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Novel mutations in genes encoding subcortical maternal complex proteins may cause human embryonic developmental arrest

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KEY MESSAGE

The genetic basis of human early embryonic arrest is largely unknown. This study identified novel mutations in subcortical matrix complex genes *TLE6*, *PADI6* and *KHDC3L* and identified novel phenotypes of embryo development arrest corresponding to the mutant genes, which may allow genetic diagnosis for patients with recurrent failure of IVF/ICSI.

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ABSTRACT

Successful human reproduction initiates from normal gamete formation, fertilization and early embryonic development. Abnormalities in any of these steps will lead to infertility. Many infertile patients undergo several failures of IVF and intracytoplasmic sperm injection (ICSI) cycles, and embryonic developmental arrest is a common phenotype in cases of recurrent failure of IVF/ICSI attempts. However, the genetic basis for this phenotype is poorly understood. The subcortical maternal complex (SCMC) genes play important roles during embryonic development, and using whole-exome sequencing novel biallelic mutations in the SCMC genes *TLE6*, *PADI6* and *KHDC3L* were identified in four patients with embryonic developmental arrest. A mutation in *TLE6* was found in a patient with cleaved embryos that arrested on day 3 and failed to form blastocysts. Two patients with embryos that arrested at the cleavage stage had mutations in *PADI6*, and a mutation in *KHDC3L* was found in a patient with embryos arrested at the morula stage. No mutations were identified in these genes in an additional 80 patients. These findings provide further evidence for the important roles of *TLE6*, *PADI6* and *KHDC3L* in embryonic development. This work lays the foundation for the genetic diagnosis of patients with recurrent IVF/ICSI failure.

Introduction

Successful human reproduction initiates from normal gamete formation and fertilization. Following fertilization, the zygotic genome is activated and embryogenesis begins (Lee et al., 2014). Embryos subsequently undergo cleavage, go through the morula and blastocyst stages, and finally establish pregnancy. Failure at any step of this process will lead to infertility. Assisted reproductive technologies (ART), including IVF and intracytoplasmic sperm injection (ICSI), are an innovative treatment for infertility (Zegers-Hochschild et al., 2009). However, some patients undergo several failed IVF/ICSI cycles without any successful pregnancy for unknown reasons. Oocyte maturation arrest is one of the reasons for ART failure, and it has previously been shown that mutations in *TUBB8* cause this phenotype (Chen et al., 2017; Feng et al., 2016a, 2016b; Huang et al., 2017). Compared with oocyte maturation arrest, embryonic developmental arrest is another common reason for recurrent IVF/ICSI failure (Betts and Madan, 2008). Embryonic arrest at the cleavage stage is referred to as preimplantation embryo lethality, while a hydatidiform mole is a kind of aberrant pregnancy in which non-viable or abnormal embryos (sometimes showing an arrested phenotype) lead to hydropic degeneration of the chorionic villi and excessive proliferation of the trophoblast.

It is known that maternal-effect factors play essential roles in the process of embryogenesis. In mice, defects in a few maternal-effect genes result in abnormal embryogenesis, but in humans the genetic basis of embryonic developmental arrest is poorly understood (Kim and Lee, 2014; Zhang and Smith, 2015). Among maternal-effect factors, the subcortical maternal complex (SCMC) is a multiprotein complex located in the subcortex of the oocyte and embryo and is essential for embryogenesis. It mainly consists of five proteins, including maternal antigen that embryos require (MATER), factor located in oocytes permitting embryonic development (FLOPED), peptidylarginine deiminase, type VI (PADI6), transducin-like enhancer of split 6 (TLE6), and Filia, which are encoded by *NLRP5*, *OOEP*, *PADI6*, *TLE6* and *KHDC3L*, respectively. Recently, mutations in *PADI6* and *TLE6* that are responsible for human preimplantation embryo lethality have been identified (Alazami et al., 2015; Maddirevula et al., 2017; Xu et al., 2016). In addition, mutations in *KHDC3L* have been reported to cause recurrent hydatidiform mole (RHM) (Parry et al., 2011). However, these mutations could only account for a small number of cases, and other new mutant genes or novel mutations in SCMC genes responsible for these phenotypes still need to be identified.

To identify novel mutations in SCMC genes, the genetic reasons for these in four patients from four independent families were investigated by whole-exome sequencing.

Materials and methods

Human subjects

Patients from four families and an additional 80 female infertility patients were recruited from the Ninth Hospital Affiliated to Shanghai Jiao Tong University, the Zhongshan Hospital Affiliated to Fudan University in China, and the Clinic of Reproductive Medicine 'Nadiya' in Ukraine from 2015 to 2017. The four patients and 80 additional female infertility patients were diagnosed with primary infertility caused by embryonic arrest; all had normal menstrual cycles and hormone levels. These patients had no obvious structural rearrangements or aneuploidy upon clinical chromosomal analysis. In this study, control oocytes with first polar body (PB1) were obtained from the germinal vesicle stage or metaphase I oocytes during in-vitro maturation culture in the IVF/ICSI cycles, from women pursuing IVF/ICSI because of a sperm problem in their male partner. The morphology of a pronuclear and cleaving stage embryo developed from a control ovulated oocyte with PB1, originating from a couple treated with ICSI for male infertility, was assessed 1 and 3 days after fertilization. All control oocytes and embryos were from the Ninth Hospital Affiliated to Shanghai Jiao Tong University. For ovarian stimulation the controls and patients were injected with 225 IU human menopausal gonadotrophin (HMG) (Livzon Pharmaceutical Group) once a day for 8 successive days and then a single dose of 2000 IU human chorionic gonadotrophin (HCG) (Livzon Pharmaceutical Group) was administered 34 h before the oocytes were retrieved. The oocytes/embryos were cultured in an incubator at below 37°C, 6% CO_2 and 5% O_2. For ICSI treatment, the spermatozoa were injected into oocytes by a microinjection system under a microscope.

All studies on human subjects were approved by the Fudan University Institutional Medical Review Board (No 148, approved on 24 February 2017), Ninth Hospital Affiliated to Shanghai Jiao Tong University Medical Review Board (No 20161207, approved on 5 December 2016) and the Clinic of Reproductive Medicine 'Nadiya' Medical Review Board (No 1/2015, approved on 10 March 2015).

The observation of oocytes/embryos and immunostaining

All of the live oocytes and embryos were evaluated under a light microscope. For immunostaining, one oocyte with PB1 from control and

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