A Novel Mutation in NLRP7 Related to Recurrent Hydatidiform Mole and Reproductive Failure

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Abstract

Background: Hydatidiform mole (HM) is an abnormal human pregnancy with excessive trophoblastic proliferation and abnormal embryonic development, dividing into two complete HM (CHM) and partial HM (PHM) groups. One subcategory of the CHMs is recurrent and familial, which is known as biparental HM (BiHMs) or recurrent HM (RHM). NLRP7, KHDC3L and PADI6 are maternal-effect genes involved in RHMs. NLRP7 is a major gene responsible for RHMs. This study was performed on patients with molar pregnancies and miscarriage. The aim of this study was to genetic screen for mutations in NLRP7 and KHDC3L genes in an affected woman with previous history of 5RHM and the sibling with history of miscarriage.

Materials and Methods: In this experimental study, DNA was extracted from blood samples. KHDC3L and NLRP7 were polymerase chain reaction (PCR) amplified. The PCR products were purified and Sanger sequenced.

Results: In this study, there is no mutation in KHDC3L gene but a novel mutation was identified in the NACHT domain of NLRP7 gene. Patient with five recurrent moles had this mutation in the homozygous state while her sister with one miscarriage and one normal child showed this mutation in the heterozygous state.

Conclusion: In this study, we identified a new mutation in NLRP7 gene of a patient with recurrent HM. Following egg donation, this patient has a normal boy. The sister of this patient with heterozygous mutation has a spontaneous abortion and one normal child that confirm the impact of a defective allele of NLRP7 on reproductive wastage in a recent finding.

Keywords: Hydatidiform Mole, KHDC3L, NLRP7

Introduction

Hydatidiform mole (HM) is an abnormal human conception with a defect in fetal development and growth (1). HM is divided into two categories, complete HM (CHM) and partial HM (PHM). CHMs are commonly androgenetic diploid conceptions (2) and PHMs are mostly dispermic triploid conceptions (3). Both CHM and PHM have an extra set of the paternal genome, therefore, paternal genes are more expressed and consequently show excessive trophoblastic proliferation (4). In most of the cases, HM is sporadic, however, in a subgroup of CHM, it is recurrent and familial condition which is known as biparental HM (BiHMs) or recurrent HM (RHM) (OMIM 231090). Occurrence of at least two moles in the same woman is referred to recurrent type and this form is inherited in an autosomal recessive fashion. Frequency of RHMs in the Middle and Far East is reported about 2.5% up to 9.4% of all HMs, which is twice or more compared to Western countries (5-9).

So far, three maternal-effect genes, NLRP7, KHDC3L and recently PADI6, have been identified to be responsible for RHMs (10-12). It is suggested that these three genes function in setting genomic imprinting process (13). NLRP7 mutations have been reported in 48-80% of RHMs cases (14-19), while mutations in KHDC3L was only reported in 10-14% of these patients with no NLRP7 mutations (10, 20, 21). Homozygote or compound heterozygote mutations of these three genes have been observed in most of the affected women (22). There is still a few fractions of RHM patients with the unidentified responsible gene. NLRP7 is the principal gene responsible for RHMs, identified by Murdoch and colleagues in
2006. NLRP7 as the candidate of maternal-effect gene is responsible for RHMs and reproductive disorders such as spontaneous abortions and stillbirths (11).

NLRP7, which encodes a protein with 1037 amino acids, is a member of the CATERPILLER protein family with four conserved and functional pyrine, 9-10 leucine-rich repeats, NACHT-associated domain (NAD) and a NACHT domain (Fig.1A) (23, 24). About 48% of intronic sequences of NLRP7 gene contain Alu repetitive elements. It is believed that Alu repeats act as a hot spot for INDEL mutations (20). To date, 60 pathogenic point and INDEL mutations have been reported in NLRP7 (20, 25). In this study, a new mutation was identified in NLRP7 gene in a patient with recurrent HM. This patient has a normal boy using egg donation. Also, the sister of this patient with heterozygous mutation has a spontaneous abortion and one normal child.

Materials and Methods

In this experimental study, two sisters with molar pregnancies and miscarriage referred to the Infertility Center in Shiraz University of Medical Sciences, Shiraz, Iran. In the patient, as proband, five moles were reported without any normal child. Patient’s sister represented one normal child and one miscarriage. Proband was diagnosed as Bi-HMs because she has more than two moles and genetic studies were performed on NLRP7 and KHDC3L genes. Genomic DNA was isolated from whole blood cells using DNA Kit (Cinaclon, Iran). Three exons and intron boundaries of KHDC3L and 11 exons and intron boundaries of NLRP7 were polymerase chain reaction (PCR) amplified using our previously designed primers and conditions (20, 26). PCR products were purified and Sanger sequenced (Eurofins, Germany). The Ethics Committee of Shiraz University of Medical Sciences approved the study protocol and patients gave written consent to participate in the study (code: IR.SUMS.REC.1396.540).

Results

The sequence of NLRP7 and KHDC3L were analysed by Chromas software (Technelysium Pty Ltd, Australia). BLAST of sequences was performed for two genes based on the reference sequences in the NCBI database (NLRP7, NG_008056.1, and KHDC3L, NG_031942.1). Sequencing analysis of NLRP7 in the patient revealed a new three nucleotides deletion in exon 4 in a homozygous state (Fig.1B). Sequence analysis of the patient’s sister with one spontaneous abortion and one normal child showed a heterozygous deletion status for these three nucleotides (Fig.1C). Normal sequence is provided in Figure 1D. This deletion is expected to remove amino acid Threonine in codon 185 (c.555_557delCAC, p.Thr185del). The mutation was evaluated by parameters of Mutation Taster (www.mutationtaster.org) and it was regarded as disease-causing alteration. In addition, the mutation was analysed by PROVEAN parameter (http://provean.jcvi.org). Variants with a score equal to or below -2.5 are considered "deleterious," and variants with a score above -2.5 are considered "neutral." PROVEAN score was estimated -13.000 for this mutation. This means that the mutation is deleterious.

In addition, Threonine in codon 185 is conserved in various species using multiple sequence alignment by Clustal Omeg (www.ebi.ac.uk/Tools/msa/clustalo/) (Fig.1E). Histopathology of the molar tissue for the patient is provided in Figure 2. Excessive proliferation of trophoblastic tissue has been observed around chorionic villi, while fetal tissues were clearly absent.
Discussion

In this study, a new mutation in the homozygous state has been identified within the NACHT domain of NLRP7 protein, suggesting the importance of this domain in normal function. This study on a patient with a homozygous mutation in NLRP7, while she has a healthy boy via ovum donation, add further evidence that pathology of RHM is restricted to the oocyte and normal ovum is able to rescue defects of these patients for normal pregnancies. To date, four cases of ovum donation in patients with a mild missense mutations in NLRP7 have been reported (27, 28). Investigations on healthy reproductive male individual with a homozygous mutation in NLRP7 show that function of this gene is not necessary for normal sperm, in contrast to ovum (14, 15). The sister of indicated patient with heterozygous mutation has a spontaneous abortion and one normal child, confirming the impact of the defective allele of NLRP7 on reproductive wastage, reported in recent finding (25).

Conclusion

We report a new mutation in NLRP7 gene, related to RHM and spontaneous abortion in homozygous and heterozygous states, respectively. Regarding this study and four previous reports, patients with homozygous mutation in NLRP7 are able to live birth with egg donation. In contrast to four previously reported cases with a mild missense mutations, investigation on this new patient shows that more deleterious mutations with severe functional effect are also good candidate for egg donation.

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Authors’ Contribution

M.F., Z.A.; Contributed to conception and design. J.F.; Contributed to all experimental work. M.F., J.F., V.R.; Contributed to interpretation of data and drafted the manuscript. M.A.-J.; Contributed to interpretation of the molar tissue. M.M., B.N.-J.; Contributed to consulting and detection of patients through clinical specification also performed editing and approving the final version of this manuscript for submission. All authors read and approved the final manuscript.

References

1. Savage P, Williams J, Wong SL, Short D, Casalboni S, Catalano K, et al. The demographics of molar pregnancies in England and Wales from 2000-2009. J Reprod Med. 2010; 55(7-8): 341-345.
2. Kajii T, Ohama K. Androgenetic origin of hydatidiform mole. Nature. 1977; 268(5621): 633-634.
3. Zaragoza MV, Surti U, Redline RW, Millie E, Chakravarti A, Hasold TJ. Parental origin and phenotype of triploidy in spontaneous abortions: predominance of diandry and association with the partial hydatidiform mole. Am J Hum Genet. 2000; 66(6): 1807-1820.
4. Fisher RA, Hodges MD. Genomic imprinting in gestational trophoblastic disease a review. Placenta. 2003; 24 Suppl A: S111-S118.
5. Kim JH, Park DC, Bae SN, Namkoong SE, Kim SJ. Subsequent reproductive experience after treatment for gestational trophoblastic disease. Gynecol Oncol. 1998; 71(1): 108-112.
6. Yagyu EG, Ahyan A, Engeleni MH. Pregnancy outcome after hydatidiform mole, initial and recurrent. J Reprod Med. 1994; 39(4): 297-299.
7. ACOSTA-SISON H. The chance of malignancy in a repeated hydatidiform mole. Am J Obstet Gynecol. 1959; 78: 876-877.
8. Kronfol NM, Iliya FA, Hajji SN. Recurrent hydatidiform mole: a report of five cases with review of the literature. J Med Liban. 1969; 22(4): 507-520.
9. Boufettal H, Couillin P, Mahdouaoui S, Noun M, Hermes S, Samouh N. Complete hydatidiform mole in Morocco: epidemiological and leucine-rich study. J Gynecol Obstet Biol Reprod (Paris). 2011; 40(5): 419-429.
10. Parry DA, Logan CV, Hayward BE, Shires M, Landolfo H, Diggle M, et al. Mutations causing familial biparental hydatidiform mole implicate c stimulating as a possible regulator of genomic imprinting in the human genome. Am J Hum Genet. 2011; 89(3): 451-465.
11. Murdoch S, Djuric U, Mazhar B, Seoud M, Khan R, Kuick R, et al. Mutations in NALP7 cause recurrent hydatidiform moles and reproductive wastage in humans. Nat Genet. 2006; 38(3): 300-302.
12. Qian J, Nguyen NMP, Rezaei M, Huang B, Tao Y, Zhang X, et al. Biallelic PADI6 variants linking infertility, miscarriages, and hydatidiform moles. Eur J Hum Genet. 2018; 26(7): 1007-1013.
13. Zhang P, Dixon M, Zucelli M, Hambili F, Levkov L, Hovatta O, et al. Expression analysis of the NLRP gene family suggests a role in human preimplantation development. PLoS One. 2008; 3(7): e2755.
14. Kou YC, Shao L, Peng HH, Rosetta R, del Gaudio D, Wagner AF, et al. A recurrent intragenic genomic duplication, novel mutations in NLRP7 and imprinting defects in recurrent hydatidiform moles. Mol Hum Reprod. 2008; 14(1): 33-40.
15. Wang CM, Dixon PH, Decordova S, Hodges MD, Sebire NJ, Ozalp S, et al. Identification of 13 novel NLRP7 mutations in 20 families with recurrent hydatidiform mole; missense mutations cluster in the leucine-rich region. J Med Genet. 2009; 46(8): 569-575.
16. Hayward BE, De Vos M, Talati N, Abdollahi MR, Taylor GR, Meyer E, et al. Genetic and epigenetic analysis of recurrent hydatidiform mole. Hum Mutat. 2009; 30(5): E629-E639.
17. Slim R, Bagga R, Chebaro W, Srinivasan R, Agarwal N. A strong founder effect for two NLRP7 mutations in an Indian population: an intriguing observation. Clin Genet. 2009; 76(3): 292-295.
18. Qian J, Cheng Q, Murdoch S, Xu C, Jin F, Chebaro W, et al. The genetics of recurrent hydatidiform moles in China: correlations between NLRP7 mutations, molar genotypes and reproductive outcomes. Mol Hum Reprod. 2011; 17(10): 612-619.
19. Estrada H, Buentello B, Zenteno JC, Fiszman R, Aguinaga M. The p.L750V mutation in the NLRP7 gene is frequent in Mexican patients with recurrent molar pregnancies and is not associated with recurrent pregnancy loss. Prenat Diagn. 2013; 33(3): 205-208.
20. Reddy R, Nguyen NM, Sarrabay G, Rezaei M, Rivas MC, Kavasogluz A, et al. The genomic architecture of NLRP7 is Alu rich and predisposes to disease-associated large deletions. Eur J Hum Genet. 2016; 24(10): 1445-1452.
21. Reddy R, Akouy E, Phuong Nguyen NM, Abdul-Rahman OA, Dery C, Gupta N, et al. Report of four new patients with protein-truncating mutations in C6orf221/KHDC3L and colorization with NLRP7. Eur J Hum Genet. 2013; 21(9): 957-964.
22. Nguyen NM, Slim R. Genetics and epigenetics of recurrent hydatidiform moles: basic science and genetic counselling. Curr Obstet Gynecol Rep. 2014; 3: 55-64.
23. Messaed C, Akouy E, Djuric U, Zeng J, Saleh M, Gilbert L, et al. NLRP7, a nucleotide oligomerization domain-like receptor protein, is required for normal cytokine secretion and co-localizes with Golgi and the microtubule-organizing center. J Biol Chem. 2011; 286(50): 43313-43323.
24. Ginger H, Biswas A, Zimmer N, Messaed C, Oldenburg J, Slim R, et al. NLRP7 inter-domain interactions: the NACHT-associated domain is the physical mediator for oligomeric assembly. Mol Hum Reprod. 2014; 20(10): 990-1001.
25. Soellner L, Begemann M, Degenhardt F, Geipel A, Eggertmann M, Mangold E. Maternal heterozygous NLRP7 variant results in re-
current reproductive failure and imprinting disturbances in the offspring. Eur J Hum Genet. 2017; 25(8): 924-929.

26. Rezaei M, Nguyen NM, Foroughinia L, Dash P, Ahmadpour F, Verma IC, et al. Two novel mutations in the KHDC3L gene in Asian patients with recurrent hydatidiform mole. Hum Genome Var. 2016; 3: 16027.

27. Fisher RA, Lavery SA, Carby A, Abu-Hayyeh S, Swingler R, Sebire NJ, et al. What a difference an egg makes. Lancet. 2011; 378(9807): 1974.

28. Akoury E, Gupta N, Bagga R, Brown S, Déry C, Kabra M, et al. Live births in women with recurrent hydatidiform mole and two NLRP7 mutations. Reprod Biomed Online. 2015; 31(1): 120-124.