Analysis of the chromosomal region 19q13.4 in two Chinese families with recurrent hydatidiform mole

J.Zhao¹, J.Moss³, N.J.Sebire³, Q.C.Cui², M.J.Seckl⁴, Y.Xiang¹ and R.A.Fisher⁴,⁵

¹Department of Obstetrics and Gynecology and ²Department of Pathology, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Science, Beijing 100730, China, ³Department of Histopathology, Charing Cross Hospital, London W6 8RF and ⁴Department of Oncology, Division of Surgery, Oncology, Reproductive Biology and Anaesthetics, Faculty of Medicine, Imperial College London, Charing Cross Campus, London W6 8RF, UK

To whom correspondence should be addressed at Department of Oncology, Division of Surgery, Oncology, Reproductive Biology and Anaesthetics, Faculty of Medicine, Imperial College London, Charing Cross Campus, Fulham Palace Road, London W6 8RF, UK.
E-mail: r.fisher@imperial.ac.uk

BACKGROUND: Familial recurrent hydatidiform mole is an extremely rare autosomal recessive condition in which affected individuals have a predisposition to molar pregnancies that are diploid but biparental, rather than androgenetic, in origin. A gene for this condition has been previously mapped to a 1.1 Mb region of chromosome 19q13.4. However, investigation of further families is needed to refine the location of the specific gene(s) involved.

METHODS: We have recently identified two novel Chinese families in which four affected women had recurrent pregnancy loss including 14 complete hydatidiform moles (CHM). Fluorescent microsatellite genotyping was used to determine the origin of CHM in both families. Using a panel of polymorphic microsatellite markers, genotyping and haplotype analysis of the 19q13.4 chromosomal region was performed in both families.

RESULTS: Genotyping of CHM from affected individuals confirmed their biparental origin and diagnosis of familial recurrent hydatidiform mole in both families. However, no significant homozygosity for the 19q13.4 candidate region was found in affected members of either family. CONCLUSION: Genotyping and haplotype analysis has shown that a mutation in 19q13.4 is unlikely to be responsible for recurrent CHM in the two Chinese families investigated and provides further evidence to support the hypothesis that, although extremely rare, this condition shows genetic heterogeneity.

Key words: biparental complete hydatidiform mole/familial hydatidiform mole/genomic imprinting/recurrent hydatidiform mole

Introduction

Familial recurrent hydatidiform mole (HM) is an extremely rare autosomal recessive condition in which affected individuals have an inherited predisposition to molar pregnancies (Seoud et al., 1995; Fisher et al., 2004a). Molar pregnancies, which may be complete (CHM) or partial (PHM) hydatidiform moles, are characterized by oedematous swelling of the placental villi, trophoblastic hyperplasia and abnormal fetal development (Szulman and Surti, 1978). In sporadic molar pregnancies this characteristic pathology reflects the presence of two paternal contributions to the molar genome (Kajii and Ohama, 1977; Wake et al., 1978; Jacobs et al., 1982; Lawler et al., 1982) and the consequent disruption to the expression of imprinted genes (Fisher et al., 2002; Thaker et al., 2004). In sporadic CHM the genome is diploid but androgenetic having only paternal chromosomes (Kajii and Ohama, 1977; Wake et al., 1978). In contrast, CHM in families with recurrent HM are diploid, but biparental, in origin having both a maternal and paternal contribution to the genome (Helwani et al., 1999; Sensi et al., 2000; Fisher et al., 2002; Hodges et al., 2003). In these conceptues the characteristic pathology does not arise from the presence of two paternal contributions to the molar genome. Instead it is thought to be a consequence of an inherited failure to set a maternal imprint in the ovum with subsequent overexpression of genes that are normally transcribed from the paternal allele (Judson et al., 2002; El-Maarri et al., 2003). The gene involved in this condition is thus likely to be an important regulator of genomic imprinting.

Previous studies of affected families have identified a 1.1 Mb candidate region on chromosome 19q13.4 in which a gene for this condition is located (Moglabey et al., 1999; Sensi et al., 2000; Hodges et al., 2003). Investigation of further families has confirmed these reports (Panichkul et al., 2005; R.A.Fisher et al., unpublished data) but failed to refine the very gene-rich region on 19q13.4 (Grimwood et al., 2004) in which the gene for this condition is located.

We have recently identified two novel families of Chinese origin in which two sisters in each family had a history of reproductive loss, including several HM. In this report we describe genetic analysis of the 19q13.4 locus in these families.
and provide evidence to support the hypothesis that this unusual condition may show genetic heterogeneity.

Materials and methods

Families

Ch01 and Ch02, both reportedly non-consanguineous, are shown in Figure 1. Family Ch01 comes from the An Hui province of China. The proband, II:3, 22 years old at the time of her first pregnancy, had a history of a CHM in 1993 followed 5 years later by a second pregnancy resulting in the delivery of an apparently normal female. However, the infant survived for only 3 days. Cause of death is uncertain as no autopsy was performed. Her third and fourth pregnancies, 15 months and 3 years later, were diagnosed as CHM on ultrasound and terminated at 10 and 14 weeks respectively. Prophylactic chemotherapy was given after the first of these two CHM. Following the last CHM the patient developed lung metastases and, after surgery, a diagnosis of invasive mole was made for which the patient received further chemotherapy.

The proband has only a single sib, an elder sister, II:1, who was also found to have a history of molar pregnancies. The first of her five pregnancies in 1994 at age 25 was confirmed on pathological diagnosis to be a CHM. Two years later the patient developed lung metastases. A clinical diagnosis of choriocarcinoma was made for which the patient subsequently underwent chemotherapy. A second, ectopic, pregnancy 4 years later was followed by two miscarriages, for which a pathological diagnosis was not available. A fifth pregnancy in 2003, diagnosed as a CHM, was followed by prophylactic chemotherapy.

Family Ch02 comes from the Zhe Jiang province of China. The proband, II:9, 21 years old at the time of her first pregnancy, had a history of 12 pregnancy losses with no normal pregnancies during a 16 year period from 1986. Her first four pregnancies together with the most recent were diagnosed as CHM on the basis of histopathology. Following the first two CHM the patient was given a single dose of prophylactic chemotherapy. Following both the fourth and fifth recognized CHM the patient developed lung metastasis and was treated with chemotherapy for invasive mole. The proband has five sibs, an elder brother II:2, three elder, and one younger sister, II:4, II:5, II:7 and II:11 respectively. Her sister II:5, 22 years old at the time of her first pregnancy, was also found to have had a history of four CHM with no normal pregnancies between 1983 and 1991. Her second and third molar pregnancies were treated with prophylactic chemotherapy while the fourth was treated following the development of lung metastasis 15 months after termination. The proband’s three other female sibs had no history of HM and have all achieved normal full-term pregnancies.

Genetic diagnosis of molar pregnancies

Blood samples were available from the proband in family Ch01 although no tissue was available from any of her molar pregnancies.

Figure 1. Pedigree structure of families Ch01 and Ch02. Reproductive outcome in affected members (filled circles) is represented by CHM (complete hydatidiform mole), EP (ectopic pregnancy), SA (spontaneous abortion) or END (early neonatal death).
However, formalin-fixed paraffin-embedded blocks of molar tissue were available from the most recent molar pregnancy of her affected sister together with blood samples from the sister and her partner. In family Ch02, blood samples and formalin-fixed paraffin-embedded blocks of molar tissue were available from the proband, her partner and her most recent molar pregnancy. DNA was prepared from parental blood using standard techniques. In order to prepare DNA from molar tissue, areas of molar vesicles were first microdissected from haematoxylin and eosin-stained sections of tissue using a PixCell II Laser Capture Microdissection system (Arcturus Engineering, Mountain View, CA, USA). Maternal tissue, present in the same sections, was also captured in the sample tested from family Ch01. DNA was subsequently prepared by digestsing captured tissue in 20 μl of digestion buffer [50 mmol/l Tris (pH 8.5), 1 mmol/l EDTA, 0.5 % Tween 20], containing 200 μg/ml of protease K, overnight at 37°C followed by heat inactivation of the protease K at 95°C for 8 min. PCR amplification and fluorescent microsatellite genotyping was carried out as previously described (Fisher et al., 2000) using DNA from the patient, her husband, captured maternal and molar tissue. Parental contributions to the molar tissue were determined using ABI 310 PRISM GeneScan software (Applied Biosystems, Warrington, UK).

Genotyping of the 19q13.4 locus
Additional blood samples for genotyping were available from the proband in family Ch01 and the parents and two unaffected sisters, II:4 and II:11, of the proband in family Ch02. DNA was prepared from these samples using standard techniques. PCR amplification and fluorescent microsatellite genotyping of the 19q13.4 chromosomal region was carried out in these individuals using a series of markers identified from the Genome Database and 10 novel primer pairs designed to span additional polymorphic repeats within the 19q13.4 locus (Hodges et al., 2003).

Results
Genetic diagnosis of molar pregnancies
Pathological review of the HM from both families confirmed the diagnosis of CHM (Figure 2). Informative microsatellite markers together with allele sizes for the patients, their partners and the molar tissue for II:1 (family Ch01) and II:9 (family Ch02) are shown in Table I. In the first case, genotyping of the maternal tissue showed it to be identical to that of the patient (data not shown), confirming the identity and integrity of the section used. For three informative microsatellite markers, the CHM tissue in this case was clearly disomic with one maternal and one paternal contribution to the genome consistent with a diploid, biparental origin. No identifiable areas of maternal tissue were present in the case from family Ch02. DNA prepared from molar tissue in this case was of poor quality such that only small microsatellite markers could be successfully amplified. Although there was a degree of allelic imbalance for some markers, there was clearly a maternal contribution to the genome in addition to a single paternal allele, consistent with a biparental genotype, for six informative markers tested. A single marker, D6S273 appeared to be trisomic in the molar tissue having two maternal alleles and a single paternal allele. Following analysis of two further markers on chromosome 6 (Table I), a microsatellite marker in TNFα, mapping close to D6S273, was also found to be trisomic while D6S260, a more telomeric marker, showed a disomic genotype with an equal contribution to the molar DNA from each parent.

![Figure 2](https://academic.oup.com/humrep/article-abstract/21/2/536/614184)

**Figure 2.** Photomicrograph of products of conception from the most recent complete hydatidiform mole (CHM) of the proband in family Ch02, demonstrating chorionic villi with moderate hydrops, abnormal budding architecture and trophoblastic hyperplasia. The villi also lacked expression of p57KIP2 not shown. The features are those of a CHM (original magnification ×250).

### Table I. Informative microsatellite markers in patients, their partners and hydatidiform mole (CHM) tissue in families Ch01 and Ch02

<table>
<thead>
<tr>
<th>Polymorphic microsatellite</th>
<th>Patient</th>
<th>CHM</th>
<th>Partner</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family Ch01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D8S1110</td>
<td>(II:1)</td>
<td>(III:5)</td>
<td>(II:2)</td>
</tr>
<tr>
<td>D20S481</td>
<td>274–282</td>
<td>278–282</td>
<td>270–278</td>
</tr>
<tr>
<td>D20S481</td>
<td>239–243</td>
<td>231–243</td>
<td>231–251</td>
</tr>
<tr>
<td>D21S1270</td>
<td>176–188</td>
<td>172–188</td>
<td>172–192</td>
</tr>
<tr>
<td>Family Ch02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D3S1262</td>
<td>107</td>
<td>107–114</td>
<td>114–118</td>
</tr>
<tr>
<td>D6S273</td>
<td>131–133</td>
<td>129–131–133</td>
<td>125–129</td>
</tr>
<tr>
<td>D7S669</td>
<td>122–132</td>
<td>130–132</td>
<td>128–130</td>
</tr>
<tr>
<td>D9S43</td>
<td>71–84</td>
<td>71–80</td>
<td>80–84</td>
</tr>
<tr>
<td>D10S189</td>
<td>184</td>
<td>178–184</td>
<td>178</td>
</tr>
<tr>
<td>D11S2345</td>
<td>128</td>
<td>124–128</td>
<td>122–124</td>
</tr>
<tr>
<td>D6S260b</td>
<td>166</td>
<td>164–166</td>
<td>164–172</td>
</tr>
</tbody>
</table>

*Allele sizes in base pairs.

Additional polymorphic markers for chromosome 6.

Genotyping of the 19q13.4 locus
The genotypes of the individuals tested in Ch01 and Ch02 are shown in Figure 3a and b respectively. Using the available data, haplotypes of the family members were constructed to minimize recombination frequency. DNA was not available from the parents of the affected sisters in family Ch01. However, the genotypes of the two sisters were consistent with inheritance of the same chromosomal haplotype from one parent together with a different chromosomal haplotype from the other parent. Only markers D19S924 and AAAT11138, neither within the candidate region, were homozygous in both sisters.

In family Ch02 the proband and her unaffected sib II:4 shared their maternally inherited haplotype while inheriting a...
different haplotype from their father, whereas her unaffected sister, II:11, shared neither haplotype. Two markers in the candidate region, D19S880 and GT17173, were homozygous in the proband. GT17173 was an isolated homozygous marker, flanked by markers for which the affected individual was heterozygous. D19S880 was contiguous with two other homozygous markers, D19S418 and D19S926, centromeric to the candidate region.

Discussion

In the majority of HM the underlying genetic defect is an excess of paternal genomes. This results in overexpression of paternally transcribed genes and, in the case of CHM, loss of expression for genes that are normally transcribed from the maternally derived genome (Fisher and Hodges, 2003). Most PHM are triploid conceptuses arising by dispermy (Jacobs et al., 1982; Sensi et al., 2000; Hodges et al., 2003).

Figure 3. Genotypes and haplotypes for 26 markers within the 1913.4 locus for (A) Ch01 and (B) Ch02. Markers D19S418 and AAAT11138 flank the centromeric and telomeric ends of the minimum region of homozygosity previously identified in familial recurrent hydatidiform mole (Sensi et al., 2000; Hodges et al., 2003). Marker order is based on assembly of sequenced human chromosome 19 genomic clones as previously described (Hodges et al., 2003).
Lawler et al., 1982). CHM are usually androgenetic diploids arising by dispermic fertilization of an anucleate oocyte (Ohama et al., 1981) or fertilization of an anucleate oocyte by a single sperm that undergoes reduplication to restore the diploid chromosome complement (Kajii and Ohama, 1977; Wake et al., 1978).

However, occasional conceptions that are pathologically CHM have been found to have an apparently normal, biparental genotype. These unusual biparental CHM (BiCHM) are often associated with families having an inherited predisposition to molar pregnancies (Helwani et al., 1999; Sensi et al., 2000; Fisher et al., 2002; Hodges et al., 2003; Panichkul et al., 2005) and are thought to result from an autosomal recessive condition in which affected women fail to set the correct genomic imprint in the ovum (Judson et al., 2002; El-Maarri et al., 2003). These BiCHM are consequently similar to AnCHM with respect to over-expression of paternally transcribed genes. Not all pregnancies in affected women necessarily result in CHM. Pregnancy outcome may be variable with PHM, stillbirths, frequent miscarriages and occasional live births. However, the majority of women with this condition have recurrent BiCHM with no normal pregnancies (reviewed in Fisher et al., 2004a).

Linkage studies in a large consanguineous Lebanese family with familial recurrent HM initially mapped the gene responsible to a 15.2 cM interval on chromosome 19q13.4 (Moglabey et al., 1999). In the same study, a similar region of homozygosity for 19q13.4 was found in a second, reportedly non-consanguineous, European family with the same condition. This suggests that familial recurrent HM is sufficiently rare that affected individuals are likely to be homozygous by descent even when there is no reported consanguinity in the immediate family. Investigation of non-consanguineous families with familial recurrent hydatidiform mole is likely to be useful in refining the location of the candidate gene. Further mapping of two affected European families narrowed the candidate region to a 1.1 Mb gene-rich region flanked by D19S418 and AAAT11138 at the centromeric and telomeric ends respectively (Sensi et al., 2000; Hodges et al., 2003). Investigation of further families with this condition is needed to refine the region and identify the specific gene involved.

In this report we describe genotyping of the 19q13.4 region in two Chinese families. Although recurrent HM has been previously reported in Chinese women (Thavarasah and Kanagalingam, 1988; Huang et al., 2003), to the best of our knowledge this is the first report of familial CHM of biparental origin in families of Chinese origin. A diagnosis of familial recurrent HM was likely given the presence of two sisters in each family with several molar pregnancies. This was confirmed by genotyping tissue from the most recent CHM from one sister in each family and showing them to be of biparental, rather than androgenetic, origin. In family Ch02 the CHM examined appeared to be trisomic for two markers mapping close together on chromosome 6. For one marker the additional allele was clearly maternal in origin while the alleles for the second marker were consistent with an additional contribution from the patient. The significance of this is unclear. However, CHM have been described which, although essentially androgenetic, contain some extra chromosomal material from the maternal genome (Fisher et al., 2004b). This case may represent an unusual BiCHM that has inherited additional chromosome material from both maternal chromosomes 6.

HM is a premalignant condition, ~1 in 10 women with a CHM going on to develop either invasive mole or choriocarcinoma. Although there have been few confirmed cases of BiCHM, it has been suggested that the risk of persistent trophoblastic disease (PTD) is similar following BiCHM to that following the more usual AnCHM (Fisher et al., 2004a). In this study, all four patients developed lung metastases after at least one of their molar pregnancies and were treated with chemotherapy confirming that, like AnCHM, BiCHM have a significant risk of progressing to PTD.

As with other families with recurrent HM the pattern of inheritance in the two families was consistent with an autosomal recessive condition. To investigate the involvement of the 19q13.4 chromosomal locus in these families, genotyping was carried out with a panel of microsatellite markers for this region. In family Ch01, the genotypes of the two sisters were consistent with their having inherited two different chromosomal haplotypes for this region from at least one parent suggesting that the causative gene in this family is unlikely to map to the 19q13.4 region.

In family Ch02, haplotype analysis of the proband showed that she had inherited a different combination of genes from her parents to either of her two unaffected sisters. Two markers, GT17173 and D19S880, in the candidate region did show homozygosity in the affected sister but were not consecutive. GT17173 was an isolated homozygous marker of an allele size previously found to be common in both Caucasian and Asian controls. D19S880 was one of three consecutive homozygous markers, two of which were outside the candidate region. The allele sizes for these three markers, 89 base pairs (bp) (D19S926), 81 bp (D19S418) and 187 bp (D19S880), are common alleles in Caucasian populations, occurring at a frequency of 0.4, 0.7 and 0.1 respectively. Since the parents themselves are homozygous for two of these markers, these alleles are also likely to be common in Chinese populations. While homozygosity by descent cannot be excluded for this small region of 19q13.4 in this family, it is more likely that these markers are homozygous by state. Although we cannot exclude the possibility that the affected individual in Ch02 is a compound heterozygote for mutations in the 19q13.4 region, heterozygosity across most of this region argues in favour of a different chromosomal location for the mutated gene in this family.

Heterozygosity across the 19q13.4 region in these families was not totally unexpected. Although most families with recurrent HM have shown large regions of homozygosity on chromosome 19 (Moglabey et al., 1999; Sensi et al., 2000; Fisher et al., 2002; Hodges et al., 2003; Panichkul et al., 2005) two consanguineous families with this condition, but no evidence of homozygosity for this region, have been previously described (Judson et al., 2002; Slim et al., 2005).

Lack of homozygosity for the 19q13.4 region in two further families supports the hypothesis that the condition shows genetic heterogeneity with mutations in two or more different loci giving rise to the same phenotype. Identification of further families is needed to perform linkage analysis, refine the location and identify the specific genes underlying this condition.
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