

# Founder Effect of *KHDC3L*, p.M1V Mutation, on Iranian Patients with Recurrent Hydatidiform Moles

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## What's Known

- Recurrent hydatidiform mole (RHM) is an unusual pregnancy.
- *NLRP7* and *KHDC3L* are two maternal-effect genes as the cause of RHMs.

## What's New

- We found a founder effect in the *KHDC3L* gene in the Iranian population.

## Abstract

**Background:** Recurrent hydatidiform moles (RHMs) are an unusual pregnancy with at least two molar gestations that are associated with abnormal proliferation of trophoblastic tissue and a failure in the embryonic tissues development. Three maternal-effect genes, including *NLRP7*, *KHDC3L*, and *PADI6* have been identified as the cause of RHMs. The present study aimed to understand the association of a founder mutation with the incidence and prevalence of a disease in different individuals of a population.

**Methods:** 14 unrelated Iranian patients with recurrent reproductive wastage, including at least two HMs, entered this study. In order to find a possible mutation in *KHDC3L*, all the 14 samples were Sanger sequenced. For haplotype analysis, three single nucleotide polymorphisms (SNPs) were selected with highest Minor Allele Frequency along *KHDC3L*.

**Results:** A common *KHDC3L* mutation with the same haplotype was identified in four out of 14 patients with RHM. Regarding the present study, c.1A>G is the highest reported mutation in *KHDC3L* so far and is also the first report of the homozygous state that has led to RHM.

**Conclusion:** c.1A>G mutation in *KHDC3L* is the highest reported mutation around the world. Our data also demonstrated the presence of founder effects for this particular mutation in Iranian populations. These data suggest that the high frequency of this mutation is potentially responsible for a higher rate of RHM in Iran.

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**Keywords** • Haplotypes • *KHDC3L* protein, human • Hydatidiform mole • Founder effect • Mutation

## Introduction

Hydatidiform mole (HM) is an abnormal human conception with a disorder in fetal growth, which is represented in two types, complete (CHM) and partial (PHM).<sup>1</sup> One subcategory of CHMs is recurrent and familial (OMIM 231090), which is known as biparental hydatidiform moles (BiHMs) or Recurrent hydatidiform moles (RHMs). This type is a rare and unusual pregnancy that is associated with hyperproliferative vesicular trophoblast and abnormal fetal development. By definition, RHM is the occurrence of at least two hydatidiform moles in the same individual.<sup>2</sup> HM occurs once in 600-1000 pregnancies in Western

countries, from which 0.6–2.6% is RHM.<sup>1</sup> With unknown reason, this percentage is higher, 2.5% up to 9.4%, in some parts of the world including the Middle East.<sup>2</sup> Based on several studies in western countries, it was shown that 1% to 2% of patients with a prior mole had a second one as well.<sup>3</sup> Three maternal-effect genes as the cause of RHMs have been reported; these include *NLRP7*, *KHDC3L*, and, recently, *PADI6*, which are inherited in an autosomal recessive manner.<sup>4,6</sup> It is suggested that these genes are involved in the setting of genomic imprinting.<sup>7</sup> Using detail mapping analysis, *NLRP7* (NACHT, leucine-rich repeat, and PYD containing 7) was identified as the first maternal effect gene, which is responsible for 48-80% of BiHMs cases.<sup>8-13</sup>

In 2011, Parry and his colleagues discovered *KHDC3L* (KH domain containing 3-like) as a second gene responsible for RHMs.<sup>6</sup> In their study,<sup>6</sup> p.M1V mutation was reported only in one allele of a patient and was not reported anywhere in the world. Only 10-14% of BiHMs patients with no *NLRP7* mutations reflected mutation in *KHDC3L*.<sup>6, 14, 15</sup> Still, a fraction of patients with no recessive mutations in these three known genes has other molecular reason, which remains to be discovered in future studies. *KHDC3L* contains three exons that encode a protein with 217 amino acids.<sup>4</sup> *KHDC3L* protein is a member of the KHDC1 (KH homology domain containing 1) protein family. Unlike other proteins in this family (*KHDC1*, *DPPA5*, *OOEP*), *KHDC3L* protein possesses an atypical KH domain that cannot bind to an RNA molecule. *KHDC3L* transcripts are expressed in a variety of human tissues, including hematopoietic cells, all oocytes stages, and preimplantation embryos.<sup>2</sup> To date, six pathogenic mutations have been reported in *KHDC3L* (c.1A>G, c.322\_325delGACT, c.3G>T, c.299\_302delTCAA, c.17\_20delGGTT, and c.349+1G>A).<sup>6, 15, 16</sup>

Qian and colleagues identified *PADI6* gene in 2018 as the third maternal-effect gene and a member of the subcortical maternal complex as a cause of FBHM. They did not observe any mutations in *NLRP7* and *KHDC3L* genes in 24 Chinese women with RHM. They performed exome sequencing, and two missense variants were identified in a compound heterozygous state in *PADI6* gene (c.1793A>G, p.Asn598Ser and c.2045G>A, p. Arg682Gln). They also demonstrated the co-localization of *NLRP7* and *PADI6* in human oocytes and early embryos.<sup>5</sup>

In order to find a possible reason for the high frequency of RHM in Iran, *KHDC3L* mutation was studied in 14 unrelated patients and their relatives. The present study was an investigation towards haplotype analysis of a particular mutation in *KHDC3L* gene using single

nucleotide polymorphisms (SNPs) sequence analysis in four unrelated women with RHMs and reproductive failure.

## Materials and Methods

### Patients

Patients were referred to the Infertility Research Center, Shiraz University of Medical Sciences, Shiraz, Iran. Based on the disease history, 14 unrelated patients were considered in this study. All of the patients had at least two molar pregnancies in their history. Histopathology and hematoxylin-eosin staining of a section of the molar tissue were done by an expert pathologist. All of the patients and their family members gave written informed consent for participation in this study. The referral of patients to the infertility center, collecting their blood samples, and genetic analysis of their DNA lasted about one year. The study was approved by the Ethics Committee of Shiraz University of Medical Sciences (IR.SUMS.REC.1396.540), Shiraz, Iran.

### Preparation of Genomic DNA and PCR

Genomic DNA was extracted from peripheral blood cells by Cinnapure DNA kit (Cinnagen, Iran). The concentration of genomic DNA was measured by NanoDrop (ND1000, USA) and stored at -20 °C until use. PCR amplification of *KHDC3L* was performed in two fragments using our previously designed primers and conditions.<sup>16</sup> The PCR amplifications were carried out in 25  $\mu$ l volume, consisting of 12.5  $\mu$ l Taq DNA Polymerase 2x Master MixRed (Ampliqon, Odense M, Denmark), 9.5  $\mu$ l dH<sub>2</sub>O, 1  $\mu$ l of each primer (10 pmol/ $\mu$ l), and 1  $\mu$ l DNA template (50-100 ng). The reaction conditions were as follows: Primary denaturing temperature at 95 °C for 4 min, 35 cycles of PCR denaturation at 94 °C for 25 s, annealing temperature at 60 °C (for both fragments) for 20 s, extension temperature at 72 °C for 60 s, and a final extension stage of 72 °C for 5 min. Four microliters of the PCR products were visualized on 1% agarose gel containing Erythro-Gel (Arashteb, Iran).

### Sequencing and Mutation Analysis

PCR products were purified and Sanger sequenced by Eurofins Company (Germany). The sequences were analyzed using Chromas software (Technelysium Pty Ltd) and compared with the reference sequence NM\_001017361.2 for *KHDC3L*.

### SNPs Selection

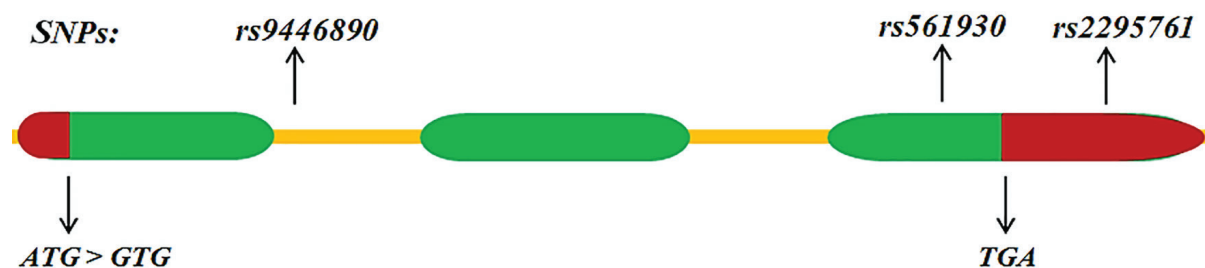
Founder mutation is a mutation that exists in the genome of a distinct population. This

mutation can be transmitted to other generations and affect many people in the population with the same founder effect. Haplotype studies are a method of choice for finding events about the relationship between the variants and the origin of a founder mutation in populations.<sup>17, 18</sup> The existence of the same haplotype among people is rare because linkage disequilibrium (LD) relationships may be different from population to population.<sup>19-21</sup> Therefore, if an identical haplotype is found in the population, it has been shown that the origin of this haplotype is due to the founder effect. In this study, three single nucleotide polymorphisms with the highest minor allele frequency within *KHDC3L* gene were selected for the haplotype analysis. These three SNPs, which have been obtained from NCBI dbSNP database, included rs9446890 (G/C), MAF: <0.3790 located at intron 1,

rs561930 (C/G), MAF: <0.4884 located at exon 3 and rs2295761 (T/C), MAF: <0.0823 located at 3'-UTR of the *KHDC3L* gene (figure 1). The location of these three SNPs mentioned above is 229, 1079, and 1433 bps downstream of the mutation site, respectively.

### Results

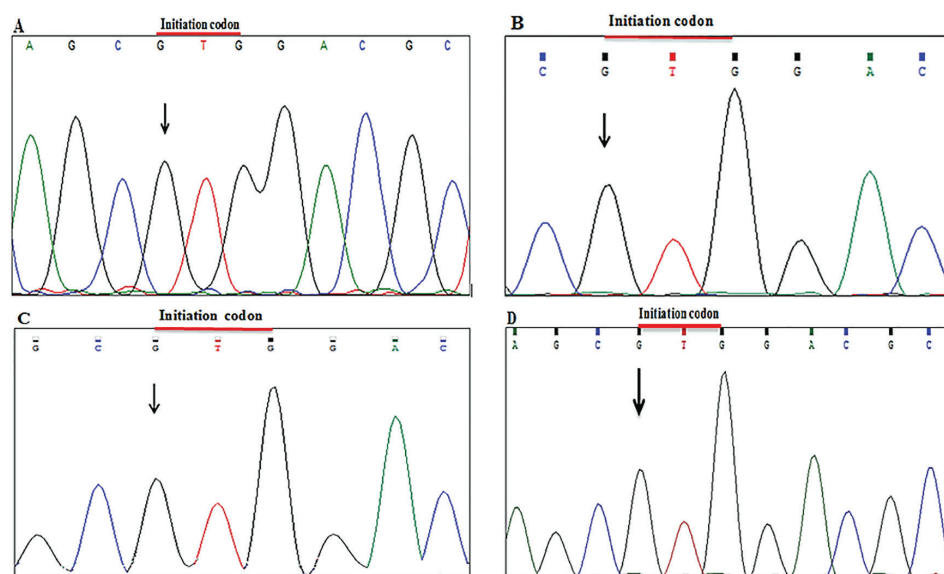
*KHDC3L* gene, including exons, introns, 230 bp in 5'-UTR, and 480 bp in 3'-UTR, was sequenced. Out of 14 patients in this study, only four patients (table 1) from separate families showed a common homozygous mutation (c.1A>G, p.M1V) (figure 2) in exon one of *KHDC3L* gene. Other ten patients did not show mutation in *KHDC3L*. In 28 chromosomes from the 14 unrelated patients in this study, five different haplotypes were identified (table 2).



**Figure 1:** Schematic diagram of *KHDC3L* structure is depicted. Three exons (green), two introns (orange), 5' and 3' UTR (red), and the locations of three SNPs that are used in this study are displayed.

**Table 1:** Summary of the reproductive outcome and identified mutation in four patients

Patients	Reproductive outcome	Mutation
1	4 CHM	<i>KHDC3L</i> , c.1A>G, p.M1V
2	2 CHM	<i>KHDC3L</i> , c.1A>G, p.M1V
3	2 CHM	<i>KHDC3L</i> , c.1A>G, p.M1V
4	5 CHM	<i>KHDC3L</i> , c.1A>G, p.M1V



**Figure 2:** Sequence chromatograms of the mutation in initiation codon are depicted. (A-D) Mutation (c.1A>G) in four patients are in a homozygous state and are shown with black arrows.

**Table 2:** Haplotype diversity in fourteen samples

Polymorphism	rs9446890 (G/C)	rs561930 (C/G)	rs2295761 (T/C)	Number of haplotypes
Reference sequence NM_001017361.2	G	C	T	-
P1	C	C	T	2
P2	C	C	T	2
P3	C	C	T	2
P4	C	C	T	2
S3, S8, S8, S9, S9	C	C	T	5
S1, S2, S4, S6, S7, S10	G	C	T	6
S1, S2, S4, S7, S10	C	G	T	5
S3, S5, S5	C	C	C	3
S6	C	G	C	1

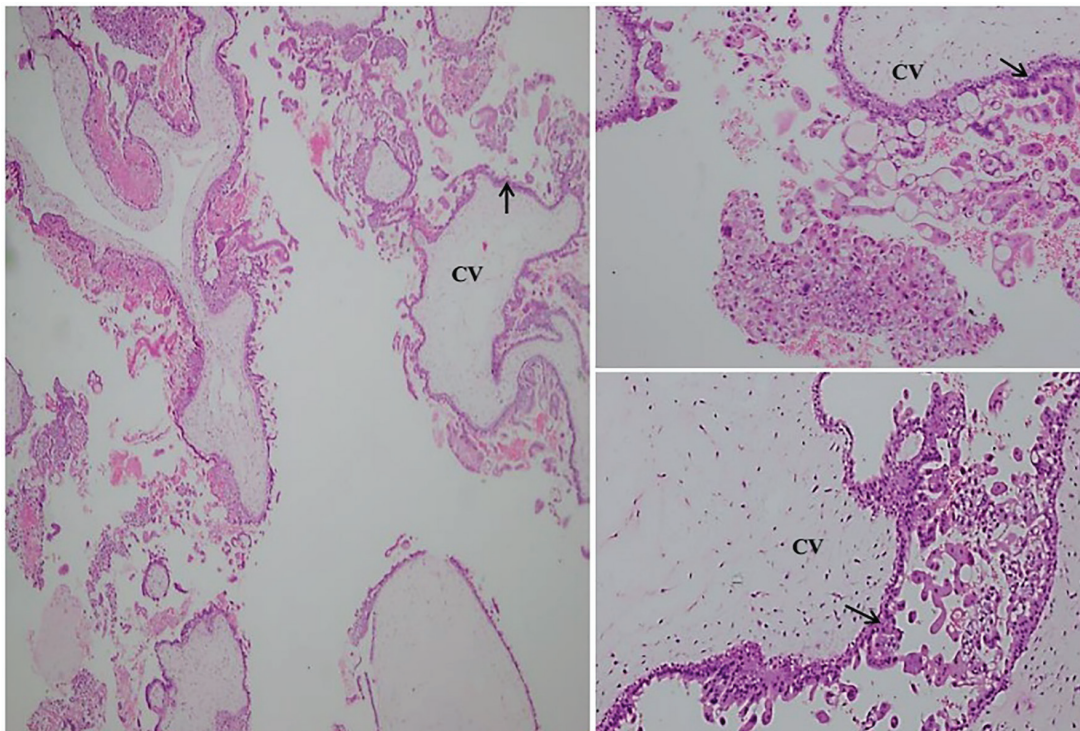
P: Patient; S: Sample

The sequence haplotype analysis of the four unrelated patients with c.1A>G, p.M1V missense mutation showed the same haplotype (C/C/T) (table 2). In addition, five alleles of patients, without this particular mutation, also showed this haplotype. The most prevalent haplotype (G/C/T) was the same as the reference haplotype for *KHDC3L* gene (NM\_001017361.2). This haplotype was identified in six chromosomes. Three other haplotypes (C/G/T, C/C/C, and C/G/C) with the lowest frequency were also identified in 5, 3, and 1 chromosomes, respectively. Trophoblastic proliferation was observed in molar tissue of patient 1 (figure 3) (paraffin tissue block was not available for the rest of the patients).

## Discussion

In the present study, we found a founder effect in the affected women with RHM. To date, six different pathogenic mutations in *KHDC3L* gene have been identified in three different studies. Each of the five previously reported mutations was only observed in one separate family and only one deletion mutation, c.322\_325delGACT, was present in four different families.<sup>6, 15, 16</sup>

The first study was performed by Parry and colleagues, leading to the identification of the *KHDC3L* gene as a cause for FBHM. They reported three mutations in *KHDC3L* gene. In a Pakistani family with two related women, a homozygous c.3G>T mutation was observed



**Figure 3:** The histopathology of molar tissue for patient 1 is illustrated. Enlarge placental villi with cistern formation and excessive trophoblastic proliferation of tissue (arrow) is clearly seen around chorionic villi (CV). The chorionic villi are enlarged, edematous with cistern formation, avascular, and have multilayer circumferential proliferation trophoblast. Some of the trophoblast show atypical nuclei. No gestational sac and fetal part are seen in the specimen of the product of conception. left  $\times 40$ , right  $\times 100$ .

in the start codon (ATG change to ATT). Using a gene-transfer approach, it was shown that ATT codon was ignored and the second codon of ATG in downstream was recognized as the start codon for translation. As a result of this mutation, 13 amino acids in N terminal were eliminated, which was pathogenic and led to FBHM. c.322\_325delGACT; p.Asp108Ilefs\*30 mutation was the second mutation that was observed in a Tunisian woman in a homozygous state. Following four base pair deletion in exon 2, the frameshift occurred and 110 amino acids of C terminal were deleted. The third mutation was c.1A>G observed in a woman with Asian origin in a compound heterozygous state. In order to identify the second mutation in this patient, a cloning strategy was used and c.322\_325delGACT mutation was identified.<sup>6</sup>

In a second study by Reddy and colleagues, c.299\_302delTCAA, p.Ile100Argfs\*2 mutation was identified in a homozygous state in a patient with African-American origin. She had seven moles with three partners in her history. c.322\_325delGACT mutation in a homozygous state in two unrelated patients with Turkish and Indian origins was also observed in Reddy's study. Because c.322\_325delGACT mutation was reported in four patients of different countries, Reddy and colleagues, using haplotype analysis, demonstrated that this mutation was an old founder effect in Indian, Tunisian, and Turkish patients and had a common origin.<sup>15</sup>

In the third study, Rezaei and colleagues reported two new mutations in *KHDC3L*. One of these mutations, c.17\_20delGGTT, p.Arg6Leufs\*7, in a homozygous state in exon 1, was observed in an Iranian woman with 11 HMs. This mutation was the third reported deletion mutation in *KHDC3L* gene. The other mutation, c.349+1G>A, was a splicing mutation in a homozygous state observed in an Indian woman with 6 HMs.<sup>16</sup>

In our study, p.M1V, c.1A>G mutation in the start codon of *KHDC3L*, was identified in four unrelated Iranian patients with a history of RHM in a homozygous state. While Parry and colleagues have previously reported this mutation in a compound heterozygous state,<sup>6</sup> it is worth mentioning that our report is the first study in which c.1A>G mutation in a homozygous state leads to RHM. Due to the presence of this particular mutation in four unrelated families in different provinces of Iran, we sought, in the present study, to identify whether this particular mutation had the same origin or single mutational event. For this purpose, haplotype analysis was performed. For haplotype analysis, three SNPs with high MAF were selected in different locations

of *KHDC3L* gene (figure 2). In terms of population genetics, the importance of recognizing the common origin of a mutation in different patients from different cities and provinces meant that the frequency of this mutation in the population could be high. Since mutation in *KHDC3L* gene causes infertility in women, the detection of this mutation was prioritized. Moreover, genetic testing of this mutation is suggested in pre-marital tests, especially consanguineous marriages with a history of infertility.

## Conclusion

In this study, c.1A>G mutation was identified for the first time in a homozygous state in four unrelated Iranian patients with RMH. This represents a high frequency compared to other previously reported mutations in *KHDC3L* gene around the world. In addition, in the present study, the presence of eight alleles with the same haplotype demonstrated a strong founder effect and a common origin for c.1A>G mutation. According to the present data, this particular mutation can potentially be expected to be responsible for a higher rate of RHM in the Iranian population.

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**Conflict of Interest:** None declared.

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