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Case Report

Recurrent hydatidiform mole: when to stop ?

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Summary

Recurrent hydatidiform mole (RHM) is defined as two or more repeated molar pregnancies in the same patient. Familial recurrent hydatidiform mole (FRHM) is a rare condition in which the patient has relatives with the same condition and mutations. Maternal mutations in the *NLRP7* gene are mostly observed in RHM. The authors report a patient from Turkey with a history of seven molar pregnancies who had an aunt with similar obstetric history and *NLRP7* mutations.

Key words: Familial hydatidiform mole; *NLRP7*; Ovum donation; Genomic imprinting.

Introduction

Hydatidiform mole (HM) is the most common gestational trophoblastic disease and the only one that can be recurrent, which indicates the patient's genetic predisposition. There is nonexistent or abnormal embryonic development, excessive trophoblastic proliferation, and cystic degeneration of chorionic villi. There is a difference in the incidence of HM among countries, ranging from 11.5/1,000 deliveries in Indonesia to less than 1/1,000 delivery in the United States. Women with a history of one HM seem to have a tenfold risk of repeat HM compared with women who have no history of HM [1]. HM is classified into partial (PHM) and complete (CHM) subtypes according to histopathologic and genetic criteria [2]. CHM is mostly diploid with two copies of the paternal genome, while PHM is mostly triploid with two different copies of the paternal genome and one copy of the maternal genome. Most cases of CHM are sporadic and are androgenetic, with two sets of paternal chromosomes. Rarely, CHM are diploid with both a maternal and paternal chromosome complement (BiCHM). Affected women have an autosomal recessive condition that presents as a history of recurrent HM (RHM).

RHM is seen in 1% to 2% of cases [3]. RHM may be non-familial or familial. In familial cases of RHM, two maternal gene mutations, a major gene *NLRP7* and a minor gene KHDC3L, have been identified. *NLRP7* mutations may also be responsible for causing recurrent spontaneous abortions, stillbirths, and intrauterine growth restriction [4]. Herein, the authors present a woman with RHM in seven consecutive pregnancies and parents with *NLRP7* mutations and a relative with a similar obstetric history.

Case Report

A 25-year-old woman who was otherwise healthy presented with a history of seven recurrent molar pregnancies and no living children. There was no history of infertility in her family. There was a history of consanguinity between the patients' parents, but no consanguinity between the patient and her husband. The patient and her partner had normal karyotypes, who both originated from a small rural region. The patient's aunt had a similar obstetric history, but the authors could not obtain her pathologic or genetic results.

The patient had seven consecutive pregnancies between 2008 and 2017; three pregnancies were documented as CHM and four were PHM on histopathologic examination. All surgical procedures and examinations were performed in different hospitals. The patient received contraception for one year after each pregnancy. The patient did not develop persistent trophoblastic disease after evacuation of any of the seven molar pregnancies. Unfortunately, no genetic analysis was performed on any of her molar pregnancies. After she presented to this hospital, she was referred for genetic counseling. Genetic testing for mutations in the *NLPR7* gene was performed on genomic DNA from the patient, her parents, and her brother. A homozygous NM_139176.3(*NLRP7*): c.2487_2488insC(p. Ile830Hisfs) frame shift mutation was detected in the patient and her father. Heterozygous NM_139176.3(*NLRP7*): c.2487_2488insC(p. Ile830Hisfs) frame shift mutation was detected in her mother and brother. Informed consent was obtained from the patient included in the study.

Materials and Methods

Genomic DNA was extracted from venous whole blood samples (from leukocytes) using a Qiamp DNA blood mini kit. The promotor region, splice site, and all coding regions (11 exons) of the *NLRP7* were amplified using polymerase chain reaction (PCR). The primers used in the PCR and sequencing reactions are listed in Table 1. The PCR reactions were performed under universal conditions in a volume of 50 μL. Electrophoresis of 5 μL of the final PCR reaction

Table 1. — *PCR conditions of primers used in NLRP7 mutation analysis*

Exon		Primer sequences (5'- 3')	T (°C)	Size (bp)
1	Forward	AGCCCACTGAAGCCCAATTACA	58.7	348
	Reverse	AGCATCCTCGCACCAACCATTA		
2	Forward	CTTGGCACACAGGAAACTGTGGTT	60	392
	Reverse	TACCCATCATCTCAGCCTTTGCCA		
3	Forward	ACCATGCCTGGCTGACACTTTA	58	221
	Reverse	TGCACCTTGCATGCTCTCAA		
4	Forward 4-1	CCTGGCCCTCATTTCTGGTA	60	371
	Reverse 4-1	TTGCAGCTGAGGTAGAACGC		
	Forward 4-2	CTGGACAGACTGCAACCTCAGC	60	427
	Reverse 4-2	GTGTCTCAGGAAATAGGCCCTCC		
	Forward 4-3	TTCCAAGCATCCTAGCCCAA	56	406
	Reverse 4-3	AGAGTCGTGCACACAATCCA		
	Forward 4-4	AGCAGCCGATCTACGTAAGGGTG	60	417
	Reverse 4-4	TGTCTCCGTCCAGGAACAGACG		
	Forward 4-5	TCCAAAGGCTGCTACTCCTT	56	236
	Reverse 4-5	AAAGTGGCCTCCAACTCCTT		
	Forward 4-6	TCGCTAACGAGAAGAGAGCCAA	58	432
	Reverse 4-6	TGACAGTAAGCGACAGGGCAAA		
5	Forward	ATGTGGTTTGCAGATGCCCA	57.4	313
	Reverse	AGTCATCGTTCAGGGTCTTCCT		
6	Forward	ACCCGGCCAAGAACTTCTAA	56	402
	Reverse	AATACATTCCCTGTCTGGGACG		
7	Forward	ACGCCTTTGCATTCCAGACT	58	367
	Reverse	TGGCGCAGTAAGTCAGGTGTTA		
8	Forward	AGCAGGTTGGAGTTGTGGAA	56	360
	Reverse	TGGCCTCTGCCTGTTCTTTA		
9	Forward	TAACTGCTTCACAGGGCGTT	56	353
	Reverse	ATCAAGCCTGGAAGACCGAA		
10	Forward 10-1	ACACGCTTGAGCCACTACCT	60	205
	Reverse 10-1	TTGTCTTAGAACCCCAAAGAGC		
	Forward 10-2	GCTTGAAACTCTGGACCTGG	60	642
	Reverse 10-2	GGGCAACAGTGAGACTCCAT		
11	Forward 11-1	TTCAGGCATCCTGGGTAGTT	55.5	278
	Reverse 11-1	ACCCTCTGACCTGCATTCAT		
	Forward 11-2	TTGATTGCAATGCTTCCGGG	56	462
	Reverse 11-2	TCACATAGATGCACCTGGCA		

volume was performed on 2% agarose gel to test the amplification reaction.

After the amplification reaction control, PCR products were purified using the QIAquick gel extraction purification kit for DNA sequencing. Fifty nanograms of the purified PCR product were used for cycle sequencing with the BigDye Terminator V3.1 cycle sequencing Kit in a final volume of 20 μL. The amplified products were purified with Sephadex spin columns and sequenced on an Avant automated DNA capillary sequencing system. Samples were run using the POP 7 polymer, with dye set Z and analyzed using the Genescan Version 3.7, Proseq and BioEdit software programs. All sequences were compared with the reference genomic sequences (NM_139176) for mutation detection.

Discussion

Hydatidiform mole incidence is approximately one in 500-1,000 pregnancies. The risk of having an HM in a subsequent pregnancy is only about 1% [3]. Familial RHM is a rare autosomal recessive condition in which affected patients have a predisposition to multiple CHMs. Unlike sporadic forms, they are biparenteral and have a normal diploid genotype.

The exact mechanisms leading to molar pregnancies are unknown. Approximately 80% of women with FRHM have been found to have mutations in the maternal effect gene *NLRP7*, which is located on chromosome 19q13.3–q13 [4]. The exact role of *NLRP7* in CHM is unknown. It may have a role in controlling the timing of oocyte growth or in transducing signals required to initiate imprint establishment [5].

In the present patient, 11 exons of *NLRP7* gene (Transcript: NLRP7-209 ENST00000592784.5) were sequenced by designing deep intronic primers using the Sanger sequencing method. In the genetic analysis, the homozygous NM_139176.3 (*NLRP7*):c.2487_2488insC (p.Ile830Hisfs) frame shift mutation that was detected in the patient was identified as number rs766731093 in the dbSNP database (https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=766731093), and as number CI109386 in the HGMD database (HGMD: http://www.hgmd.cf.ac.uk/ac/all.php). The minor allele frequency (MAF) value is reported as G=0.00005/6. The mutation taster result for NM_139176.3 (*NLRP7*):c.2487_2488insC (p.Ile830Hisfs) mutation was determined as "disease causing." In the segregation analysis of the mutation of the patient (hotspot Sanger sequencing analysis of the parents and the brother of the patient), it was detected that her mother and brother were heterozygous and her father was homozygous NM_139176.3 (*NLRP7*):c.2487_2488insC (p.Ile830Hisfs) mutation carriers. According to the HGMD database and Buyukkurt *et al.,* [6] the homozygous NM_139176.3 (*NLRP7*):c.2487_ 2488insC (p.Ile830Hisfs) mutation is associated with CHM.

No treatment for women with RFHM and *NLRP7* mutations has been described. There is also a possibility of malignant transformation in future pregnancies [7, 8]. To prevent malignancy and toxic effects of chemotherapy, avoiding further pregnancies should be recommended to these patients. *NLRP7* is believed to have a role in oocyte growth; therefore, ovum donation might be an alternative treatment for these patients. It should be kept in mind that *NLPR7* can be expressed in the uterus and even ovum donation can fail. However, with close follow up during pregnancy, there is a better chance of a healthy offspring with ovum donation rather than spontaneous pregnancy [7, 9].

With this case, the present authors want to recommend genetic counseling for patients with a history of RHM. Genotyping should be performed in molar pregnancies for the confirmation of biparenteral and diploid hydatidiform moles for the diagnosis of familial RHMs. Genetic testing for mutations in the *NLPR7* gene should be performed on genomic DNA from the patient. Detailed information should be provided to the patients about FRHM and mutations. These patients are usually anxious and desperate to have a healthy pregnancy. The possibility of having a normal off spring with assisted reproductive cycles using donated ovum should be explained and offered to patients with FRHM and maternal gene mutations.

Conflict of Interest

The authors declare no competing interests.

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