Novel Biallelic TLE6 Variants Induce Preimplantation Embryonic Lethality That Cannot be Rescued by IVF or ICSI

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Research

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Abstract

Background: Preimplantation embryonic lethality is a rare cause of primary female infertility. Transducin-like enhancer of split 6 (TLE6) is a maternal effect gene which encodes a component of the subcortical maternal complex located in the oocytes and early embryos. It has been reported that biallelic variants in TLE6 can lead to preimplantation embryonic lethality. However, the incidence of TLE6 variants in patients with preimplantation embryonic lethality is not fully understood.

Methods: Whole-exome sequencing and bioinformatics analyses were used to analyze a cohort of 28 patients with preimplantation embryonic lethality. We retrospectively analyzed the process and outcome of their attempts at in vitro fertilization and intracytoplasmic sperm injection.

Results: In this study, four patients (14.29 %, 4/28) from three unrelated families in a cohort of 28 individuals with preimplantation embryonic lethality were identified as carrying biallelic TLE6 variants, including two homozygous variants and one compound heterozygous variant. These novel frameshift variants in TLE6 caused truncation of the TLE6 protein likely impairing its function. Immunofluorescence staining of oocytes for TLE6 indicated that it is localized in the oocyte cytoplasm, and that TLE6 was almost absent in the oocytes of the patients carrying biallelic TLE6 variants compared with normal control oocytes. A retrospective analysis showed that the four affected individuals underwent a total of nine in vitro fertilization and intracytoplasmic sperm injection attempts, in which a total of 119 metaphase II oocytes were retrieved, but none of them obtained high-quality blastocysts or obtained a successful pregnancy. However, one of these patients became pregnant on the first attempt using donated oocytes.

Conclusions: Biallelic TLE6 variants cause preimplantation embryonic lethality that cannot be rescued by in vitro fertilization or intracytoplasmic sperm injection. Thus, oocyte donation may be the preferred treatment for patients harboring biallelic TLE6 variants.

Background

Infertility affects about 10–15% of couples worldwide and has become an increasingly common health problem [1]. In recent years, assisted reproductive technology (ART) has become an important treatment for many women suffering from infertility. Recent evidence from ART and embryo research suggests that preimplantation embryonic lethality (PEL) (OMIM:616814) may be a rare cause of primary female infertility [2]. Women with PEL have normal ovarian follicle development and ovulation, while suffering from recurrent failures of in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) attempts due to fertilization failure and early embryonic arrest. It is challenging to identify genes that cause preimplantation embryonic lethality in humans.

Maternal effect genes (MEGs) are vital during embryonic cleavage stages. After fertilization, zygotic genome is transcriptionally quiescent and early embryo development relies on MEGs that encode many of the RNAs and proteins required for early divisions, chromatin remodeling, epigenetic reprogramming and transcriptional activation cascades [3]. Subcortical maternal complex (SCMC), present in the oocytes and early embryos, contains multiple proteins encoded by MEGs, and has been identified as being important for
preimplantation mouse embryogenesis [4]. The SCMC appears to be functionally conserved throughout mammalian species. It consists of at least eight proteins, including OOEP, NLRP5, TLE6, KHDC3L, PADI6, ZBED3, NLRP2, and NLRP7 [5–8]. In mice, knockout of certain MEGs, namely Nlrp2, Mater, Padi6, Floped, and Tle6, leads to infertility or subfertility owing to embryonic arrest [8, 10–13]. Recently, some MEGs have been identified in humans by way of whole-exome sequencing (WES) in a limited number of clinical cases. For example, TLE6 (OMIM: 616814) variants have been shown to result in the earliest known PEL in human [14]. Furthermore, biallelic variants in PATL2 (OMIM: 617743), WEE2 (OMIM: 617996), PADI6 (OMIM: 617234), NLRP5 (OMIM: 611689), and NLRP2 (OMIM: 609364) have been identified as the causes of a spectrum of PEL phenotypes, including oocyte maturation arrest, fertilization failure, and early embryonic arrest [6, 15–17]. However, variants in these genes can only explain a few cases, and the genetic basis of PEL is still largely unclear.

In the current study, we identified novel biallelic variants in TLE6 in four affected individuals from three unrelated families by WES and genetic analyses, which further expands the variant spectrum of TLE6. Our study also showed that the TLE6 variants accounted for 14.29% of patients in a small cohort of women affected with PEL. These findings not only provide a foundation for genetic diagnoses but also render efficient clinical decision-making for women with PEL.

**Methods**

**Study subjects**

We recruited 28 women affected with PEL from the First Affiliated Hospital of Anhui Medical University. Subjects with common causes of infertility, including chromosome anomalies, anovulation, tubal pathology, uterine abnormalities, cervical factors, or male factors, were excluded. We retrospectively analyzed the process and outcome of affected individuals’ IVF/ICSI attempts. Peripheral blood samples for DNA extraction were obtained from the affected individuals, their available family members, and control subjects. This study was approved by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University. All of the subjects gave their informed consent to participate.

**WES and genetic analyses**

Genomic DNA was extracted from peripheral blood of the subjects using DNeasy Blood and Tissue kit (Qiagen, Germany). Whole-exome capture was performed using SureSelectXT Human All Exon Kit (Agilent Technologies, CA, USA) following the manufacturer’s instructions, and sequencing was carried out on the HiSeq X-TEN platform (Illumina, CA, USA). Allele frequencies of the variants were searched using the Exome Aggregation Consortium (ExAC) database (http://exac.broadinstitute.org/), the 1000 Genomes Project database (http://www.1000genomes.org/), and the Genome Aggregation Database (gnomAD) (http://gnomad.broadinstitute.org/). The candidate variants and their parental origins were confirmed via Sanger sequencing.

**Evaluation of embryo phenotypes**
We used a light microscope (IX-71, Olympus, Japan) to observe morphologies of the embryos at different stages of development. Quality of each embryo was evaluated at several predefined timepoints during embryo development according to the conventional guidelines as described previously [18].

**Immunofluorescence**

Immature (germinal vesicle stage or metaphase I) and unfertilized (metaphase II, MII) oocytes were donated by the affected individuals and control subjects pursuing IVF/ICSI due to male infertility. These immature oocytes were matured *in vitro* following previously described methods [19]. Oocyte immunofluorescence staining was performed to assess TLE6 localization. Briefly, oocytes from all subjects (control as well as patients) were fixed with 4% paraformaldehyde for 30 min. The oocytes were then processed with a membrane permeabilizing solution (0.1% Triton X-100 in PBS) for 30 min, followed by blocking with 5% donkey serum for 1 h. Further, the oocytes were incubated with a primary mouse anti-TLE6 antibody (1:50, sc-515065, Santa Cruz Biotechnology, CA, USA) overnight at 4 °C. The oocytes were then incubated with Alexa Fluor 488 AffiniPure Donkey anti-mouse IgG (H + L) (1:100, 715-545-150, Jackson ImmunoResearch Laboratories, PA, USA) and protected from light for 1 h at room temperature. Following this, the oocytes were incubated with DAPI for 10 min to label DNA. Finally, the oocytes were visualized using an LSM800 confocal laser-scanning microscope (Zeiss, Germany).

**Results**

**Clinical characteristics of patients**

The clinical information of the four patients carrying the biallelic *TLE6* variants are listed in Table 1, and their family pedigrees are shown in Fig. 1A. Their menstrual cycles, karyotypes, transvaginal sonography, and sex hormone levels revealed no abnormalities. Furthermore, their husbands also showed normal semen parameters (sperm concentration, motility, and sperm morphology) as well as karyotypes. These patients had been unable to get pregnant despite years of trying (Table 1).
### Table 1
Clinical laboratory evaluation for the patients carrying variants in *TLE6*

| Patients | II-3 in family 1 | II-1 in family 2 | II-1 in family 3 |
|----------|------------------|------------------|------------------|
| Gene     | TLE6             | TLE6             | TLE6             |
| cDNA variant | c.1631_1632delCA | c.475_476delCT   | c.798_799insG    | c.222G > C |
| Protein alteration | p.Pro544Argfs*5 | p.Leu159Aspfs*14 | p.Gln267Alafs*54 | p.Gln74His |
| Variant type | Frameshift | Frameshift | Frameshift | Missense |
| Zygosity | Homozygous | Homozygous | Heterozygous | Heterozygous |
| Exon | Exon 17 | Exon 7 | Exon 12 | Exon 5 |
| Allele frequency | 1KGP NA | NA | NA | NA |
| | ExAC NA | NA | NA | NA |
| | gnomAD NA | 4.0 × 10^{-6} | NA | NA |
| Karyotype | Female 46, XX | 46, XX | 46, XX | 46, XX |
| | Male 46, XY | 46, XY | 46, XY | 46, XY |
| Female sex hormone | | | | |
| FSH (mIU/ml) | 7.34 | 6.68 | 8.81 | 4.19 |
| LH (mIU/ml) | 5.14 | 3.45 | 4.64 | 2.45 |
| E2 (pmol/L) | 138.00 | 156.63 | 296.00 | 169.00 |
| P (nmol/L) | 4.30 | 0.76 | 5.62 | 1.59 |
| T (nmol/L) | 1.40 | 0.47 | 2.90 | 2.80 |
| PRL (ng/ml) | 13.39 | 17.49 | 13.32 | 13.83 |
| Male semen parameters | | | | |
| Sperm concentration (10^6/ml) | 28.7 | 59.2 | 20.5 | 7.8 |

*a* The GenBank accession numbers of *TLE6* is NM_001143986.1; *b* Full length protein has 572 amino acids. Abbreviation: 1KGP, 1000 Genomes Project; ExAC, Exome Aggregation Consortium; gnomAD, Genome Aggregation Database; NA, not available; FSH, follicle stimulating hormone; LH, luteinizing hormone; E₂, estradiol; P, progesterone; T, testosterone; PRL: prolactin.
| Patients                        |       |       |       |       |
|--------------------------------|-------|-------|-------|-------|
| Progressive motility (%)       | 27.8  | 47.7  | 29.8  | 23.9  |
| (%)                            |       |       |       |       |
| Normal sperm morphology (%)    | 7     | 5     | 4     | 3     |

The GenBank accession numbers of *TLE6* is NM_001143986.1; Full length protein has 572 amino acids. Abbreviation: 1KGP, 1000 Genomes Project; ExAC, Exome Aggregation Consortium; gnomAD, Genome Aggregation Database; NA, not available; FSH, follicle stimulating hormone; LH, luteinizing hormone; E₂, estradiol; P, progesterone; T, testosterone; PRL: prolactin.

In family 1, Subjects I-1 and I-2 raised four daughters. Three of four sisters (II-2, II-3, and II-4) in this family had infertility for several years. Two of the affected sisters (II-3 and II-4) underwent several IVF/ICSI attempts in the Reproductive Medicine Center of the First Affiliated Hospital of Anhui Medical University. The proband (II-3, 37 years old) had undergone two IVF/ICSI attempts. A total of 71 oocytes (including 44 MII oocytes) were retrieved in the two attempts. Total fertilization and total blastocyst development rates in both attempts were 23.9% and 11.8%, respectively. After cultivation, majority of her embryos were arrested at the early stages. No high-quality blastocysts formed in these two IVF/ICSI attempts, and only two low-scoring blastocysts were available for transfer. Although the proband underwent one frozen-thawed embryo transfer cycle, she failed to obtain a successful pregnancy (Table 2).
Table 2
Clinical outcomes of the four patients following IVF/ICSI

|                      | family 1 | II-1 in family 2 | II-1 in family 3 |
|----------------------|----------|------------------|------------------|
|                      | II-3     | II-4             |                  |
| Female age (years)   | 37       | 33               | 32               | 32               |
| Male age (years)     | 37       | 34               | 31               | 41               |
| No. of IVF/ICSI cycles | 2        | 4                | 1                | 2                |
| No. of oocytes retrieved | 71       | 92               | 5                | 24               |
| No. of MII oocytes   | 44       | 55               | 3                | 17               |
| Fertilization rate (%) | 17/71(23.9) | 38/92(41.3) | 1/5(20.0) | 5/24(20.8) |
| Cleavage rate (%)    | 17/17(100.0) | 38/38(100.0) | 1/1(100.0) | 5/5(100.0) |
| Blastocyst development rate (%) | 2/17(11.8) | 6/38(15.8) | 0                | 1/5(20.0) |
| No. of high-quality blastocysts | 0      | 0                | 0                | 0                |
| No. of frozen-thawed embryos transfer cycles | 1      | 3                | 0                | 1                |
| Number of embryos transferred | 2      | 6                | 0                | 1                |
| Implantation rate (%) | 0      | 0                | 0                | 0                |
| Clinical pregnancy | NO       | NO               | NO               | NO               |

Annotations: TLE6, transducin-like enhancer of split 6; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; MII, metaphase II.

The other affected sister (II-4, 33 years old) in family 1 had undergone four IVF/ICSI attempts. A total of 92 oocytes (including 55 MII oocytes) were retrieved in the four attempts. The total fertilization rate and total blastocyst development rate in the four attempts were 41.3% and 15.8%, respectively. No high-quality blastocysts were obtained in these IVF/ICSI attempts, while only six embryos developed into low-scoring blastocysts and were frozen. The affected individual underwent three frozen-thawed embryo transfer cycles, none of which was successful (Table 2). Finally, the affected individual obtained high-quality blastocysts via oocyte donation and established pregnancy successfully.

In family 2, the proband (II-1, 32 years old) had undergone an IVF attempt in which five oocytes (including three MII oocytes) were obtained. The fertilization rate was 20.0%. The zygote was arrested during further blastocyst culture without blastocyst formation (Table 2).

In family 3, the proband (II-1, 32 years old) had undergone two IVF/ICSI attempts, which also resulted in 24 retrieved oocytes (including 17 MII oocytes). The total fertilization rate and total blastocyst development rate in the two attempts were 20.8% and 20.0%, respectively. One of the zygotes formed a low-scoring blastocyst,
while the others showed developmental arrest on day 3. The patient underwent a frozen-thawed embryo transfer cycle but failed to establish pregnancy (Table 2).

**Identification of bi-allelic variants in TLE6**

We recruited 28 affected individuals with preimplantation embryonic lethality and identified four affected individuals (accounting for 14.29% of the cohort) from three unrelated families carrying biallelic variants TLE6 (NM_001143986.1) by whole-exome sequencing and bioinformatics analyses. Two (II-3 and II-4 in family 1) of the four affected individuals were sisters from a non-consanguineous family in which three-quarters of the sisters were diagnosed with primary infertility, both of them carrying a homozygous TLE6 frameshift variant c.1631_1632delCA (p.Pro544Argfs*5). In family 1, Sanger sequencing verified that the parents and one fertile elder sister were heterozygous carriers, while the other three sisters were homozygous for the variant, indicating a recessive inheritance pattern (Fig. 1). Another homozygous frameshift variant in TLE6 (c.475_476delCT, p.Leu159Aspfs*14) was identified in the proband (II-1 in family 2) from a consanguineous family. Sanger sequencing confirmed that the proband’s parents were both heterozygous carriers (Fig. 1). Moreover, we identified a compound heterozygous variant in TLE6 (c.222G > C, p.Gln74His; c.798_799insG, p.Gln267Alafs*54) in the proband (II-1 in family 3) from another non-consanguineous family and the two variants were also inherited from her heterozygous parental carriers, respectively (Fig. 1).

These TLE6 variants were absent in the ExAC database, the 1000 Genomes Project database and the gnomAD database, except that the TLE6 frameshift variant c.475_476delCT was found at extremely low allele frequency (4.0 × 10⁻⁶) in the general population in the gnomAD (Table 1). The three TLE6 frameshift variants c.1631_1632delCA, c.475_476delCT, and c.798_799insG were all loss-of-function (LoF) variants that caused impaired function of the gene-encoded protein. Positions of the four TLE6 variants and conservation of mutant residues in their expressed protein among different species are shown in Fig. 2. Only the TLE6 frameshift variant c.1631_1632delCA (p.Pro544Argfs*5) was located within the C-terminal WD40 repeat domain, while the other three variants were to the left of the WD40 repeat domain (Fig. 2a). The positions of these four variants are highly conserved in primates (Fig. 2b).

**Phenotypic spectrum of patients with TLE6 variants**

We used light microscopy to observe the development and morphology of the embryos from family 1 member II-4 for five consecutive days in her last ICSI attempt. Five of the embryos on day 3 were arrested, whereas the others had a high percentage of fragmentation, and all of them failed to form blastocysts (Fig. 3a). An immunofluorescence analysis showed that an immunofluorescence signal for TLE6 was observed in the cytoplasm in the control oocytes, similar to previous reports of the localization of other SCMC component proteins in human oocytes, such as PADI6 and NLRP2 [6, 17]. However, the TLE6 signal was much weaker in oocytes from the affected individual (II-4 in family 1) compared with control oocytes (Fig. 3b). These data suggest that the biallelic variants in TLE6 result in degradation of the TLE6 protein in oocytes.

**Discussion**
In this study, using WES and bioinformatics analyses, we identified biallelic \textit{TLE6} variants in 4 from a total of 28 infertile women with PEL, accounting for 14.29\% of the cohort. Moreover, the three novel \textit{TLE6} frameshift variants in the present study were all LOF variants that cause protein truncation as well as impaired function. By immunofluorescence staining, we found that the biallelic variants in \textit{TLE6} impaired the stability of the TLE6 and resulted in its degradation in oocytes.

TLE6, also known as Groucho family member 6 (GRG6), is part of the SCMC that is necessary for mammalian embryonic development [20–21]. Research has shown that high levels of cAMP in the oocyte maintain an increase in cAMP-dependent protein kinase (PKA) activity, which causes meiotic prophase I arrest. Following the luteinizing hormone surge during ovulation, PKA activity reduces due to a decrease in cAMP levels, leading to a resumption of meiosis, while PKA activity increases throughout the process of meiosis from the time of germinal vesicle breakdown (GVBD) until the MII arrest [22]. TLE6 is a substrate of PKA during mouse oocyte maturation. Inhibition of PKA activity can lead to delays in GVBD dynamics, abnormal spindle and chromatin structures, and a reduced ability of oocytes to undergo MII [22]. Therefore, it has been speculated that the PKA-dependent phosphorylation of TLE6 during GVBD may be relevant to oocyte maturation and subsequent embryonic development. In mouse models, knockout of the \textit{Tle6} gene has no effect on folliculogenesis, oogenesis, and ovulation, but instead results in embryonic development arrest at the two-cell stage, and some embryos showed significant fragmentation at embryonic day 2.5 and embryonic day 3.5, eventually leading to female mice infertility [12–13]. Further studies indicate that the SCMC might regulate spindle assembly by controlling formation of the F-actin cytoskeleton to ensure symmetric division of mouse zygotes, while the absence of TLE6 affects the formation of F-actin cytoskeleton due to destruction of the integrity and function of the SCMC, and thus, results in asymmetric cleavage as well as early embryonic arrest [13].

Alazami et al. first identified a homozygous \textit{TLE6} variant c.1529C > A (p.S510Y) in affected individuals from two Saudi families, which resulted in the earliest known human PEL phenotype, including fertilization failure and early cleavage failure. Their further research showed that the \textit{TLE6} variant not only caused a significant reduction in the PKA-mediated TLE6 phosphorylation but also impaired its binding to other component proteins of the SCMC [14]. Wang et al. found the \textit{TLE6} variant c.1133delC (p.A378Efs*75) being responsible for embryonic developmental arrest on day 3, similar to the phenotype of \textit{Tle6}\textsuperscript{Null} mice [23]. Furthermore, another study also found that three patients carrying biallelic \textit{TLE6} variants had fertilization failure and early embryonic arrest in several IVF/ICSI attempts. Two of them obtained a very low number of low-quality embryos but failed to establish pregnancy [24]. Recently, using time-lapse imaging, Zheng et al. found that the \textit{TLE6} missense variant c.1564G > C (p.Asp522His) is associated with direct cleavage (zygotes directly cleaved into more than two blastomeres) [25].

Phenotypes of infertile women harboring biallelic \textit{TLE6} variants in this study are similar to the previously reported clinical cases [14, 23–25]. These four patients underwent a total of nine IVF/ICSI attempts in which the number of retrieved oocytes and frequency of oocyte maturity were not clearly abnormal; however, the fertilization rate and blastocyst development rate were very low. The overwhelming majority of the embryos had developmental arrest on the third day, so that none of these patients obtained high-quality blastocysts or established pregnancy successfully. One of the patients (II-4 in family 1) got pregnant successfully at the
first attempt using donated oocytes. Therefore, these biallelic TLE6 variants are responsible for PEL, which cannot be rescued by IVF or ICSI. In contrast, subject II-1 in family 1, who had a heterozygous variant in TLE6, had normal fertility and two healthy children. Furthermore, in our cohort of control women pursuing IVF/ICSI due to male infertility, we also found that a subject harboring a heterozygous missense variant in TLE6 established pregnancy successfully via sperm donation. These clinical cases combined with Sanger sequencing can help further understand the inheritance pattern of the TLE6 mutant gene.

In addition, we used light microscopy for five consecutive days to observe the development and morphology of the embryos from one patient (II-4 in family 1) who carried the homozygous TLE6 frameshift variant c.1631_1632delCA (p.Pro544Argfs*5), and found that three embryos had a high percentage of fragmentation during culture, whereas the other five embryos were arrested on the third day, showing similar phenotype in embryogenesis between infertile women carrying TLE6 variants and Tle6Null female mice. Thus, combined with previous reports, we speculated that the TLE6 missense/frameshift variants lead to embryo fragmentation by disrupting the F-actin and spindle dynamics. In addition, our study is the first to assess the expression levels of the TLE6 protein in the oocytes of affected individuals. Immunofluorescence staining showed that the biallelic variants in TLE6 in the present study resulted in the degradation of TLE6 in the oocytes of affected individuals. From this, we speculate that TLE6 protein degradation might then affect the stability and function of the SCMC, eventually leading to preimplantation embryonic lethality.

There are a few limitations associated with the current study. First, the exact molecular mechanism of PEL could not be completely elucidated owing to the paucity of human oocytes and embryos. It will be worthwhile to study the molecular mechanism using knock-in mice for each variant in future. Second, sample size of affected women was limited in the present study, so the incidence of TLE6 variants in patients with PEL requires further research.

In conclusion, this study extends the spectrum of variants in TLE6 and shows that biallelic TLE6 variants are responsible for preimplantation embryonic lethality, which cannot be rescued by IVF or ICSI. Thus, oocyte donation may be the best option for a successful pregnancy in patients with primary infertility who harbor biallelic TLE6 variants.

**Abbreviations**

Transducin-like enhancer of split 6 (TLE6)

Assisted reproductive technology (ART)

Preimplantation embryonic lethality (PEL)

*in vitro* fertilization (IVF)

Intracytoplasmic sperm injection (ICSI)

Maternal effect genes (MEGs)
Subcortical maternal complex (SCMC)

Whole-exome sequencing (WES)

Metaphase II (MII)

Loss-of-function (LoF)

Protein kinase (PKA)

Germinal vesicle breakdown (GVBD)

Declarations

1. Ethics approval and consent to participate: This study was approved by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University (number: PJ2020-13-10). The clinical research was performed in accordance with the Helsinki Declaration. Informed consent was obtained from each participant.

2. Consent for publication: All the participants gave consent for its publication.

3. Availability of data and materials: All data generated or analysed during this study are included in this published article.

4. Competing interests: The authors declare that they have no competing interests.

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6. Authors' contributions: MZ wrote the manuscript. CL and ML analysed the data. BC, HZ, YL and MZ conducted the experiments. YG, TW, QX and YZ collected sample and data. HW, XH and YX designed and directed the study. ZZ, PZ and ZW revised the manuscript. YC was responsible for the study supervision. All authors read and approved the final manuscript.

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Figures

Figure 1

Identification of biallelic TLE6 variants in the human individuals. Pedigrees structure of three families harboring variants in TLE6, circles represent females, squares represent males and black circles denote patients with primary infertility. The double line represents consanguinity, equal signs indicate infertility and the arrow indicates the proband. Sanger sequencing verification is shown below the pedigrees. Red arrows indicate the mutated positions.
Figure 2

a Schematic representation of the TLE6 gene and domain structure of its protein product. Black arrows indicate previously reported variants, while red arrows represent the novel variants identified in this study. Orange box represents a cluster of seven WD40 domain repeats. b Sequence alignment displays conservation of mutant residues in TLE6 among different species.
Figure 3

a Phenotype of control embryos from a subject underwent the ICSI attempt owing to male infertility and embryos from the patient II-4 in family 1. The light microscope was used to observe the morphologies of embryos on day 1, day 2, day 3, and day 5 during cultivation. b The morphologies and immunofluorescence staining results of an oocyte from the patient II-4 in family 1 and a normal oocyte from a control individual. Oocytes were stained with DAPI (blue) in order to visualize the DNA and immunolabeled with antibodies against TLE6 (green). Scale bar: 20 µm.