Homozygous RRM2 Variant Might Lead to Early Embryo Developmental Arrest: A Case Report

Xiong Wang
Qindao University Medical College Affiliated Yantai Yuhuangding Hospital

Zhen-teng Liu
Qindao University Medical College Affiliated Yantai Yuhuangding Hospital

Yan-wei Sha
Xiamen City Health and Family Planning Commission

Xiao-yan Liu
Qindao University Medical College Affiliated Yantai Yuhuangding Hospital

Hai-ling Wang
Xiamen University Medical College

Cui-fang Hao
Qingdao Women and Childrens Hospital

Jian-feng Wu
Xiamen University

Jiahui Wang (jiahui.wang@outlook.com)
Qindao University Medical College Affiliated Yantai Yuhuangding Hospital

Case Report

Keywords: infertility, gene mutation, early embryo development arrest, ribonucleotide reductase subunit M2, maternal effect

DOI: https://doi.org/10.21203/rs.3.rs-50066/v1

License: ©  This work is licensed under a Creative Commons Attribution 4.0 International License.  Read Full License
Abstract

Background: Early embryonic developmental stagnation is one of the reasons that affect the outcome of in vitro fertilization-embryo transfer, leading to the depletion of available embryos or failure after transplantation. It has been shown that defects in the ribonucleotide-reductase lead to cell cycle arrest, developmental delay, and high mutation rates.

Case presentation: Two female patients, who were siblings from an inbreeding family, suffered primary infertility of unknown causes and early embryonic developmental arrest during IVF treatment. A total of 39 oocytes were obtained from the patients in collectively 5 IVF/ICSI cycles, of which 37 were mature eggs, only 2 transplantable embryos were formed, and no pregnancy was achieved. Whole genome sequencing and Sanger sequencing were adopted to identify and confirm variations that might cause early embryo developmental stagnation in this family. We identified a homozygous variant c.262C>T:p.His88Tyr in ribonucleotide-diphosphate reductase subunit M2 (RRM2) in both patients and their parents each carried a heterozygous allele. Pedigree analysis showed an autosomal recessive inheritance pattern. Function of this variant was predicted by online databases, which indicated it to be a potential pathogenic mutation.

Conclusions: We identified RRM2 as a potential causative gene for early embryonic developmental stagnation. It was also suggested that RRM2 might be a maternal effect gene.

Background

Infertility is one serious issue that threatens the human reproductive health. According to statistics, on a worldwide scale 15% of married couples are infertile, and infertility factors affect about half of men and women (1). The development of assisted reproductive technology brings hope to many infertile patients. Studies have shown that 40%-70% of human embryos in in vitro fertilization (IVF) cycles are living embryos, whereas the others are arrested at different developmental stages (2). Stagnant embryos are usually discarded, and live embryos are frozen, continue to be cultured into blastocysts, or directly transplanted back into the patient's uterus. However, in some cases the development of all embryos from a patient are arrested at early developmental stages, leading to failed IVF attempt, which cannot be rescued by multiple embryo transplantation or single sperm microinjection. The cause of such condition is speculated to relate to genetic factors.

At present, research on the genetic factors of human embryonic stagnation is very limited. Up to now, there are only two reported gene mutations that could cause human embryo development stagnation and infertility. A homozygous variation in TLE6 gene was first reported to account for the stagnation of early embryonic development in human, which was identified by exome sequencing in an Arab inbreeding family (3). TLE6 is a maternal effect gene. In the early stage of human embryonic development, especially from fertilization to the 8-cell stage, gene transcription is not fully initiated. The basic biological processes of cells including protein synthesis and regulation of embryo development depend almost entirely on the RNA stored in the egg. Therefore, the mutation of maternal effect genes may affect the development and survival of fertilized eggs and embryos. In another study, homozygous mutations in PADI6 gene in a Chinese inbreeding family resulted in the stagnation of fertilized eggs and embryos from the female patient (4). In the meantime, compound heterozygous mutations of PADI6 gene were found in 2 of 36 sporadic cases of early embryonic development arrest (4). Interestingly, PADI6 is also a maternal effect gene, which again indicated the important role of maternal factors in early embryonic development.

In this study, we reported the identification of a homozygous variation NM_001034: exon3: c.262C>T:p.His88Tyr in ribonucleotide-diphosphate reductase subunit M2 (RRM2) in two infertile sisters from an inbreed family, who both suffered primary infertility and early embryo developmental arrest. RRM2 was also identified as a potential maternal effect factor.

Case Presentation

Patients

Two female patients P1 and P2 (Table 1), who were siblings from an inbreeding family (Fig. 1A), were diagnosed of primary infertility. Both patients were in good physical condition, and had regular sexual intercourse of 2–3 times a week. Through physical examination, ultrasonography, hystero-salpingography and laparoscopic exploration, we found no abnormalities in the patients’ vulva, vagina, cervix, ovary, fallopian tubes, and uterus. Periodic development of follicles was monitored by transvaginal ultrasound. Blood gonadal hormone was detected on the second day after menstruation, and no abnormalities were observed in all indicators in either patient. The patients and their husbands all had normal karyotype. The reproductive system of both patients’ husband was well developed, and no abnormalities were found in semen analysis. The two couples had sought medical advice elsewhere but failed to identify any potential cause of infertility and had been treated with empirical Chinese herbal medicine. Both patients received in vitro fertilization and embryo transfer (IVF-ET) in our institution (Table 2).
Table 1
Basic information of the two patients.

|          | P1       | P2       |
|----------|----------|----------|
| Age (yrs)| 38       | 35       |
| Length of infertility (yrs)| 11       | 7        |
| Menstruation cycle (days)| 30       | 35       |
| Menstrual period (days)| 5        | 7        |
| Age of menarche (yrs)| 14       | 17       |
| BMI      | 25.4     | 25.6     |

Table 2
Summary of the patients’ assisted reproduction treatment outcome.

| Patient | Cycle | Fertilization method | Total oocytes obtained (N) | Immature oocytes (N) | Mature oocytes (N) | Unfertilized (N) | 2PN | 1PN | Multi prokaryotic (N) | Cleavage (N) | Transplantable embryos (N) |
|---------|-------|----------------------|-----------------------------|----------------------|--------------------|------------------|-----|----|----------------------|--------------|-----------------------------|
| P1      | 1     | IVF                  | 5                           | 0                    | 5                  | 1                | 4   | 0  | 0                    | 3            | 2                           |
|         | 2     | IVF                  | 5                           | 0                    | 5                  | 2                | 1   | 2  | 0                    | 0            | 0                           |
|         | 3     | ICSI                 | 7                           | 1                    | 6                  | 2                | 4   | 0  | 0                    | 0            | 0                           |
|         | 4     | IVF                  | 6                           | 0                    | 6                  | 1                | 3   | 0  | 2                    | 0            | 0                           |
| P2      | 5     | IVF                  | 16                          | 1                    | 15                 | 5                | 6   | 2  | 2                    | 0            | 0                           |
| Total   |       |                      | 39                          | 2                    | 37                 | 11               | 18  | 4  | 4                    | 3            | 2                           |

A total of 39 oocytes were obtained from the patients in collectively 5 IVF/ICSI cycles, of which 37 were mature eggs. Two embryos were formed during three IVF cycles plus one ICSI cycle of P1, both of which failed to develop after transplantation. P2 went through one IVF cycle, in which no transplantable embryo was formed, and gave up treatment. The outstanding problem was that the fertilization rate of mature eggs (18/37, 48.6%) and the cleavage rate of 2PN cells (3/18, 16.7%) were both low.

**Exome sequencing identified homozygous RRM2 variation**

We performed whole exome sequencing as previously described (3) on the peripheral blood sample of P1 and focused on the identification of homozygous variations. The average depth of whole exome sequencing was greater than 100×. Through screening the gene polymorphisms with allele frequencies greater than 1% in the dbSNP, 1000 Genome and ESP6500 databases, we found a list of genes containing homozygous variations (Table 3), among which only RRM2 is closely related to embryonic development potential. The homozygous variant in RRM2 (NM_001034:exon3:c.262C > T;p.His88Tyr) was confirmed by Sanger sequencing as previously described (5) in both patients (Fig. 1B, 2A), and their parents each carried a heterozygous allele. Pedigree analysis showed an autosomal recessive inheritance pattern (Fig. 1A).
Table 3
List of homozygous gene variations identified by whole exome sequencing.

| Gene   | Chromosome | Variation                                                                 | Exonic function          |
|--------|------------|----------------------------------------------------------------------------|--------------------------|
| IGFN1  | chr1       | NM_001164586:exon12:c. 6119C > T:p.Ala2040Val                             | nonsynonymous SNV        |
| OR8G2  | chr11      | NM_001291438:exon1:c. 850G > A:p.Gly284Arg                                | nonsynonymous SNV        |
| DCHS1  | chr11      | NM_003737:exon2:c.99_100insCTG:p.Gly34delinsLeuGly                        | nonframeshift insertion  |
| DACH1  | chr13      | NM_004392:exon1:c.244_249del:p. 82_83del,                                 | nonframeshift deletion   |
|        |            | NM_080759:exon1:c.244_249del:p. 82_83del,                                 |                           |
|        |            | NM_080760:exon1:c.244_249del:p. 82_83del                                  |                           |
| RIN3   | chr14      | NM_024832:exon10:c.2899_2901del:p. 967_967del                             | nonframeshift deletion   |
| MKL2   | chr16      | NM_001308142:exon9:c.761C > G:p.Pro254Arg,                                | nonsynonymous SNV        |
|        |            | NM_014048:exon9: c.761C > G:p.Pro254Arg,                                  |                           |
| KIAA0430 | chr16    | NM_001184999:exon21: c.4181_4182insTGTCTGAAA:p.Lys1394delinsAsnValValLys, | nonframeshift insertion  |
|        |            | NM_001184999:exon21: c.4172_4173insTGTCTGAAA:p.Lys1391delinsAsnValValLys, |                           |
|        |            | NM_014647:exon21: c.4181_4182insTGTCTGAAA:p.Lys1394delinsAsnValValLys      |                           |
| COQ7   | chr16      | NM_016138:exon1:c.50C > T:p.Pro17Leu                                      | nonsynonymous SNV        |
| PKD1L2 | chr16      | NM_001076780:exon4:c.706_707del:p.Asn236fs                                | frameshift deletion      |
| GSDMA4 | chr17      | NM_178171:exon3:c.382G > T:p.Val128Leu                                    | nonsynonymous SNV        |
| PLIN4  | chr19      | NM_001080400:exon3:c.2580A > T:p.Lys860Asn                                 | nonsynonymous SNV        |
| RRM2   | chr2       | NM_001034:exon3:c.262C > T:p.His88Tyr,                                    | nonsynonymous SNV        |
|        |            | NM_001165931:exon3:c.442C > T:p.His148Tyr                                 |                           |
| NCL    | chr2       | NM_005381:exon4:c.774_776del:p. 258_259del                                | nonframeshift deletion   |
| MAP3K1 | chr5       | NM_005921:exon14:c.2822_2824del:p. 941_942del                             | nonframeshift deletion   |
| MUC3A  | chr7       | NM_005960:exon11:c.4384C > T:p.His1462Tyr                                 | nonsynonymous SNV        |
| MEOX2  | chr7       | NM_005924:exon1:c.228_230del:p. 76_77del                                  | nonframeshift deletion   |

In silico analysis of RRM2:c.262C > T:p.His88Tyr variant

In silico analysis predicted that RRM2:c.262C > T was a disease-associated mutation in human (Table 4). The allele frequency of RRM2:c.262C > T in the world population was very low, but was 10-fold higher in the East Asian population. However, homozygotes of this variant has not been documented in either 1000 Genomes or Exome Aggregation Consortium (ExAC) database. The c.262C > T point mutation in RRM2 resulted in a substitution of histidine by tyrosine at the 88 amino acid. As histidine is a hydrophilic, positively-charged basic amino acid while tyrosine is a hydrophobic, neutral amino acid with aromatic residue, this substitution might affect the three-dimensional protein conformation, attenuating the stability and function of RRM2 protein (Fig. 2B). This histidine of RRM2 is highly conserved among different species (Fig. 2C), indicating its functional importance. The heterozygous variant of RRM2:c.262C > T carried by the patients' parents suggested that heterozygote of this variant is not pathogenic.
This study also suggested that RRM2 might be a potential cause of early embryonic development stagnation in two sisters who had multiple failed assisted reproduction attempts. 

Ribonucleotide reductase (RR) consists of two subunits (RRM1 and RRM2), and is a rate-limiting enzyme in deoxynucleotide production for DNA synthesis (7). As optimal cell concentration of deoxyribonucleotides is essential for DNA synthesis, replication, and repair (8), RR plays an important role in cell proliferation. RR defects often lead to cell cycle arrest, developmental delay, and abnormally increased RR activities led to high frequency of gene mutation (9). Functionally, RRM1 controls substrate specificity, and RRM2 regulates the overall catalytic activity (10). RRM2 is widely expressed in human organs, with highest expression level in secondary oocyte (data from Bgee data Base for Gene Expression Evolution).

RRM2 could regulate zygotic genome activation (ZGA) through the yes-associated protein (YAP). In early mammalian embryos, genomic transcription is quiescent until ZGA occurs 2–3 days after fertilization. The coding gene of YAP, Yap1, was found to be highly expressed in human and mouse oocytes and early embryos (11, 12), and maternally accumulated YAP in oocyte is essential for ZGA (1). The embryos of Yap1 knockout female mice presented prolonged 2-cell stage and slower development into the 4-cell stage. Rrm2, the mouse homologous gene of human RRM2, and Rpl13 were found to be the target genes of Yap1, followed by invasion of the matrix, and proliferation and differentiation of endometrial stromal cells (15). It has been shown that Rrm2 is strongly expressed in decidual tissues and is up-regulated by progesterone and DNA damage in mouse (7). RRM2-specific inhibitors effectively reduced the weight of implantation sites and deciduoma (7). In the future, functional study by generating genetically modified mouse in the Rrm2 gene would help supporting the pathogenicity of this variant.

In summary, this study identified a homozygous variation of RRM2:exon3:c.262C>T:p.His88Tyr, which might alter RRM2 protein conformation and attenuate its function, and might be a potential cause of early embryonic development stagnation in two sisters who had multiple failed assisted reproduction attempts. This study also suggested that RRM2 might be a maternal effect gene. Further animal study is still required to confirm the role of RRM2 in early embryo development.

List Of Abbreviations
Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Yantai Yuhuangding Hospital for use of human sample. Informed consent was obtained from all participants for enrolling in the study prior to their inclusion in the study.

Consent for publication

Informed consent was obtained from all participants for the use of personal information for publication.

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due to privacy and ethical restrictions but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

No funding was received.

Authors’ contributions

XW, CFH, and JFW conceived of this study. XW, ZTL and XYL performed medical examinations and IVF-ET treatment and collected information. YWS, HLW and JFW carried out gene sequencing and genetic analysis. XW and JW analyzed data and wrote the manuscript.

Acknowledgements

Not applicable.

References

1. Dada R, Gupta NP, Kucheria K. Molecular screening for Yq microdeletion in men with idiopathic oligozoospermia and azoospermia. J Biosci. 2003;28(2):163–8.
2. Gardner DK, Lane M. Culture and selection of viable blastocysts: a feasible proposition for human IVF? Hum Reprod Update. 1997;3(4):367–82.
3. Alazami AM, Awad SM, Coskun S, Al-Hassan S, Hijazi H, Abdulwahab FM, et al. TLE6 mutation causes the earliest known human embryonic lethality. Genome Biol. 2015;16:240.
4. Xu Y, Shi Y, Fu J, Yu M, Feng R, Sang Q, et al. Mutations in PADI6 Cause Female Infertility Characterized by Early Embryonic Arrest. A Am J Hum Genet. 2016;99(3):744–52.
5. Li L, Zhou X, Wang X, Wang J, Zhang W, Wang B, et al. A dominant negative mutation at the ATP binding domain of AMHR2 is associated with a defective anti-Mullerian hormone signaling pathway. Mol Hum Reprod. 2016;22(9):669–78.
6. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405–24.
7. Lei W, Feng XH, Deng WB, Ni H, Zhang ZR, Jia B, et al. Progesterone and DNA damage encourage uterine cell proliferation and decidualization through up-regulating ribonucleotide reductase 2 expression during early pregnancy in mice. J Biol Chem. 2012;287(19):15174–92.
8. Reichard P. From RNA to DNA, why so many ribonucleotide reductases? Science. 1993;260(5115):1773–7.
9. Wang C, Liu Z. Arabidopsis ribonucleotide reductases are critical for cell cycle progression, DNA damage repair, and plant development. Plant cell. 2006;18(2):350–65.
10. Eriksson S, Graslund A, Skog S, Thelander L, Tribukait B. Cell cycle-dependent regulation of mammalian ribonucleotide reductase. The S phase-correlated increase in subunit M2 is regulated by de novo protein synthesis. J Biol Chem. 1984;259(19):11695–700.
11. Gallardo TD, John GB, Shirley L, Contreras CM, Akbay EA, Haynie JM, et al. Genomewide discovery and classification of candidate ovarian fertility genes in the mouse. Genetics. 2007;177(1):179–94.

12. Yan L, Yang M, Guo H, Yang L, Wu J, Li R, et al. Single-cell RNA-Seq profiling of human preimplantation embryos and embryonic stem cells. Nat Struct Mol Biol. 2013;20(9):1131–9.

13. Yu C, Ji SY, Dang YJ, Sha QQ, Yuan YF, Zhou JJ, et al. Oocyte-expressed yes-associated protein is a key activator of the early zygotic genome in mouse. Cell Res. 2016;26(3):275–87.

14. Strumpf D, Mao CA, Yamanaka Y, Ralston A, Chawengsaksophak K, Beck F, Rossant J. Cdx2 is required for correct cell fate specification and differentiation of trophectoderm in the mouse blastocyst. Development. 2005;132(9):2093–102.

15. Wang H, Dey SK. Roadmap to embryo implantation: clues from mouse models. Nat Rev Genet. 2006;7(3):185–99.

Figures

(A) Pedigree of the patients' family. m: mutated allele of RRM2; +: normal allele; green dots: normal phenotype; red triangle: embryonic development failure phenotype.

(B) Control

P1

P2

Mother

Figure 1

(A) Pedigree of the patients’ family. m: mutated allele of RRM2; +: normal allele; green dots: normal phenotype; red triangle: embryonic development failure phenotype.
Figure 1

(A) Pedigree of the patients' family. m: mutated allele of RRM2; +: normal allele; green dots: normal phenotype; red triangle: embryonic development failure phenotype.
Figure 2

(A) Diagram of the RRM2 protein. (B) Diagram showing the substitution of the histidine by tyrosine in RRM2 protein of the patients. (C) Homology analysis of RRM2 protein among different species.
Figure 2

(A) Diagram of the RRM2 protein. (B) Diagram showing the substitution of the histidine by tyrosine in RRM2 protein of the patients. (C) Homology analysis of RRM2 protein among difference species.