Genetic evidence that placental site trophoblastic tumours can originate from a hydatidiform mole or a normal conceptus

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Summary The genetic origin of two placental site trophoblastic tumours was established using a Y chromosome-specific and locus-specific minisatellite probes. A gestational origin was confirmed for both tumours. In one case the origin of the tumour was consistent with derivation from a normal female conceptus while the other was shown to arise from a homozygous complete hydatidiform mole, an abnormal conceptus more usually associated with the development of choriocarcinoma.

Placental site trophoblastic tumours (PSTT) are rare gestational trophoblastic tumours. They resemble the intermediate trophoblast of the placental bed and are distinguished from the more common gestational trophoblastic tumour, choriocarcinoma, by having few multinucleated syncytial cells, abundant cytoplasmic human placental lactogen (hPL), low level of human chorionic gonadotrophin (hCG) production (Kurman et al., 1984) and relative resistance to standard trophoblastic disease chemotherapy (Lathrop et al., 1988).

Although choriocarcinoma can follow any type of pregnancy approximately half follow pregnancy with complete hydatidiform mole, an unusual conceptus which has two paternal sets of chromosomes and is therefore androgenetic in origin (Kajii & Ohama, 1977). PSTT, however, generally follow term pregnancies or non-molar abortions (WHO, 1983) although a small number have been described in pateints with a clinical history of hydatidiform mole (Lathrop et al., 1988; Dessau et al., 1990).

Genetic studies have confirmed the origin of choriocarcinoma from normal term pregnancy or homozygous or heterozygous complete hydatidiform moles (Fisher et al., 1988; Chaganti et al., 1990; Fisher et al., 1992), but have also shown that the causative pregnancy in trophoblastic tumours is not always the antecedent pregnancy (Fisher et al., 1992). Thus a clinical diagnosis of post-term or post-mole PSTT is not conclusive evidence of the origin of the tumour.

We report here two cases of PSTT in which the genetic origin has been established using a Y chromosome-specific probe CY84 (Wolfe et al., 1985) and a panel of locus-specific minisatellite probes, which identify highly polymorphic restriction fragment length polymorphisms (RFLPs) of the DNA (Wong et al., 1987; Armour et al., 1990). One case was shown to originate in a normal conceptus and the other from a pregnancy with hydatidiform mole.

Patients and methods

S.J. was a 28 years old Caucasian who had four full term normal deliveries of a male and three females, the last delivery of a female child being 16 months previously. She presented with irregular vaginal bleeding and pathological examination of tissue following dilatation and curettage showed PSTT (Figure 1). A hysterectomy performed 6 weeks later confirmed the histological diagnosis of PSTT.

J.D. was a 29 year old Caucasian with an obstetric history

of one full term normal delivery 5 years previously and a hydatidiform mole 16 months prior to presentation. She presented with a 6 month history of vaginal bleeding. When hysterectomy was performed 2 months after presentation pathological examination showed a PSTT.

Pathological diagnosis of the tumours was carried out following routine histological examination. Tumours were characterised immunohistochemically with a monoclonal antibody to hCG (INN 13-Serotec) and a rabbit polyclonal antisera to hPL using an avidin biotin peroxidase technique (Kardana et al., 1988). Fresh tissue was available for DNA analysis from the primary tumour in both cases. Tumour tissue was cut into small pieces and then snap frozen in liquid nitrogen. Seven μ cryostat sections were cut from each piece and stained with haematoxylin and eosin. Areas comprising mainly tumour cells were identified and corresponding areas dissected out from the frozen tissue. DNA was prepared from this tissue using standard techniques. Ten ml samples of heparinised blood were used to prepare DNA from the patient and her partner.

RFLPs of the DNA, defined by the locus-specific minisatellite probe MS1 (Wong et al., 1987), were examined in HinfI digested parental and tumour DNA as previously described (Wong et al., 1987; Fisher et al., 1989). The probe was subsequently stripped from the DNA and rehybridisation carried out successively with probes MS8, MS31, pλg3, MS43 and MS621 (Wong et al., 1987; Armour et al., 1990). In the case JD parental and tumour DNA was reprobed with three additional locus-specific minisatellite probes MS605, MS622 and MS620 (Armour et al., 1990). The chromosomal location, heterozygosity and allelic length range of these probes are shown in Table I.

To examine tumours for Y chromosome-specific sequences, 5 μg of DNA was digested with EcoRI, hybridised with CY84, and examined for the presence or absence of the 5.5 kb male-specific band (Wolfe et al., 1985).

Results

No Y chromosome-specific sequences were identified in either of the two tumours. RFLPs identified with the locus-specific minisatellite probes are shown in Table II.

S.J.

Examination of DNA from tumour tissue with probes MS1, MS31, MS43 and MS621 showed the presence of a paternal band in the tumour tissue confirming gestational origin. Of these probes the maternal sample was heterozygous for RFLPs identified with MS1, MS31, and MS621. All three probes demonstrated the presence of both maternal alleles in the DNA prepared from the tumour. However, the relative
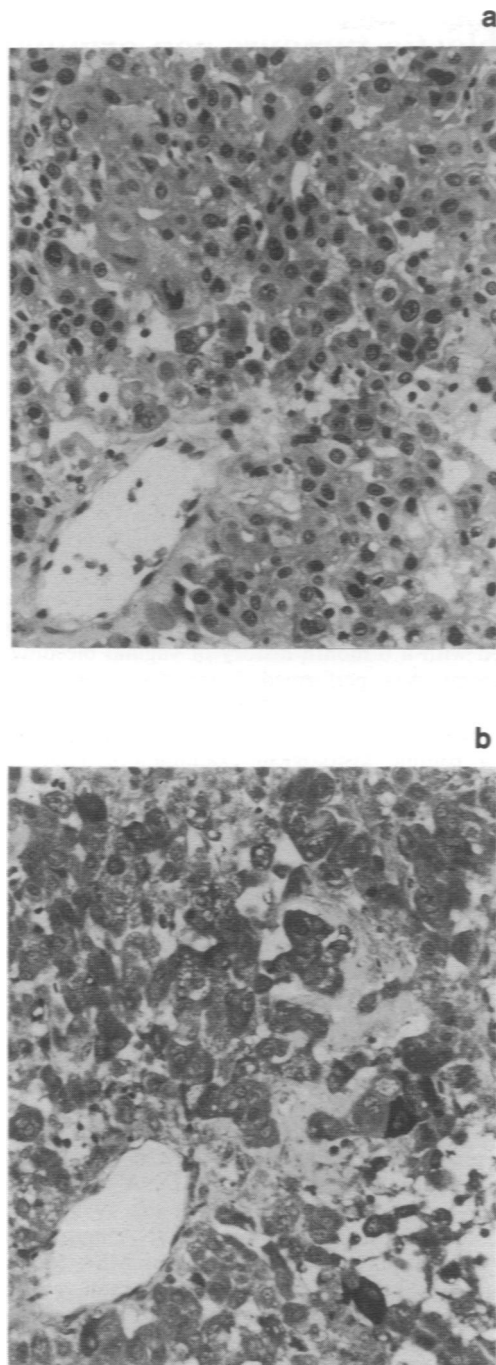
Figure 1 Appearance of the tumour in hysterectomy specimen from patient S.J. **a**, Haematoxylin and eosin. **b**, Indirect immunoperoxidase for human placental lactogen (hPL). × 250. Most cells are positive for hPL with a few cells being strongly positive.

intensity of the two maternal alleles in the tumour was different to those in the maternal tissue itself (Figure 2) indicating that one allele was there at an increased dosage due to its presence both in the tumour genome and the DNA from infiltrating host cells. Results with MS8 and pλg3 were uninformative due to sharing of alleles by the parents.

J.D.

The probes MS1 (Figure 3) and MS31 identified a paternal contribution in this tumour. However, due to sharing of parental alleles probes MS8, pλg3, MS43 and MS621 were uninformative in this respect. Three additional probes, MS605, MS622 and MS620 used to examine this case all showed a single paternal band in the tumour indicating that the tumour was androgenetic and therefore had originated from a pregnancy with hydatidiform mole. Although MS43A was uninformative in terms of parental origin the father was heterozygous while the tumour had only a single band compatible with paternal origin (Figure 3). Similarly pλg3, MS31 and MS621 identified hererozygous alleles in the father only one of which was present in the tumour. The tumour was thus homozygous for eight unlinked autosomal loci for which the father was heterozygous. Absence of Y chromosome-specific sequences in this tumour suggests that it was also homozygous for the X chromosome.
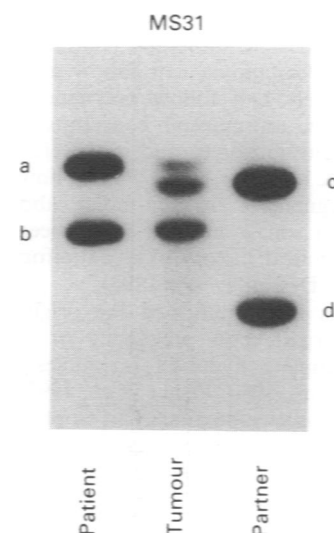

Figure 2 RFLPs detected with MS31 in DNA from the patient, tumour tissue and her partner in case S.J. A paternal contribution to the tumour **c** is indicated. The increased intensity of the maternal allele **b** relative to allele **a** in the tumour sample compared to that in the patient DNA indicates the presence of a maternal contribution **b** to the tumour genome in addition to maternal DNA **ab** derived from host tissue infiltrating the tumour.

Table I   Locus-specific minisatellite probes used to examine DNA from cases of PSTT

Probe	MS1	MS8	MS31	pλg3	MS43[a]	MS621	MS605	MS622	MS620
Chromosomal location	1p33-p35	5q35-qter	7p22-pter	7q36-qter	12q24.3-qter	5p	6q	10q	15q
Heterozygosity (%)	99.4	85.1	98	97.4	95.9	92	87	83	91
Allelic length range (kb)	2.0–22.0	2.4–9.5	3.5–13	0.6–20	3.5–16	–	–	–	–

[a]The probe MS43 identifies two very closely linked polymorphic loci MS43A and MS43B (Royle *et al.*, 1988). The details given are for the major polymorphism MS43A. References: Wong *et al.*, 1987; Jeffreys *et al.*, 1988; Royle *et al.*, 1988; Armour *et al.*, 1990.

Table II Restriction fragment length polymorphisms identified in cases of PSTT

Probe	MS1	MS8	MS31	pλg3	MS43A	B	MS621	MS605	MS622	MS620
Case S.J.										
patient	ab*	a	ab	ab	ab	a	ab	–	–	–
tumour	ad (ab)**	a	bc (ab)	ab	ab	ab	ac (ab)	–	–	–
partner	cd	ac	cd	bc	ac	ab	bc	–	–	–
Case J.D.										
patient	ab	ab	ab	ab	ab	a	abc⁺	ab	ab	ab
tumour	d	a	d	b	b	a	a	c	d	c
partner	cd	a	cd	bc	bc	a	ad	cd	cd	cd

*The letters a, b, c, d are used to differentiate between different band sizes within a case and do not represent specific polymorphisms. **RFLPs given in brackets represent those of infiltrating host cells. ⁺Three bands present in the maternal sample suggest the presence of a HinfI restriction site within the RFLP giving rise to two bands at one locus.
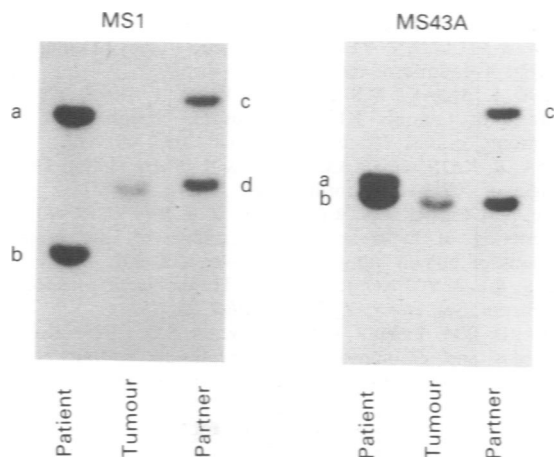

Figure 3 RFLPs detected with MS1 and MS43 in DNA from the patient, tumour tissue and her partner in case J.D. The patient and her partner are heterozygous for both loci although they have an RFLP b in common at the MS43A locus. The single band d identified in the tumour with probe MS1 is clearly of paternal origin while the single band b identified at the MS43A locus is compatible with a paternal origin.

Discussion

Three types of gestational trophoblastic tumours are now recognised, invasive mole, a relatively benign tumour following pregnancy with hydatidiform mole, choriocarcinoma and PSTT. PSTT is a relatively rare lesion previously known by a variety of names such as chorioma, atypical choriocarcinoma and trophoblastic pseudotumour (Scully & Young, 1981). It resembles the trophoblast of the placental bed at the site of implantation and is therefore composed of mainly intermediate trophoblast in contrast to choriocarcinoma which exhibits a dimorphic pattern of cytotrophoblast and syncytiotrophoblast which resembles villous trophoblast. Absence of extensive haemorrhage and predominant interstitial rather than intravascular infiltration also distinguish PSTT from choriocarcinoma.

Genetic studies have shown that choriocarcinoma may derive from a normal conceptus having both maternal and paternal contributions to the genome (Wake et al., 1981; Chaganti et al., 1990; Fisher et al., 1992) or from a pregnancy with complete hydatidiform mole which is androgenetic, having only a paternal contribution to the nuclear genome (Fisher et al., 1988; Fisher et al., 1992). One study also demonstrated that the immediately antecedent pregnancy is not always the causative pregnancy, one patient with a post-mole tumour having had an intervening pregnancy with normal full term delivery of twins (Fisher et al., 1992).

Thus a clinical diagnosis of post-term or post-mole tumour may not always be biologically correct.·

Few cases of PSTT have previously been examined genetically. Two cases have been reported as diploid (Eckstein et al., 1985; Lathrop et al., 1988) in contrast to the more aneuploid karyotypes usually described in choriocarcinomas (Makino et al., 1965; Wake et al., 1981; Sheppard et al., 1985; Lawler & Fisher, 1986). We are not aware of any studies where the origin of PSTT has been examined. Unlike choriocarcinoma, where approximately half occur following molar pregnancies, most reported cases of PSTT have followed term pregnancies or non-molar abortions (WHO, 1983; Lathrop et al., 1988). The present study was undertaken to examine the genetic origin of two PSTT using DNA analysis, and to determine the nature of the causative pregnancy in each case. In both cases examination with locus-specific probes established the presence of a paternally derived band confirming their gestational origin.

Further interpretation of results in case S.J. was complicated by the presence of a high proportion of DNA from infiltrating host cells. DNA from intervening host cells will have RFLPs identical to those of the patient and is suggested by the presence of both maternal bands in the tumour track in addition to one or more paternal bands. In case S.J. the relative difference in intensity of the two maternal bands in the tumour track compared with the patient sample (Figure 2), suggests that one allele is present both in the contaminating host and the tumour DNA. The presence of both a maternal and paternal contribution to the tumour, in the absence of Y chromosome-specific sequences would be consistent with an origin from a normal female conceptus.

In the case J.D., only a single band was seen with each minisatellite probe used and in all tests was paternal or compatible with a paternal origin. No maternal contribution to the tumour genome was identified. An origin from an androgenetic complete mole was thus highly likely. Complete hydatidiform moles may be homozygous deriving from the doubling of a haploid sperm following fertilisation of an enucleate egg (Lawler et al., 1979; Jacobs et al., 1980) or heterozygous arising by dispermy (Ohama et al., 1981). The former are female while the latter may be male or female. Choriocarcinoma has been shown to follow both types of hydatidiform mole (Fisher et al., 1988). Homozygosity in the tumour J.D. for eight independently segregating markers heterozygous in the father, together with the absence of Y-specific sequences makes it statistically unlikely that the tumour followed a heterozygous complete mole ($P < 0.002$) and it is therefore most likely that the PSTT arose from a homozygous complete mole.

Attempts were made to establish the origin of a third case of PSTT in a patient (A.H.) with a history of hydatidiform mole. However, pathological examination of the tumour showed large numbers of infiltrating cells and several preparations of DNA from tumour tissue resulted in DNA which were mostly of host origin. The use of sections from formalin-fixed, paraffin-embedded blocks in combination

with new techniques such as polymerase chain reaction technology which require very small amounts of DNA (Shibata *et al.*, 1988) may enable the origin of this case, and other cases where fresh materal is not available to be resolved.

DNA analysis has made it possible to confirm that two tumours with the pathology of PSTT were gestational in origin and to show that PSTT may originate not only in a normal term pregnancy but also after a complete hydatidiform mole, the latter being more usually associated with the gestational trophoblastic tumour, choriocarcinoma.

Thus gestational tumours following hydatidiform mole may be invasive mole, choriocarcinoma or PSTT.

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