RGS12 as a Novel Maternal-Effect Gene Causes Arrest at the Pronuclear Stage of Human Zygote

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Research

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Abstract

Background: Early embryonic arrest is one of the major causes of female infertility after in vitro fertilization (IVF), but the causal gene of arrest at the pronuclear (PN) zygote stage is largely unknown.

Results: To understand this process, we recruited a family characterized by recurrent PN arrest during IVF cycles and performed whole-exome sequencing. The missense variant c.C1630T (p.R544W) in RGS12 was responsible for a phenotype characterized by paternal transmission. RGS12 controls Ca\textsuperscript{2+} oscillation, which is required for oocyte activation after fertilization. Single-cell transcriptome profiling of PN-arrest zygotes revealed defective established translation, RNA processing and cell cycle, which explained the failure of complete oocyte activation. Furthermore, we identified proximal genes involved in Ca\textsuperscript{2+} oscillation–cytostatic factor–anaphase-promoting complex (Ca\textsuperscript{2+} oscillation–CSF–APC) signaling, including upregulated CaMKII, ORAI1, CDC20, and CDH1 and downregulated EMI1 and BUB3. The findings indicated abnormal spontaneous Ca\textsuperscript{2+} oscillations leading to oocytes with prolonged low CSF and high APC level, which resulted in defective nuclear envelope breakdown and DNA replication. The changes in levels of critical genes were confirmed by examining other independent PN-arrest zygotes. However, the PN-arrest zygote phenotype was not consistent with that of RGS12-deficient mice, thereby indicating species-specific functions between human and mouse.

Conclusion: Our findings expand our knowledge of the genetic determinants of human early embryonic arrest at the PN stage and provide guidance for selecting clinically infertile individuals with PN-arrest zygotes for Ca\textsuperscript{2+} intervention.

Background

In vitro fertilization (IVF) is now routine for treating infertile women and has brought an estimated at least 8 million babies into the world globally. Indeed, the earliest embryonic development after fertilization is a complex process, including the formation of spermatozoa and oocyte pronuclei (two-pronuclear [2PN] zygote), cytoskeletal rearrangements, pronuclear union, and initiation of cleavage of the zygote. About 5% of fertilized human oocytes present early developmental arrest at the PN stage after IVF trials [1]. Homozygous mutations in TLE6 (MIM: 612399) and PADI6 (MIM: 10363) have been reported to cause embryonic arrest at the 2- to 4-cell stage with normal cleavage in consanguineous families [2, 3]. However, the crucial gene responsible for PN-arrest zygotes remains largely unknown.

Material And Methods

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Guangdong Medical University Affiliated Hospital (YS2018010) and written informed consent was obtained from participants. We confirmed that patients gave written informed consent for the use of abandoned zygotes and peripheral blood for research on the
arrest mechanism of pronuclear (PN) stage, with no monetary payment. All procedures used in the present study were performed in accordance with the relevant guidelines and regulations.

**Family recruitment**

Families were recruited through the Reproductive Medicine Center at the Affiliated Hospital of Guangdong Medical University based on the observation of PN arrest during regular in vitro fertilization treatment of two siblings. Eligible families and controls were enrolled after signing a written informed consent. Peripheral blood samples were taken for DNA extraction.

**Patients, ovarian stimulation, oocyte retrieval, and the IVF/ICSI procedure**

*In vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) were performed according to the laboratory routine insemination procedures on the day of oocyte retrieval (Day 0). The presence of two pronuclei (PN) was observed 16–18 hr after insemination or injection, then the zygotes were cultured in 25 µL pre-equilibrated cleavage medium. The embryos were cultured in incubators at 37 °C under 6% CO₂. Embryo morphology was evaluated 42–46 hr (Day 2) and 68–72 hr (Day 3) after insemination. Male and female pronuclei that continued to separate on Day 2 and 3 without fusion were defined as PN-arrest zygotes.

**Whole-exome sequencing and data**

Germline genomic DNA was subjected to exome capture (60 Mb) with the Agilent SureSelect Human All ExonV6 kit according to the manufacturers' instructions (Agilent, Santa Clara, CA). Paired-end sequencing, resulting in 150 bases from each end of the fragments, was performed with a HiSeq PE150 Genome Analyzer (Illumina) at Novogene Bioinformatics Technology (Beijing). Sequencing reads were mapped to the reference genome (GRCh37, UCSC hg19) by using the Burrow-Wheeler Aligner (BWA) and were analyzed by using the Genome Analysis Toolkit (GATK, v3.1) for calling single nucleotide variants, insertions and deletions. The 1000 Genomes, Exome Sequencing Project (ESP6500), Exome Aggregation Consortium (ExAC) and an in-house database were used to annotate the minor allele frequency (MAF) for each variant. *In silico* analysis, Sort Intolerant from Tolerant (SIFT), Polymorphism Phenotyping v2 (PolyPhen-2), MutationAssessor, and GERP++ (Genomic Evolutionary Rate Profiling) were used to predict the impact of each nonsynonymous variant.

**Variant filtering**

The pipeline was designed to filter heterozygous variants: 1) shared by both affected individuals; 2) absent in other unaffected family members; 3) not previously reported or reported to have a frequency < 0.1% in the public databases: 1000 Genomes, ESP6500, ExAC and in-house databases; 4) frameshift, nonsense, splice-site and missense variants predicted to be damaging in at least 3 of the 4 algorithms: SIFT, PolyPhen-2, MutationAssessor, and GERP++.
Sanger sequencing validation and segregation analysis for candidate variants

The variants were then validated by Sanger sequencing in the affected individuals and other family members. PCR amplification and Sanger sequencing were conducted. The RGS12 gene-specific primers to generate the variation were

5′-CAGGTTCTGGGACCTAAACAAG-3′ (forward)
and 5′-GACTGTGCAAGCTGGTGACT-3′ (reverse).

Variants were evaluated for co-segregation based on an autosomal-dominant mode of inheritance.

RNA library preparation and sequencing

The single-cell RNA-seq method used was described previously[4]. Briefly, zygotes were transferred into lysate buffer by using a mouth pipette and a reverse transcription reaction was performed on the whole-cell lysate according to the manufacturer’s instructions. The terminal deoxynucleotidyl transferase was used to add a poly (A) tail to the 3′ end of the first-strand cDNA, then 20 + 10 cycles of PCR were performed to amplify the single-cell cDNA. The libraries were sequenced on HiSeq PE150 Genome Analyzer platform at Annoroad Gene Technology (Beijing; http://www.annoroad.com).

Transcript alignment and assembly

Overall read quality was checked by using FASTQC v.0.11.5. The raw sequence data, in the form of FASTQ files, were aligned to the human genome (GRCh38, Ensembl Homo_sapiens) by using HISAT2 (v. 2.1.0) and SAMTOOLS (v1.3.1). Read count and Fragments Per Kilobase Millon mapped reads (FPKM) for each sample were generated by using HTSeq v0.6.0.

Differential expression analysis

HTSeq read counts were uploaded into RNA-seq 2G (http://52.90.192.24:3838/maseq2g/) for DESeq2 analysis. Normalization was performed with default settings (“normalize count by DESeq” / “normalize logged by Loess”). The P-values were adjusted by using the Benjamini and Hochberg method for controlling the false discovery rate (FDR). Genes with FDR P < 0.05 and fold change > 2 or < 0.5 were considered differentially expressed.

Functional enrichment analysis

RNA-seq normalized data (FPKM) were subjected to principal component analysis (PCA) by using an unsupervised approach to observe the whole clustering profile. Gene Ontology (GO, biological processes) and pathway enrichment was performed by using DAVID (http://david.abcc.ncifcrf.gov/) with the Benjamini and Hochberg FDR to adjust the P value. The significantly enriched GO categories were visualized by using REVIGO[5] (http://revigo.irb.hr/). The network of enriched terms was evaluated by using Metascape [6] (http://metascape.org/). To infer the transcription factor regulatory network of this
study, we used all 1,665 human transcription factors in the human TFDB 3.0 (http://bioinfo.life.hust.edu.cn/AnimalTFDB#!/).

RGS12 -knockout mice

RGS12-knockout mice on a C57BL/6J background were from Cyagen Biosciences (Guangzhou, China). All animal studies were approved by Institutional Animal Care and Use Committee of Shantou University Medical College.

Data availability

All RNA-seq data sets that were generated in this study have been deposited in Gene Expression Omnibus. Human oocyte, preimplantation embryo RNA-seq data were obtained from GSE44183 [7]. The RNA-seq data for normal PN zygotes (n = 22) were downloaded from GSE6548 [8]. The list of differentially expressed genes from RNA-seq data for validation were downloaded from a previous publication [9].

Results

Identification of variant in RGS12 responsible for phenotype of PN arrest of human zygote

To identify novel PN-zygote arrest-specific genes, we recruited a family with multiple infertile individuals who presented recurrent visible PN zygotes with second polar-body emission that failed to complete PN fusion after 24 to 68 hr during IVF trials (Fig. 1a and Supplementary Table S1). We used whole-exome sequencing (WES) with 2 individuals, an unaffected sibling, and their parents (Fig. 1b). Given the pedigree structure, we used an autosomal-dominant inheritance pattern and identified heterozygous, rare, potential pathogenic variants co-segregated with PN zygote arrest. Initially, 13 candidate genes were filtered by WES (Supplementary Table S2), and only the missense variant c.C1630T (minor allele frequency [MAF] = 0.00018 in ExAC database) resulting in a p.R544W of regulator of G protein signaling-12 (RGS12) was confirmed by Sanger sequencing results available for relatives and was characterized by paternal transmission (Fig. 1d).

RGS12 is the largest protein in the regulators of the G-protein signaling (RGS) family, and is a negative regulator of specific G-protein–coupled receptor (GPCR) signals [10]. It has the highest expressive levels in testis and ovary [11]. RGS12 has PDZ, PTB, RGS, RBD domains and GoLoco motifs (Gαi) [12]. It interacts with G protein via the RGS domain to inhibit cyclical activation of PLCζ-IP3 and intracellular Ca\(^{2+}\) release from endoplasmic reticulum and with the tyrosine-phosphorylated N-type Ca\(^{2+}\) channel by binding its PTB domain to regulate extracellular Ca\(^{2+}\) influx [12]. RGS12 p.R544W is located between the RGS and PTB domains and presents a non-conserved pattern between human and mouse (Fig. 1c), so it might have species-specific functional effect.
Identification of the molecular landscape underlying PN arrest zygote caused by RGS12 mutation

To describe the molecular landscape underlying the PN-arrest zygote (Fig. 2a), we performed single-cell RNA sequencing of PN-arrest zygotes (n = 3) from patient III-3 to explore the transcriptional profiles of PN-arrest zygotes by comparison with normal PN zygotes (n = 22, GSE65481)[8]. We found significant upregulation of 1415 genes (fold change > 2, \( P < 0.001 \)) and downregulation of 1545 genes (Fig. 2b and Supplementary Table S3). Differentially expressed genes (DEGs) were enriched in the Gene Ontology (GO) terms (biological processes) RNA processing (false discovery rate [FDR] = 1.13 × 10\(^{-21}\)), translational elongation (FDR = 1.25 × 10\(^{-16}\)), intracellular transport (FDR = 1.63 × 10\(^{-15}\)), and cell cycle (FDR = 4.11 × 10\(^{-13}\)), which indicates that the oocyte-specific transcription and translation machinery is not complete established (Fig. 2c-d). Pathway enrichment analysis revealed that DEGs in PN-arrest zygotes were also mainly involved in RNA processing and translation, such as ribosome (FDR = 6.40 × 10\(^{-22}\)) and spliceosome (FDR = 1.52 × 10\(^{-5}\)) (Fig. 2f). The switch from oocyte to embryo transition is driven by a maternal stockpile of mRNA and translational machinery that are “packed” into the oocyte. Furthermore, oocyte activation after fertilization includes changes to oocyte coverings to prevent polyspermy, release of oocyte meiotic arrest, generation of haploid female and male pronuclei, changes in maternal mRNA and protein populations, and cytoskeletal rearrangements. Our transcriptional prolife results implied that the PN-arrested zygotes had properties of failure of complete oocyte activation after fertilization.

Mutant RGS12 affects Ca\(^{2+}\) oscillations during oocyte activation after fertilization

Oocyte activation events present different Ca\(^{2+}\) requirements: 1) for cortical granules and blocking polyspermy; 2) inducing the resumption of meiosis including second meiotic polar body extrusion and initiating recruitment of maternal mRNAs; and 3) promoting pronuclear formation and initiation of embryonic mitosis. On oocyte activation, after PN formation, Ca\(^{2+}\) signalling continues to play a role in PN fusion and DNA synthesis for initiation of embryonic mitosis during the oocyte-to-embryo transition.

\( RGS12 \) suppresses Ca\(^{2+}\) oscillations by inhibiting the activity of G proteins. \( RGS2 \)-depleted oocytes were found to undergo spontaneous Ca\(^{2+}\) release, causing slight and consistent first Ca\(^{2+}\) oscillations after fertilization [13]. Therefore, we suspected that a loss-of-function effect of \( RGS12 \) p.R544W caused spontaneous and abnormal Ca\(^{2+}\) oscillations after fertilization as well.

Mature oocytes await fertilization while arrested at MII, which is maintained by maturation promoting factor (MPF) consisting of cyclin B1/CDK1 subunits. Cytostatic factor (CSF) mediates MPF stabilization by inhibiting anaphase-promoting complex (APC), which would otherwise destroy cyclin B. Fertilization breaks the MII arrest via cytoplasmic Ca\(^{2+}\) oscillation and triggers the APC, which mediates the degradation of cyclin B and thus inactivation of MPF. To confirm the spontaneous and abnormal Ca\(^{2+}\)
oscillations, we traced the transcriptional change in genes that participate in vital processes from Ca\(^{2+}\) oscillation, CSF and APC (Fig. 2e). Results are as follows:

Sperm-induced Ca\(^{2+}\) oscillations prevent subsequent fertilization by inducing cortical granule release, which modifies the zona pellucida. The sperm binds to ZP3, which is consistent with the G-protein activation, and causes transient Ca\(^{2+}\) influx [14]. PN-arrest zygotes showed 9.54-fold increased ZP3 expression, which indicates sustained ZP3-evoked Ca\(^{2+}\) entry by Ca\(^{2+}\) influx and activation of G protein. Furthermore, the plasma membrane Ca\(^{2+}\) channel ORAI1 mediates Ca\(^{2+}\) influx of oocytes after fertilization. In PN-arrest zygotes, ORAI1 showed 7.2-fold upregulation, which further confirmed the Ca\(^{2+}\) influx. The frequency and duration of Ca\(^{2+}\) oscillations are strictly temporal and spatial; otherwise, the inordinate Ca\(^{2+}\) oscillation during oocyte activation usually leads to impaired oocyte-to-embryo transition [15]. Especially, high-frequency Ca\(^{2+}\) oscillations via increased PLC\(\zeta\) cause efficient oocyte activation (pronuclei formation) and cleavage stage arrest [16]. In PN-arrest zygotes, the expression change of sperm-specific PLC\(\zeta\) was not observed, whereas upregulation of PLCD3 and PLCH2 and downregulation of PLC\(\beta1\) was identified. Lack of PLC\(\beta1\) disrupted amplitude Ca\(^{2+}\) oscillation with normal duration and frequency after fertilization [17]. These results indicate a spontaneous and abnormal Ca\(^{2+}\) oscillation in PN-arrest zygotes.

Next, we explored further evidence to support spontaneous and abnormal Ca\(^{2+}\) oscillation and its effects. CAMKII (CAMK2A) links Ca\(^{2+}\) oscillations and inactivates the MPF as well as translation and degradation of maternal mRNAs. Emission of the second polar body is driven largely by the early CaMKII-driven meiosis-resumption events [18]. We found upregulation of CAMK2A in PN-arrest zygotes, which implies the existence of prolonged Ca\(^{2+}\) oscillations. CaMKII activation by Ca\(^{2+}\) oscillations leads to activation of the APC via inhibition of CSF activity, which suppresses APC via EMI1 working with MOS. As a consequence of abnormal Ca\(^{2+}\) oscillations, EMI1 (FBXO5) was significantly downregulated (12.25-fold) in PN-arrest zygotes, which indicates the lower CSF level and possible high APC level.

The activation of APC is regulated by two activators, CDC20 and CDH1. CDC20 and CDH1 expression was 2.7- and 10.3-fold increased, respectively, in PN-arrest zygotes. CDC20 and CDH1 bind to APC7, whose level was also increased in PN-arrest zygotes. The mitotic checkpoint complex (MCC), composed of CDC20, MAD2, BUBR1, CENPE and BUB3, acts as an APC inhibitor [19], but we found downregulated BUB3 and CENPE in PN-arrest zygotes. Downregulation of EMI1 and BUB3 and upregulation of CDC20, CDH1, and APC7 implied continually increased APC level in PN-arrest zygotes.

Activation of APC/CDC20 and APC/CDH1 mediates cyclin-A and -B1 destruction and alters the substrate specificity[20]. The catalytic center of APC is formed by APC11 and APC2 along with APC10 and the co-activators CDC20 or CDH1 for substrate recognition. APC10 is crucial for cyclin-B1 substrate recognition but not cyclin-A destruction [21]. Cyclin A is an extremely efficient APC substrate, requiring minimal amounts of CDC20 for its destruction [20]. We found the expression of APC10 and cyclin A2 (CCNA2) downregulated, with no change in CCNB1 expression. CCNA2 is required for timely nuclear-envelope
breakdown (NEBD) [22]. We also found downregulation of GMNN (geminin), an APC substrate and essential for regulation of DNA replication for zygotes.

Each pronucleus undergoes DNA replication and NEBD before their chromosomes eventually intermingle and enter the first mitosis. CDC7/DBF4 initiate DNA replication, whereas geminin is essential to prevent DNA re-replication [23]. CDC7/DBF4 and geminin are substrates of APC/CDC20 and APC/CDH1 [24]. Furthermore, depletion of EMI1 leads to geminin and cyclin A degradation due to unopposed APC/C activity. In PN-arrest zygotes, CDC7/DBF4 expression was decreased ~ five-fold. Therefore, continuous APC disrupted NEBD and DNA replication in PN-arrest zygotes.

Normally, sufficient Ca\(^{2+}\) oscillations promote APC to prevent MPF activation, which continues for about 4 hr, thus allowing the oocyte to enter interphase (marked by PN formation) [25]. In turn, the pronucleus results in cessation of Ca\(^{2+}\) oscillations to trigger the process of NEBD [26]. The partially activated oocyte does not progress further and is arrested again in the PN stage, described as a new metaphase-III arrest [27], caused by CaMKII activation by Ca\(^{2+}\) oscillations [28]. Here we show that spontaneous and abnormal Ca\(^{2+}\) oscillation increased APC level by the mutant RGS12, leading to defective NEBD and DNA replication after 24 to 68 hr in IVF trials. The trigger for the oocyte-to-embryo transition is oocyte activation. APC also contributes to the change from meiosis in the oocyte to mitosis in the embryo [29]. Ca\(^{2+}\) ionophores such as A23187 improve embryonic development of fertilized human oocytes with PN arrest, but the success rates are still poor. Therefore, our evidence supports that the PN arrest is due to spontaneous and abnormal Ca\(^{2+}\) oscillation causing prolonged APC activation. Hence, a precise pattern of Ca\(^{2+}\) oscillations after fertilization should be evaluated for further treating optimal oocyte activation.

Validation of Ca\(^{2+}\) oscillation–CSF–APC signaling in PN arrest zygotes

To validate the CSF, APC and MPF levels and their key genes in PN-arrest zygotes, we integrated the zygote transcriptome data from this study with previous work by Suo et al. [9] We identified 589 common genes enriched in the GO terms translational elongation (FDR = 5.88 \times 10^{-34}) and translation (FDR = 2.45 \times 10^{-17}) and confirmed the incomplete oocyte activation. The key components of Ca\(^{2+}\) oscillation, CSF and APC signaling, EMI1, CCNA2, CDC7/DBF4, and GMNN were also identified (Fig. 3a-d). To investigate the master regulators and construct the transcriptional regulatory network in the PN-arrest zygotes, we used the ARACNe method to analyze transcription factors. Only the transcription factor MAX was upregulated (Fig. 3e), which indicates that MYC-MAX may play a critical role in the cell cycle entry of PN-arrest zygotes.

We observed upregulation of 2- to 4-cell arrest-specific genes TLE6 and PATL2, which indicates that the RGS12 mutation caused an earlier embryonic development arrest (Fig. 3e). Furthermore, we found downregulation of SYCP3 and TUBB8, which indicates defective cytoskeletal rearrangements. We did not find a disruption of zygotic arrest 1 (ZART1), a oocyte-specific maternal-effect gene for mouse oocyte-to-embryo transition [30].
Loss of RGS12 causes minor defects in PN arrest and is compatible with mouse development

To determine whether RGS12 deficiency caused PN arrest, RGS12-deficient (RGS12−/−) mice containing both RGS and RBD1 domains of Rgs12 were generated. Like RGS2−/− females[31], RGS12−/− mice are viable and fertile and have high blood pressure due to increased Ca²⁺ release in response to vasoconstrictors, which act through GPCR (data not shown). The first cleavage occurred in 24/104 (23%) in vitro-fertilized embryos from RGS12−/− females as compared with Rgs12+/− mice (30/100, 30%) versus two-cell embryos observed in 105/139 (76%) of embryos from RGS12 wild-type females (Supplementary Fig. 1). Ca²⁺ oscillations are largely species-specific, with different species possessing specific patterns of amplitude, duration and frequency over time[16]. Our data indicate that RGS12 has only a minor role in PN and that loss of RGS12 is compatible with mouse embryonic development. Discussion

Maternal genes play a critical effect in the earliest stages of embryonic development. Although Zar1 was first identified as oocyte-specific maternal-effect gene that functions at the oocyte-to-embryo transition in mice[30], it could not be utilized in human PN-arrest zygotes. Regarding to TUBB8 mutations cause female infertility by oocyte meiotic spindle assembly and maturation, homozygous mutation c.322G > A (p.Glu108Lys) of TUBB8 had been observed in a sporadic patient with phenotype of PN-arrest zygote after IVF by Sanger sequencing strategy[32]. However, nothing is known about the genetic cause of phenotype of human PN-arrest zygotes.

To the best of our knowledge, this is the first report to describe variant in the RGS12 responsible for female infertility characterized by arrest at the PN stage during multiple IVF. The genetic basis for infertility characterized by abnormalities in human oocyte development and early embryogenesis (2- to 4-cell) had been described[2, 3, 33]. Variant in RGS12 extends the genetic causes of infertility. We have proposed that the data presented here provide the basis for developing diagnostic strategy that use the Sanger sequencing for the identification of women with mutations in RGS12.

RGS12 controls Ca²⁺ oscillations, which provides an important spatially restricted Ca²⁺ signal required for complete oocyte activation after fertilization, and triggers the CSF-APC signaling to switched from meiotic to mitotic process. Our single-cell transcriptome sequencing data revealed unique features in translation, RNA processing and cell cycle impairments of failure of complete oocyte activation, and uncovered Ca²⁺ oscillation–CSF–APC signaling pathway that mutant RGS12 exerted its maternal effect on PN-arrest. The genes involved PN-arrest of Ca²⁺ oscillation–CSF–APC signaling pathway were validated in the other data[9]. The partially activated oocyte do not progress further and get arrested again in PN stage had been described as a new metaphase-III arrest[27]. The key genes underling PN-arrest zygote (Fig. 4) improve the understanding of why and how RGS12 mutation causes the phenotype.

Conclusions

In conclusion, we have identified an RGS12 variant as the potential cause of female infertility characterized by arrest at the PN stage during multiple IVF. This gene should be further screened in
individuals with infertility caused by arrest at the PN stage during IVF. These findings expand our knowledge of the genetic basis of human early embryonic arrest and provide the basis for genetic diagnoses of clinically infertile individuals with this phenotype.

**Abbreviations**

APC: anaphase-promoting complex; BUB3: mitotic checkpoint protein; CaMKII: Calcium/calmodulin-dependent protein kinase II; CCNA2: cyclin A2; CDC: cell division cycle; CDH1: cadherin 1; CENPE: centromere protein E; CSF: cytostatic factor; EMI1: F-box protein 5; GMNN: geminin DNA replication inhibitor; ICSI: intracytoplasmic sperm injection; IVF: in vitro fertilization; ORAI1: calcium release-activated calcium modulator 1; PN: pronuclear; RGS12: regulator of G protein signaling 12.

**Declarations**

**Ethical Approval and Consent to participate**

This study was approved by the Ethics Committee of Guangdong Medical University Affiliated Hospital (YS2018010) and written informed consent was obtained from participants.

**Consent for publication**

Not applicable.

**Availability of supporting data**

All data generated through this study are included in this article.

**Competing interests**

The authors declare no competing financial interests.

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**Authors' contributions**

T.M. and G.Z. conceived and designed the study. G.Z. analyzed data and wrote the manuscript. C.Z. managed the mice. S.Z, X.X, J.C, J.W. and S.G. performed the experiments. R.M. and G.Z. interpreted the results.

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Figures

Figure 1

Structure of RGS12 and sites of pathogenic mutations associated with pronuclear (PN)-arrest zygotes. a, Morphology of zygotes on days 1–3 after IVF. Most zygotes from affected individuals remained at the one-cell stage, and maternal and paternal pronuclei were separated. b, Pedigrees of the family affected by infertility due to arrest at PN zygote stage. Squares denote male family members, circles denote female members, and solid symbols represent affected members. Equal signs indicate infertility. MT, mutant type; WT, wild type. c, Conservation of amino acid residues affected by mutations in different species, structure of RGS12, and known domains of the gene product. Exons are red vertical bars, introns are...
dashed lines, and open rectangles at each end are noncoding exons. d, Sanger sequencing confirmation of RGS12 variant (c.C1630T) in the family members.

**Figure 2**

Transcriptome profile of PN arrest zygote by single-cell RNA-sequencing. a, 3-D principal-component analysis (PCA) of the transcriptome of human oocytes, preimplantation embryos and PN-arrest zygotes. Cells of different preimplantation stages form distinct clusters, and PN-arrest zygotes had a specific...
transcriptome character. b, Scatter plots comparing expression of genes with fold change > 2, false
discovery rate (FDR) < 0.05. c, REVIGO scatter plot showing the cluster representatives in a 2-D space
derived by applying multidimensional scaling to a matrix of semantic similarities for the Gene Ontology
(GO) terms (biological processes). d, Metascape enrichment network showing intra-cluster and inter-
cluster similarities of enriched GO terms. Cluster annotations are shown in color code. e, Comparative
analysis of genes in the Ca2+ oscillation–CSF–APC signaling pathway by average RNA-sequencing of
normalized read counts for human PN and PN-arrest zygotes. FDR value, Data are mean ±SD. **, p<0.001;
***, p<0.0001. f, Significantly enriched KEGG pathways in PN-arrest zygotes; length of column indicates
the –log10 P value.
Figure 3

Validation of transcriptome profile in other PN-arrest zygotes. a, Venn diagram shows overlapped differentially expressed genes between previous study by Suo et al[9] (PN arrest I) and our study (PN arrest II) for PN arrest groups each compared with their control group. b, REVIGO scatterplot summarizes the overrepresented GO terms (biological processes) for representative subsets of terms. c, Metascape enrichment network of the intra-cluster and inter-cluster similarities of enriched GO terms. d, Metascape
interactome network formed by all GO terms, confirming the defective RNA processing and translation in both PN-arrest zygote groups. e, Relative expression of particularly interesting DEGs. FDR value, Data are mean ±SD. **, p<0.001; ***, p<0.0001. f, Significantly enriched KEGG pathways shared in PN-arrest zygotes.

Supplementary Files

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