

Recurrent Triploid and Dispermic Conceptions in Patients with *NLRP7* Mutations

Rima Slim^{a,b,*}, Asangla Ao^{b,a}, Urvashi Surti^c, Li Zhang^b, Lori Hoffner^c, Jocelyne Arseneau^d, Annie Cheung^e, Wafaa Chebaro^{a,b}, and Anita Wischmeijer^f

^aDepartment of Human Genetics, McGill University Health Center, Montreal H3G 1A4, Canada

^bDepartment of Obstetrics and Gynecology, McGill University Health Center, Montreal H3G 1A4, Canada

^cMagee-Womens Research Institute and Department of Pathology, University of Pittsburgh, 300 Halket Street, Pittsburgh, PA 15213, USA

^dDepartment of Pathology, McGill University Health Center, Montreal H3G 1A4, Canada

^eDepartment of Pathology, The University of Hong Kong, Queen Mary Hospital, Hong Kong, China

^fDepartment of Medical Genetics, Policlinico Sant'Orsola-Malpighi, University of Bologna, Bologna, Italy

Abstract

To understand the mechanisms leading to hydatidiform mole formation in patients with *NLRP7* mutations, we used a combination of various approaches to characterize five products of conception, from two patients, shown by flow cytometry to contain non-diploid cells. We demonstrate that four of these conceptions are triploid and two of them originated from fertilization with more than one sperm. We show that three of these triploid conceptions fulfill the histopathological criteria of partial hydatidiform mole and one fulfills the histopathological criteria of spontaneous abortion. Our data demonstrate that oocytes from one patient with *NLRP7* mutations are not able to prevent polyspermic fertilization and highlight the importance of using several approaches to characterize the genetic complexity of molar tissues and reproductive wastage. Altogether, our previous and current data show the association of *NLRP7* mutations with several types of hydatidiform moles and with triploid spontaneous abortions.

Keywords

hydatidiform mole; *NLRP7*; polyspermic fertilization; triploid diandry; dispermy

*Correspondence to: Rima Slim, Montreal General Hospital Research Institute, L3-121, 1650 Cedar Avenue, Montreal, P.Q., Canada, H3G 1A4, Tel.: (514) 934-1934 Ext: 44550, Fax.: (514) 934-8261, rima.slim@muhc.mcgill.ca.

1. Introduction

Hydatidiform mole (HM) is an abnormal human pregnancy characterized by absence of, or abnormal, embryonic development and hydropic degeneration of chorionic villi. *NLRP7* (NOD-like receptor, pyrin domain containing 7) is a gene responsible for an autosomal recessive form of recurrent hydatidiform moles (RHMs) (1). Women with recurrent moles have usually two defective alleles. However, so far, seven patients have been found to have a single defective allele by sequencing the 11 *NLRP7* exons (2, 3). To date, it is not clear how *NLRP7* defects lead to this condition. *NLRP7* is a cytoplasmic protein expressed in a wide range of normal human tissues including Fallopian tubes, endometrium, as well as oocytes at the germinal vesicle and metaphase II stages and early embryonic cleavage stages, (1, 4) (and unpublished data). We previously reported early cleavage abnormalities during *in-vitro* and *in-vivo* development of embryos from patients with *NLRP7* mutations and variants (2). We have also shown the presence of *NLRP7* mutations, not only in women with diploid biparental moles, as observed so far in all characterized molar tissues from familial cases of RHMs, but also in two patients with diploid androgenetic monospermic moles and in one patient with a triploid partial mole (2). Here, we show the recurrence of triploid conceptions in two patients with heterozygous *NLRP7* mutations and document the diandric origin of two products of conceptions (POCs) from one patient.

2. Cases

2.1. History and mutations

The reproductive outcomes of the two patients, 636 and 647, and their *NLRP7* mutations were previously reported (2) and are summarized in Figs. 1 and 2. Patient 636 is heterozygous for c.2156C>T, p.Ala719Val (A719V), and patient 647 is heterozygous for c.467G>A, p.Arg156Gln (R156Q) (2). Both patients are of European ancestry and their two missense mutations, A719V and R156Q, were not found, respectively, in 100 and 150 women of European descent from the general population (2).

2.2. Parental contribution to the products of conception

Flow cytometry on five of their POCs revealed that four contain triploid cells and one is diploid (Figs. 1 and 2). Tissue sections from the available paraffin blocks were examined independently by two pathologists who agreed on the diagnosis of three out of five POCs, two triploid partial HMs (PHMs) and one diploid complete HM (CHM). For one of the remaining POCs, both pathologists did not reach a conclusion on whether to classify it as hydropic spontaneous abortion (HSA) or PHM. For the other, one pathologist diagnosed it as PHM, while the other as HSA (Figs. 1 and 2) (Supplementary materials).

p57^{KIP2} immunohistochemistry demonstrated its nuclear expression in the cytotrophoblast and mesenchyme stromal cells of the villi of the four triploid POCs, 730, 994, 720 and 722 and the absence of its expression in the villi of the diploid POC 721 (Figs. 1 and 2), which is in agreement with their histopathological diagnoses.

Fluorescent microsatellite genotyping was performed on DNA extracted from chorionic villi using Pinpoint solution (Zymo Research, Orange CA) as previously described (5). This

analysis revealed the dispermic contribution to the two POCs from patient 636 (Fig. 1B). In patient 647, genotyping data showed paternal contributions to the three POCs (Supplementary materials). However, because of the absence of the paternal DNA and the limited amount of tissues from the three POCs, we could not reach a conclusion as to whether the diploid POC 721 is biparental or androgenetic dispermic and whether the two triploid POCs, 720 and 722 are diandric or digynic.

Fluorescent in situ hybridization (FISH) was performed using probes (Vysis Inc) from seven chromosomes and confirmed the triploid nature of the four POCs, 730, 994, 720 (Supplementary materials) and 722 and the diploid nature of POC 721 (Figs 1 & 2). In POC 730, two cell lines one, triploid XYY, and another, diploid XX, were observed (Fig. 1C). XYY and XX cells were both present within the same chorionic villous with XX cells being less frequent and only in the villous stroma. Taking into consideration the presence of two paternal alleles at several autosomes in this POC and the presence of the paternal X chromosome alleles, two scenarios are possible (Fig. 1D). The first one involves dispermic fertilization with two spermatozoa, one carrying an X and one carrying a Y chromosome, followed by the endoduplication of the two paternal pronuclei and asymmetric first cell division leading to the generation of two cell lines, diploid androgenetic monospermic (XX) and triploid diandric monospermic (XYY). The second scenario involves the fertilization with three spermatozoa with one carrying an X and two, each carrying a Y chromosome, followed by asymmetric first cell division and the generation of two cell lines, diploid androgenetic monospermic (XX) and triploid diandric dispermic (XYY) (Fig. 1D). In POCs 730, 722, and 721, some cells confined to the extravillous trophoblast displayed polyploidy (Figs 1 and 2). Polyploid cells in the extravillous trophoblast are known to occur in normal as well as molar conceptions.

3. Discussion

In the last 30 years, the accepted model of androgenetic complete mole formation has been the fertilization of an empty oocyte by one or two haploid spermatozoa. Consequently, most scientists in this field, including us, were characterizing the parental contribution to moles using only one, and sometimes two, methods. However, based on various observations and mainly on data from assisted reproductive technologies, Golubovsky (6) rejected the empty oocyte model and proposed a nice model implying dispermic fertilization and the formation of triprounuclear zygotes at the origin of various types of triploidies, aneuploidies, heteroploidies, and some genotypic types of moles. In line with Golubovsky's proposal are our observations that oocytes from patients with heterozygous *NLRP7* mutations were not empty, but abnormalities in their embryos started at the first zygotic cell and during early cleavage stages (2). In a different independent study, Niemann et al (7) reached a similar conclusion and proposed that fertilization of a single nucleated oocyte by one, two, or three spermatozoa followed by abnormal first zygotic cell division are at the origin of several twin pregnancies of diploid moles (monospermic or dispermic) and a co-existing normal fetus. Therefore, data from various groups looking at the pathology of moles from different perspectives are now converging toward several mechanisms leading to different types of moles, but all involving a single nucleated oocyte and some occurring around the time of fertilization and/or during early embryonic cleavage. The latter would be expected to

generate very complex aneuploidies and mosaicisms that cannot be detected by any single method and would require a combination of several methods.

Our histopathological analysis confirms interobserver variability in the classification of some moles into CHM and PHM and in distinguishing PHMs from hydropic spontaneous abortions (8) and indicates that in the same patient, under the same genetic background (for *NLRP7* mutation and for other genes), the histopathological type of the mole is greatly influenced by its genotype. Recently, we have shown that embryos from patients with *NLRP7* mutations and variants have early cleavage abnormalities during *in vivo* and *in vitro* post-zygotic development (2). Now, we show the occurrence of triploid diandric conceptions in a patient with one *NLRP7* mutation, which suggests a pre-existing defect in her oocytes at the time of fertilization. Altogether, these data suggest that some oocytes from patients with heterozygous *NLRP7* mutations are defective at several levels.

To date, three cases of recurrent triploidies have been described (9–11), but a clear conclusion on the parental contribution to the POCs was reached in only one of these cases where recurrent triploid digyny was demonstrated in two POCs by karyotype and DNA genotyping analyses (10). Our report is the first demonstrating the recurrence of triploid diandry in a patient with a heterozygous *NLRP7* mutation. Altogether, our previous and current data suggest that *NLRP7* may be a susceptibility gene for several types of hydatidiform moles and triploid spontaneous abortions. Since triploid conceptions are common and represent 1–2 % of all recognized pregnancies in the first trimester, additional data are needed to further delineate the extent of *NLRP7* involvement in triploid conceptions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Glossary

NLRP7	NOD-like receptor, pyrin domain containing 7
POC	product of conception

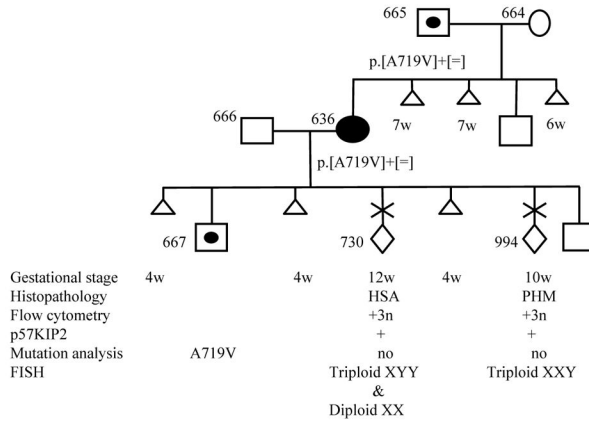
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MoCa94

A



B

Parental contribution to two products of conception from patient 636

Locus	Chromosome	Patient 636		1st POC 730			2nd POC 994			Partner 666	
D6S273	6	148	142	n.a.			148	144	150	144	150
D6S248	6	170	170	<i>170</i>	<i>144</i>	<i>154</i>	<i>170</i>	<i>144</i>	<i>154</i>	144	154
LH1	6	135	165	<i>135</i>	<i>152</i>	<i>154</i>	n.a.			152	154
D10S2325	10	3	3	3	2	3 2 1			2	1	
Th01	11	126	134	126	134	n.a.			126	134	
D11S1338	11	177	183	177	183	185	n.a.			183	185
D13S796	13	154	154	<i>154</i>	<i>159</i>	<i>163</i>	154	159	159		
D13S1317	13	139	155	155	137	139	n.a.			137	139
D13S742	13	234	242	234	244	n.a.			226	244	
D15S123	15	148	154	<i>148</i>	<i>156</i>	<i>158</i>	148	156	158	156	158
D18S1145	18	164	174	164	174	178	n.a.			174	178
D19S112	19	122	130	<i>122</i>	<i>114</i>	<i>126</i>	122	114	126	114	126
D21S268	21	131	143	<i>131</i>	<i>135</i>	<i>137</i>	<i>143</i>	<i>135</i>	<i>137</i>	135	137
D21S167	21	152	154	152	154	156	n.a.			154	156
D21S1888	21	180	188	180	188	174	n.a.			174	188
AMEL	X & Y	188(X)	188(X)	188(X)	193(Y)	188(X)	193(Y)	188(X)	193(Y)	188(X)	193(Y)
DXS1214	X	154	158	158	154	n.a.			154	158	
DXS1235	X	166	166	166	162	166	166	162	162	162	162
DXS1237	X	168	170	168	172	172	168	172	172	168	172

n.a. indicates not available; POC, indicates product of conception. Only informative markers displaying three alleles are shown.

C

POC ID	730, 2 cell lines		994
	Cell line 1	Cell line 2	
X cen (DXZ1)	1	2	2
Y cen (DYZ3)	2	0	1
18 cen (D18Z1)	3	2	3
8CEP*	3	2	3
11CEP*	3	2	n.a.
Interpretation	Triploid XYY	Diploid XX	Triploid XYY

Numbers indicate the number of detected copies for the designated chromosomes. *Polyloid cells (2%) were observed in the extravillous trophoblast.

D

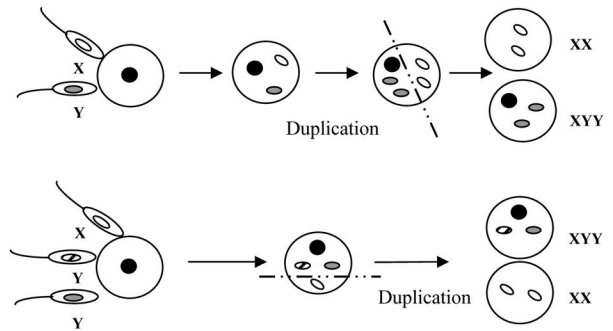


Fig. 1.

Characterization of two POCs from patient 636 heterozygous for one *NLRP7* mutation illustrating the importance of using various approaches. **A.** The reproductive outcomes are shown by chronological order from left to right. **B.** Italicized genotypes are informative and display two paternal alleles. **C.** Summary of the FISH analysis with probes from five chromosomes on the two POCs from patient 636. **D.** Illustration of the two scenarios that may have led to the mosaicism in POC 730.

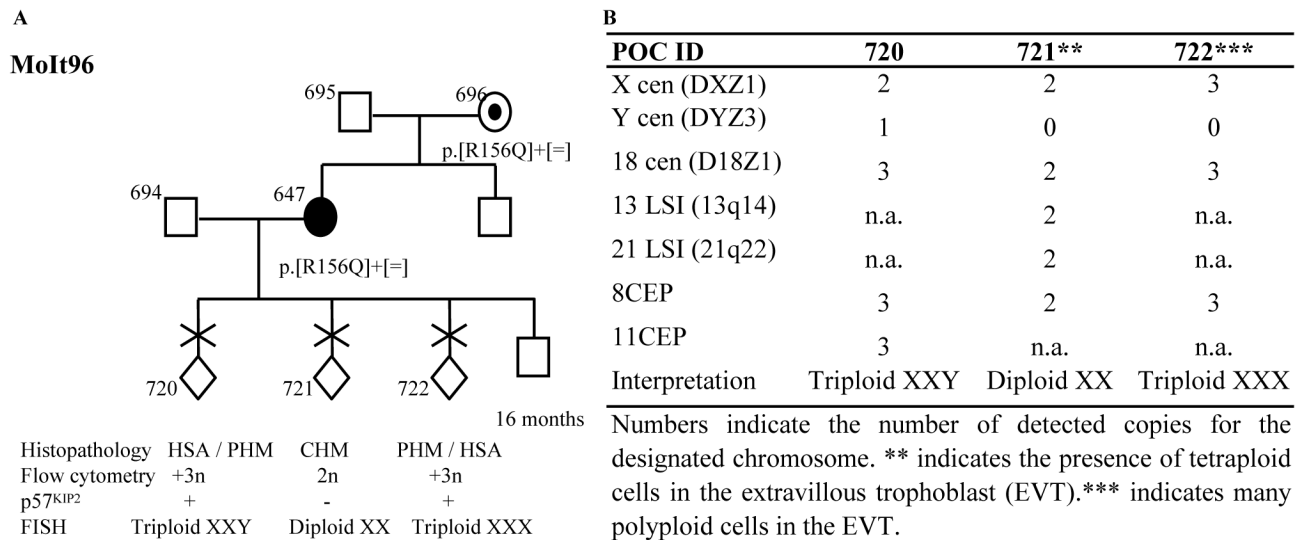


Fig. 2.

A. Characterization of three POCs from patient 647 heterozygous for one *NLRP7* mutation.

The reproductive outcomes are shown by chronological order from left to right.

Histopathological diagnoses of pathologists 1 and 2 are separated by a “/”. **B.** Summary of the FISH analysis with probes from 7 chromosomes on the three POCs from patient 647.