IJBPAS, January, 2015, 4(1): 15-23

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

MUTATIONS STUDY iN NALP7 GENE fOR HYDATIDIFORM MOLE DISEASE aMONG IRANIAN WOMEN

VAHIDEH KEYVANI[1], ZOHREH YOUSEFI[2], DOR MOHAMMAD KORDI-TAMANDANI[1], MOHAMMAD AMIN KERACHIAN[* 3, 4]

1: Department of Biology, University of Sistan and Baluchestan, Zahedan, Iran

2: Department of Obstetrics and Gynecology, Women's Health Researches Center, Mashhad University of Medical Sciences, Iran

3: Department of Medical Genetics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

4: Medical Genetics Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

***Corresponding Author: E Mail: Kerachianma@mums.ac.ir**

ABSTRACT

Hydatidiform moles (HMs) are abnormal human pregnancies with cystic degeneration of the chorionic villi and abnormal fetal development. Recurrent hydatidi form moles (RHMs) are a rare clinical entity in which molar tissues are diploid and have bi parental contribution to their genome. It has been previously found that a maternal recessive locus, 19q13.4 is responsible for familial hydatidi form moles. Extensive research work on families with HM led to the identification of mutations in the NALP7 gene as being responsible for this condition. Mutations in this gene have been shown to be responsible for HMs associated with other forms of reproductive wastage in women from several ethnic groups.

DNA was extracted from blood samples of patients and normal individuals. The NALP7 gene region was then amplified by specific primers and analyzed for mutations using HRM technique and sequencing in both patient and control samples.

HRM analysis showed differences in some women with HM. These samples were then subjected to sequencing that demonstrated some mutations in NALP7 gene.

Here, we suggest that mutations in NALP7 are not strongly associated with HMs among Iranian women.

Keywords: Mole Hydatidi form, DNA Mutation, High Resolution Melting, NALP7 Gene

INTRODUCTION

Hydatidi form mole (HM), is an abnormal human pregnancy characterized by unusual hyperplasic trophoblast and hydropic villi, and faulty fetal development **[1]**. Based on the histopathology of the evacuated molar tissues, HMs are separated in two types: complete hydatidi formmoles (CHMs) and partial hydatidiform moles (PHMs). CHMs are characterized by hydropic degeneration of all villi and absence of embryo, cord, and amniotic membranes. In CHMs, all the villi are enlarged with cisternae, avascular (no fetal vessels), and surrounded by areas of excessive trophoblasticproliferation. PHMs are characterized by focal trophoblastic proliferation with a mixture of normal-sized villi and edematous villi. The trophoblastic proliferation is less pronounced than in complete moles. An embryo, cord, and amniotic membranes are usually present in partial moles **[2-4]**. At the karyotype and genotype scales, most sporadic CHMs are diploid androgenetic, but may also have any of the following genotypes, diploid biparental, tetraploid androgenetic, aneuploid (nontriploid/tetraploidaneuploid), triploid diandric or dygenic, or mosaic with two cellular populations **[5, 6]**. HMs are relatively common and occur in approximately 1 in every 1500 pregnancies in Europe and North America. This incidence varies between ethnic groups and is 2 to 10 times higher in some countries of Latin America, the Middle East, and the Far East with the highest frequencies being in Mexico, Iran, and Indonesia **[2]**. Several studies have been performed to correlate the higher incidence of moles in particular racial groups with genetic and various environmental factors such as food preferences, vitamin A deficiency, and viral factors. These studies demonstrated that women of Asian origin are at higher risk of developing moles than others **[7, 8]**. In Japan, the incidence is 2 per 1,000 pregnancies, which is three times greater than the incidence in North America and Europe. These variations may result from differences in reporting, and also, the high incidence historically reported in some areas may be related to socioeconomic, nutritional, or

genetic factors **[9]**.Classical genetic mapping studies demonstrated that almost all of the familial cases are mapped to a gene-rich region of chromosome 19q13.4 **[10]**. Linkage analysis, homozygosity mapping, and subsequent screening of candidate genes led to the identification of the NALP7 gene, which has been shown to have a causal role in recurrent hydatidiform mole and possibly other reproductive wastage **[9]**. Mutations in NALP7 gene have been found in women from several ethnic groups in Asia. NALP7 is a cytoplasmic protein belonging to a group of proteins made up of an N-terminal pyrin (PYD) domain, a NACHT domain, and a C-terminal leucine-rich repeat (LRR) domain. Little is known about the function of NALP7; however, some members of the NLRP family have been implicated in the inflammatory processes and apoptosis **[11]**. In an *in vitro* study, the wild-type NALP7 protein acted as an inhibitor of interleukin-1b, a pro inflammatory cytokine abundantly expressed in the female reproductive tract. Mutations in NALP7 resulting in impaired cytokine secretion could impair the implantation and development of the embryo by altering inflammatory pathways in the uterus. The impaired inflammatory response of patients withNALP7 mutations could make them tolerant to delay the rejection of abnormal molar conceptions **[9]**. Mutations inNALP7 gene is the main genetic cause of HM known so far. Previous researches have revealed that prevalence of RHMs has increased in Iran, but there is no report about genetic causes of this disease in Iranian women with RHMs. The aim of this study is, for the first time, to investigate the mutations in hot spot regions of NALP7 gene in Iranian women.

MATERIALS AND METHODS

Subjects

Inclusion criteria for this study included all patients with hydatidiform mole who have been infected at least once the hydatidiform mole in the period of 2011 to 2013, referred to Ghaem General Hospital, Mashhad, Iran. The exclusion

criteria for patients are non-genetic causes of the disease, such as severe anemia. The genetic laboratory at Ghaem General Hospital collected information about clinical histories of patients in order to optimize the knowledge, and the research study of these pathologies. All patients were given written informed consent for NALP7 mutation screening with the specific information about the lack of current therapeutic benefit. We performed a case-control study. All 35 patients presenting with at least one molar pregnancies were offered a mutation analysis

for mutation in the NALP7 gene with HRM technique.

Analysis of the NALP7 gene

Genomic DNA was extracted from peripheral blood samples with standard salting out procedure of 35 patients and36 healthy individuals. The NALP7 gene region was amplified by specific primers that designed with primer3software (Table 1) then the PCR products were analyzed for mutations using melting curve of HRM technique.DNA sequencing was performed at Gene Fanavaran Company in Iran in both patient and control groups. Nine primer pairs were designed for amplification of the 5 exons in LRR domain, a hot spot region, and the exon intron junction sequences of NALP7 using the reference sequences derived from the NCBI genomic data bank. To ensure a unique pairing of primers for all primers by the software BLAST searches were performed in the human genome.

RESULTS

From 2011 to 2013, 40 cases were referred to the Ghaem General Hospital, as possible molar pregnancies, of which35 were confirmed by pathology studies. Among the 35 HM patients that ascertained by a pathologist, 33 cases had partial (PHM) moles. And two patients were presented with recurrent hydatidiform moles (RHM). A genetic analysis of NALP7 was conducted to assess the involvement of this gene in the patho physiology of these partial and complete cases. HRM analysis has demonestrated shifts in HRM melting curves between patients with HM compared to control groups **(Figure 1)**. Later, the samples were recruited to sequencing. The results of sequencing demonstrated some variations in NALP7 gene in one patient (45 years old) with complete hydatidiform mole. This patient was investigated independently in another time with backward sequencing and we confirmed her variation c.2775A>Gand c.2682T>C in exon nine of NALP7 gene **(Figure 2)**. These mutations were not found in our healthy control group.

DISCUSSION

NALP7 probably has a role as a maternal effect gene. Maternal effect genes are defined as genes whose products are needed in the oocytes to support early embryonic development until the activation of the embryonic genome. Such genes are not supposed to affect ovulation and fertilization, but their absence would lead to early embryo arrest **[12]**. Another possible role of NALP7 is after implantation and is supported by the following observations. This gene is transcribed in human endometrium and has been shown to play a role in IL-1b secretion,

a cytokine involved in decidualization and trophoblast invasion **[13, 14]**. The NALP7 inflammatory pathway is part of the cellular immune response, a well-known cause of repeated spontaneous abortions and various forms of reproductive failure. To date, 43 different mutations in NALP7 have been reported by different groups in women with RHMs and reproductive wastage from various populations demonstrating that NALP7 is a major cause for this condition. Many of these reports are related to Asian regions that some examples are given **(Table 2)**. Wang *et al*. **[15]** analyzed the NALP7 gene in affected individuals from 20 families with a confirmed diagnosis of familial RHM and identified 16 different mutations in 17 of the families. Affected members from 14 of the 17mutation-positive families were homozygous for the identified mutation, although only 1 family reported consanguinity. This research group noted that 3 different mutations had been identified at codon 693 (R693W, R693P, and R693Q), suggesting that spot 693, located in the C-terminal LRR domain, demonstrates a hot spot for mutation in the NALP7 gene. In this study, we for the first time in Iran, determined the gene NALP7 with HRM technique and sequencing in a total of 35 unrelated Iranian patients and found mutations in only one of them. The patient was homozygous recessive for both mutations. Comparing these data with those of Pakistani **[16]** and Indian patients **[17]** with HMs revealed two distinctive features of the Iranian population. First, NALP7 is not a major gene for RHM and reproductive wastage in Iran, since only 3% of Iranian patients with at least one HMs have NALP7 mutations when compared with 81 and 84% of Pakistani and Indian patients, respectively. And these variations were silent mutations, thus, there is more likely no change in protein sequence and its function. Mutations identified in this study, previously been reported in Pakistan as polymorphism, could be considered as founder mutation resulting from migration from Pakistan to Iran. The relatively low incidence of NALP7 mutations in this study could be due to the fact that there is a difference in epigenetic factors in particular nutrition, lifestyle, and even varying weather conditions among different ethnic groups or there might be mutations in other genes such as C6orf221, Ki-67, P53, Bcl-2. Also, in addition to LLR domain other domains of this gene should be examined for mutation detection analysis which has not been performed in this study and a large scale sample study is also recommended.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGEMENTS

We thank Dr. Simin Hiradfar at Mashhad Health Reference Laboratory, for her technical assistant. This work was supported by a grant from the Vice Chancellor for Research at Mashhad University of Medical Sciences (Grant No.901002).

REFERENCES

[1] Muhlstein, J., *et al*., The spectrum of NLRP7 mutations in French patients with recurrent hydatidiform mole. European Journal of Obstetrics & Gynecology and Reproductive Biology, 2011. 157 (2): p. 197-199.

[2] Slim, R. and A. Mehio, The genetics of hydatidiform moles: new lights on an ancient disease. Clinicalgenetics, 2007. 71(1): p. 25-34.

[3] Szulman, A.E. and U. Surti, The syndromes of hydatidiform mole. II. Morphologic evolution of thecomplete and partial mole. Am. J Obstet. Gynecol, 1978. 132(1): p. 20-7.

[4] Zaragoza, M.V., *et al*., Early complete hydatidiform moles contains inner cell mass derivatives. Am. J. MedGenet, 1997, 70(3): p. 273-7.

[5] Vejerslev, L.O., *et al*., Hydatidiform mole: Genetic markers in diploid abortuses with macroscopic willous enlargement. Cancer genetics and cytogenetics, 1987. 26 (1): p. 143-155.

[6] Deveault, C., *et al*., NLRP7 mutations in women with diploid androgenetic and triploid moles: a proposed mechanism for mole formation. Human molecular genetics, 2009. 18(5): p. 888-897.

[7] Tham, B., *et al*., Gestational trophoblastic disease in the Asian population of Northern England and North Wales. BJOG: An International Journal of Obstetrics & Gynaecology, 2003. 110(6): p. 555-559.

[8] Matsuura, J., *et al*., Complete hydatidiform mole in Hawaii: an epidemiological study. Geneticepidemiology, 1984. 1(3): p. 271-284.

[9] Hoffner, L. and U. Surti, The genetics of gestational trophoblastic disease: a rare complication of pregnancy. Cancer genetics, 2012. 205(3): p. 63-77.

[10] Moglabey, Y.B., *et al*., Genetic mapping of a maternal locus responsible for familial hydatidiform

moles. Human molecular genetics, 1999. 8(4): p. 667-667.

[11] Martinon, F., *et al*. NALP inflammasomes: a central role in innate immunity. in Seminars in immunopathology. 2007. Springer.

[12] Tong, Z.-B., *et al*., Mater, a maternal effect gene required for early embryonic development in mice. Naturegenetics, 2000. 26(3).

[13] Strakova, Z., S. Srisuparp, and A.T. Fazleabas, IL-1beta during in vitro decidualization in primate. J Reprod Immunol, 2002. 55(1-2): p. 35-47.

[14] Karmakar, S. and C. Das, Regulation of trophoblast invasion by IL-1beta and TGF-beta1. Am J Reprod. Immunol, 2002. 48(4): p. 210-9.

[15] Wang, C.M., *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): p. 569-75.

[16] Hayward, B.E., *et al*., Genetic and epigenetic analysis of recurrent hydatidiform mole. Human mutation, 2009. 30 (5): p. E629-E639.

[17] Slim, R., *et al*., A strong founder effect for two NLRP7 mutations in the Indian population: an intriguing observation. Clin Genet, 2009. 76(3): p. 292-5.

Table 1: Characteristics and sequence of primers

Sequence	Primer	Exon	Length	TM
5'-AGATGAACGTGTGTTCTCCTG -3'	Forward	Exon5 Primer pair 1	198bp	58°C
5'-CTACGGGTTACGTGGTCACA -3'	Reverse			59°C
5'-GCGGATTCTTTGTGACCACG -3'	Forward	Exon5 Primer pair 2	84bp	60°C
5'-GTCTTCCTTGCAAGATGAGCTTC-3'	Reverse			60°C
5'-AGTGGGAACGCACGATGATG -3'	Forward	Exon6 Primer pair 1	96bp	60°C
5'-AGAGCCGTGACCGTGAGA -3'	Reverse			60°C
5'-CCAAGTGGAATCTCTTCTGCT -3'	Forward	Exon6 Primer pair 2	154bp	57°C
5'-CATCAGCATCATCGTGCGTT -3'	Reverse			59°C
5'-CTCAAAGCCAACCAGTCCCT -3'	Forward	Exon7 Primer pair 1	140bp	60°C
5'-CCATGGGAAGAGGAGACTTACG -3'	Reverse			60°C
5`- CTCTTTTCACAGGTTGGGAGGT- 3`	Forward	Exon7 Primer pair 2	116bp	60°C
5`-GGAGCACATTGGCTGAGAGA-3`	Reverse			59°C
5'-GCAGGTTGGAAAACTGTCGT -3'	Forward	Exon8	195bp	59°C
5'-CACACCCAGCAGGGACTTAC -3'	Reverse			60°C
5'-GGATAACAGGCTTCTCTTCCTT-3'	Forward	Exon9 Primer pair 1	170bp	57°C
5'-ATGCCTGACAGAGAATCCACA -3'	Reverse			59°C
5'-AGCTCGTGGATTGTGGATTCT -3'	Forward	Exon9 Primer pair 2	70bp	59°C
5'-CTACCGTAGGTGTTTTAGGTTACA -3'	Reverse			58°C

Table 2: Mutations and reproductive outcomes of the different groups

No	Ethnicity	Region	Mutation DNA	Protein
1	Pakistan	Intron 7 donor splice site	c.[2471G>A]	-
2	Indian	Exon 5	c.[2078G>C]	Arg693-to-pro
3	Indian	Exon 9	c.[2738A>G]	Asn913-to-ser
4	Chinese	Exon 4	c.[1294C>T]	Arg432-to-ter
5	Chinese	Exon 5	c.[2078G>A]	Arg693-to-gln

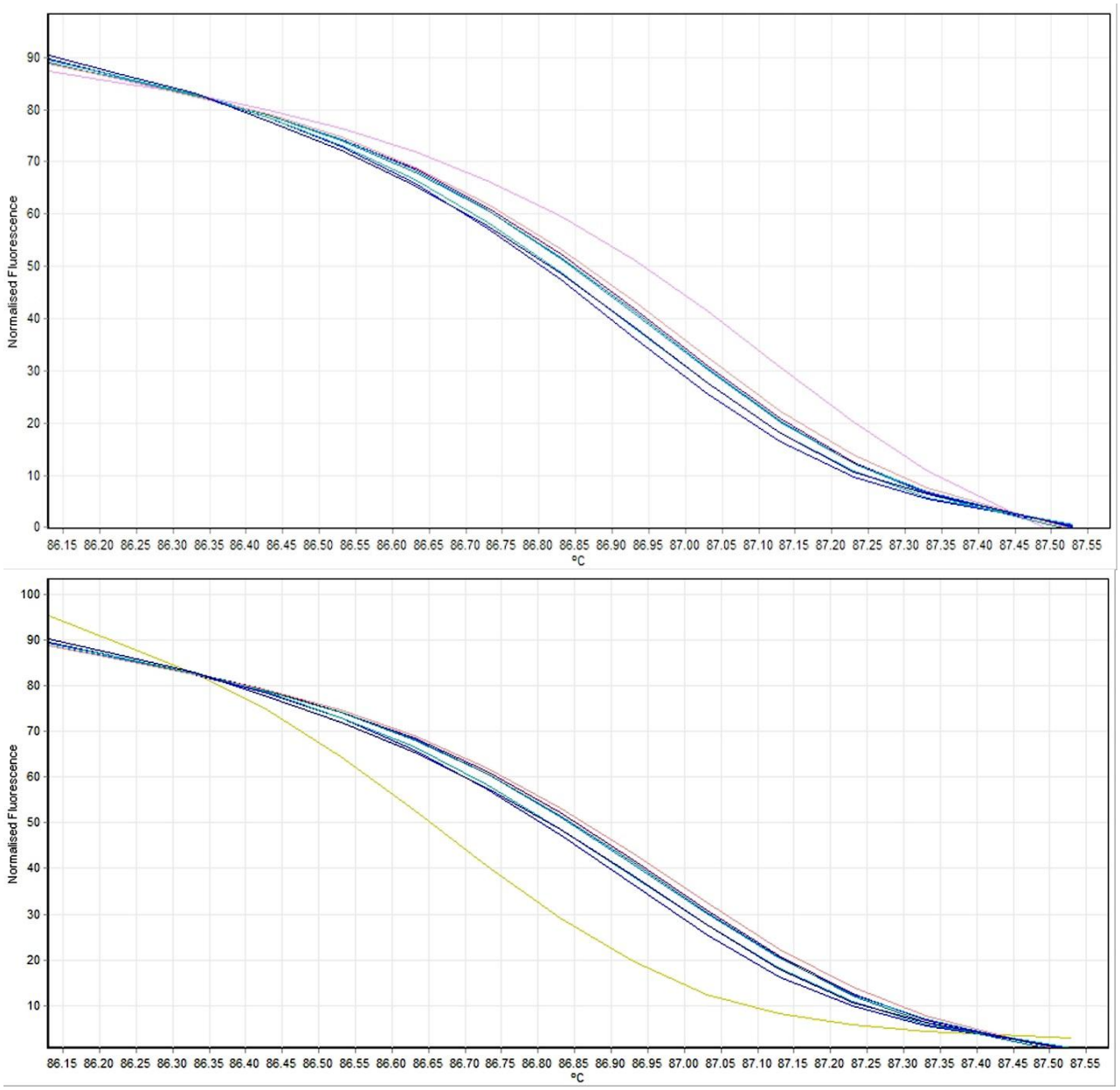

Figure 1: Normalized Melting Curves. Based on temperature shifting, genetic variances among samples are observed in exon 9 (Section A. homozygous patient allele: yellow curve, normal healthy persons as negative controls:blue curves. Section B. homozygous patient allele: pink curve, normal healthy persons as negative controls:blue curves; 209x97mm (300 x 300 DPI)

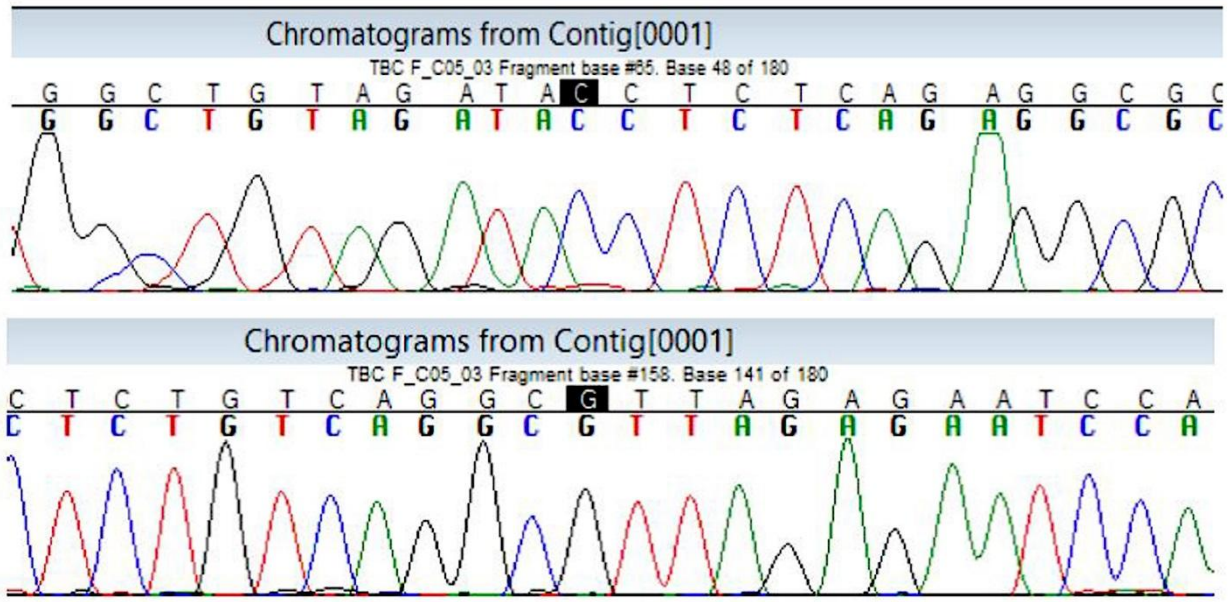

Figure 2: Chromatogram obtained from homozygous mutant sequence in exon 9 (Section A. T>C change &Section B. A>G change); 211x63mm (300 x 300 DPI)