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Paternal Hemizygoty in 11p15 in Mole-like Conceptuses

Two Case Reports

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Abstract: Hydatidiform mole is an abnormal human pregnancy characterized by the fetus being absent or nonviable, and the chorionic villi being vesicular and with trophoblastic hyperplasia. Most often, the mole phenotype is seen in conceptuses with an excess of paternally inherited genome set(s) relative to maternally inherited genome set(s), suggesting that the phenotype is caused by an excess of genome with a paternal imprinting pattern. However, it is unknown if correct parental origin of every imprinted gene is crucial for normal early differentiation or if abnormal parental imprinting of only one, or some, gene(s) can cause the mole phenotype.

Two conceptuses included in the Danish Mole Project stood out since they presented with vesicular chorionic villi and without signs of fetal differentiation, and had apparently biparental diploid genomes, and no mutations in *NLRP7* or *KHDC3L* were detected in the mothers. These conceptuses were subjected to a centralized histopathological revision and their genetic complements were scrutinized using fluorescence in situ hybridization, and DNA-marker and array comparative genomic hybridization analyses. Both conceptuses showed dysmorphic chorionic villi with some similarities to hydatidiform moles; however, no definite fetal trophoblast hyperplasia was observed. Both conceptuses showed paternal hemizygoty of 11pter-11p15.4, most likely in nonmosaic state.

Our findings suggest that the product of one (or a few) maternally expressed gene(s) on the tip of chromosome 11 is necessary for normal early embryonic differentiation. However, since the present two cases did not exhibit all features of hydatidiform moles, it is likely that abnormal

parental imprinting of genes in other regions contribute to the phenotype of a hydatidiform mole.

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Abbreviations: Array CGH = array comparative genomic hybridization, BWS = Beckwith–Wiedemann syndrome, FISH = fluorescence in situ hybridization, HM = hydatidiform mole, PM = paternal–maternal, PMD = placental mesenchymal dysplasia, PP = paternal–paternal, PPM = paternal–paternal–maternal, SNP = single-nucleotide polymorphism, UPD = uniparental disomy.

INTRODUCTION

Hydatidiform mole (HM) is an abnormal human pregnancy, characterized by vesicular swelling of the chorionic villi and hyperplasia of the trophoblastic layer. Most HMs are either diploid androgenetic (paternal–paternal [PP]), or triploid, with two chromosome sets originating from the father and one from the mother (paternal–paternal–maternal [PPM]). However, rare diploid HMs with biparental genomic markers (paternal–maternal [PM]) have been reported.^{1–6} Some of these rare conceptuses seem to have a paternal epigenotype on the maternal allele of one or more imprinted genes, due to mutation in both alleles of *NLRP7* or *KHDC3L* in the mother.^{7–10} In other cases, diploid moles with biparental genetic markers are mosaics, one cell line being diploid androgenetic and the other diploid biparental (PP/PM).^{11–13} PP/PM mosaicism is often associated with placental mesenchymal dysplasia (PMD), where the placenta may be enlarged and cystic, but without trophoblastic hyperplasia.^{14–16} Due to the morphologic similarities between HM and PMD, the latter has also been named "pseudopartial mole".¹⁷ In PP/PM mosaics, the abnormal phenotype is likely to be caused by the androgenetic cell line. Thus, it seems that a mole/a mole-like phenotype is seen in conceptuses with an excess of genome with a paternal imprinting pattern, relative to genome with maternal imprinting pattern.

However, it is possible that the correct imprinting pattern of every imprinted gene is not crucial for normal early differentiation, and a molar or mole-like phenotype may be caused by abnormal imprinting pattern of only one, or some, genes. No such genes have yet been identified, although some well defined syndromes in liveborn individuals are caused by abnormalities restricted to one or a few imprinted genes. For instance, with Wiedemann syndrome (BWS), in which the placenta often display PMD,¹⁸ is most often related to reduced function of one, or more, maternally expressed genes in 11p15.5.¹⁹

Since 1986, we have collected samples in the Danish Mole Project from placentas suspected of being HMs, because of vesicular chorionic villi visible to the naked eye. On histological review, most of these have been classified as HMs, whereas none of these has hitherto been classified as PMD.²⁰

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TABLE 1. Two cases of conceptuses with paternal hemizygoty of 11p15.4-pter: Family and fertility histories, clinical presentation, and morphological observations

	Case 101	Case 635
Family and fertility history, apart from present pregnancy	The parents had 3 children, 4 miscarriages, and 1 stillbirth wk 30 with a very small fetus. See pedigree (Figure 1).	The parents had 1 child and no miscarriages. Family history inconspicuous.
Ultrasound	ULS week 15 + 6, due to previous miscarriages: "Gestation sac 3 cm. Scattered echoes. No fetus."	Routine ULS week 12: "Suspicion of mole, complete HM or partial HM"
Termination	Week 16 + 1 (1986)	Week 13 + 1 (2001)
Se-hCG at diagnosis (time to se-hCG undetectable)	Not measured (19 days)	6379 IU/mL (35 days)
Macroscopic inspection of unfixed evacuated tissue	Gynaecologist: "Very suspect of mole. No fetal parts"	Gynaecologist: "Mole. No fetal parts"
Local histopathologist	Sample forwarded for Danish Mole Project: Vesicles ≤ 4 mm Partial HM/non-HM? Hydropic villi without vessels. Some fjords. No distinct trophoblastic hyperplasia. No nucleated RBC	Sample forwarded for Danish Mole Project: Vesicles 0.5–5 mm Complete HM Large, round, edematous villi. Some cisterns, some trophoblast inclusions. No fjords. Slight trophoblastic hyperplasia, multifocally circumferential. Villous vessels without nucleated RBC
Histopathological review 2003	Partial (complete?) HM Small edematous/vesicular villi. Focal, slight trophoblastic hyperplasia. Few invaginations. No nuclear debris. No fetal parts	Partial HM Small edematous/vesicular villi Almost no trophoblastic hyperplasia. Few invaginations. No nuclear debris. No fetal parts
Centralized histopathological review 2014	Nonmolar conception Focally hydropic and scattered irregular dentate villi Trophoblastic inclusions Some circumferential trophoblastic sprouts, but no definite florid abnormal trophoblast hyperplasia	Nonmolar conception Hydropic villi with few cisterns Scattered dentate villi and inclusions Occasional empty vessels Some circumferential trophoblastic sprouts, but no definite florid abnormal trophoblast hyperplasia

HM = hydatidiform mole, RBC = red blood cells, ULS = ultrasound scanning.

We here report the presence of a deletion on the maternal chromosome 11 affecting the region 11pter-11p15.4 in two unrelated diploid biparental conceptuses without a fetus and with dysmorphic chorionic villi with some phenotypic similarities to HMs. This raises the possibility that one (or a few) gene(s) crucial for normal early embryonic differentiation is located within this region.

MATERIALS AND METHODS

In the Danish Mole Project, unfixed samples from conceptuses presenting with vesicular villi on ultrasound or by visual inspection of aborted tissue, are collected consecutively. At the genetic laboratory, the samples are inspected macroscopically, and only samples with at least 10 vesicular chorionic villi, with diameters >1 mm, are included. In the period April 1986 to June 2003, samples from 270 conceptuses were received and classified as HMs by a centralized histopathologic review.²⁰

Of the 270 conceptuses, 162 were diploid. In 1 of the diploid conceptuses, the parental origin could not be determined. 150 cases were androgenetic (PP), including 1 case (case 497), which, in the original study, was erroneously classified as diploid biparental due to contamination with maternal DNA (data not shown),²⁰ and 8 cases that were part of multiple pregnancies comprising one or two normal pregnancies and a diploid androgenetic HM.²¹ In the remaining 11 diploid cases, genetic markers from both parents were demonstrated; none of these cases were part of a multiple pregnancy. However, 8 were mosaics with two cell lines, one androgenetic and one biparental (PP/PM). In the last 3 of these 11 cases, one biparental (PM) cell line, only, was identified.¹² One of these 3 cases (case 131) was part of an extensively studied family in which the mother carries mutations in both alleles of *NLRP7*.^{5,22–24}

The remaining two diploid cases with one biparental cell line (cases 101 and 635) seemed "unexplained", with no

mutations detected in *NLRP7* or *KDHC3L*.²⁴ Details about these two cases are given in Table 1.

Clinical data were obtained from a questionnaire filled in by the parents of the conceptuses, and from the histopathological and medical records.

Data on the morphology were retrieved from the original pathology reports made by the local pathologists, and from the centralised review performed previously ("review 2003").²⁰ A renewed centralized revision was made by three of us (HL, AG, and NS). Histopathological evaluation was performed on hematoxylin and eosin-stained sections. Immunohistochemical stainings were performed on representative formalin-fixed paraffin-embedded tissue sections using a mouse monoclonal antibody directed against the p57^{KIP2} protein, (clone 25B2 at 1:100; Novocastra). The presence of distinct nuclear staining was evaluated in the villous stromal cells and the cytotrophoblasts.

Ploidy was determined by karyotyping of uncultured and/or cultured cells, and/or by measurement of the nuclear DNA contents by flow cytometry of unfixed nuclei, using chicken and trout erythrocytes (that both are nucleated and in diploid state have quantities of DNA per nucleus that are different from the quantity of DNA in a human diploid cell) as controls (DNA-ploidy).²⁵

DNA was prepared from parental leukocytes using standard techniques. DNA from conceptuses was prepared by digesting unfixed vesicular villi in lysis buffer (tris(hydroxymethyl)aminomethane hydrochloride 10mM, ethylenediaminetetraacetic

acid 1 mM, NaCl 150mM, sodium dodecyl sulfate 0.5%, pH 10.5) containing proteinase K (1 mg/mL) for 2 hours at 56°C. DNA was subsequently extracted with phenol and chloroform, precipitated with sodium acetate and ethanol, and resuspended. Analysis of the parental origin of the genome of the conceptuses using a multiplexed analysis of 16 microsatellite markers (AmpFISTR) was performed according to the manufacturer's guidelines. 10 ng template DNA was used.

Global DNA array analysis was carried out using the Genome-Wide Human SNP Array 6.0 (SNP6) (Affymetrix, Santa Clara, CA). In brief, 500 ng genomic DNA was hybridized to the array using the Human mapping SNP6.0 assay kit and following the manufacturer's protocol. Staining and washing of the arrays were carried out on a Fluidics Station 450 (Affymetrix) and the arrays were then scanned with a GeneChip scanner 3000 7G system (Affymetrix). Genotyping was performed with the Geno-typing Console V4.1 software (Affymetrix) using the Birdseed V2 algorithm, including an extra 30 reference samples to improve the accuracy of genotype calls. Genotyping call rate were above 98.5% for all samples. The copy number analysis was performed using the SNP6 array data and the software Partek Genomics Suite (MO) V6.11 using the copy number baseline files generated from 270 SNP6 HapMap samples distributed by Partek. All files and genomic positions are from the hg19 version of the human genome. Genotypes from 640 SNPs from the 3.5 Mb 11pter-11p15.4 region were extracted in Genotyping Console identifying the parental origin of the

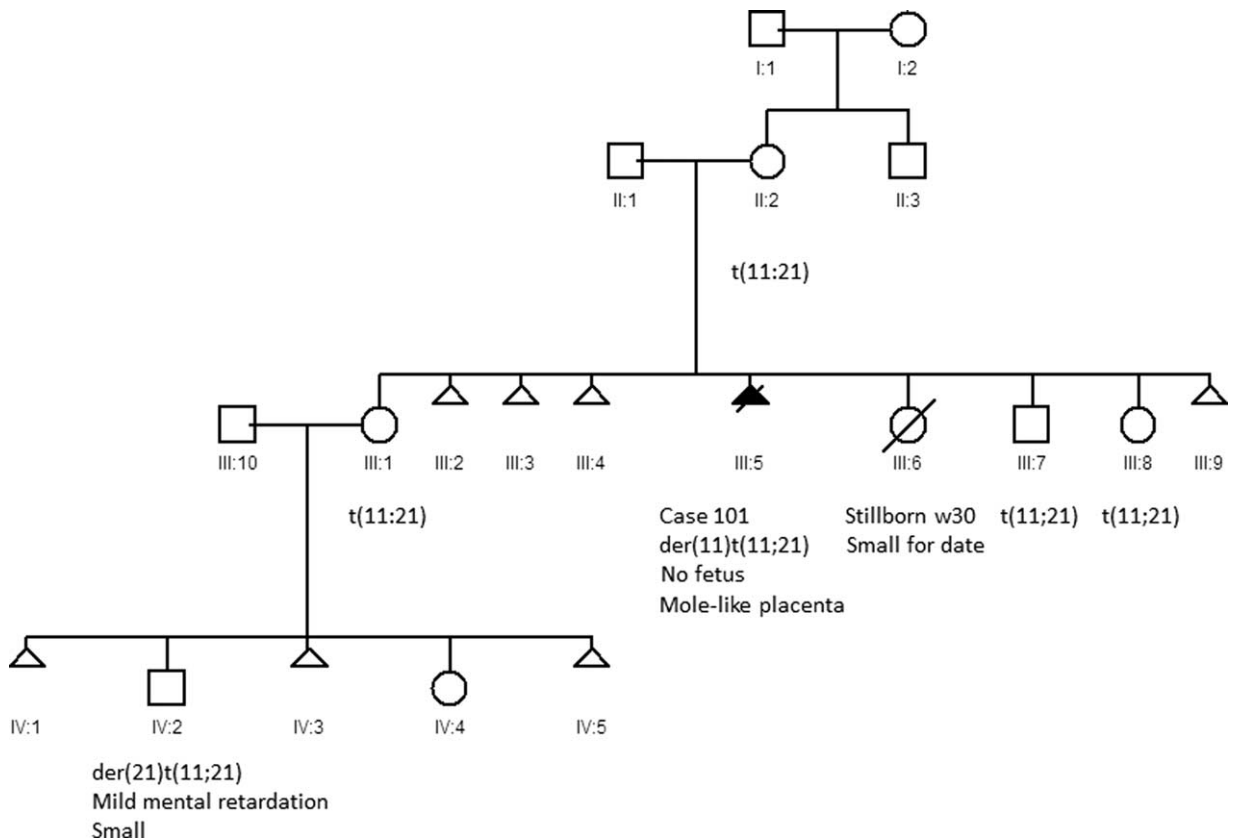


FIGURE 1. Pedigree of the family of case 101. Case 101 (III:5) that had the karyotype 46,XY,der(11)t(11;21)(p15.4;q22.3)mat was included in the Danish Mole Project as vesicular chorionic villi were observed. No information about the morphology of the other miscarriages of II:2 and III:1 was available. IV:2 has the karyotype 46,XY,der(21)t(11;21)(p15.4;q22.3)mat and has mild developmental delay and mild dysmorphic features. IV:4 has a normal karyotype.

deleted region in each of the two cases using the SNPTrio workflow in Partek. After filtering all non-informative SNPs in each trio, 40 informative SNPs remained in the 101 trio and 32 informative SNPs remained in the 635 trio in the region of interest.

Structural chromosome abnormalities were explored using fluorescence in situ hybridization (FISH) with probes specific for the centromeres and telomeric regions of the short arms of chromosomes 11 and 12.

The regional Committee on Biomedical Research Ethics approved the study. All participants gave informed consent.

RESULTS

Two independent miscarriages - case 101 and case 635 - were included in the Danish Mole Project, because vesicular chorionic villi were observed. In both cases, previous histopathologic examinations had classified the conceptuses as HMs, and in both cases we identified biparental diploidy and an

unbalanced translocation causing paternal hemizyosity in chromosome 11pter-11p15.4.

Details on the fertility and the family histories of the parents, the clinical presentations, and the morphologic observations are given in Table 1 and Figure 1.

Centralized Histopathological Review, 2014

At a renewed centralized revision, both pregnancy products showed morphologically abnormal, dysmorphic, chorionic villi with scattered dentate villi and inclusions. Hydropic changes and/or few well formed cisterns were seen as well. Empty vessels were noted, but no nucleated fetal red cells. There was no definite florid abnormal trophoblast hyperplasia (Fig. 2). In both cases, immunohistochemical analyses of p57^{KIP2}, which, in cytotrophoblasts and villous stromal cells, is normally expressed from the maternal allele of the gene *CDKN1C* located in 11p15.5, were negative in these cells. Although some of the observed features are seen in HMs,

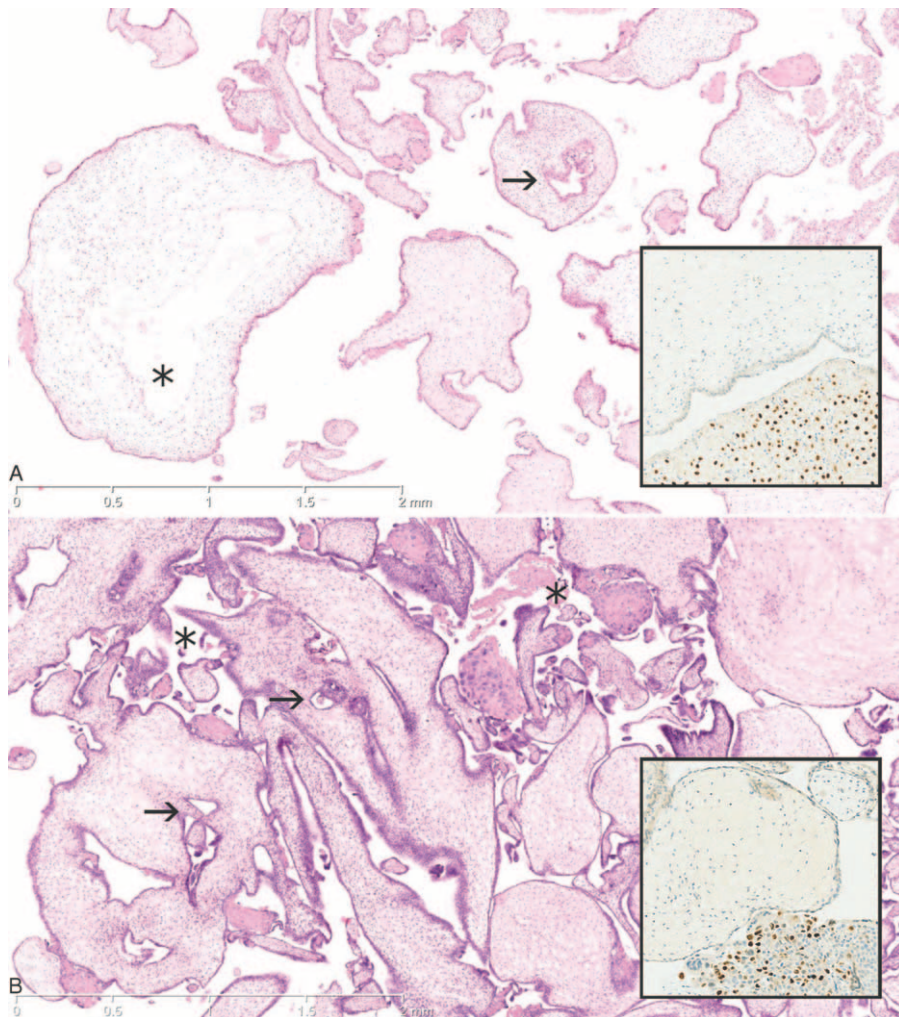


FIGURE 2. Photomicrographs of the two pregnancy products with morphologically abnormal chorionic villi (cases 101 and 635). A, Case 101 showing focal hydrops and a few well formed cisterns (*) and inclusions (→). B, Case 635 showing scattered dentate villi (*) with inclusions (→). In both cases, there was no definite trophoblastic hyperplasia, and immunohistochemical staining for p57^{KIP2} (insets) was negative in both cytotrophoblasts and villous stromal cells. Maternal decidua and intermediate trophoblast served as positive internal controls. Original magnifications: x25 for photos and 100× for insets.

TABLE 2. Two cases of paternal hemizyosity of 11pter-11p15.4: Genetic observations in the conceptuses and the parents

	Karyotype	Microsatellite Analysis (AmpFISTR)*	SNP, Copy Number Analysis	FISH	Conclusion
Case 101					
Conceptus	Diploidy [†]	TH01: 173 [‡]	Del(11)(pter-3.3Mb) Dup(21)(45.5 Mb-qter)	NA	46,XY,der(11)t(11;21)(p15.4;q22.3)mat
Mother	46,XX	TH01: 177/185	Normal	t(11;21)	46,XX,t(11;21)
Father	46,XY	TH01: 173/187	Normal		46,XY
Case 635					
Conceptus	46,XY	TH01: 177 vWA: 176/180/180	Del(11) (pter-3.5 Mb) Del(11) (pter-3.5 Mb)		46,XY,der(11)t(11;12)(p15.4;p13.31)mat
Mother	46,XX	TH01:173/185 vWA: 180	Normal	Normal	46,XX
Father	46,XY	TH01: 173/177 vWA: 176	Normal	Normal	46,XY

Two Cases of Paternal Hemizyosity of 11pter-11p15.4. FISH = fluorescence in situ hybridisation, SNP = single-nucleotide polymorphism.

*Only results indicating deviation from diploid, biparental genome are given.[‡]Determined by flow cytometric quantification of the nuclear DNA contents.[†]Further the alleles observed in the Amelogenin locus indicated the presence of both X and Y chromosomes.

the lack of definitely abnormal trophoblast proliferation precluded the diagnosis HM. As the few vessels noted were inconspicuous, there was no suspicion of PMD.

Genetic Analyses

Table 2 summarizes the results of the genetic analyses. In both conceptuses, analyses of ploidy and parental origin of the genome suggested diploidy, with a normal biparental origin of the genome. However, analyses of 16 microsatellite markers (AmpFISTR) indicated that there were not balanced biparental contributions to all parts of the genome: In both cases, there was no maternal allele for the locus *THO1* (11p.15.5), and in case 635, an exceptionally high peak representing the maternal allele was observed for the locus *vWA* (12p13.32).

Genome-wide SNP6 analysis of case 101 confirmed the deletion on the maternally inherited chromosome 11 (Fig. 3). The breakpoint was mapped to cytoband 11p15.4, close to the border to 11p15.5, at genomic position 3.3 Mb (hg19) (between *MRGPRE* and *ZNF195*). In addition, a maternal gain was identified at chromosome 21q22.3-qter, with a breakpoint at genome position 45.5 Mb (hg19), suggesting that this conceptus had a maternal derivative chromosome 11, resulting from a translocation between 11p15.4 and 21q22.3.

In case 635, the predicted deletion on the maternal chromosome 11 and the gain of maternal chromosome 12 were also confirmed by SNP analysis. The breakpoints were mapped to cytoband 11p15.4 at genome positions 3.5 Mb (hg 19) (between *ZNF195* and *ART5*), and at cytoband 12p13.31 at genome position 8.4 Mb (hg19) (between *FAM90A1* and *LOC389634*), respectively. For both conceptuses, the paternal hemizyosity in 11p15 was confirmed using all informative SNPs in the region. In the two cases, the breakpoints on chromosome 11p15 were remarkably similar, but not identical, with the deletion in case 101 being the smallest. The minimal chromosome region deleted in both conceptuses encompasses 105 genes including at least 11 genes that are subject to genomic imprinting (See Table, Supplemental Digital Content 1, <http://links.lww.com/MD/A478>).

In the mother of case 101, FISH with probes specific for the centromere and p-telomere of chromosome 11 confirmed a

reciprocal translocation between chromosomes 11 and 21. FISH with probes for the centromeres and the p-telomeres of chromosomes 11 and 12 disclosed no cytogenetic abnormalities in the parents of case 635 (Fig. 4).

In none of the two conceptuses did we observe signs of mosaicism, neither by analyses of microsatellite markers nor in the SNP analysis.

DISCUSSION

A HM is a conceptus defined by its phenotype hallmarked by vesicular swelling of the chorionic villi and trophoblastic hyperplasia.^{26,27} In 1991, Moscoco suggested the name PMD for the phenotype of a placenta with hyperplasia and edematous/cystic changes of the stem villi, dilated vessel on the fetal surface of the placenta, and no trophoblastic hyperplasia.¹⁴

Most HMs seem to be diploid androgenetic (PP) or triploid, with an extra chromosome set from the father (PPM), whereas tetraploid HMs, aneuploid HMs, and diploid biparental HMs caused by deficient imprinting are rare.²⁸ However, both in placentas classified as HMs and in placentas classified as PMD, mosaicism for two diploid cell lines - one androgenetic and one biparental (PP/PM) - have been observed.^{11-13,15,16}

In the present study, we performed whole genome analysis of DNA markers in two diploid conceptuses with no fetus and villous dysmorphism reminiscent of HM, and found paternal hemizyosity for overlapping regions in 11pter-11p15.4. These cases fulfilled the initial inclusion criteria of the Danish Mole Project, as macroscopically visible vesicular chorionic villi were observed; and at previous histopathological examinations, these were classified as HMs. Review by specialist pathologists independently classified the villous morphology of these conceptuses as dysmorphic, but likely nonmolar, concordant with low and rapidly declining maternal serum-human chorionic gonadotropin (hCG) values. There were no findings suggestive of PMD.

We cannot exclude that haploinsufficiency for genes in the deleted region on chromosome 11 influenced the phenotype. Further, as one of these conceptuses harbored an extra maternal copy of a segment of chromosome 21 and the other had an extra maternal copy of a segment of chromosome

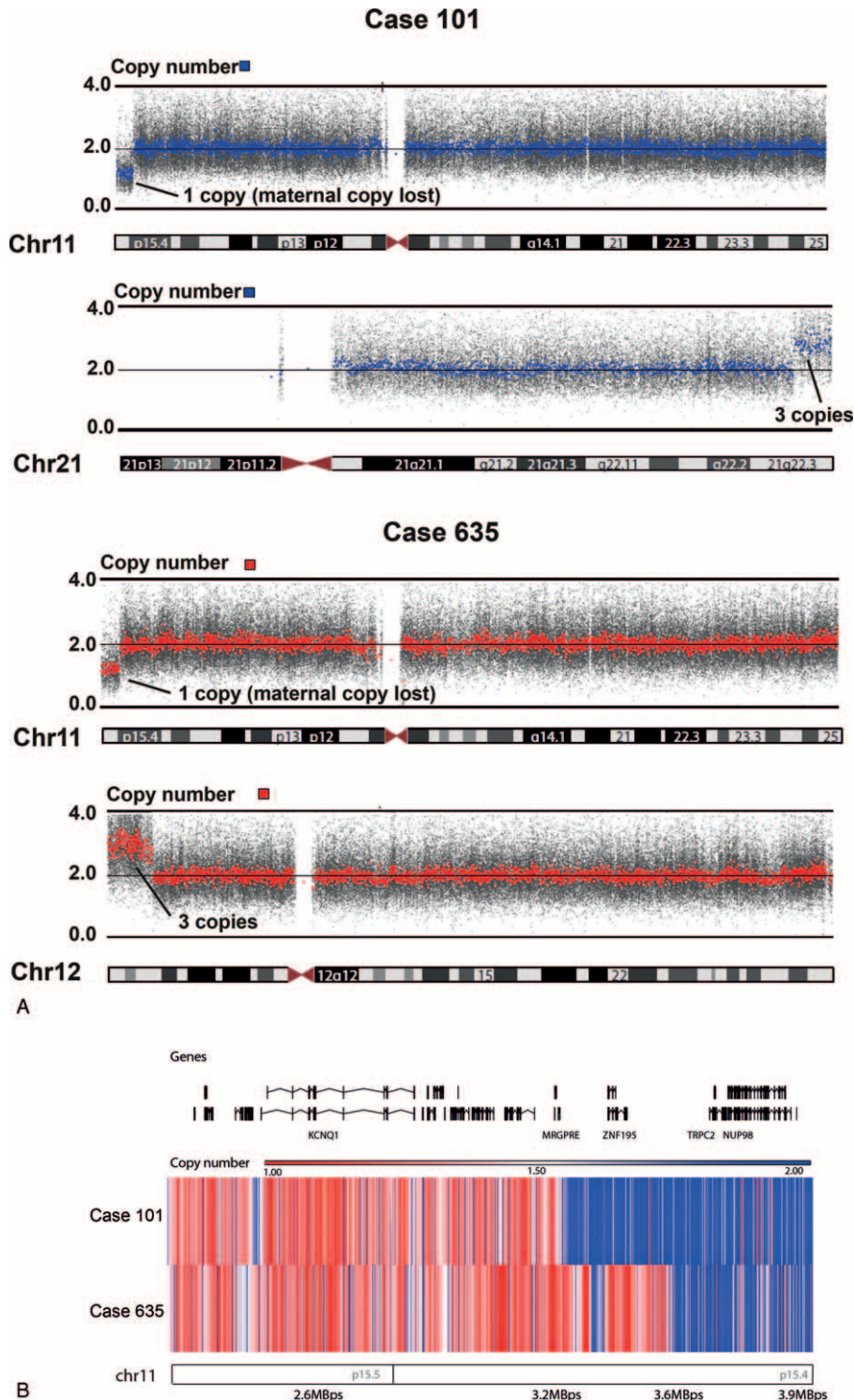


FIGURE 3. Results of SNP6 analyses. Genomic profiles of two cases determined by SNP array. A, Case 101: deletion of 11pter-11p15.4, gain of 21q22.3-qter; case 635: deletion of 11pter-11p15.4, gain of 12pter-12p13.31. B, Zoom in on the breakpoints on chromosome 11. Red bars: probes showing deletion; blue bars: probes showing normal copy number. Note that the deletion in case 635 was slightly larger than in case 101, including one additional gene (*ZNF195*). SNP = single-nucleotide polymorphism.

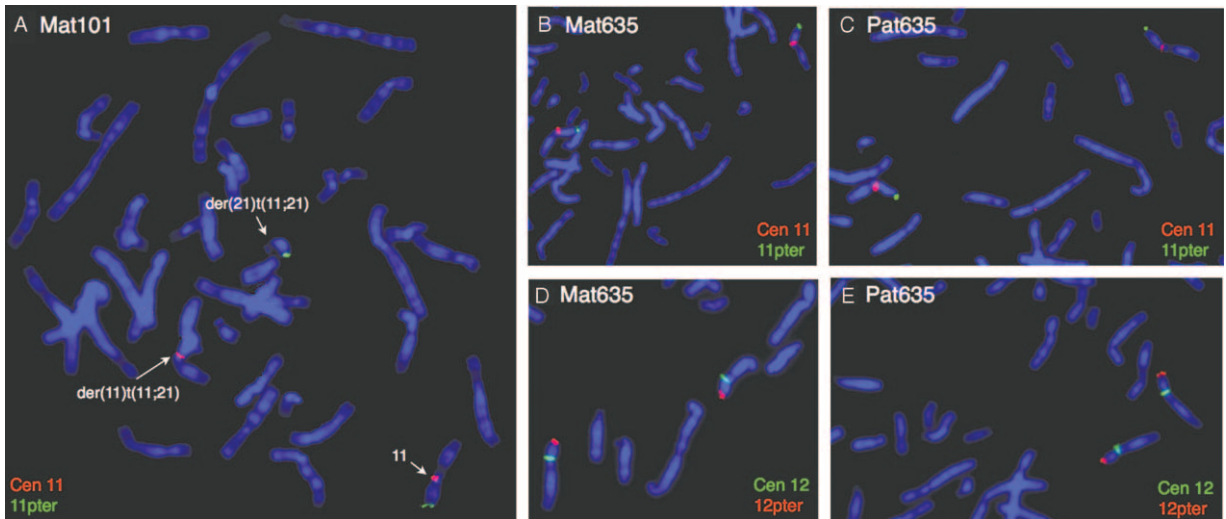


FIGURE 4. FISH in families 101 and 635. A, Metaphase from the mother of case 101; probes specific for the centromere (red) and p-telomere (green) of chromosome 11 identifying the two derivative chromosomes due to t(11;21)(p15.4;q22.3). B and C, Metaphases from the mother and father of case 635; probes specific for the centromere (red) and p-telomere (green) of chromosome 11 identifying normal chromosomes 11. D and E, Metaphases from the mother and father of case 635; probes specific for the centromere (green) and p-telomere (red) of chromosome 12 identifying normal chromosomes 12. FISH=fluorescence in situ hybridization.

12, we cannot exclude that trisomy for sequences on chromosome 12 or 21 also contributed to the phenotype. However, as the phenotype in the two cases had similarities to the phenotype of HMs that is correlated to a relative excess of the paternal genome, and as the excess maternal contribution to

the genomic affected different regions in the two conceptsuses, it is likely that the absence of a fetus and the placental dysmorphism in these two cases were caused by deficiency of the product of one (or more) maternally expressed gene(s) in 11p15.

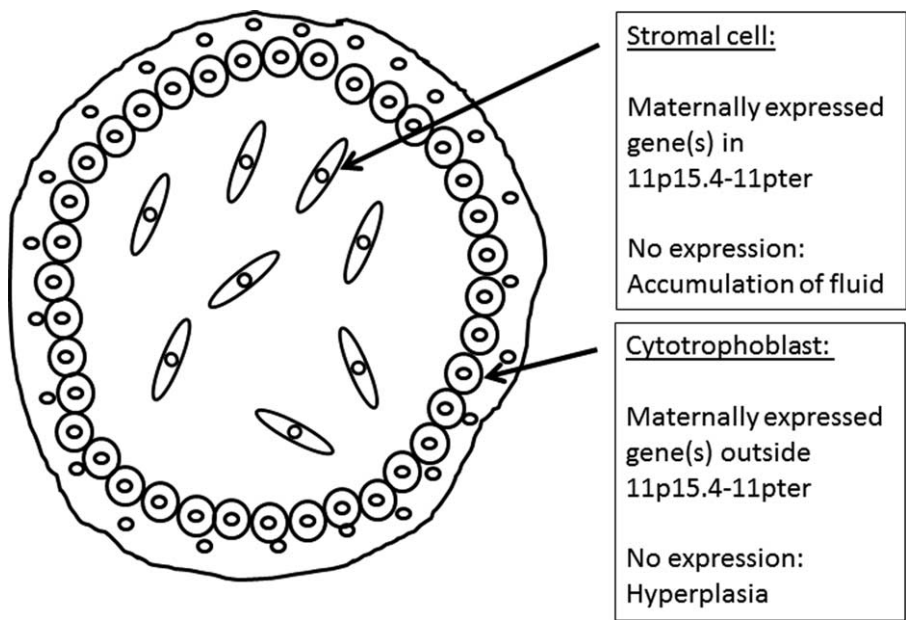


FIGURE 5. Model of genes possibly involved in the molar phenotype. Schematic representation of a cross-section of a chorionic villus. The observations in cases 101 and 635 indicate that absence of the product from maternally expressed gene(s) in 11pter-11p15.4 can cause vesicular chorionic villi. In mosaic PP/PM conceptuses suspected of HM, Lewis et al observed p57^{KIP2}-negative stromal cells (ie, stromal cells having an androgenetic genome, PP) and p57^{KIP2}-positive cytotrophoblasts (ie, cytotrophoblasts having a normal biparental genome, PM) in conceptuses without trophoblastic hyperplasia; whereas in conceptuses with trophoblastic hyperplasia, both the stromal cells and the cytotrophoblasts were p57^{KIP2}-negative (ie, androgenetic, PP).¹⁹ Thus, it is possible that absence of maternal expression of one or more gene(s) in 11pter-11p15.4 in stromal cells can cause accumulation of fluid in the villi, whereas the trophoblastic hyperplasia may be influenced by absent maternal expression in cytotrophoblasts of genes outside this region.

Imprinting errors in 11p15 are implicated in BWS. In approximately 20% of patients with BWS, the syndrome is ascribed to paternal uniparental disomy (UPD) of the entire chromosome 11, or to a part of the chromosome including 11p15.5 (UPD11pat).²⁹ Importantly, in all cases published, the abnormality was present in a mosaic state, and the severity of the phenotype in mosaic UPD11pat seems to correlate with the frequency of the cells with UPD.^{30,31} PMD has repeatedly been observed in the placenta of fetuses with BWS, and this placental phenotype may be especially frequent among those cases caused by mosaicism for UPD11pat.³² A case of a conceptus surviving to gestational week 34, with PMD and fetal overgrowth, showed mosaicism for paternal hemizygosity of 11p15.5. This case also suggests that loss of maternal 11p15.5 might be associated with PMD.²⁹

Nonmosaic deletion of 11p15.5 seems to be unreported in liveborn individuals.³³ Further, by October 2014, no such case were registered in Decipher³⁴ or in Danish Cytogenetic Central Registry that holds data on all cytogenetic analyses performed for clinical reasons in Denmark (Jan Hansen, personal communication).

The present two cases of paternal hemizygosity for 11pter-11p15.4 most likely were nonmosaic: in both cases, no sign of a normal cell line was noted in the genetic analyses, and furthermore, in one case, the deleted chromosome was inherited. As no fetal differentiation was observed, it seems likely that the product of one (or a few) maternally expressed gene(s) on the tip of chromosome 11 is necessary for early embryonic differentiation to take place, as already suggested by others.¹⁹ These may include genes expressing transcripts directly translated into essential proteins, or transcript repressing paternal transcription, the deletion thereby indirectly causing an overexpression of genes from the paternally inherited genome.

As the two present cases did not show trophoblastic hyperplasia, our findings also indicate that loss of maternally expressed gene(s) in 11pter-11p15.4, only, is not sufficient for all the features of the molar phenotype to evolve. Possibly abnormal parental (paternal) origin or abnormal imprinting in a number of loci may contribute to a more or less pronounced molar phenotype ("polyepigenetic" etiology). Interestingly, in mosaic PP/PM conceptuses with some suspicion of HM and no trophoblastic hyperplasia, Lewis et al¹³ observed p57^{KIP2}-positive cytotrophoblasts and p57^{KIP2}-negative stromal cells, whereas in the cases with trophoblastic hyperplasia, also the cytotrophoblasts were p57^{KIP2}-negative. Thus, it is possible that absence of maternal expression of one or more gene(s) in 11pter-11p15.4 in stromal cells can cause a vesicular appearance of the villi, whereas trophoblastic hyperplasia may be caused by absence of maternal expression of one or more gene(s) outside this region in the cytotrophoblasts (Fig. 5). Such a mechanism may also explain the seemingly conflicting observations previously reported in HMs, showing aneuploidy for chromosome 11: two diploid androgenetic conceptuses, trisomic due to a retained maternal chromosome 11 had the phenotype of complete HM,^{35,36} and a triploid diandric mole that was paternal disomic for chromosome 11 also showed the phenotype of complete HM.³⁷ Furthermore, this mechanism may explain some of the inconsistencies between classification of HMs using morphologic criteria and the classification using genetic criteria.²⁰

Immunostaining of p57^{KIP2}, encoded by the gene *CDKN1C* localized in 11p15.5, has been shown to be a method for discrimination of complete and partial HMs with high sensitivity and specificity. Rare misclassifications have been attributed to

aneuploidy for maternal chromosome 11 in HMs, with two paternal chromosome sets or mosaicism PP/PM.³⁸ The observation of a borderline molar villous morphology in the present two conceptuses with paternal hemizygosity for 11p15.5 illustrates another reason why inspection of the morphology of aborted tissue by a skilled histopathologist cannot be replaced by a simple scoring of tissue immunostained for p57^{KIP2}.

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