

Molecular genotyping of placental site and epithelioid trophoblastic tumours; female predominance

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Introduction

Gestational trophoblastic diseases (GTD), representing a range of proliferative disorders that originate from the placenta, fall into two main types, the premalignant conditions complete (CHM) and partial hydatidiform mole (PHM) and the gestational trophoblastic neoplasms (GTN), invasive mole, choriocarcinoma, placental-site trophoblastic tumour (PSTT) and epithelioid trophoblastic tumour (ETT).

While CHM and PHM are identified on the basis of histopathological findings (Sebire 2010), tissue from IM is rarely seen as most are treated successfully with chemotherapy following early detection by human chorionic gonadotrophin (hCG) monitoring subsequent to a molar pregnancy. Most other GTN are diagnosed clinically with primary or metastatic disease following an unrecognised molar pregnancy or non-molar pregnancy and diagnosis confirmed on the basis of histopathological features (Sebire and Lindsay 2010).

Choriocarcinoma, occurs in approximately 1 in 30,00 to 40,000 pregnancies in Europe and North America (Smith 2003). Histopathologically, these tumours show differentiation towards villous trophoblast and are characterised by proliferation of mononuclear cytotrophoblast-like and multinucleated syncytiotrophoblast-like cells surrounding necrotic and haemorrhagic tissue (Sebire and Lindsay 2010).

PSTT, first described as a separate entity in 1976 (Kurman et al 1976) and recognised as a malignant trophoblastic tumour in 1981 (Scully and Young

1981) are biologically unique from choriocarcinoma due to their relatively slow growth rate, lower human chorionic gonadotropin (hCG) serum levels, later onset of metastatic potential and greater resistance to chemotherapy. These tumours are characterized by differentiation towards implantation site-trophoblast and appear as a monomorphic population of large polyhedral cells with irregular hyperchromatic nuclei (Sebire and Lindsay 2010).

More recently a third tumour, epithelioid trophoblastic tumour (ETT), has been established as a rare variant of GTN with its own distinct morphology and immunohistochemical features (Shih and Kurman 1998). ETT is characterised by transformation of the chorionic-type intermediate trophoblastic cells. Clinically these tumours closely exhibit similar behaviour to PSTT. Occasionally mixed tumours, having elements of both choriocarcinoma and PSTT or both PSTT and ETT have been described (Shih and Kurman 2001).

Choriocarcinoma may follow any type of pregnancy but most commonly occurs after an abnormal pregnancy, approximately 40 - 50% following a pregnancy with a HM (Bagshawe et al 1976; Lurain et al 1982). In contrast, reports suggest that PSTT and ETT are more likely to follow a normal term pregnancy (Shih and Kurman 1998; Chang et al 1999; Baergen et al 2006; Palmer et al 2008; Schmid et al 2009) with a strong bias towards presenting after a female pregnancy (Hui et al 2000; Hui et al 2007). Molecular genotyping of twenty PSTT found the presence of one or more X chromosomes together with the absence of a Y chromosome in all cases suggesting that the presence of paternally derived X chromosome may be

required for the pathogenesis of PSTT. A subsequent study of the Y chromosomal complement in GTN did identify a single case of PSTT (7%) and three ETT (18%) with Y chromosomes but confirmed that the majority of both PSTT and ETT were female (Yap et al 2010).

The antecedent pregnancies were not reported in these studies and one explanation for the very high numbers of female pregnancies might be that, like choriocarcinoma, the causative pregnancy in a large proportion of PSTT and ETT might be a CHM. Since approximately 90% of CHM are female (Fisher et al 1989; Cheung et al 1994) then a bias towards female tumours would be expected. Although few PSTT and ETT are reported to occur following a HM, it has been clearly shown that in choriocarcinoma at least, the causative pregnancy may not be the antecedent pregnancy (Fisher et al, 1995, Shahib et al 2001, Fisher et al 2007).

The aim of this study was to investigate a large series of PSTTs and ETTs, treated at a national trophoblastic disease centre, to further elucidate the relationship between the nature and sex of both the antecedent and causative pregnancies and the development of these rare tumours.

Materials and methods

Patient Cohort

All PSTT and ETT cases referred to the Charing Cross Hospital Trophoblastic Tumour Screening and Treatment Centre were identified from the electronic data bases of the unit. Between January 1976 and January 2016 ninety two cases with a confirmed diagnosis of PSTT, ETT or mixed PSTT/ETT were

referred to Charing Cross Hospital, the first two cases being unusual trophoblastic tumours that were diagnosed as PSTT on pathological review in 1981. The site and histopathological diagnosis of the tumour, together with available obstetric history including the nature and sex of the antecedent pregnancy and time interval until diagnosis was recorded for each case.

Histopathology

The histopathology of all cases was reviewed at the trophoblastic disease unit, by specialist histopathologists with expertise in GTD, using criteria based on the World Health Organisation classification of tumours of the female reproductive organs (Hui et al 2014). Confirmatory immunohistochemical staining with markers such as placental alkaline phosphatase, human placental lactogen and P63 were carried out as appropriate.

Molecular Genotyping

DNA extraction

Tumour and adjacent maternal tissue were dissected independently from 3-5 unstained 5 μ sections of formalin fixed paraffin embedded tissue with reference to a consecutive haematoxylin and eosin stained section. Following deparaffinisation of the tissue, DNA was prepared by digesting the tissue in a buffer comprising 50mM Tris pH 8.5; 1mM EDTA pH 8.0; 0.5% Tween 20 and 200 μ g/ml proteinase K at 56 $^{\circ}$ C for 3 hours, the volume determined by the amount of tissue available. Following heat inactivation of the proteinase K at 95 $^{\circ}$ C for 8 min, the solution containing DNA was stored at -20 $^{\circ}$ C until use. In nine cases DNA was prepared from children of antecedent pregnancies. In

these cases saliva samples were collected in an Oragene DNA collection kit (DNA Genotek, Ottawa, Canada) and DNA prepared according to manufacturer's instructions. In 3 cases DNA was prepared from a 200 µl blood sample from the patient's partner using a QIAamp DNA mini kit (Qiagen, UK) as per the manufacturer's protocol.

Fluorescent microsatellite genotyping

1 µl DNA from tumour and maternal tissue (or 20ngs DNA from saliva) was amplified with primers for 15 short tandem repeat (STR) loci on 13 chromosomes, together with the amelogenin locus, using an AmpFISTR Identifiler Plus kit (Applied Biosystems, Warrington, UK). PCR products were resolved by capillary electrophoresis using an ABI 3100 Genetic Analyser and genotypes determined using GeneMapper version 4.0 software (Applied Biosystems, Warrington, UK). The genotype of the tumour tissue was compared with that of the maternal tissue to determine the gestational origin of disease. In cases where the parental origin of the X chromosome was identified, 1 µl of DNA from the tumour and maternal tissue, together with 20 ngs of DNA from her partner, were amplified with primers for two polymorphic dinucleotide repeats DXS451 and DXS984.

Statistical analysis

Frequencies of female genotypes between groups were examined using comparison of proportions test (modified chi squared test) using StatsDirect (UK). $P < 0.05$ was considered significant.

Ethical approval

The project was approved (project no, R14032) by the Tissue Management Committee of the Imperial College Healthcare NHS Trust Research Tissue Bank (project no. R14032), which is approved by NRES to provide deemed ethics for projects accessing material and data stored within the Research Tissue Bank.

Results

Patient characteristics

Ninety-two patients were identified from the electronic databases at Charing Cross Hospital with a diagnosis of PSTT, ETT or a mixed tumour containing elements of PSTT and or ETT. The majority of tumours were morphologically PSTT (66%), approximately 25% ETT or mixed PSTT/ETT and a smaller number of mixed tumours containing elements of choriocarcinoma (Figure 1; Table 1).

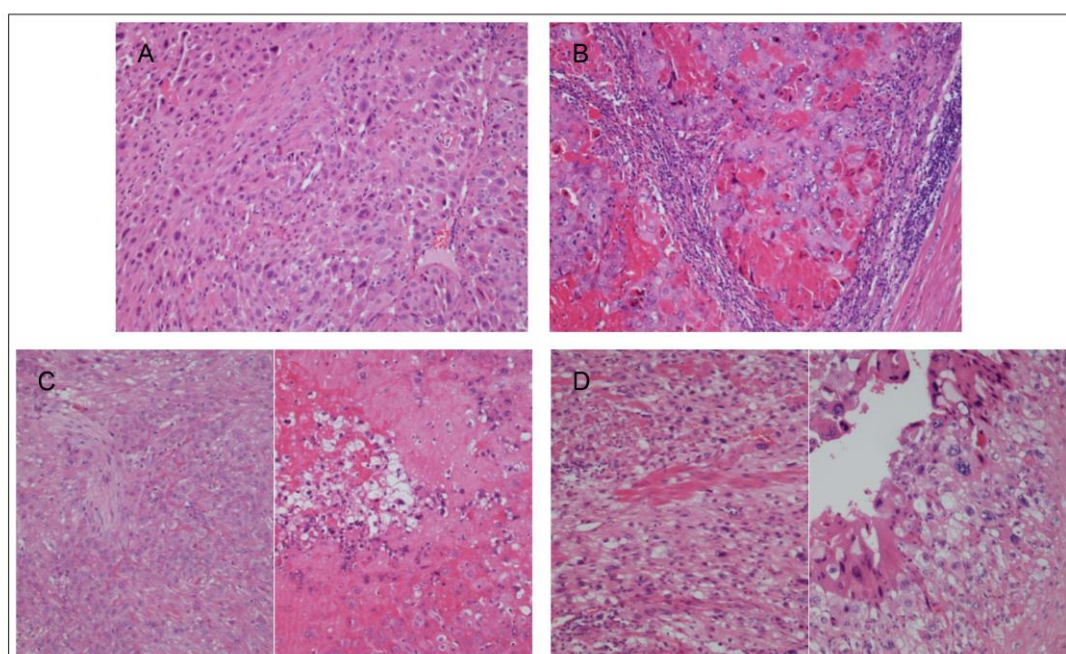


Figure 1. Photomicrographs of haematoxylin and eosin stained placental site trophoblastic, epithelioid and mixed trophoblastic tumours showing representative morphological features. A. Typical PSTT exhibiting sheets of monomorphic interstitial trophoblast cells with eosinophilic cytoplasm and minimal atypia. B. ETT demonstrating extravillous trophoblast with areas of hyalinisation. C. A mixed PSTT (left panel) and ETT (right panel). D. A mixed PSTT (left panel) with choriocarcinoma (right panel, demonstrating more marked atypia and focal syncytiotrophoblastic differentiation). (original magnifications x100).

Pathology	Cases	Age at diagnosis (years)		Time since antecedent pregnancy (years)	
		Mean	Range	Mean	Range
PSTT	61 (66%)	33.3	20 - 54	2.68	0.1 - 21.5
ETT	11 (12%)	36.5	15 - 49	3.27	0.9 - 13.3
Mixed PSTT/ETT	11 (12%)	37.4	28 - 45	6.34	1.0 - 18.9
Mixed PSTT/CC	8 (9%)	37.2	23 - 59	4.98	0.9 - 18.5
Mixed ETT/CC	1 (1%)	47	-	3.15	-
Total	92	34.7	15 - 59	3.40	0.1 - 21.5

Table 1. Characteristics of patients included in the study

Antecedent Pregnancy

The antecedent pregnancy as recorded in patient notes showed that this was a full term normal delivery in over half (64%) of cases. In 19 (21%) cases the tumour was preceded by a molar pregnancy, the majority of these being morphologically CHM. In the remaining 15% of cases the antecedent pregnancy was miscarriage (6.5%), termination of pregnancy (7.5%) or still birth (1%) (Table 2). The sex of the antecedent pregnancy was not recorded

in eleven cases. In the remaining singleton pregnancies a significantly greater proportion were reported as female, 35 of 45 (78%) than male, 10 of 45 (22%) ($p < 0.0001$; 95% confidence interval 0.629-0.889). In one of two cases in which the antecedent pregnancy was twins, both infants were female and the other one male and one female.

	Cases	<u>Live birth</u>				<u>HM</u>		<u>Other</u>		Still Birth
		Female	Male	NK	twins	CHM	Other	Miscarriage	TOP	
PSTT	61	22	7	9	M/F	8	HM -2 PHM -1	5	5 *	1
ETT	11	4		1		5			1	
Mixed PSTT/ETT	11	6	2		F/F	1		1		
Mixed PSTT/CC	8	4	1	1		1			1	
Mixed ETT/CC	1					1				
Total	92	36	10	11	2	16	3	6	7	1

Table 2. Antecedent pregnancies reported for cases in the study. M, male; F, Female; NK, Not known; HM hydatidiform mole; CHM, complete hydatidiform mole; TOP, termination of pregnancy; PHM, partial hydatidiform mole; *Includes one case of late termination for spina bifida.

Causative pregnancy

In 15 cases H and E stained slides, without further pathological material, were sent to the unit for review. In a further eight cases, histological blocks had been returned to the referring hospital following pathological review and were not available for analysis. DNA was prepared and genotyping performed for all 69 cases in which tumour tissue was available. In some cases larger alleles at some loci included in the AmpflSTR kit failed to amplify on

genotyping due to DNA degradation of archival material. Only cases in which three or more non-maternal (paternal) alleles were identified in the tumour, and hence a gestational origin confirmed, were included in the study. Contamination of tumour DNA with DNA from the patient was present to some degree in most of the tumours due to difficulties in microdissection of tumours which infiltrate the normal maternal myometrium and infiltration of the tumour tissue by maternal lymphocytes. Eight cases, including six where the tumour tissue was over 20 years old, and four cases where insufficient unique paternal alleles were identified to confirm a diagnosis due to maternal contamination, were excluded from the study. Of the 57 tumours analysed, 48 were from the primary tumour, in three cases lung metastases, two cases pelvic metastases and one case the ovary. Forty-two tumours were found to have arisen in non-molar pregnancies, having both a maternal and paternal contribution to the tumour genome (Figure 2;) and 15 in molar pregnancies (Figure 3) (Table 3).

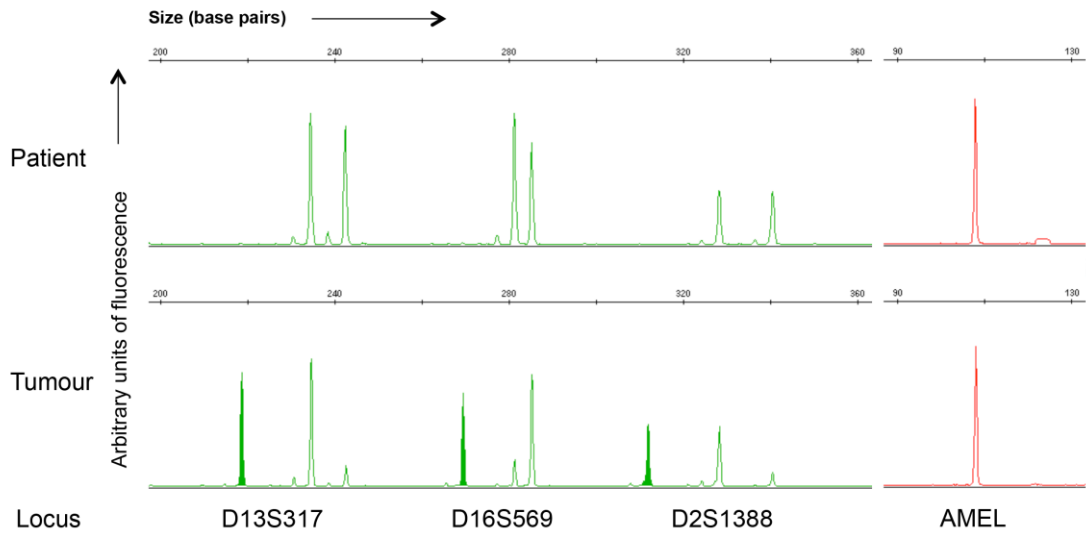


Figure 2. Partial genotypes of maternal and tumour tissue in a case of PSTT/ETT following a normal term birth. Genotyping of the tumour for STR loci D13S317, D16S569 and D2S1388 (and others not shown) show a single non-maternal allele (solid peak). Both maternal alleles (open peaks) are present in the tumour DNA, the lower peak representing maternal contamination while the higher peak represents a maternal contribution to the tumour genome, and a small proportion of maternal contamination. DNA at the AMEL locus is from the X chromosome only.

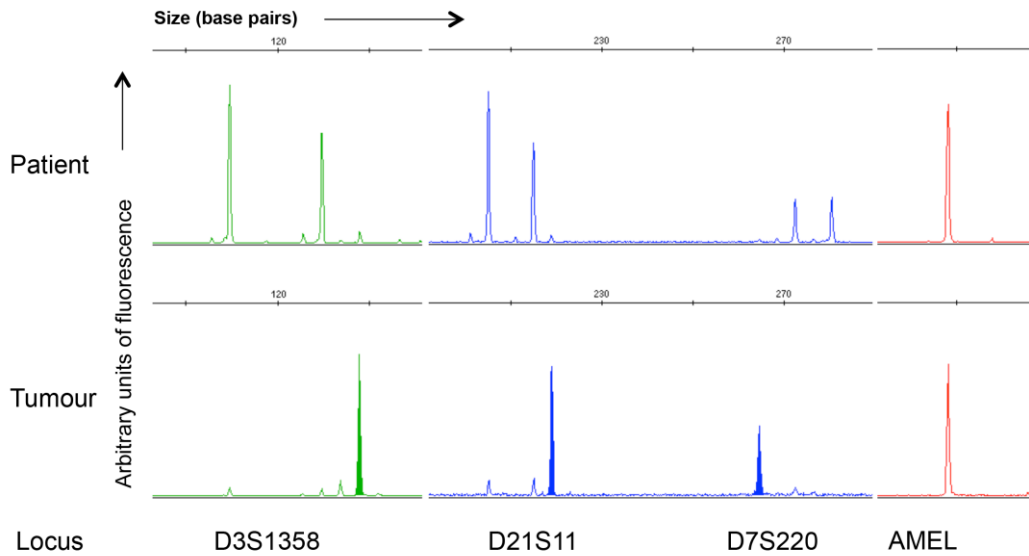


Figure 3. Partial genotypes of maternal and tumour tissue in a case of PSTT/ETT following an antecedent molar pregnancy. Genotyping of the tumour for STR loci D3S1358, D21S11 and D7S220 (and others not shown) show a single non-maternal allele (solid peak). DNA at the AMEL locus is from the X chromosome only.

Pathology	Cases	<u>Non-molar</u>		<u>Molar</u>	
		Female	Male	Female	Male
PSTT	33	22	2	8	1 PHM
ETT	8	5	-	2	1 BiCHM
Mixed PSTT/ETT	9	7	1	1	-
Mixed PSTT/CC	6	4	1	-	1
Mixed ETT/CC	1	-	-	1	-
Total	57	38	4	12	3

Table 3: Causative pregnancy identified by genotyping of maternal and tumour tissue. PHM, partial hydatidiform mole; BiCHM diploid biparental complete hydatidiform mole.

In tumours where the causative pregnancy was non-molar, a significantly greater number were female than male ($p < 0.0001$; 95% confidence interval 0.219 - 0.569) only 4 of 42 cases (9.5%) having a Y chromosomal allele present (Figure 4).

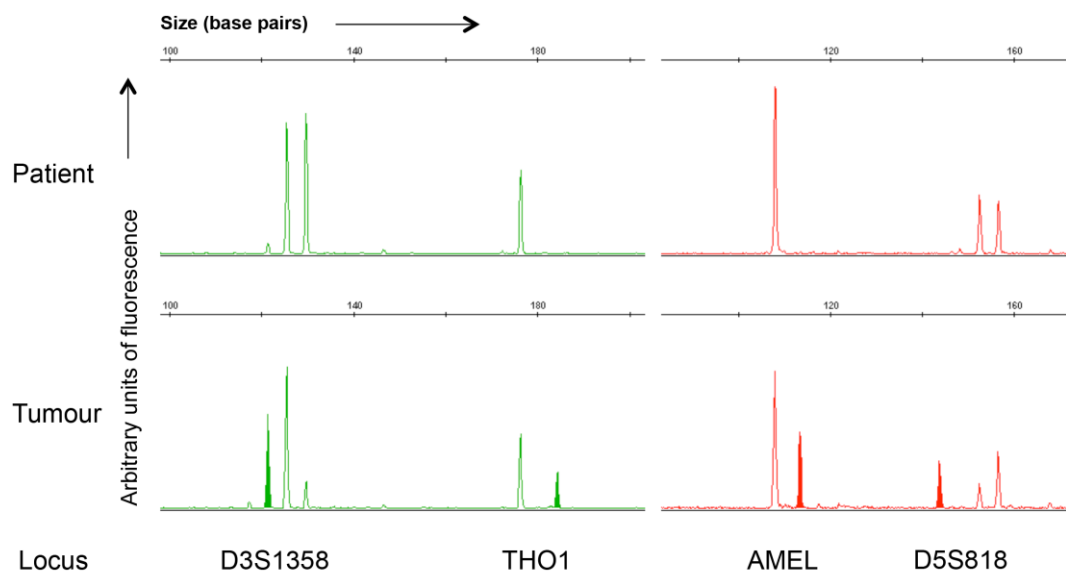


Figure 4. Partial genotypes of maternal and tumour tissue in a case of PSTT/CC following a male term birth. Genotyping of the tumour for STR loci D3S1358, THO and D5S818 (and others not shown) show a single non-maternal allele (solid peak). Both maternal alleles (open peaks) are present in the tumour DNA, the lower peak representing maternal contamination while the higher peak represents a maternal contribution to the tumour genome, and a small proportion of maternal contamination. DNA at the AMEL locus is from both the X and Y chromosome.

Three of the fifteen tumours following molar pregnancies were shown to have a Y chromosome. One of these was a tumour in which the causative pregnancy was a PHM and the other a tumour that had originated in a

dispermic CHM. In a third case a patient with familial recurrent hydatidiform mole, a condition in which women have CHM that are diploid and biparental rather than androgenetic developed a PSTT following a CHM. Although the pregnancy was diploid and biparental, due to the patient's condition, this tumour was considered to have arisen in a diploid biparental CHM with a Y chromosome. In the 12 post-mole tumours of female origin, all autosomal markers were homozygous inconsistent with a monospermic origin.

In twelve of the 14 cases where a CHM was the causative pregnancy, the antecedent pregnancy was reported as a CHM (Table 4). In five cases where tissue was available from the antecedent molar pregnancy, genotyping confirmed that the antecedent pregnancy was the causative pregnancy. In one case the molar pregnancy was not classified and sections unavailable for review while one case was reported as a miscarriage. The single case where a PHM was the causative pregnancy, the antecedent pregnancy was also reported as a PHM.

The antecedent pregnancies in all four cases in which a non-molar male conception was the causative pregnancy were full term normal deliveries of male infants. In the 38 cases in which the causative pregnancy was non-molar female, the sex of the antecedent pregnancy was unknown in 13, a miscarriage in two and termination of pregnancy in five. In the 28 cases of causative pregnancy reported to follow normal term delivery, 25 cases, including one case of female twins, were reported as female. Interestingly the causative pregnancy in three cases in which the antecedent pregnancy was reported as male had no Y chromosomal material (Table 4).

<u>Causative Pregnancy</u>		<u>Antecedent Pregnancy</u>	
	Cases		Cases
CHM Female	12	CHM	10
		HM	1
		SA	1
CHM Male	3	CHM	2
		PHM	1
Non-molar male	4	FTND male	4
Non-molar female	38	FTND female	24
		FTND NK	3
		FTND female twins	1
		FTND male	3
		SA	2
		TOP	5

Table 4: Antecedent pregnancies in cases where causative pregnancy was identified by genotyping. CHM, complete hydatidiform mole; HM, hydatidiform mole; PHM, partial hydatidiform mole; SA, spontaneous abortion; FTND, full term normal delivery; NK, Not known, TOP, termination of pregnancy.

Genotyping of the child from the antecedent pregnancy was not performed in most cases. However, in nine cases genotyping of the child was performed to confirm the time interval between the pregnancy and diagnosis, an important risk factor for PSTT and ETT. In eight of these cases the genotype of the antecedent pregnancy was consistent with that of the tumour. In one case of PSTT/ETT both the antecedent pregnancy and causative pregnancy were non-molar female conceptions. However, the genotype of the causative

pregnancy was different to that of the antecedent pregnancy (Figure 5). Subsequent genotyping of other pregnancies in the patient showed that the tumour had originated in the pregnancy with her elder sister (Figure 5).

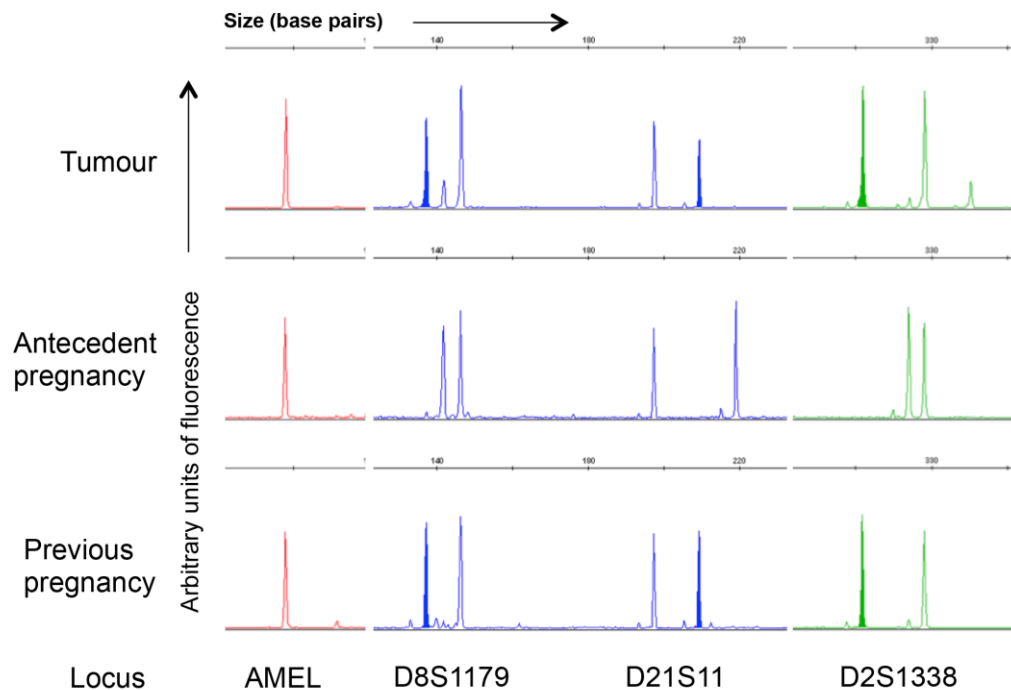


Figure 5. Partial genotypes of tumour tissue in a case of PSTT/ETT following a female term birth. Both the tumour and antecedent pregnancy are female. However, genotyping of the tumour for STR loci D8S1179, D21S11 and D2S1338 (and others not shown) identified alleles in the tumour which were not present in DNA from the child of the antecedent pregnancy (solid peaks). Genotyping of the child from the previous pregnancy identified a genotype consistent with that in the tumour.

In three cases where genotyping of the child of the antecedent pregnancy confirmed that the tumour had arisen in the antecedent male pregnancy, genotyping of two STR polymorphisms on the X chromosome in maternal,

tumour and paternal DNA confirmed that the tumour had only a single X chromosome of maternal origin (Table 5).

Case		Short Tandem Repeat	
		DXS451	DXS984
49	Patient	186	166
	PST/CC	186	166
	Partner	174	182
62	Patient	174 - 193	166 - 176
	PSTT	193	166
	Partner	178	166
85	Patient	186 - 190	166 - 170
	PSTT/ETT	186	166
	Partner	186	178

Table 5: Allele sizes (base pairs) identified following amplification with primes for dinucleotide short tandem repeats DXS451 and DXS984 in 3 tumours with a Y chromosome.

Discussion

PSTT, a rare form of trophoblastic tumour originating in intermediate trophoblast (Kurman et al 1976), represents about 0.2% of patients with gestational trophoblastic disease in the UK (Schmid et al 2009). Since the recognition of ETT a second tumour arising from intermediate trophoblast, in 1998 (Shih and Kurman 1998), the majority of these tumours, 63% are still classified as PSTT. Approximately 17% have been classified as ETT while the

remaining 20% represent mixed tumours with populations of cells resembling ETT and PSTT.

While PSTT and ETT can follow any type of pregnancy, the antecedent pregnancy is most likely to be a normal term pregnancy (Shih and Kurman 1998; Chang et al 1999; Baergen et al 2006; Palmer et al 2008; Schmid et al 2009). However, when the sex of these pregnancies has been recorded the approximate equal numbers of male and female pregnancies expected has not been observed, a review of the literature showing 21 of 23 antecedent pregnancies in a series of PSTT were female. This prompted the suggestion that the pathogenesis of PSTT might require a paternally derived X chromosome (Hui et al 2000). Subsequent molecular genotyping to identify the causative pregnancy in series of PSTT found none of the twenty cases examined to have a Y chromosome (Hui et al 2007). A further study of the Y chromosomal complement in GTN did identify a single case of PSTT (7%) and three ETT (18%) with Y chromosomes but confirmed that the majority of both PSTT and ETT were female (Yap et al 2010). Although the tumours investigated were confirmed to be gestational in the study by Hui et al (2000) the nature of the causative pregnancy was not determined in either study leading Yap et al (2010) to hypothesise that the absence of the Y chromosomal component could be due to the majority of GTN originating in CHM, in which most of the cases are genetically XX (Fisher et al 1989), rather than the recognised antecedent pregnancy. While it has been reported that the causative pregnancy in choriocarcinomas is not always the antecedent pregnancy (Fisher 1995; Shahib et al 2001; Fisher et al 2007) and we have observed post-mole choriocarcinoma in women without a history of a

recognised molar pregnancy, this has not yet been demonstrated for other types of trophoblastic tumours.

In the present study investigation of a large series of PSTT and ETT has confirmed that most antecedent pregnancies, based on patient history, and most causative pregnancies, determined by genetic analysis, are female. Of the 92 cases included in the study the majority, 61, were PSTT. While PSTT and ETT are thought to arise from different subpopulations of intermediate trophoblast (Shih and Kurman 2001), the number of ETT was small, the behavior of ETT closely resembles that of PSTT and some tumours had elements of both PSTT and ETT, the tumours were considered as one group for analysis.

The nature of the antecedent pregnancy for tumours in this study was similar to that reported in earlier studies with 64% of tumours following term pregnancies, 21% following molar pregnancies and 15% following other pregnancy losses. Most antecedent molar pregnancies were reported as CHM with two cases of undefined origin and one PHM, confirming that, like other types of GTN the major risk factor for a post-mole tumour is a pregnancy with a CHM.

Genotyping was performed in a subset of these patients, for whom pathological material was available. While the AmpFISTR Identifiler PCR assay failed to amplify some of the larger products for autosomal loci, due to degradation of the FFPE material, the polymorphic products generated for the X and Y loci of 106 and 110 base pairs respectively, amplified consistently. However, only the 57 cases where three or more informative loci confirmed the presence of non-maternal alleles in the tumour, and therefore a

gestational origin, were included in the study. Genotyping of these cases demonstrated that the causative pregnancy in most PSTT and ETT is not, as previously hypothesized (Yap et al 2010) a CHM since 74% of tumours were shown to originate in non-molar pregnancies with both a maternal and a non-maternal contribution to the tumour genotype. Of the 15 tumours that did originate in molar pregnancies, the causative molar pregnancy was a PHM in one while the remaining 14 were CHM. Of these one was unusual in that it had originated in a diploid biparental CHM in a patient with familial recurrent hydatidiform mole, a genetic disorder in which women have an inherited predisposition to molar pregnancies (Fisher et al 2004; Nguyen and Slim 2014). While most CHM are monospermic, approximately 20% are dispermic (Fisher et al 1989). The relevant malignant potential of the two types of CHM remains controversial (Bynum et al 2016). The fact that only a single CHM in the study was dispermic, having a heterozygous genotype while twelve were homozygous for all alleles analysed and therefore monospermic, does not support the hypothesis that dispermic CHM have a more malignant potential than monospermic CHM.

The present study did demonstrate that both antecedent and causative pregnancies that originated in non-molar pregnancies were mostly, but not exclusively, female. In tumours following singleton non-molar pregnancies the sex of the antecedent pregnancy was reported to be male in 10 of 46 (22%) and where the causative pregnancy was identified 4 out of 42 (10%) apparently failing to support the hypothesis that a paternally derived X is essential for the development of PSTT. In order to confirm that where the causative pregnancy was male these tumours did not also harbour a second

male-derived X chromosome, genotyping of two polymorphisms on the X chromosome was performed in three cases where paternal DNA was available. No unique paternal alleles were found in any of the three tumours, only a single maternal allele being present for all informative markers. A paternally derived X does not therefore appear to be a requirement for tumour development. While there were fewer cases of ETT or mixed tumours than PSTT, the proportion of male to female tumours appeared similar in the different groups. Histopathological review revealed no differences between male and female tumours of the same morphological type.

Amongst causative pregnancies fewer male pregnancies were observed than in the antecedent group, only 4 of 42 (10%) non-molar pregnancies being male. This may reflect the fact that “non-molar pregnancies” comprise slightly cases in the two groups. The antecedent pregnancy group includes only term pregnancies for which the sex is known whereas in the causative pregnancy group non-molar pregnancies include term births and other reproductive loss. For three cases of PSTT with a male antecedent pregnancy, the tumour was female on genotyping, suggesting some tumours in the study had lost the Y chromosome during tumour progression, a feature of a number of different malignancies (Hunter et al 1993; Brunelli et al; 2003; Bianchi et al 2009). While it is not possible to confirm loss of the Y chromosome during progression in these cases without knowing the genotype of the child for the antecedent pregnancy, it is interesting that the mean time interval between the antecedent pregnancy and diagnosis for the three tumours that have apparently lost their Y chromosome is 7.16 years (range 3.90 - 8.85 years) compared to the mean interval between the antecedent pregnancy and

diagnosis in the four cases where both antecedent and causative pregnancy were male, 2.98 years (range 1.38 - 5.78 years).

The genotype of the antecedent pregnancy is not available in most cases. However, since the demonstration that the time interval between the antecedent pregnancy and diagnosis of PSTT is the single most important prognostic factor for women with these tumours (Schmid et al 2009), genotyping of the child of the antecedent pregnancy has been performed in a small number of cases. In 13 of 14 cases, 5 CHM and eight non-molar pregnancies, the genotype of the tumour was consistent with the antecedent pregnancy. In only one case of a mixed PSTT/ETT was the antecedent pregnancy not the causative pregnancy. This provides confirmation that in PSTT and ETT, like choriocarcinoma, the antecedent pregnancy may not be the causative pregnancy. However, this is a rare event and most PSTT and ETT are therefore unlikely to have arisen in unrecognised molar pregnancies. Although only 25% of PSTT and ETT arise in molar pregnancies, given that only one in 600 pregnancies in the UK are HM, (Savage et al 2013), this represents a considerable malignant potential for molar pregnancies. Since the abnormal pathology of HM reflects the fact that most have two copies of the paternal genome and therefore exhibit aberrant expression of imprinted genes it is likely that this also drives the development of post-mole tumours. We hypothesise that tumours originating in non-molar pregnancies may also arise as a result of aberrant expression of imprinted genes in the placental tissue and, given the high propensity for tumours to follow female pregnancies, that the defect might also involve over expression of genes on the X chromosome one of which is normally inactivated in female pregnancies

(Morey and Avner 2010). It may be the presence of two active X chromosomes that is important for tumour development rather than the presence of a paternal X chromosome per se.

Since a greater proportion of choriocarcinoma do follow CHM, they are expected to be predominately female. In an earlier report in which the sex of a variety of trophoblastic tumours was examined, only 5% of choriocarcinoma were male. This would suggest that in the order of 90% of choriocarcinoma follow CHM, a figure much higher than any cited in the literature. To address this we analysed the sex of a series of tumours that were pathologically choriocarcinoma and for which genotyping was performed to determine whether they were gestational or non-gestational tumours. Among 13 tumours that were confirmed to be gestational and to have originated in non-molar pregnancies, only three were male, suggesting there may also be a bias towards an origin in female pregnancies in choriocarcinoma following non-molar pregnancies.

The mechanism by which the small number of tumours deriving in male pregnancies arise remains obscure but the presence of a Y chromosome may be a favourable prognostic indicator in a tumour following a non molar pregnancy. All nineteen patients with a post-mole tumour remain alive and well, as do 10 women with tumours arising in tumours with an antecedent male pregnancy while only 28 of 37 patients with an antecedent female pregnancy remain alive and well.

In conclusion this study has shown that PSTT and ETT can develop in male pregnancies and that a male derived X chromosome is not required for

tumour development. While the study has confirmed that the majority of PSTT, ETT and mixed tumours originate in female pregnancies this is because most develop in non-molar pregnancies that are female rather than because they arise in unrecognised pregnancies with CHM. We have shown that PSTT/ETT may arise in a pregnancy other than the antecedent pregnancy but that this is a rare event and the antecedent pregnancy is usually the causative pregnancy. We provide preliminary data to suggest there is also a predominance of female tumours amongst post-term choriocarcinoma and that the mechanisms leading to malignant development may therefore be similar in different types of GTN. While GTN following non-molar pregnancies is rare, there is a 13.6% per cent chance of GTN following a CHM (Savage et al 2013) and identifying women at risk is important in the clinical management of these women. Epigenetic investigation of tumours arising in non-molar pregnancies may provide insights into the underlying mechanisms that give rise to GTN and provide useful markers to enable earlier identification of those CHM that will progress to GTN.

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