Recurrent triploid digynic conceptions and mature ovarian teratomas: are they different manifestations of the same genetic defect?

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Abstract

Miscarriages affect 15% of clinically recognized pregnancies. Recurrent miscarriage (RM) is defined by the occurrence of at least two consecutive pregnancy losses and affects 1% to 5% of couples trying to conceive. In an attempt to categorize patients with RM and identify the mechanisms leading to their miscarriages, we first used flow cytometry to assess the ploidy of 93 products of conception (POCs) from 53 patients with RM (≥ 3 miscarriages). We identified a single patient with four triploid POCs. We then used fluorescent in situ hybridization to confirm the triploidies and fluorescent microsatellite genotyping with distal and pericentromeric markers to determine their parental origin and the mechanisms leading to their formation. We found that all four triploidies are digynic and due to a failure in meiosis II (MII) suggesting a genetic predisposition. Upon further investigation into the family, we found a remarkable history of ovarian cysts and dysfunctions on the maternal side. Notably, one maternal cousin had a mature ovarian teratoma that we analyzed and found an identical mechanism at its origin, a failure in MII. The identification of two patients in the same family with two different manifestations, digynic triploid conceptions and mature ovarian teratomas, both resulting from the failure of MII, suggests an inherited genetic susceptibility towards an error in MII segregating in the family and that may manifest in the form of a triploid digynic miscarriage as well as a mature ovarian teratoma. Our findings may facilitate the future identification of causative mutations for MII defects.

45 Key words: recurrent miscarriages / recurrent triploidy / digyny / meiosis II failure /
46 polycystic ovary syndrome / mature ovarian teratoma

Introduction

A miscarriage is a failure of pregnancy before 20 weeks of gestation.[1] It may manifest in other forms, such as a chemical pregnancy, ectopic pregnancy, blighted ovum, arrested pregnancy, inevitable miscarriage, or molar pregnancy. Miscarriages affect 15% of clinically recognized pregnancies.[2-4] Recurrent miscarriage (RM) is defined by the occurrence of at least two consecutive miscarriages and affects 1% to 5% of couples trying to conceive. RM is clinically and genetically highly heterogeneous.[5]

The most common cause of miscarriages is chromosomal aneuploidies in the products of conception (POCs), and accounts for over 50% of the cases.[4] At the clinical level, various other abnormalities such as infection, parental karyotype anomalies, endocrine, pelvic anatomical, thrombophilic, and autoimmune abnormalities may be found in women with RM. Despite all of these known causes, in about 50% of the cases no abnormalities can be identified in the patients even after comprehensive clinical and laboratory evaluations.[1,6-8] Such patients are diagnosed with RM of unexplained clinical origin (or unexplained RM).

Recent studies have explored the role of copy number variants (CNVs), which are submicroscopic chromosomal changes (longer than 1kb),[9] in the genetic predisposition to pregnancy loss. They concluded that CNVs may contribute to pregnancy loss if they are very close to or overlap genes that play a role in pregnancy and affect their expression. CNVs can be detected in 5% of miscarriages that have a normal karyotype.[10] However, it is still unknown if these CNVs are clinically significant since only few studies have been done.

At the genetic level, there are no identified major genes that play causal roles in a substantial fraction of RM in humans. One gene that codes for the synaptonemal complex protein 3 (SYCP3), a component of the synaptonemal protein complex that forms between homologous chromosomes in prophase of meiosis I (MI), was shown to play a causal role in female and male meiosis. In one study, mutations in *SYCP3* were found in three patients with RM; however, subsequent studies did not replicate these findings in other populations, indicating that *SYCP3* mutations underlie only a minority of cases of RM.[11-15] Variants in approximately one hundred genes have been shown to be associated with RM in some populations and studies, but many of these associations were not replicated when tested in other populations or in meta-analyses.[1,16,17] The main factors that have hampered the identification of causative or susceptibility genes for RM lie in the difficulty in dividing the patients into homogeneous categories and the high genetic heterogeneity of this clinical entity.[18]

To reduce the genetic heterogeneity of RM and facilitate the identification of new causative genes responsible for them, we searched for a unique and recurrent mechanism for RM by performing flow cytometry on 93 formalin fixed paraffin embedded (FFPE) POCs from 53 patients with at least three miscarriages. We found one patient (from now on called the proband) with four triploid POCs, a medical history of polycystic ovary syndrome (PCOS), and a family history of ovarian cysts in five relatives, including one mature ovarian teratoma. In this report, we describe a comprehensive genotyping characterization of the four miscarriages from the proband and one mature ovarian teratoma from her cousin. We demonstrate a failure of maternal meiosis II (MII) at the origin of the four triploid POCs as well as the mature ovarian teratoma. Our data argue in

favor of a common genetic susceptibility to failure of MII at the origin of both the triploid conception and the mature ovarian teratoma.

Materials and Methods

Patients recruitment and retrieval of archived tissues

The study was approved by the McGill Institutional Review Board (IRB# A01-M07-03A). All patients answered a comprehensive questionnaire about their medical histories and provided written consent to participate in the study and to retrieve their FFPE POCs from various pathology departments. The patients were recruited from the Recurrent Pregnancy Loss Clinic at the Royal Victoria Hospital of the McGill University Health Centre from May 2006 to August 2014. The proband of this study was among our cohort of 53 patients with at least three RM. Her reproductive history included ten miscarriages followed by a live birth and another miscarriage, all from spontaneous conceptions. The first ten miscarriages occurred between the ages of 27 and 31, the live birth at the age of 34, and the 11th miscarriage at the age of 36. The proband underwent comprehensive assessments that included blood karyotypes for her and her partner, thrombophilia, immunology, endocrinology, and pelvic anatomy workup (see Supplementary Material I for the list of tests). The proband is not obese and the results of her tests were all normal with the exception of her history of oligomenorrhea and polycystic ovary revealed by sonography. Based on these results, the proband was diagnosed with PCOS and treated with metformin to regularize her menstrual cycles. The proband also indicated a family history of miscarriages and infertility in her maternal aunts.

Archived FFPE blocks were retrospectively retrieved from the hospitals where the dilation and curettages (D&Cs) were performed. Out of the eleven miscarriages that the proband had only four required D&Cs and we were able to retrieve their archived FFPE tissues.

Flow cytometry

Flow cytometry was performed on FFPE tissues that were prepared according to standard histopathological methods. Cellular preparation for flow cytometry was performed according to a modified version of Hedley's protocol.[19] Two sections of 60 µm were cut from each FFPE block and placed in 15 ml Falcon tubes. The sections were deparaffinised twice for 10 minutes in 5 ml of xylene and rehydrated in a sequence of 5 ml of 100%, 100%, 95%, 70%, and 50% ethanol for 10 min each at room temperature, and the washed twice in 10 ml Milli-Q water for 10 min. Five ml of cold citrate solution (10 mmol/L, pH 6.0) was added to each tube and the tubes were incubated at 80°C for 2 h. They were then allowed to cool to room temperature for 15 min and rinsed with PBS-1X. The proteins were digested in 1 ml of 5 mg/ml pepsin (Sigma) in 0.9% NaCl (adjusted to pH 1.5 with HCl) for 30 min with intermittent vortexing every 10 min. The cellular suspension was then rinsed with PBS-1X and suspended in propidium iodide (PI) solution (0.1 mg/µl, Sigma-Aldrich, St Louis, MO) and 50 µl RNase (1 mg/ml) and incubated at 37°C for 30 min. Finally, they were filtered through a 48 µm mesh nylon filter and analyzed using a BD FACS Canto II at the Immunophenotyping Core facility of the McGill University Health Centre. Data files were analyzed using FCSalyzer (Wien, Austria).

Fluorescent in situ hybridization

Fluorescent in situ hybridisation (FISH) was performed on 4 μm sections. Slides were hybridised with probes from the centromeres of three chromosomes, X, Y and 18, as previously described.[20] For each POC, more than 100 cells from different microscopic fields were scored with each probe.

Microsatellite genotyping

Depending on the amount of chorionic villi (CV) in the paraffin blocks, 5-12 serial 10 μm sections were prepared from the blocks that contained the largest amount of CV that are separated from maternal tissues. These sections were mounted on slides and stained with hematoxylin and eosin (H&E). Under a stereomicroscope, maternal tissues that are in close proximity to the CV were removed and discarded while the CV were then removed from the slides using Kimwipes and forceps and used for DNA extraction using the Qiagen QIAamp DNA FFPE Tissue Kit (Catalogue number 56404, Hilden, Germany). Extracted DNA was quantified using a Nanodrop and loaded on an agarose gel to evaluate its quality and the required quantity for multiplex fluorescent microsatellite genotyping with the PowerPlex 16 HS System (Promega, Corporation, Fitchburg, Wisconsin, USA). The reaction consisted of short tandem repeat (STR) multiplex PCR assays that amplify DNA at 15 different STR loci and a fragment from the Amelogenin gene that distinguishes the X and Y chromosomes. In addition, 13 pericentromeric markers mapped at less than 5 Mb from the centromeres of several chromosomes were selected from the Marshfield genetic map

(http://www.bli.uzh.ch/BLI/Projects/genetics/maps/marsh.html) and used to determine whether the triploid conceptions or the MOT originated from failure of MI or MII, as previously described by Zaragoza et al..[21] Primer sequences for the pericentromeric markers and PCR conditions are provided in Supplementary Table 1. DNA from the POCs and their available parents was amplified and the PCR products were resolved by capillary electrophoresis using an Applied Biosystems 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA) at the Centre for Applied Genomics (http://www.tcag.ca). The data were analyzed with PeakScanner version 1.0 (Applied Biosystems, Foster City, CA, USA) and the POC alleles were compared to the parental alleles to determine their origin.

Results

Four triploid conceptions in a patient with RM

We performed flow cytometry to determine the ploidy of 93 POCs from 53 patients with RM. Twenty-two of these patients had at least two FFPE POCs from their miscarriages that were analyzed by flow cytometry. Out of these 22 patients, only one, the proband, had three triploid POCs. A representative flow cytometry result is shown in Fig. 1a. To confirm the triploidies with a second method, we performed FISH on tissue sections from her three triploid conceptions using centromeric probes from three chromosomes, X, Y, and 18. The FISH analysis confirmed the triploidy of the three POCs (Fig. 1b-d) and revealed the absence of mosaicism based on the analysis of 100 nuclei from each POC. Updating the reproductive history of the proband revealed that in

addition to her ten miscarriages, she had an additional one that required D&C after a live birth. Analysis of the 11th miscarriage by flow cytometry demonstrated again its triploid genotype. In conclusion, the flow cytometry and FISH analyses demonstrated that the proband's four miscarriages are triploid.

Maternal origin of the four triploid conceptions

To determine the parental origin of the four triploidies, we performed fluorescent microsatellite genotyping using the PowerPlex 16 HS System (Promega Corp., Madison, WI). This analysis demonstrated that all four triploidies are digynic (i.e. maternal origin of the extra set of chromosomes) based on the presence of two different maternal alleles (Fig. 2a, markers D18S51 and FGA) or two doses of one maternal allele in the POCs (Fig. 2a, marker D16S539) at four to nine markers from different chromosomes for each of the four triploidies (Fig. 2 and Supplementary Table 2).

Meiosis II failure in the four triploid conceptions

To determine whether these triploid POCs were caused by errors affecting the proper completion of MI or MII, we genotyped their DNA with pericentromeric markers mapped at less than 5 Mb from the centromeres of several chromosomes (Supplementary Table 1). Briefly, pericentromeric markers were used to minimize the chances of recombination and to determine whether maternal heterozygosity on homologous chromosomes was maintained (non-reduction) or reduced to homozygosity (reduction) in the triploid digynic POCs.[21] If the triploidy resulted from failure of MI, one would expect

non-reduction at all pericentromeric markers for which the mother is heterozygous, while if the triploidy resulted from failure of MII, one would expect reduction to homozygosity at all pericentromeric markers that are heterozygous in the mother. The results of this analysis are shown in Table 1. For example, at marker D1S534, the proband is heterozygous and has two alleles, 208 bp and 212 bp, but only one of them, the 208 bp allele, is transmitted to POC 1. This indicates reduction to homozygosity. Similar reduction to homozygosity is observed at four to six markers in each of the four POCs from the proband. These data demonstrate that MI must have taken place (i.e. that the homologous chromosomes have separated) and that the triploidy originated after MI completion and from the failure of MII in the separation of the sister chromatids. Interestingly, our analysis demonstrated that all four triploidies of the proband resulted from failure of MII.

Revisiting the medical and family history of the patient

The presence of the same mechanism, failure of MII, at the origin of the four triploidies suggested a strong genetic predisposition and prompted us to enquire about the reproductive history of her family members. The reproductive history of the paternal side is not very severe; the highest number of miscarriages in her paternal relatives was two and occurred only once in one of her cousins. Another paternal cousin had one miscarriage and two live births. The family history on the maternal side was remarkable and very complex, as shown in the pedigree, Fig. 3. It included unilateral or bilateral ovarian cysts in five relatives, adenomyosis in two, uterine fibroids in two, ulcerative colitis in one, at least two miscarriages in three members, infertility treatments in four

relatives, three of which were able to have normal pregnancies and one who never achieved pregnancy. All the ovarian cysts in the five members were benign and required surgeries - cystectomy in the younger members and total hysterectomy in post-menopausal members with adenomyosis or uterine fibroids or salpingo-ovariectomy in post-menopausal members with no fibroids. The pathology report of the ovariectomy of one aunt indicated an ovary with a hemorrhagic cyst and that of the cystectomy in the cousin indicated a diagnosis of a mature ovarian teratoma (MOT). We note that the cousin who had a MOT had also mild hirsutism. FFPE tissues from the latter MOT was accessible and its analysis is described below. The other ovarian cysts were not available because they were removed over ten years ago and most hospitals keep FFPE tissues only for ten years. No POCs from other family members on both sides required D&C and therefore could not be analyzed.

Meiosis II failure at the origin of the mature ovarian teratoma

MOTs, also known as dermoid cysts, are derived from non-ovulated and unfertilized female germ cells that have undergone parthenogenetic activation followed by embryonic cleavage and tissue differentiation within the ovarian follicle. There, they give rise to a benign tumor with disorganized well-differentiated tissues originating from the three germ cell layers, ectoderm (e.g. skin, brain), mesoderm (e.g. muscle, fat), and endoderm (e.g. mucinous/ciliated epithelium). Based on their genotypes, MOTs are classified depending on the time of their parthenogenetic activation during meiosis (i.e. before or after meiosis I or II). The histopathology of the cousin's MOT is shown in Fig. 4. To determine its genotype, we separated the MOT tissues under a stereomicroscope with

extreme care in taking only the MOT tissues without contamination with the maternal ovarian epithelium. We then extracted the DNA and performed fluorescent microsatellite genotyping using the Powerplex 16 HS system and pericentromeric markers as described for the POCs. Distal markers from the Powerplex genotyping showed that the MOT displays the two maternal alleles at six out of the eight markers for which the mother is heterozygous, which demonstrates that meiotic recombination during meiosis I had taken place (homologous chromosomes separated), and that an apparently normal level of recombination occurred (Table 2). We then used five pericentromeric markers that are heterozygous in the mother and found that they were all reduced to homozygosity in the MOT, which demonstrated, similar to the four triploidies observed in the proband, a failure of MII at the origin of the MOT (Fig. 5).

Discussion

We used flow cytometry to assess the ploidy of 93 POCs from 53 patients with ≥3 miscarriages. Among these patients, 23 had at least two POCs included in the analysis. We identified one patient with recurrent triploid conceptions. Using FISH and microsatellite genotyping with distal and pericentromeric markers, we confirmed the triploidies in this patient and demonstrated that her four available triploid POCs are digynic and originated from the failure of MII. Recurrent triploidies of maternal origin are rare and so far only six such cases have been reported,[22-27] but a demonstration of the maternal origin in at least two POCs from the same patient has only been shown in two cases.[24,27] In the latter paper by Filges et al., the authors nicely demonstrated the recurrence of maternal triploidy due to failure of MII in six conceptions from the same

patient.[27] They suggested an autosomal dominant genetic susceptibility transmitted from the mother, who had 18 miscarriages and two live births.

Further investigation into our proband's family revealed a strong family history of polycystic ovaries (PCO), ovarian cysts that required surgeries, oligomenorrhea, infertility, adenomysosis, endometriosis, and fibroids, all on the maternal side. All of the aforementioned conditions can be grouped into two categories of abnormalities affecting the ovaries and/or the endometrium. The relationship between PCO, ovarian cysts, oligomenorrhea and infertility is well-documented[28,29] and PCO is the most frequent abnormality observed in women seeking assisted reproductive technologies.[30] Adenomyosis, endometriosis, and uterine fibroids are all characterized by abnormal growth and have been repeatedly shown to be associated with subfertility. In addition, one study showed an association between endometriosis and uterine fibroids.[31] Among the many ovarian tumors that are associated with PCOS, MOTs are the most common and constitute 35% of ovarian tumors associated with PCOS.[32] Also, one third of women of fertile age with endometrial adenocarcinoma have PCO (Sorensen 1987). It is unfortunate that the pathology reports of only two ovarian cysts and archived tissues from one were available, but this is part of the many challenges of working with reproductive conditions in humans where tissues from different generations (separated by 25 years on average) cannot be accessed. The analysis of the available MOT revealed that it also resulted from a failure of MII.

Based on genetic analyses, MOTs are classified into five types of origins according to the times of their occurrence during female meiosis. Type-I originate from a primary oocyte that fails to undergo MI; Type-II, originate from a secondary oocyte that

297 fails to undergo MII; Type-III, from an ovum that undergoes endoreduplication; Type-IV,
298 originate from an oogonium that does not undergo meiosis (pre-meiotic), and Type-V,
299 from two ova that fuse together.[33] Familial cases of MOTs are very rare and to date,
300 approximately ten familial cases have been described.[34-43] All reported cases display a
301 dominant mode of inheritance, and there are no genes known to cause, or predispose to,
302 MOTs in humans.

303 In mice, ovarian teratomas have been observed in eight different models
304 (reviewed in [44]). In most of these models, the pathology of ovarian teratomas is attributed
305 to the asynchronic growth between the oocytes and the somatic components of the
306 follicles and/or meiotic abnormalities. In one of the models, transgenic expression of a
307 missense mutation in the *Fshr* (follicle stimulating hormone receptor) in granulosa cells
308 is associated with, in addition to the teratomas, hemorrhagic cysts.[45] In another model,
309 the LT/Sv strain, the ovarian teratomas were shown to occur in oocytes that completed
310 MI and failed to undergo MII, which is a similar mechanism to that of the MOT in the
311 cousin. Moreover, oocytes from these LT/Sv mice lead to triploid digynic embryos in
312 30%-50% of their conceptions, which is similar to our observations in the proband.[46] The
313 *Foxo3a* mouse model of MOT is also in favor of their association with triploidy. It was
314 shown that the oocytes of these mice, which have a homozygous missense mutation in
315 *Foxo3a*, are less likely than those of wild type mice to have a polar body at ovulation[47]
316 which may lead to digynic triploid conceptions if these oocytes are fertilized. In
317 conclusion, these two mouse models seem to recapitulate the main features observed in
318 the described family, MOTs and triploid digynic conceptions.

What is fascinating about the present familial case is the finding that the analyzed MOT shares the same mechanism with the recurrent triploidies, which is the failure of MII. Do they also share the same genetic predisposition? It is definitely a possibility, but one that is difficult to confirm due to the fact that we have a single MOT; as such, its MII (Type-II) origin might be coincidental. However, we would like to point out that of the several studies that have classified MOTs according to their genetic origins, only two, to the best of our knowledge, used short tandem repeat (STR) markers and distinguished between all five possible genetic origins. Based on these studies that include a total of 102 MOTs, Type-II teratomas accounted for 17 (16.7%) of all the 102 MOTs (Supplementary Table 3). Furthermore, the occurrence of MOT that became very large and ruptured in several members is not common among patients with PCOS, and makes this family very rare. These observations support a common underlying genetic predisposition. Nevertheless, it remains a complex family with many unsolved mysteries.

Three possible modes of inheritance could explain the transmission of the defect in this family. Because all ovarian abnormalities are on the maternal side, the most likely mode of transmission for this defect is an autosomal dominant mode from the maternal side. Such a model implies reduced penetrance to explain the lack of miscarriages, MOT, or ovarian disorders (oligomenorrhea or infertility) in the proband's mother. We also note that in 64% of patients, MOTs are asymptomatic and are diagnosed incidentally while the patients are being examined for something else.[49] It is therefore possible that the proband's mother had small ovarian cysts that did not require medical attention and consequently went unnoticed, a suggestion that is corroborated by the fact that she had a small number of offspring, which could be due to subfertility. Another possible mode of

inheritance is a digenic or polygenic etiology consisting of at least two genetic defects, one from the maternal side, responsible for PCOS, ovarian cysts, and the miscarriages observed on the maternal side, and another segregating from the paternal side and increasing the severity of the miscarriages observed in the proband. Under this hypothesis, both defects could be contributing, alone or with other genetic factors, to the proband's severe phenotype of eleven recurrent miscarriages and PCOS. Another possible mode of transmission could be a recessive monogenic defect in the proband segregating from both of her parents; however, this would not explain the PCOS and ovarian disorders because they are absent from the paternal side. Nevertheless, it is possible that a homozygous or compound heterozygous mutation explains the severe recurrent miscarriages observed in the proband, since both sides, maternal and paternal have had smaller number of miscarriages.

To the best of our knowledge, our report is the first to describe the co-occurrence of the two entities in the same family and suggests a possible inherited genetic susceptibility for a failure of MII that may manifest in the form of triploid digynic conceptions or MOTs. Further studies of such rare familial cases are needed to confirm the relationship between triploid conceptions and MOTs.

Authors' roles

Y.K. implemented and performed flow cytometry, performed microsatellite genotyping, and wrote the manuscript. N.M. recruited patients and retrieved samples. NMP.N. helped in all technical aspects of this study. W.B. referred patients and reviewed their clinical

and laboratory findings. J.A. reviewed the histopathology of the teratoma. R.S. supervised data analysis and interpretation and helped in the writing of the manuscript. All authors read the final version of the manuscript, contributed to its critical revisions, and approved the final version.

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Conflict of interest

None declared.

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385 Tables

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Table 1. Genotyping with pericentromeric markers demonstrating reduction to homozygosity at informative markers in the proband

Marker	Start Position (Mb, hg38)	Centromere location (Mb, hg38)	Proband	POC 1	POC 2	POC 3	POC 4	Partner
D1S534	119.1	122.5-124.8	**208/212**	**208**/204 - R	**208**/198 - R	**208**/198 - R	**208**/198 - R	198/204
D3S2462	96.4	91.6-93.7	**236/238**	**238**/236 - UI	**238**/236 - UI	**238**/236 - UI	NA	236/242
D4S3355	52.8	49.7-51.7	**131/144**	NA	**144**/135 - R	**144**/135 - R	**131**/135 - R	135
D6S402	62.3	58.6-59.8	**109/113**	**113**/117 - R	**113**/115 - R	**109**/117 - R	**113**/115 - R	115/117
D7S1485	57.3	58.2-60.8	**204/212**	**204**/212 - UI	**204**/198 - R	**204**/212 - UI	NA	198/212
D8S1115	42.9	44.0-45.9	**161**	NA	NA	NA	NA	161
D11S1983	58.7	51.1-54.3	**225**	NA	NA	NA	NA	229/236
D12S2080	33.3	34.8-37.2	**182**	**182**/186 - UI	NA	**182**/186 - UI	NA	186/194
D14S122	20.9	16.4-18.2	**193/200**	**200**/204 - R	**193**/204 - R	**200**/219 - R	NA	204/219
D18S869	22.5	15.8-20.6	**178/190**	190 - R	178/190 - UI	178 - R	NA	178/190
D20S484	31.5	26.6-28.5	**182/186**	**186**/194 - R	**182**/186 - UI	**186**/194 - R	186 - R	186/194

Mb stands for megabases; POC, for product of conception, NR, for non reduction; UI, for uninformative; R, for reduction; NA, for not available. Maternal alleles are bolded; double-dose alleles are underlined.

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Table 2. Multiplex microsatellite data on the cousin and mature ovarian teratoma (MOT)

Marker	Location	Cousin with the MOT	MOT
D21S11	21q11--21	215/233	215/233
TH01	11p15.5	162	162
D3S1358	3p	120/136	120/136
FGA	4q28	341/350	341/350
TPOX	2p23-2pter	269	269
D8S1179	8q	226/230	226
vWA	12p12-pter	146/158	146/158
Amelogenin	X & Y	104	104
D16S539	16q24-qter	295	295
D7S820	7q11.21-22	224/228	224/228
D13S317	13q22-q31	188/191	188
D5S818	5q23.3-32	131/134	131/134

Legends to Figures

Figure 1. a) Flow cytometry result on one POC from the proband. The red peak is the diploid peak and represents the ploidy of the maternal endometrial tissues present in the FFPE block. The yellow peak is the triploid peak and represents the ploidy of the chorionic villi. b-d) Fluorescent in situ hybridization confirming the triploidies of three POCs from patient 1138. Centromeric probes from chromosomes X, Y, and 18 were used and show the presence of three X chromosomes and three copies of chromosome 18 in all three POCs.

Figure 2. Multiplex genotyping results for the four POCs of the proband reveal a digynic origin for all. All the markers show that the POCs received two alleles from the mother and one from her partner. The multiplex assay is quantitative and depending on the heights of the peaks allows determining if there are one or two doses of an allele. For example, in a), in POC1, markers FGA and D18S51 show two different maternal alleles and marker D16S539 shows two doses of one maternal allele.

Figure 3. Partial pedigree of the maternal side of the proband. The arrow indicates the proband; asterisk indicates the cousin with the MOT. For simplicity, the rest of the family is not shown, and consists of six females and two males. All of these eight additional members have one to three live births and no miscarriages. Symbol, "=" under the two subjects refers to infertility but no medical information is available about their infertility.

Figure 4. Hematoxylin and eosin staining showing in a) a complete view of the MOT, b) a 10x magnification of the respiratory endothelium present in the MOT, and c) a 10x magnification of cartilage tissue.

Figure 5. a) Table showing pericentromeric markers tested on the maternal cousin of the proband and her mature ovarian teratoma (MOT). Allelic sizes are shown in base pairs. b) Representative pericentromeric markers for the MOT from the maternal cousin of the proband. All markers show that the MOT received only one of the alleles from mother, and there is therefore reduction to homozygosity, implying normal completion of MI and separation of homologous chromosomes and a failure of MII. Note that the lower peaks represent the second maternal alleles and are due to the presence of contaminating maternal tissues, mostly immune white blood cells inside the MOT and are much smaller than the high peaks present in all the cells of the teratoma.

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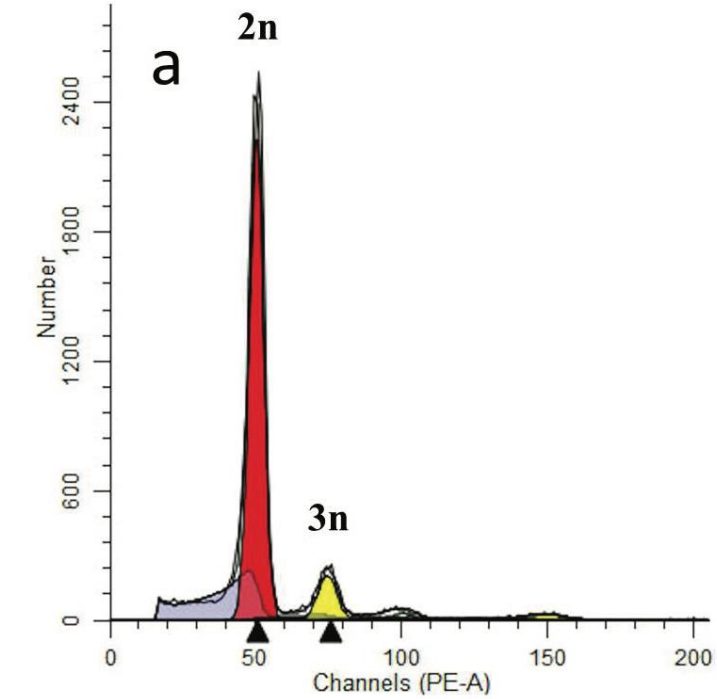
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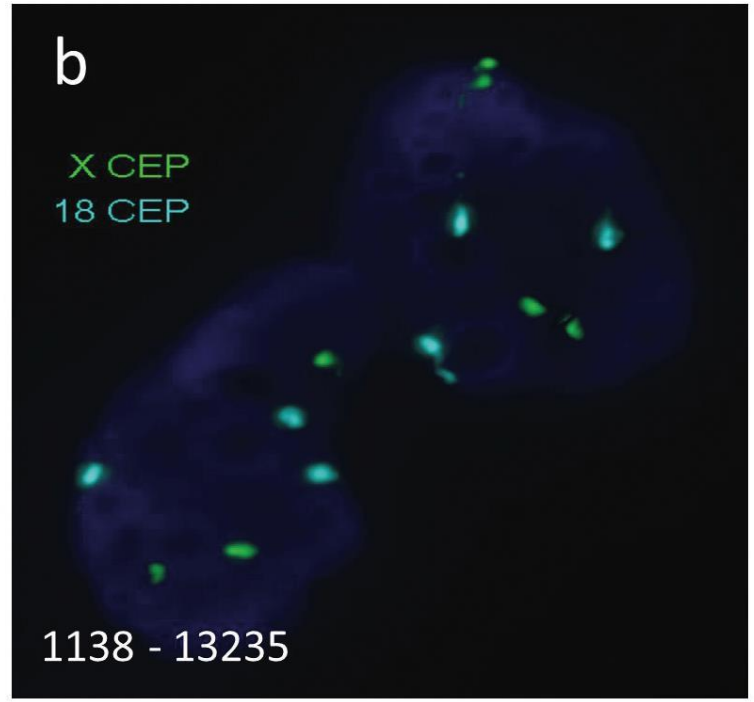
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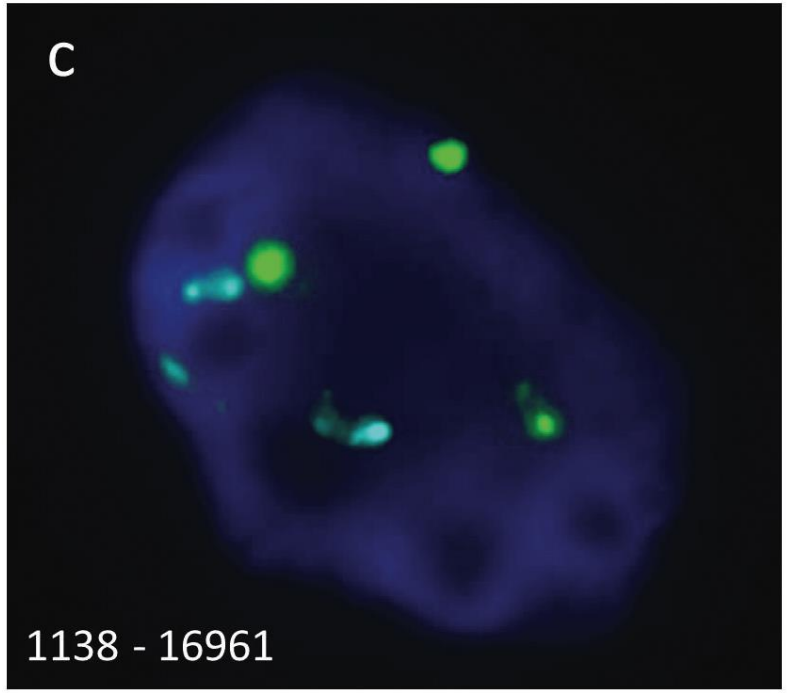
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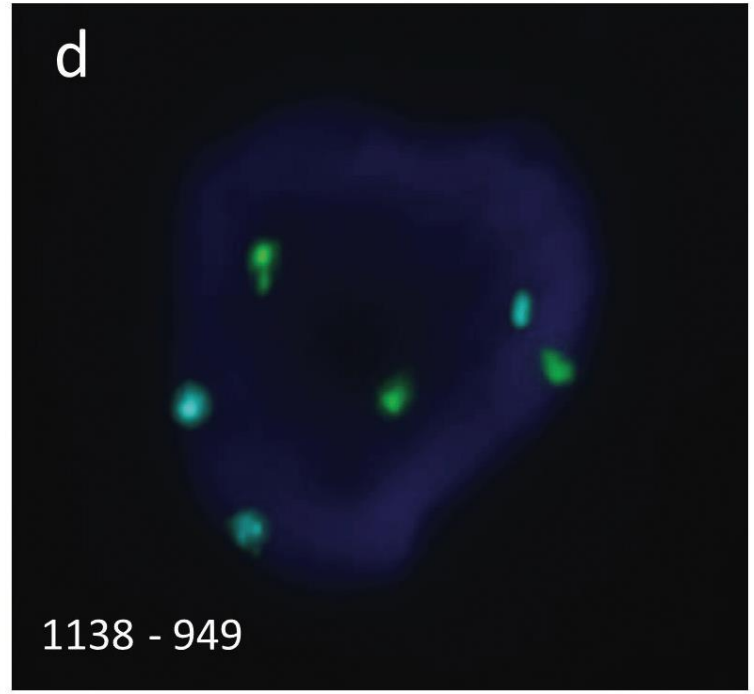
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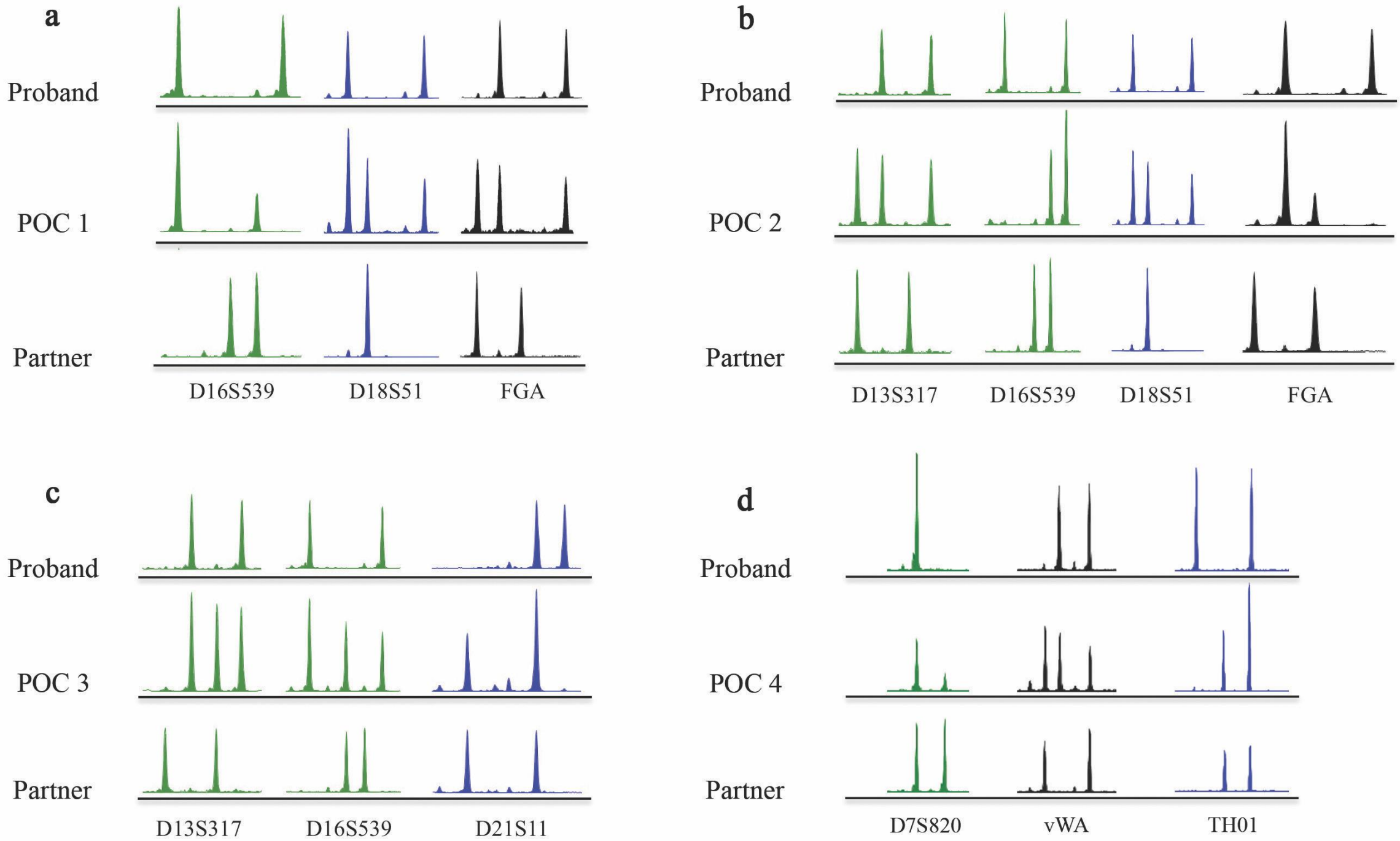
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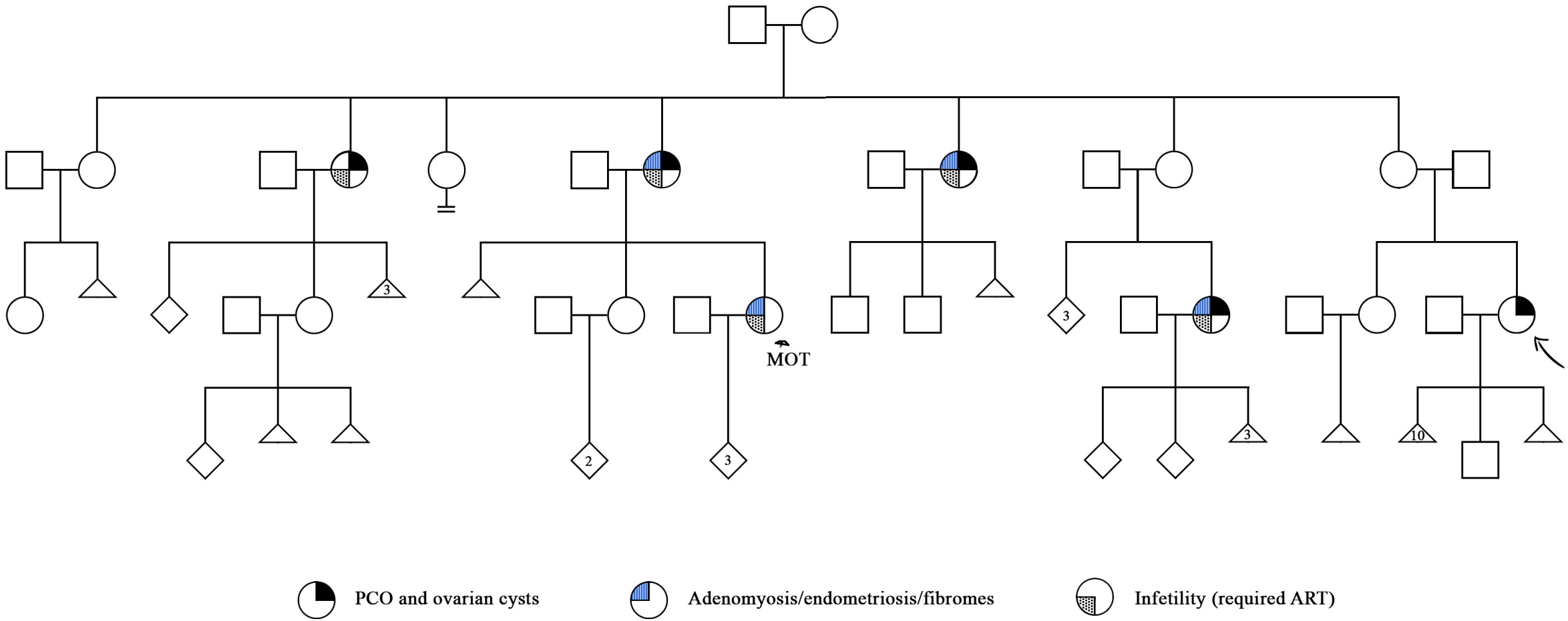
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MOT
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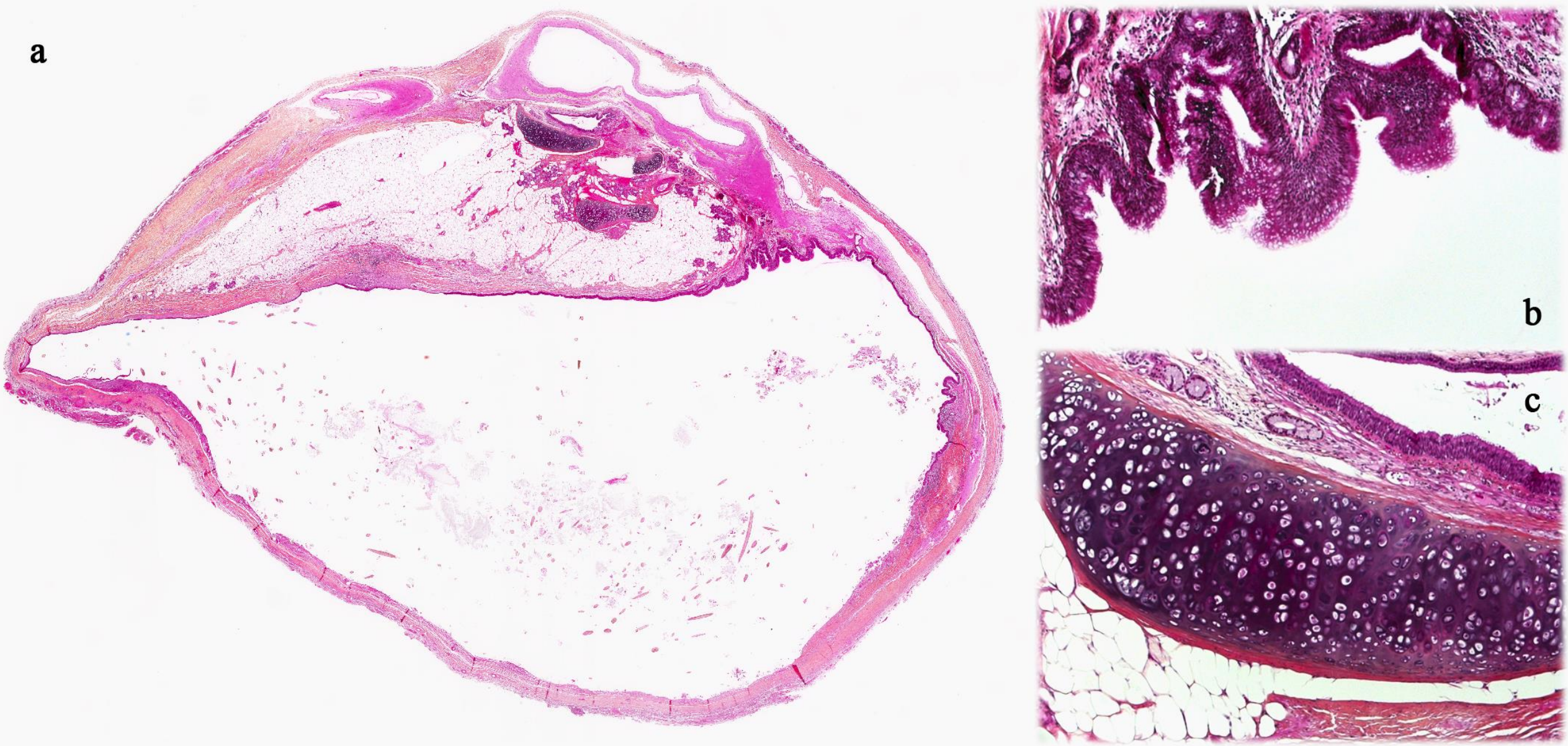
PCO and ovarian cysts
Adenomyosis/endometriosis/fibromes
Infetility (required ART)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a

Marker	Start Position (Mb, hg38)	Centromere location (Mb, hg38)	Cousin with teratoma	Teratoma
D1S534	119.1	122.5-124.8	199/208	199
D3S2462	96.4	91.6-93.7	238/240	238
D8S1115	42.9	44.0-45.9	138/164	164
D14S122	20.9	16.4-18.2	212/224	212
D20S484	31.5	26.6-28.5	189/193	193

Mb stands for megabases

b