3,4
D15S126	2,2	1,1	1,2	1,2
D15S11	2,2	1,2	2,2	1,2
GABRB3	1,3	1,2	1,2	1,1
D15S210	1,2	2,2	/	1,2

been studied by molecular methods so far.[1] In this family the recurrent molar pregnancies demonstrated a biparental diploid chromosomal constitution.

The data reported here confirm that in an independent family of different ethnic origin, recurrent familial molar pregnancies are biparental, diploid and derive from independent conceptions. The hypothesis of a *mole-maker phenotype*, due to homozygous genotype for autosomal recessive mutations in the women experiencing recurrent HMs, is supported in this family by the finding in two sisters of high recurrence of HM in the absence of normal pregnancies (Figure 1).

Sharing of both chromosome 19 haplotypes by the two sisters is in accordance with the localisation of the gene indicated by Moglabey *et al*.[1] Interestingly, the two sisters were homozygous for the whole haplotype, excepted for the D19S418 locus. This extended homozygosity supports the hypothesis of a common ancestor for the parents, while the heterozygosity at the most centromeric marker suggests a narrowing of 2.8 cM of the candidate region proposed by Moglabey.[1]

The 19q13.3–13.4 region is very densely endowed with genes and has been suggested to harbour imprinted genes.[1] Considering the etiology of sporadic partial and complete moles it should be possible that the imprinting phenomenon is also involved in familial HM. However, in sporadic holandric or tryploid HM probably more than one imprinted chromosomal region plays a pathogenetic role, whilst in familial HM it is likely that a single gene or chromosomal region is involved. If an imprinting centre or an imprinted gene were involved, a homozygous mutation in the maternal genotype would not be required and a heterozygous woman should be able to produce with equal probability normal and molar conceptuses depending on the inherited haplotype.

We are inclined to believe that an imprintor gene[13] is involved in the etiology of familial HM: a homozygous mutation in such a gene could disrupt the maternal imprinting in one or several chromosomal regions, mimicking a paternal double contribution. Although not knowing the exact timing of human imprinting, we do know, however, that somatic imprinting has to be erased in the germline and substituted with a gamete specific pattern. Some evidences from mice studies[14] indicate that specific methylation of H19 is already evident in meiotic prophase of the female germline: it is therefore possible that recessive mutations in imprinting regulator or effector genes of a woman cause an imprinting disruption in the ovum through an epigenetic modification.

We should also consider an alternative hypothesis not involving genomic imprinting: as an example, the molecular defect could be located in the complex process of foetoplacentation, regulated by complementary pairs of adhesion and receptorial molecules in maternal and trophoblast cells.[15,16] In this regard the *CD33L* gene, mapped in the 19q13 region by FISH, could prove to be an interesting candidate. It is specifically expressed in the placenta and is likely to be associated with cell–cell interaction.[17]

The identification of the gene could be of great general theoretical interest, but also of practical value for the women suffering from this rare condition. In fact, if an epigenetic effect on the conceptus genome were confirmed, an ovum donation (where allowed) could be effective in solving the problem. Otherwise, if the defect lies in some other molecule with a pivotal role in the regulation of decidual–trophoblast relations, then recurrent hydatidiform moles should equally arise, independently from the conceptus genome.

Acknowledgements

The authors wish to thank Fernanda Mora of Dipartimento di medicina sperimentale e diagnostica, Sezione Anatomia Patologica, Università di Ferrara, Arcispedale S Anna, Italy, for the protocol of DNA extraction from paraffin-embedded blocks

References

1 Moglabey YB, Kircheisen R, Seoud M, El Mogharbel N, Van den Veyver I, Slim R: Genetic mapping of a maternal locus responsible for familial hydatidiform moles. *Hum Mol Genet* 1999; **8**: 667–671.
2 Federschneider JM, Goldstein DP, Berkowitz RS, Marean AR, Bernstein MR: Natural history of recurrent molar pregnancy. *Obstet Gynecol* 1980; **55**: 457–459.
3 Sand PK, Lurain JR, Brewer JI: Repeat gestational trophoblastic disease. *Obstet Gynecol* 1984; **63**: 140–144.
4 Berkowitz RS, Bernstein MR, Laborde O, Goldstein DP: Subsequent pregnancy experience in patients with gestational trophoblastic disease. *J Reprod Med* 1994; **39**: 228–232.
5 Yapar EG, Ayhan A, Ergeneli MH: Pregnancy outcome after hydatidiform mole, initial and recurrent. *J Reprod Med* 1994; **39**: 297–299.
6 Ambani LM, Vaidya RA, Rao CS, Daftary SD, Motashaw ND: Familial occurrence of trophoblastic disease – report of recurrent molar pregnancies in sisters in three families. *Clin Genet* 1980; **18**: 27–29.
7 La Vecchia C, Franceschi S, Fasoli M, Mangioni C: Gestational trophoblastic neoplasms in homozygous twins. *Obstet Gynecol* 1982; **60**: 250–252.

8 Parazzini F, La Vecchia C, Franceschi S, Mangili G: Familial trophoblastic disease: case report. *Am J Obstet Gynecol* 1984; **149**: 382–383.
9 Kircheisen R, Ried T: Hydatidiform moles. *Hum Reprod* 1991; **9**: 1783.
10 Seoud M, Khalil A, Frangieh A, Zahed L, Azar G, Nuwayri-Salti N: Recurrent molar pregnancies in a family with extensive intermarriage: Report of a family and review of the literature. *Obstet Gynecol* 1995; **86**: 692–695.
11 Sunde L, Vejerslev LO, Jensen MP, Pedersen S, Hertz JM, Bolund L: Genetic analysis of repeated, biparental, diploid, hydatidiform moles. *Cancer Genet Cytogenet* 1993; **66**: 16–22.
12 Helwani MN, Seoud M, Zahed L, Zaatari G, Khalil A, Slim R: A familial case of recurrent hydatidiform molar pregnancies with biparental genomic contribution. *Hum Genet* 1999; **105**: 112–115.
13 Forejt J, Gregorova S: Genetic analysis of genomic imprinting: an Imprintor-1 gene controls inactivation of the paternal copy of the mouse Tme locus. *Cell* 1992; **70**: 443–450.
14 Davis TL, Trasler JM, Moss SB, Yang GJ, Bartolomei MS: Acquisition of the H19 methylation imprint occurs differentially on the parental alleles during spermatogenesis. *Genomics* 1999; **58**: 18–28.
15 Haynes MK, Wapner RL, Jackson LG, Smith JB: Phenotypic analysis of adhesion molecules in first-trimester decidual tissue from chorion villus samples. *Am J Reprod Immunol* 1997; **38**: 423–430.
16 MacCalman CD, Getsios S, Chen GT: Type 2 cadherins in the human endometrium and placenta: their putative roles in human implantation and placentation. *Am J Reprod Immunol* 1998; **39**: 96–107.
17 Takei Y, Sasaki S, Fujiwara T, Takahashi E, Muto T, Nakamura Y: Molecular cloning of a novel gene similar to myeloid antigen CD33 and its specific expression in placenta. *Cytogenet Cell Genet* 1997; **78**: 295–300.