Title: A fetus co-existing with a complete hydatidiform mole with trisomy 9 of maternal origin

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ABSTRACT

Complete hydatidiform mole (CHM) co-existing with a viable fetus is a rare finding in pregnancies. The accurate diagnosis often relies in ultrasonographic, histopathological and molecular techniques in the definite diagnosis. To the best of our knowledge, a liveborn fetus co-existing with CHM with trisomy 9 has not been described. The use of molecular genotyping and immunohistochemical laboratory investigations enabled the CHM to be fully characterized. Post-zygotic diploidisation of a triploid conception arising from dispermy is the proposed mechanism of its formation.

KEY WORDS

Obstetrics: Diagnostic Ultrasound and Prenatal Diagnosis; Multiple Gestation; Placental Pathology; Cytogenetics

INTRODUCTION

Complete hydatidiform mole (CHM) co-existing with a viable fetus is a rare finding in pregnancies. The accurate diagnosis often relies in ultrasonographic, histopathological and molecular techniques in the definite diagnosis. To the best of our knowledge, this is the first case report of CHM with trisomy 9 of maternal origin co-existing with a liveborn infant. The use of molecular genotyping, immunohistochemical investigations and bioinformatics enabled the origin of the complex genomic findings to be determined and the mechanism of its formation proposed.

CASE REPORT

This is the first spontaneous pregnancy of a 32 year old Chinese woman. She underwent first trimester combined Down syndrome screening at 12 weeks which showed low risk (1 in 13354). Maternal serum free β -human chorionic gonadotrophin (β -hCG) level was 51.37 IU/L at 1.0555 Multiples of Median (MoM), pregnancy-associated plasma protein A (PAPP-A) was 5.66 IU/L at 1.5307 MoM. Morphology scan was performed at 19 weeks which showed normal growth parameters and no structural anomalies in the fetus. However, the placenta showed a large focal vesicular change and scattered vesicular change in other parts of the placenta. Maternal blood was sent for second trimester

biochemical screening. The alpha fetal protein (AFP) level was 143.1 ng/mL (2.09 MoM), hCG level was 78.1 IU/ml (2.86 MoM). Chorionic villus sampling and amniocentesis was performed at 20 weeks for rapid aneuploidy detection by QF-PCR (quantitative fluorescent polymerase chain reaction), chromosome study and uniparental disomy testing. Part of the chorionic villi (CV) was sent for histopathology which showed no features of molar pregnancy. Chromosome study of cultured CV and AF samples showed normal female karyotype 46,XX and biparental inheritance. The hCG level was repeated during pregnancy at 22 weeks (61170 IU/L), 28 weeks (47768 IU/L) and at 35 weeks (60950 IU/L). Emergency lower segment Caesarean section was performed for antepartum haemorrhage, breech presentation and mild pre-eclampsia at 35 weeks. A female baby of 2.13 kg with Apgar score 8 at 1 minute and 10 at 5 minutes was delivered. The operative blood loss was 900 ml. Newborn examination was normal. The placenta weighed 1150g and appeared bulky, with a large part of it formed by multiple vesicles (Figure 1). Placental tissue was collected from the vesicular part of the placenta for histopathology and molecular studies to correlate with the prenatal investigation results. Serial hCG monitoring showed hCG <0.5 IU/L at 3 months after delivery and she remained well. The baby had normal growth and development when

last assessed upon 5 years of age.

QF-PCR study was performed on parental blood and various tissues collected from the pregnancy (Figure 2). Cultured CV and uncultured amniocytes showed normal 13, 18, 21, XX. The cultured placental villi (CHM) showed trisomy 9, XY, with a different allelic pattern compared to cultured CV and uncultured amniocytes (the latter likely representing the normal fetus). Only paternal alleles were detected in the CHM, except for the trisomy of chromosome 9, where a maternal allele was also present. Uncultured CV and uncultured placental villi showed a mosaic pattern of normal XX and paternally derived XY cells with XY cells present at ~50% of XX.

Histopathologic examination of placenta showed two populations of enlarged abnormal chorionic villi against a background of normal chorionic villi (Figure 1). One population of enlarged villi showed hydropic change and circumferential trophoblastic proliferation, morphologically resembling CHM, with immunohistochemical staining for p57 being negative in both cytotrophoblasts and villous stromal cells. The other population of enlarged villi showed thick-walled vessels and no trophoblastic proliferation, morphologically resembling placental mesenchymal dysplasia, where p57 immunostaining exhibited “discordant” pattern with positive cytotrophoblasts and

negative villous stromal cells.

The cultured placental villi (CHM) containing the additional maternal chromosome 9 and the cultured CV (fetus) were then investigated using Illumina HumanCytoSNP-12 v2.1 SNP microarray (Illumina Inc., San Diego, CA, USA). The microarray data generated from the CHM demonstrated trisomy 9 (Figure 3), while the SNP genotyping data were consistent with a dispermic origin for the CHM. Approximately 37% of the CHM genome demonstrated large regions of homozygosity, rather than 100%, as is seen in CHM that arises following endoreduplication of a single 23,X sperm. In order to investigate the relationship between the CHM and the biparental fetal cell line, the microarray genotyping data were then used in a SNPduo analysis¹. SNPduo enables the relatedness of two individuals to be determined using ‘identity by state’, which is the sharing of alleles by two individuals. This analysis showed an identical haploid set of chromosomes was shared between the CHM and the fetal cell line; meaning the paternal haploid set of chromosomes carried by the fetus were identical to one of the paternal haploid sets of chromosomes seen in the CHM. Additionally, the data were also consistent with the maternal chromosome 9 being identical in both the fetus and the CHM (Figure 3).

DISCUSSION

This is the first report of a liveborn infant co-existing with a CHM with trisomy 9. The diagnosis was not suspected until morphology scan was performed at 19 weeks of gestation. The maternal serum markers were not particularly indicative of a co-existing CHM with absence of high hCG and PAPP-A², although the predictive value of these markers remains unclear. Partial hydatidiform mole (PHM) was unlikely in view of normal sonographic fetal parameters and structures and normal karyotype upon amniocentesis. There are no data to suggest phenotypic abnormalities in a partial mole. Pregnancies in which a fetus coexisted with CHM were reported to have an increased risk of adverse obstetric and perinatal outcome. Symptoms can include bleeding which can be chronic and severe, with increased fetal loss. There is overall less than a 50% chance of live term birth and 20% early onset pre-eclampsia². More recent review of reported cases by Rohilla³ and Lin⁴ showed antepartum haemorrhage being the most common medical complication and leading to pregnancy termination. Our patient remained well until 35 weeks when delivery was warranted by antepartum haemorrhage and mild pre-eclampsia.

Morphologic assessment of hydatidiform mole could be a challenge in some cases even for experienced pathologists in which high interobserver and intraobserver variability exist⁵. Nonmolar entities such as chromosomal trisomy syndromes were also reported to exhibit some histologic features of molar pregnancy⁶. Trophoblastic hyperplasia of similar extent as that seen in molar pregnancy was found to be more frequent in trisomy 7⁷. Hence ancillary techniques such as p57 immunohistochemistry and molecular genotyping using PCR for microsatellites have been widely adopted as diagnostic adjuncts. The major challenge in interpretation of this case lies in the heterogeneity of chorionic villi. Apart from the chorionic villi resembling CHM, the coexisting population of enlarged villi with morphologic resemblance of placental mesenchymal dysplasia and “discordant” p57 immunostaining pattern are recognized features of androgenetic/biparental mosaic/chimeric conceptions⁸. An accurate diagnosis thus depends on careful correlation of histology with p57 immunohistochemistry, in the light of clinical and karyotypic information.

The CV collected at 19 weeks and placental sampling post-delivery consisted of a mixture of cells, hence giving rise to a ‘mosaic’ molecular genotyping result on the uncultured cells from these two samples. Cultured mesenchymal cells in CV were

representative of the fetus i.e. 46,XX, while cultured placental villi showed 47,XY,+9 which represented the CHM.

There are few reports of CHM described in association with autosomal trisomy. Three reports describe an additional chromosome 11 of maternal origin (one also retaining an additional maternal chromosome 6), which lead to aberrant p57 staining⁹⁻¹¹, while another describes trisomy 13 where the extra copy of chromosome 13 was paternal¹².

An aberrant or ambiguous p57 staining pattern not in agreement with a histomorphological diagnosis of CHM led to further investigations on each of these samples. Significantly, all three cases of CHM with a retained maternal chromosome arose from dispermy. Our own case is similar; the extra copy of chromosome 9 is maternal in origin and both microsatellite and SNP genotyping data are consistent with a dispermic origin of the CHM. The occurrence of postzygotic diploidization of triploids and karyotype instability and its cytogenetic implications in the formation of hydatidiform mole had been described by Golubovsky¹³, Sunde¹⁴ and Candelier¹⁵. Thus, the most likely mechanism to explain this complex result is proposed as dispermic fertilization of a single oocyte followed by abnormal division of the zygote with endoreduplication of one paternal pronucleus leading to post-zygotic diploidisation¹⁴.

This means the CHM and biparental (fetal) cell lines share one identical haploid set of paternal chromosomes. In our case, SNPduo analysis together with the microsatellite marker analysis also suggests the retained maternal chromosome 9 is identical in both the CHM and the fetal sample. In summary, the normal fetus developed from cells being euploid with one set of maternal and one set of paternal haploid chromosomes. The CHM developed from cells with two different sets of paternal haploid chromosomes (one set being identical to that in the fetus) and an additional copy of chromosome 9 of maternal origin.

This case study demonstrates the power of molecular biology and bioinformatics in determining the origin of complex genomic findings. Our data provide additional evidence for the post-zygotic diploidisation of triploid conceptions, with sharing of identical paternal haploid genomes in the biparental and CHM cell lines. Retention of a maternal chromosome in CHMs appears to be associated exclusively with a dispermic origin of the CHM and post-zygotic diploidisation. While this occurrence is rare and very few cases have been reported in the literature to date, thorough genetic evaluation of CHM co-existing with live fetus is warranted to expand the knowledge and understanding of this condition.

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DISCLOSURE

The authors declare no potential conflicts of interest.

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FIGURE LEGENDS

Figure 1. Left panel: Gross appearance of the placenta displaying multiple vesicles against a background of normal-appearing placental parenchyma. Middle panel: (H&E, magnification 20×) Histopathology examination of placenta shows two types of abnormal villi, one type showing thick-walled vessels and resembling placental mesenchymal dysplasia (indicated by arrow), while the other type showing hydropic villous stroma typical of complete mole (indicated by asterisk), coexisting with normal chorionic villi. Right panel: Immunohistochemical staining for p57 demonstrates scattered positive cytotrophoblasts in the chorionic villi resembling placental mesenchymal dysplasia (indicated by arrows), with completely negative staining in those villi resembling complete mole (indicated by asterisk).

Figure 2. Quantitative fluorescent polymerase chain reaction (QF-PCR) study on parental blood (i,ii) and various tissues of the pregnancy (iii to v) using short tandem repeat (STR) markers on chromosomes 21, 18, 13, 9, X and Y. The small panels showed the capillary electrophoresis data of 10 of the STR markers used. Small letter above the allelic peak represents maternal allele (red); paternal allele (blue); or shared maternal or paternal allele (black). The bottom panel indicates the name of the STR markers used. Results from cultured CV, same as uncultured amniocytes (iv), showed normal 13, 18, 21, XX. Results from cultured placenta (v) showed paternally derived 13, 18, 21, XY and trisomy 9. Results of uncultured CV, same as uncultured placenta (iii) showed a mosaic pattern of (iv) and (v). CV= chorionic villi.

Figure 3. Left panel: Illumina HumanCytoSNP-12 microarray on cultured placental tissue (CHM) showing trisomy 9. Right panel: SNPduo analysis comparing SNP microarray genotyping data generated from cultured placental tissue (CHM) in middle panel and cultured chorionic villi (fetus) in lower panel. The data are consistent with the biparental fetal cell line containing one paternal and one maternal chromosome 9 that is identical to that carried by the CHM. The dispermic CHM also carries a segment of chromosome 9 (paternal) that is not present in the fetal cell line (identity by state = 1).

Figure 1 20171026.tif

Figure 2 20171026.tif

Figure 3 20171026.tif

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