GYNECOLOGIC ONCOLOGY 55, 386–392 (1994)

Detection of Heterozygous XY Complete Hydatidiform Mole by Chromosome *in Situ* Hybridization

ANNIE N. Y. CHEUNG, M.R.C.PATH.,* ANITA S. Y. SIT, B.SC.,† L. P. CHUNG, D.PHIL.,*
HEXTAN Y. S. NGAN, M.R.C.O.G.,‡ KATHERINE O'HANLAN, M.D.,† L. C. WONG, F.R.C.O.G.,‡
AND H. K. MA, F.R.C.O.G.‡

*The Departments of *Pathology and ‡Obstetrics and Gynaecology, University of Hong Kong, Hong Kong; and †The Department of Obstetrics and Gynecology, Stanford University, Stanford, California 94305*

Received January 25, 1994

Complete hydatidiform mole has a substantial risk of developing persistent gestational trophoblastic disease (PTD). Whether heterozygous complete moles, arising from dispermy, have a higher risk of such progression than their homozygous counterparts is controversial. In this study, the frequency of heterozygous XY complete mole in 93 consecutive cases of histologically proven complete moles managed in Hong Kong was assessed by the technique of chromosome *in situ* hybridization (CISH) using DNA probes specific for the short arm of the Y chromosome. The incidence of Y-chromosome positive complete mole in the groups of patients with spontaneous remissions and the group with PTD with or without metastasis was also compared. The presence of Y chromosome was identified in 6 of the 93 cases (6.5%), and this incidence fell within the range reported in the world literature. Of these 93 patients, 5 patients defaulted follow-up, while 10 patients developed PTD, with evidence of metastasis in 2 of them. The presence of Y chromosome was also assessed in another 15 patients with documented metastatic PTD. It was found that CISH signals for Y chromosome were identified in 5.1% (4/78) of complete moles with spontaneous remission and 8% (2/25) with PTD with or without metastasis ($P > 0.05$). Y chromosome was detected in 5.9% (1/17) of the complete moles that developed metastasis and in 5.8% (5/86) of the complete moles that either developed spontaneous remission or developed nonmetastatic PTD ($P > 0.05$). There is no correlation between the presence of Y chromosome and development of persistent gestational trophoblastic disease. © 1994 Academic Press, Inc.

INTRODUCTION

Gestational trophoblastic disease encompasses a heterogeneous group of lesions characterized by an abnormal proliferation of trophoblastic tissue. It includes hydatidiform mole, invasive mole, choriocarcinoma, and placental site trophoblastic tumor. Hydatidiform mole is diagnosed histologically by the presence of hydropic swelling of the chorionic villi and hyperplasia of the trophoblasts. It is associated with a substantial risk of developing persistent gestational trophoblastic disease (PTD). It can be classified into complete and partial mole according to morphologic, cytogenetic, and clinicopathologic characteristics [1,2]. Nearly all complete hydatidiform moles are diploid and androgenetic. Most of the complete moles are monospermic and arise from fertilization of an empty egg by a haploid sperm followed by duplication of the sperm genome [3,4], resulting in a homozygous 46,XX karyotype. The 46,YY karyotype is not viable. It has been estimated that about 10 to 20% of the complete moles are products of dispermy and are genetically heterozygous 46,XX or 46,XY [5–7]. About 4 to 15% of complete moles have been reported to have the 46,XY karyotype [5,6,8–10]. While it was widely believed that complete moles are more likely to develop PTD than partial moles, whether the heterozygous complete mole has a higher malignant potential than its homozygous counterpart remains controversial [5,7,8,11–15].

Differentiation between homozygous and heterozygous complete moles is essential before comparison between the prognosis of each of the two groups of patients can be carried out. Some heterozygous complete moles may be identified if a Y chromosome is present or if Y bodies or Y-chromosome-specific sequences can be demonstrated [5,8,10,15]. Chromosome *in situ* hybridization (CISH) is a technique established in recent years which allows cytogenetic analysis to be applied to archival tumor sections using chromosome-specific DNA probes [16–19]. Because such analyses can be performed on nonmitotic cells, it is generally referred to as "interphase cytogenetics" and has become a powerful tool in the study of

0090-8258/94 $5.00

numerical and structural chromosome aberrations in tumor cells. In this study, we applied this technique of chromosome *in situ* hybridization for the detection of chromosome Y in a local group of patients with complete mole diagnosed and treated in our hospital. The incidence of Y-chromosome positive heterozygous complete moles in our local population and the relative risk of such cases for developing metastatic and nonmetastatic persistent gestational trophoblastic disease were assessed.

MATERIALS AND METHODS

The pathology reports of patients with a diagnosis of complete hydatidiform mole treated at the Queen Mary Hospital, University of Hong Kong, were reviewed. The slides of these cases were retrieved and the histological features reviewed and assessed using generally agreed-upon and accepted diagnostic criteria [1,2]. Ninety-three consecutive cases with definite diagnosis of complete hydatidiform moles and an additional 15 cases of complete moles which subsequently developed metastatic PTD were selected for the study. The tissues included uterine curettings and blocks from hysterectomy specimens, all of which were routinely fixed in 10% formalin and embedded in paraffin wax.

In Situ Hybridization

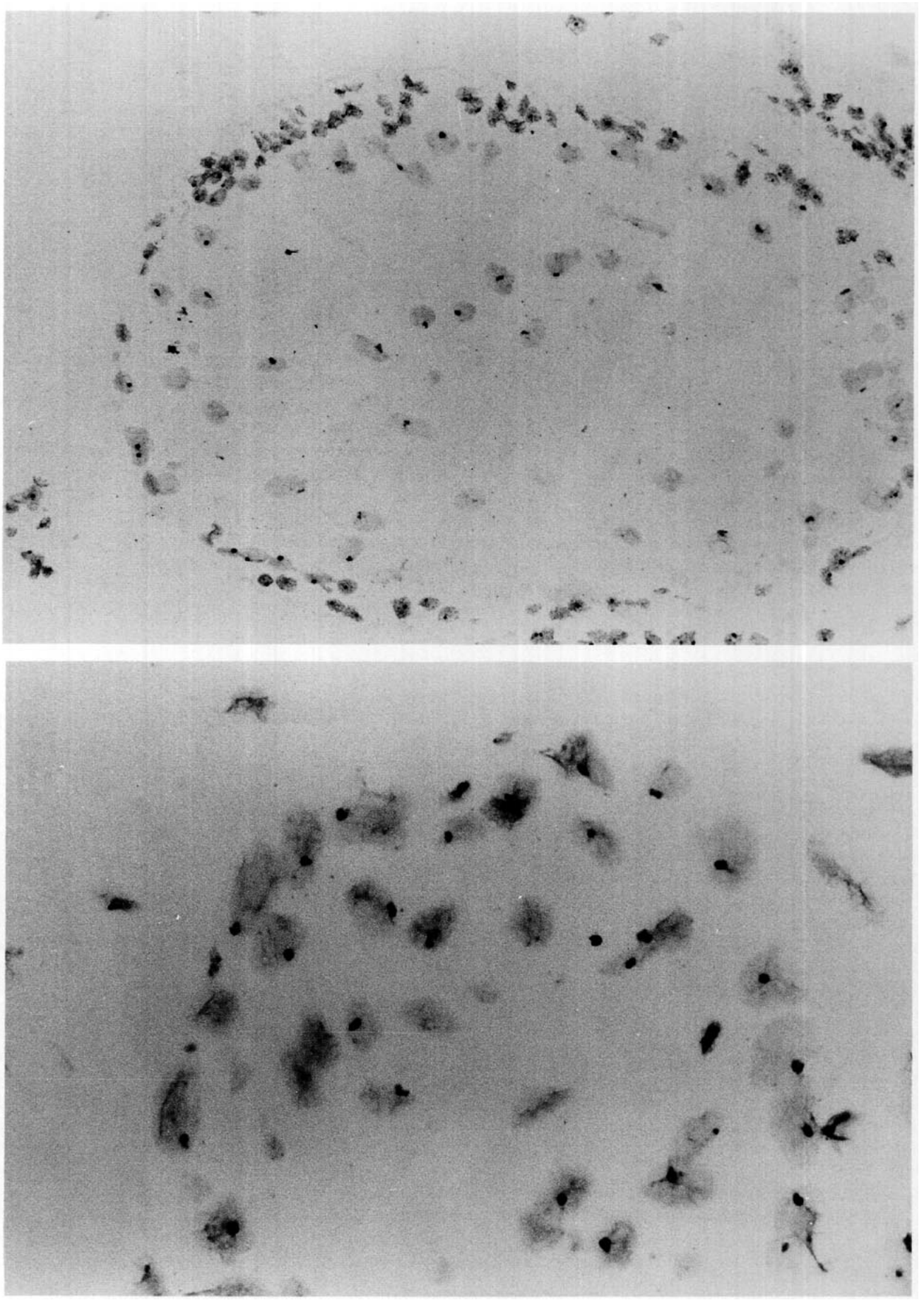
The method adopted was a modification of a protocol used to detect chromosome copy numbers in routinely processed, paraffin-embedded tissue sections by *in situ* hybridization (ISH), which has been described [16,17]. Paraffin sections 5 μm thick were mounted on 2% aminopropyltriethoxysilane (TESPA)-coated slides, dried at 60°C overnight, and deparaffinized. To permeabilize the tissue sections for penetration of the labeled DNA probes and antibodies, the slides were incubated with proteinase K (500 μg/ml) and 0.1% Triton X-100 at 50°C. The incubation time ranged from 10 to 80 min and had to be titrated in each case. The sections were then rinsed, dehydrated, and air-dried. The DNA probes specific for chromosome 16, D16Z3 (American Type Culture Collection, U.S.A., X chromosomes, DXZ1 (Oncor, U.S.A.) or pBamX5 (generous gift of Dr. AHN Hopman), and Y chromosome, DYZ5 (American Tissue Type Culture) were labeled with biotin by nick-translation method (Boehringer, U.S.A.) The labeled probe was then added to the hybridization mix and applied to the tissue sections at probe concentrations of 1 ng/μl hybridization mixture. Denaturation was performed at 90°C (12 min) followed by hybridization overnight in humidified chamber at 37°C overnight. The slides were then rinsed repetitively in 2 × SSC with 50% formamide at 37°C with intermittent agitation. Immunocytochemistry was performed using avidin, biotinylated mouse anti-avidin, rabbit anti-mouse peroxidase, and 3,3′-diaminobenzidine (Dakopatts Ltd., UK) and hydrogen peroxidase to visualize peroxidase activity. The slides were then counterstained briefly in haematoxylin and mounted [18].

Evaluation of ISH Signals

In each specimen, at least 500 nuclei were assessed and the number of chromosome-specific ISH signals was evaluated according to criteria listed in the literature [16,17]. ISH for chromosome Y was performed on all of the 93 cases. For those cases found to be positive for chromosome Y, ISH was repeated using probes for chromosome 16 and X. Heterozygous XY complete mole was diagnosed if the majority of the nuclei showed one hybridization signal specific for chromosome Y (Figs. 1 and 2), two hybridization signals for chromosome 16 (Fig. 3), and one signal for chromosome X (not shown). As Y-chromosome positive controls, paraffin sections of the lymph node of a male patient and a partial mole known to contain a copy of the Y chromosome were used. Maternal decidua was present in most of the tissue sections and acted as the internal control for normal diploid XX cells lacking the Y chromosome (Fig. 4).

CLINICAL FOLLOW-UP

After suction evacuation of the molar pregnancy, patients were monitored in a standard fashion including serial serum and urinary human chorionic gonadotrophin (β-hCG) assays [20]. In our center, persistent gestational trophoblastic disease was suspected when the patient's β-hCG levels remained the same for 4 weekly titers or if there was rising β-hCG levels for 3 consecutive weekly titers when pregnancy was excluded. Patients with suspected persistent gestational trophoblastic disease were evaluated for evidence of metastatic disease. Thorough history taking and physical examination were performed. Investigations include complete blood picture, liver and renal function tests, chest radiograph, pelvic and hepatic sonogram, pelvic and hepatic arteriogram, spinal puncture for β-hCG assay of spinal fluid, and CAT scan of brain if the spinal fluid β-hCG:serum β-hCG is greater than 1:60. If the fall of β-hCG remained unsatisfactory or if it rose, the patient was diagnosed to have PTD and treated with chemotherapy according to the WHO risk score [20]. The clinical records of all the patients with complete moles in this study were reviewed. All of the 17 patients with metastatic PTD had pulmonary metastases. In addition, one had brain metastases, one had liver metastasis, and two had vaginal metastases. Statistical analysis was performed using Fisher's exact test.



FIGS. 1 and 2. Chromosome *in situ* hybridization using biotin-labeled probe for chromosome Y. One hybridization signal was detected in the nuclei of the trophoblasts and stromal cells of a complete hydatidiform mole.

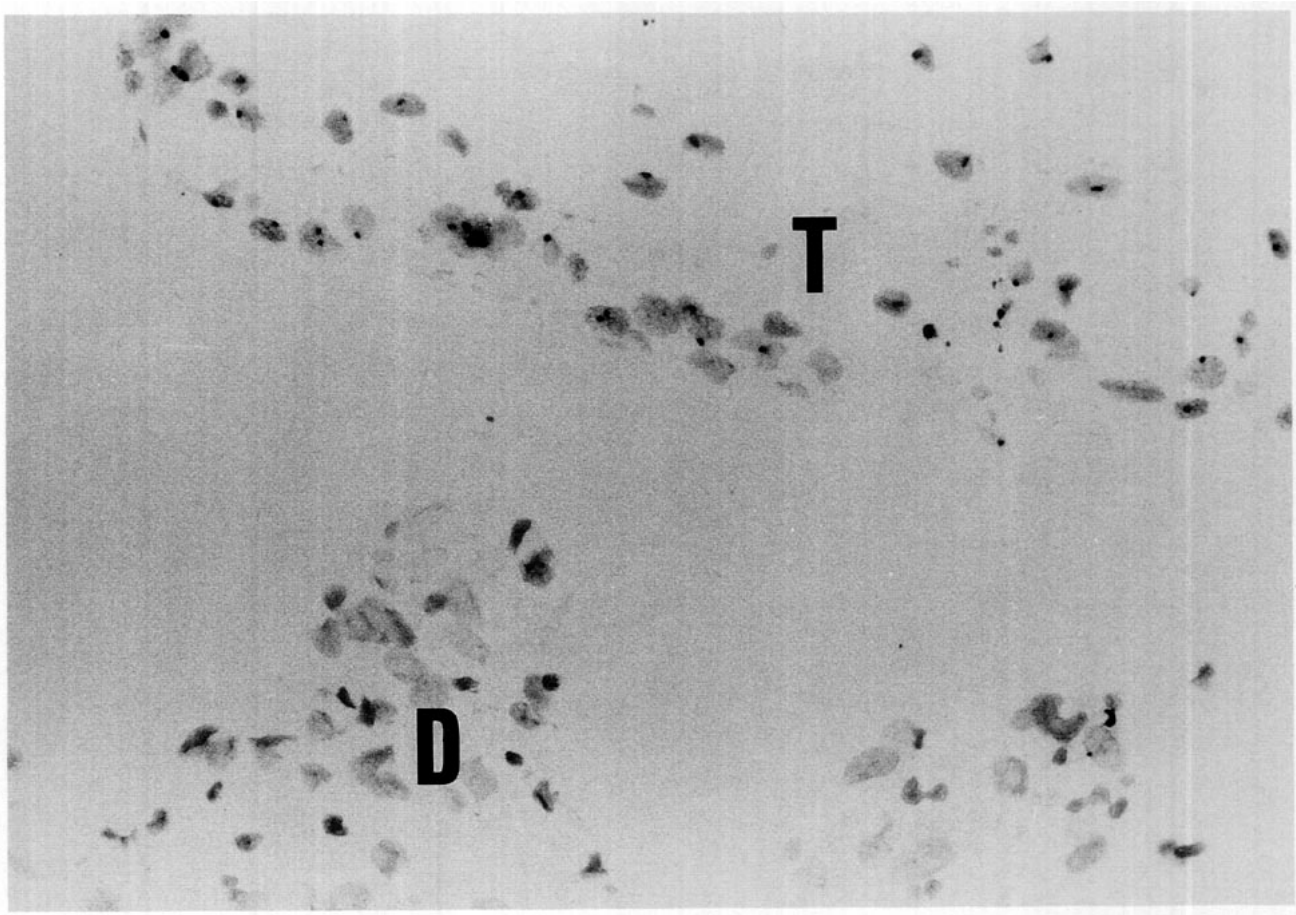


FIG. 3. While one hybridization signal for chromosome Y was evident in the trophoblasts (T), the nuclei of the decidua (D) showed no signals.

RESULTS

The sex chromosome composition of the complete hydatidiform mole included in this study is listed in Tables 1 and 2. Among the 93 cases of complete hydatidiform mole studied, 6 (6.5%) were found to be positive for Y chromosome. Five cases defaulted follow-up, including one case positive for Y chromosome. Ten of the 88 patients (11.4%) with follow-up data developed persistent gestational trophoblastic disease, 2 of which had evidence of metastasis in the lung and liver. Among these 10 patients, 1 was Y-chromosome positive (10%), while in the group of 78 patients without persistent disease, 4 were positive for Y chromosome (5.1%). In the additional 15 complete mole which subsequently develop metastatic PTD, only 1 was found to have Y chromosome. It was found that CISH signals for Y chromosome were identified in 5.1% (4/78) complete moles with spontaneous remission and 8% (2/25) with PTD with or without metastasis ($P > 0.05$). Y chromosome was detected in 5.9% (1/17) of the complete mole who developed metastasis and in 5.8% (5/86) of complete mole who either developed spontaneous remission or developed nonmetastatic PTD ($P > 0.05$).

There was no statistically significant association between the presence of Y chromosome in the group of hydatidiform moles and subsequent development of persistent gestational trophoblastic disease with or without metastasis.

DISCUSSION

In a 1984 retrospective analysis using quinacrine fluorescent staining, Davis *et al.* [8] found an unexpectedly high incidence of Y chromatin in malignant gestational trophoblastic disease. Fourteen of the 19 choriocarcinomas (73%) and 2 of the 4 invasive moles (50%) were found to be positive for Y chromatin, whereas only 9% of the 182 hydatidiform moles contained Y chromatin [8]. This technique is, however, less reliable and should be replaced by *in situ* hybridization methods. Other smaller studies on the genetics of complete moles, invasive moles, and choriocarcinoma also suggested that heterozygous complete moles, which included both 46,XX and 46,XY

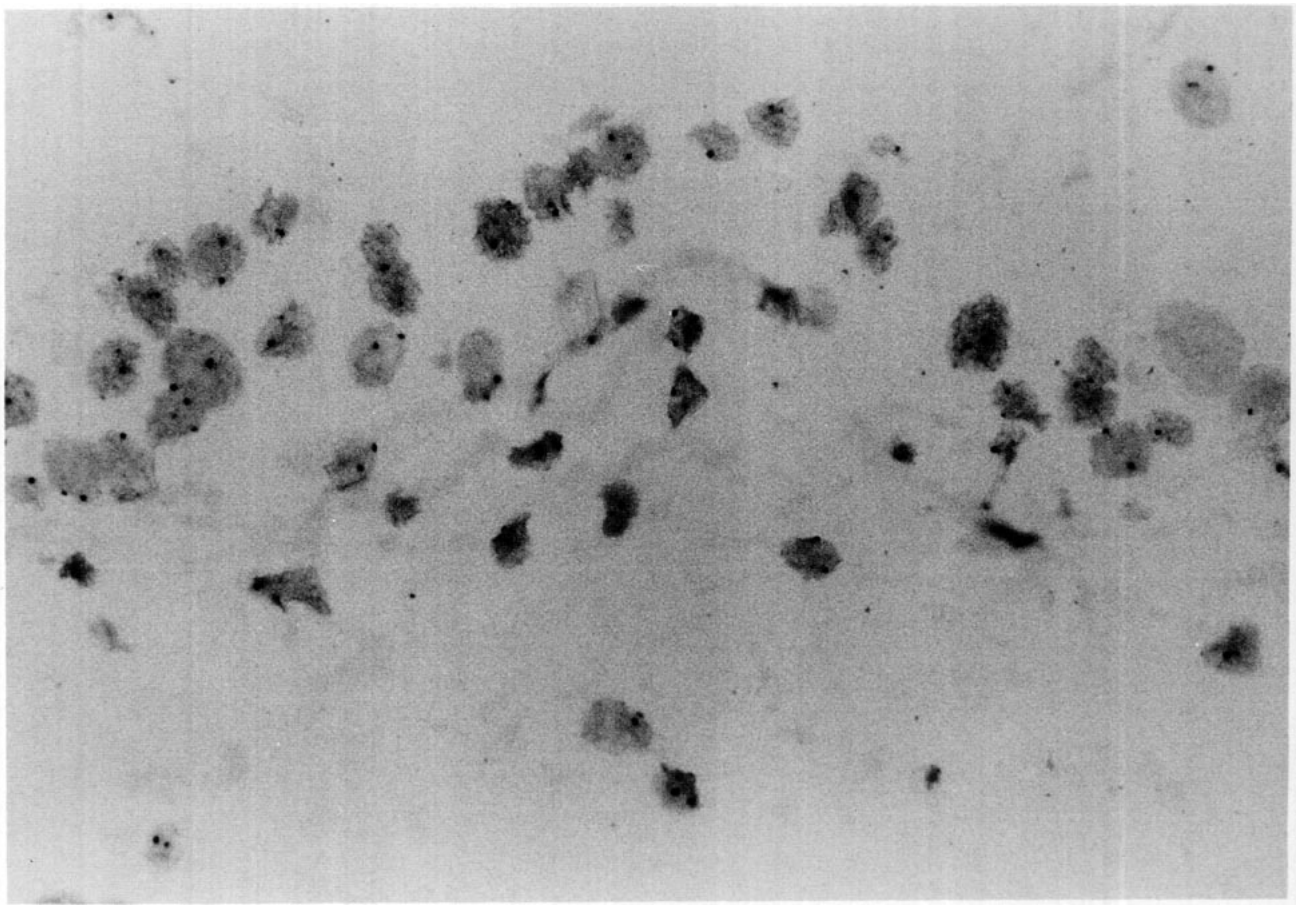


FIG. 4. Two hybridization signals were found in the majority of the nuclei of the trophoblasts and stromal cells of a complete mole as detected by DNA probes for chromosome 16.

genotypes, might have a more malignant potential than their homozygous counterparts [5,8,11,12]. However, this hypothesis was not confirmed by others [7,13–15] and the issue has remained controversial. To determine whether there is a difference in the clinical behavior of homozygous and heterozygous complete mole, it is necessary to distinguish between the two types genetically.

Unless the presence of Y chromosome, Y body, or Y-chromosome-specific sequences can be demonstrated [5,8,10,15], differentiation of a heterozygous 46,XX complete mole from a homozygous mole depends on the analysis of an adequate number of autosomal polymorphisms for which the father is heterozygous. Various methods have been used to investigate the zygosity of hydatidiform moles, including analysis of chromosomal polymorphism [4,21], biochemical polymorphism [4,9], HLA typing [22], and restriction fragment length polymorphism [10,23]. Most of these methods require fresh or frozen tissue and are difficult to apply to archival tissue. Contamination by maternal tissue with the potential problem of misinterpretation is also present in these approaches.

Cytogenetic analysis by chromosome *in situ* hybridi-

TABLE 1
Correlation of Presence of Y Chromosome with Development of Persistent Gestational Trophoblastic Disease (PTD)

	Y chromosome +	Y chromosome −	Total
PTD (metastatic and nonmetastatic)	2 (8%)	23 (92%)	25
Spontaneous remission	4 (5.1%)	74 (94.9%)	78
Total	6	97	103

TABLE 2
Correlation of Presence of Y Chromosome with Development of Metastasis

	Y chromosome +	Y chromosome −	Total
Metastasis +	1 (5.9%)	16 (94.1%)	17
Metastasis −	5 (5.8%)	81 (94.2%)	86
Total	6	97	103

zation has the advantage that it is applicable to archival paraffin-embedded material, making retrospective analysis possible. Histological details are preserved and can help in the evaluation of the signals. The necessity of bringing cells into culture for karyotyping and the problem of getting enough nuclei in metaphase for study can be avoided. Although with ISH the copy number of all chromosomes cannot be determined at the same time, the use of one or two chromosome-specific probes enables estimation of chromosome ploidy with accuracy. Moreover, a large number of nuclei can be evaluated in one histological section. Recently, chromosome *in situ* hybridization has been found to be useful in the study of hydatidiform mole [17,19]. Assessment of different cell types of the chorionic villi, including the villous and extravillous trophoblasts, and villous stromal cells is possible while it also allows discrimination between maternal decidua and trophoblasts. It is considered to be potentially useful in the differential diagnosis of complete mole, partial mole, and abortion with hydropic degeneration [19].

In this study, we identified Y chromosome containing heterozygous complete moles by the technique of *in situ* hybridization using a nonisotopic DNA probe specific for repetitive sequences at the short arm of Y chromosome. Using this technique, 6 of the 93 complete moles (6.5%) were shown to have 46,XY karyotype. This incidence fell within the range of 4 to 15% of complete moles as reported by other investigators [5,6,8–10,15].

Previous attempts to define the relationship between molar zygosity and disease progression have yielded conflicting results. According to the criteria of our center [19], 10 of the 88 patients with follow-up data (11.4%) in this study subsequently developed persistent trophoblastic disease. However, the criteria for defining persistent gestational trophoblastic disease and subsequent administration of chemotherapy vary among centers. The reported frequency of persistent disease after a molar gestation varied from 5 to 30% [20,24–26]. Such incidence is dependent on the sensitivity of the follow-up hCG assay, length of follow-up, type of primary therapy, and terminology used in reporting sequelae. Hence, the employment of persistent gestational trophoblastic disease as the index of clinical outcome was limited by its subjective definition, which varies between different centers. Comparison between different centers is thus difficult. To avoid such complication, documentation of subsequent development of metastasis, which is a more objective assessment of clinical outcome, is also utilized in our study for evaluation of the prognostic significance of Y chromosome in the development of malignant gestational trophoblastic disease. The correlation between heterozygous XY complete mole and progression to persistent trophoblastic disease with or without metastasis was also found to be statistically not significant.

Our study was confined to the assessment of heterozygous complete moles which are Y-chromosome positive, and the significance of heterozygous XX moles was not evaluated. The significance of this study is also limited by the small number of cases with documented metastasis. Application of the technique of CISH to a large number of such cases may help to elucidate the influence of sex chromosome composition in the progression of the disease. However, based on our present data, the largest study using reliable techniques, it does not appear that heterozygosity or the presence of the Y chromosome increases the risk of persistent or metastatic trophoblastic disease.

ACKNOWLEDGMENTS

This study was supported by a Committee on Research and Conference Grant (337-046-0020) from the University of Hong Kong. Miss Anita Suk Yin Sit is sponsored by the Stanford Travelling Scholar Program. We thank Miss Robyn Lukeis and Miss Annie Chan for their technical advice and Mr. Mike So for statistical analysis.

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