CASE REPORT

Two Novel Variants in *NLRP7* Gene in an Egyptian Female Patient with Consecutive Molar Pregnancies Complicated by Choriocarcinoma

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ABSTRACT

Background: Hydatidiform mole, whether complete or partial mole, is one of the most common forms of gestational trophoblastic disease. It is characterized by extreme trophoblastic proliferation and atypical embryonic growth. Though almost all of complete hydatidiform moles are diploid androgenetic, scarce cases are biparental and caused mainly by mutations in *NLRP7* and *KHDC3L* genes. *NLRP7* mutations are more common and were reported in around 50–80% of cases from diverse populations while *KHDC3* mutations were only found in 5–10% of cases.

Case description: A healthy 40-year-old Egyptian woman was referred to the Clinic of Prenatal Diagnosis and Fetal Medicine Department for counseling. She was married for 20 years to a first-degree relative and experienced 17 consecutive pregnancy losses without having any live births. Uterus ultrasound revealed endometrial thickening and subseptate uterus and in her last pregnancy failure, she complained of abdominal pain and severe shortness of breath. Immunochemistry tests were positive for β -human chorionic gonadotropin and histopathology-confirmed choriocarcinoma. Genetic testing revealed two novel heterozygous variants in the *NLRP7* gene.

Conclusion: We presented a case with 17 recurrent hydatidiform moles that was complicated by choriocarcinoma due to novel variants in the *NLRP7* gene.

Clinical significance: This is the first Egyptian case with recurrent hydatidiform mole. We identified novel *NLRP7* variants, thus expanding the mutational spectrum associated with this rare disease.

Keywords: Choriocarcinoma, Egyptian patient, *NLRP7* gene, Novel variants, Recurrent hydatidiform moles.

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BACKGROUND

Hydatidiform mole (HM) is defined as an atypical human pregnancy characterized by the absence of abnormal embryonic growth and development with hydropic degeneration of chorionic villi. It is usually not recurrent and occurs once in every 1,000–1,500 pregnancies in Western countries. However, a higher incidence (up to a tenfold increase) was noted among underdeveloped and developing countries.¹ In women with sporadic moles, 1–6% of cases encounter a second mole^{2,3} and around 10–20% go through a second nonmolar reproductive loss, commonly known as sudden spontaneous abortion.^{4,5} Concerning recurrent HM (RHM), the frequency is not yet documented but such cases are assumed to be very rare.⁶

Hydatidiform mole is divided into two types according to the histopathological level: complete HM (CHM) or partial (PHM) based on the degree of trophoblast proliferation and the presence or absence of embryonic tissues beside the chorionic villi. Regarding the karyotype and genotype levels, most of the sporadic CHMs are diploid androgenetic, but may fall into these genotypes: diploid biparental, tetraploid androgenetic or biparental, aneuploid, triploid diandric, or even mosaic with two different cellular populations.^{7,8} Among the androgenetic moles, the commonest are monospermic while 5–20% are dispermic. In addition, almost all characterized recurrent HMs from women with no family history of moles were diploid and biparental with only two reported cases where one mole in a patient and three in another were shown to be diploid androgenetic.⁹

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Recurrent HM is caused mainly by biallelic mutations in two maternal-effect genes, *NLRP7* and *KHDC3L*.^{10,11} The two genes encode proteins that play a pivotal role in maintaining the oocytes' integrity, cell-lineage differentiation, and postzygotic divisions.¹² *NLRP7* mutations are more common and were reported in around 50–80% of cases while *KHDC3* mutations were only found in 5–10% of cases. In about 10–20% of cases, no mutations were detected in the two genes suggesting the involvement of other unidentified genes.¹³

Herein, we describe an Egyptian female with 17 consecutive molar pregnancies, which were found to harbor two novel variants in the *NLRP7* gene.

CASE DESCRIPTION

A healthy 40-year-old Egyptian woman was referred to the outpatient clinic of Prenatal Diagnosis and Fetal Medicine Department, National Research Centre (NRC), for counseling. She had been married for 20 years to a first-degree relative and experienced 17 consecutive pregnancy losses with no live births. After each pregnancy loss, dilation and curettage was done and sample was taken for histopathology and results were consistent with complete vesicular mole. On examination, the patient had normal BMI and her blood pressure, glucose, and thyroid function tests were all normal. She was RH negative and the indirect Coombs test was done and showed negative results after each pregnancy loss. Cytogenetic studies were also carried out for the couple and revealed normal karyotype.

Uterus ultrasound revealed endometrial thickening and subseptate uterus. In the last pregnancy failure, the patient complained of abdominal pain and severe shortness of breath. Diagnostic workup was done and there was elevated chorionic gonadotropin levels of 2,820 mIU/mL. Immunochemistry tests were positive for β -human chorionic gonadotropin. In addition, histopathology revealed choriocarcinoma. The patient went on seven cycles of the chemotherapy regimen and was cured thereafter.

MATERIALS AND METHODS

Genomic DNA was extracted from peripheral blood lymphocytes after having a signed informed consent according to the guidelines of the Medical Research Ethics Committee of the NRC, in accordance with Declaration of Helsinki. DNA was extracted using a standard extraction procedure. The coding regions and exon/intron boundaries of the *NLRP7* and *KHDC3L* genes were amplified using specific primers designed by the Exon Primer software. Primers are available upon request from the corresponding author. Our standard PCR cycling conditions were: initial denaturation at 96°C for 5 minutes; 30 cycles of denaturation at 96°C for 30 seconds; annealing at 62°C for 30 seconds; extension at 72°C for 30 minutes, and a final extension at 72°C for 5 minutes. The PCR products were purified using Exo-SAP PCR Clean-up Kit (Fermentas, Germany) and sequenced in both directions using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and analyzed on the ABI Prism 3500 Genetic Analyzer (Applied Biosystems) according to manufacturer's instructions. The sequence data were compared with the reference genomic and cDNA sequence of the two genes, *NLRP7* (NM_001127255) and *KHDC3L* (NM_001017361).

RESULTS

The mutational analysis of the two genes revealed two novel heterozygous variants in the *NLRP7* gene while no pathogenic variants were detected in the *KHDC3L* gene. The two variants were one missense in exon 4 (c.1358T>G, p.Ile453Ser) and one frameshift in exon 7 (c.2655dupC, p.Ile886HisfsTer11) (Fig. 1). The c.2655dupC (p.Ile886HisfsTer11) was maternally inherited while the c.1358T>G (p.Ile453Ser) variant was not found in the mother's DNA and mostly inherited from the deceased father. The two variants were not found

in the public genetic databases and were predicted to be causing disease by various bioinformatic tools. According to the American College of Medical Classification (ACMG) variants classification, the c.2655dupC (p.Ile886HisfsTer11) was classified as "likely pathogenic" while the c.1358T>G (p.Ile453Ser) was classified as "variant of uncertain significance."

DISCUSSION

The *NLRP7* is a nucleotide oligomerization domain (NOD)-like receptor, pyrin containing 7, which maps to 19q13.4 and was initially recognized as the causative gene for RHMs. Studies from several groups and populations agreed that *NLRP7* is the main gene for this disorder and is mutated in 48–80% of females with a previous history of at least two HMs, reliant on patients' ascertainment criteria and populations. *NLRP7* mutations differ in their way of inheritance from other monogenetic syndromes as females with *NLRP7* mutations are healthy but they are at a high threat of reproductive failure.^{10,14} In this report, we described a new case with recurrent HM from Egypt (Fig. 2).

Normally, females older than 35 years have a twofold increased risk of molar pregnancy while those over 40 years have as high as five to tenfold increased risk.¹⁵ Nevertheless, a single study reported that recurrence of the disease (second molar pregnancy) appears to be present within 2–4 years after the first occurrence. Females should be followed up for a year if partial mole was diagnosed and for two years in case of a complete mole.¹⁶ The genetic background of HM is an important material for further investigation in many studies. *NLRP7* gene mutations were found in familial forms and were repeated in many case reports.^{11,17} Scientists found that *NLRP7* mutations were the cause for an increase in stochastic and mosaic aneuploidies during early stages of embryo development. There is a theory stating that the correlation between mutations of the *NLRP7* gene and the formation of molar pregnancy is enlightened through the inability to express inflammatory response to many antigens and stimuli.⁶

The precise role of *NLRP7* in the pathology of molar pregnancies is not fully clear, but existing data relate its protein to be involved in the inflammatory response with the trophoblastic lineage differentiation.^{18–20} Protein-truncating mutations, which are predictable to have major functional consequences on the protein, are associated with the absenteeism of embryonic tissues in the conceptions of these female patients. In contrast, missense mutations are usually associated with slighter functional consequences on the protein and the differentiation of certain embryonic tissues.²¹

Our case experienced 17 times complete vesicular mole without having any live births. She came from a rural area in Egypt where not having a child represents a social stigma. Therefore, she continuously tried to have a baby although was given several medical advices and counseling about the difficulty of having an offspring.

The mutational analysis of *NLRP7* and *KHDC3L* genes revealed two variants in the *NLRP7* gene. The two variants were one missense (c.1358T>G) in exon 4 and one frameshift (c.2655dupC) in exon 7. To our knowledge, these two variants were not described before in any case with HD. The c.1358T>G is a missense variant that results from the substitution of isoleucine by serine at position 453 (p.Ile453Ser). On the other hand, the c.2655dupC involves the duplication of one nucleotide in exon 7 and is predicted to result in early protein truncation (p.Ile886HisfsTer11). The two variants cosegregated with

Figs 1A and B: (A) Pedigree of the family; (B) Portion of the sequencing electrophoregram showing the two novel variants in the *NLRP7* gene identified in our patient. Arrow indicates site of variant

Fig. 2: DNA sequencing for two mutations; 1 missense in exon 4 (c.1358T>G, p.I453S) and 1 frameshift in exon 7 (c.2655dupC, p.I886Hfs*11)

the phenotype in our case as the frameshift variant was inherited from her mother while the missense variant was not found in the mother's DNA and we assumed that it was paternally inherited due to the lack of DNA from the deceased father.

To date, more than 60 distinct pathogenic variants have been reported in the *NLRP7* gene.^{22–25} Majority of these mutations (>60%) were protein truncating (nonsense, frameshift, or splice site) followed by missense mutations that were found in around 30–35% of cases.^{14,23–25} The c.2655dupC (p.Ile886HisfsTer11) variant found in our case is located in the C-terminal LRR domain of the gene, which harbors majority of reported mutation. In contrast, the missense variant found in our patient (c.1358T > G, p.Ile453Ser) is located in the NACHT domain of the gene. About 17 missenses have also been perceived as single heterozygous mutations or variants in females with recurrent moles. Though, the pathological consequence of these single mutations or variants is yet a topic of debate, and more data are needed to find a conclusion on their possible participation in the causation or genetic vulnerability for moles.¹³

Although *NLRP7* and *KHDC3L* are the two main causative genes of HMs, several cases were reported without having mutations in any of the two genes suggesting further genetic heterogeneity of the disorder. In this regard, Nguyen and coauthors performed whole exome sequencing for 65 women who were tested negative for

both *NLRP7* and *KHDC3L*. The study identified biallelic pathogenic variants in three new genes (*MEI1*, *C11orf80*, and *REC114*) in five unrelated women, two of them had other family members who experienced recurrent miscarriages and infertility.²⁶

Counseling and management of females with *NLRP7* mutations, egg donation, or preimplantation genetic diagnosis (PGD) have been proposed as a sole option for having a successful pregnancy. Within oocyte, the *NLRP7* protein is transcribed from the maternal genome and is essential for regular fetal growth till the embryonic genome is activated.¹² In latest studies, donor eggs having intact ooplasm lead to successful pregnancies in women with maternal effect variants in *NLRP7*.²⁷ Though aberrant *NLRP7* protein may be found in the maternal oocyte and ooplasm, PGD is focused on variants in the embryonic genome. Therefore, egg donation and PGD are possible initiators for successful pregnancy. Yet, more trials are necessary before accurate conclusions can be drawn.²⁷

CONCLUSION

We presented an Egyptian case with recurrent HM that was complicated by choriocarcinoma. What is interesting in our case is the number of molar pregnancies the patient has had, in addition to the identification of two novel variants in the *NLRP7* gene.

CLINICAL SIGNIFICANCE

This case report expands the mutational spectrum associated with this rare disease. Further, our results reinforce that *NLRP7* gene mutations are the most common etiology of HD irrespective of ethnic variability.

All authors agree on publishing this work. A signed informed consent according to the guidelines of the Medical Research Ethics Committee of the NRC was obtained prior to sampling.

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