A recurrent ZP1 variant is responsible for oocyte maturation defect with degenerated oocytes in infertile females

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Abstract
A female factor is present in approximately 70% of couple infertility, often due to ovulatory disorders. In oocyte maturation defect (OMD), affected patients have a primary infertility with normal menstrual cycles but produce no oocyte, degenerated (atretic) or abnormal oocytes blocked at different stages of maturation. Four genes have so far been associated with OMD: PATL2, TUBB8, WEE2, and ZP1. In our initial study, 6 out of 23 OMD subjects were shown to carry the same PATL2 homozygous loss of function variant and one patient had a TUBB8 truncating variant. Here, we included four additional OMD patients and reanalyzed all 27 subjects. In addition to the seven patients with a previously identified defect, five carried the same deleterious homozygous ZP1 variant (c.1097G>A; p.Arg366Gln). All the oocytes from ZP1-associated patients appeared shriveled and dark indicating that the abnormal ZP1 protein induced oocyte death and degeneration. Overall ZP1-associated patients had degenerated or absent oocytes contrary to PATL2-associated subjects who had immature oocytes blocked mainly at the germinal vesicle stage. In this cohort of North African OMD patients, whole exome sequencing permitted to diagnose 44% of the patients studied and to identify a new frequent ZP1 variant.

KEYWORDS
infertility, OMD, oocytes, zona pellucida

1 INTRODUCTION

Human infertility is defined as the inability to conceive a child after 1 year of unprotected intercourse and both male and female causes are equally described. Genetic factors are described in several types of female infertilities, including Polycystic Ovary Syndrome (PCOS), Premature Ovarian Failure or oocyte maturation defects (OMD).1 Women with OMD usually have normal menstrual cycles and hormonal levels but upon in vitro fertilization they present variable defects in oocyte maturation leading to absent, immature, atretic or unfertilizable eggs. Within the ovaries, oocytes are arrested at prophase of meiosis I until their recruitment in the pool of growing follicles, leading to the production of a fully-grown Germinal Vesicle (GV) oocyte. This latter is sensitive to hormonal stimulation, leading to meiosis resumption characterized by the GV breakdown (GVBD) and the extrusion of the first polar body, giving rise to fully mature eggs.
arrested in the metaphase stage of meiosis II (MII). Fertilization leads to a second resumption of meiosis and extrusion of the second polar body.\(^2\) In OMD, oocytes can be absent, found to be blocked in GV, MI, and MII stages or associated with a high level of atretic/dead oocyte retrieval. The causes of these defects remained obscure until recently and the recent identification of causal genes made possible by the wide use of high throughput sequencing and in particular whole exome sequencing (WES).

In 2016, heterozygote and homozygote mutations were described in TUBB8 in a Chinese cohort of females suffering of OMD.\(^3\) TUBB8 codes for a primate specific isoform of β tubulin and is necessary for the spindle formation in primate. Most of the oocytes were blocked at the MI stage, and a fraction of oocyte even reaches the MII stages. Finally, a homozygous missense mutation c.700G>C (p.Asp234His) in the oocyte-specific kinase WEE2\(^8\) was also described. Affected probands ovulated MII eggs but they were unable to reach the zygote stage after ICSI, showing a clear MII block.

Here, in order to find new candidate genes involved in OMD, we reanalyzed our previous North African cohort in which we had identified six patients with a homozygous PATL2 variant,\(^5\) enriched with four new patients. Exomic sequencing data was thus analyzed for a total of 27 subjects, 20 of which without a genetic diagnosis. This new analysis led to the identification, in five patients, of the same homozygous missense mutation in ZP1 gene, a gene coding for one of the zona pellucida (ZP) protein and previously associated with OMD.\(^9\) This variant had not been identified in our initial analysis as we had focused on loss of function variants and ZP1 had not yet been described to be associated with OMD.

### 2 | MATERIAL AND METHODS

#### 2.1 | Patients’ description

In our initial study, we had analyzed a total of 23 unrelated OMD subjects.\(^5\) Here, four additional unrelated OMD patients were recruited at the Polyclinique Les Jasmins in Tunis. All had primary infertility and had undergone at least one IVF cycle evidencing only abnormal or absent oocytes. All 27 subjects were of North African origin. All patients have given their informed consent.

#### 2.2 | WES and variants filtering

Genomic DNA was isolated from blood samples. Genetic data were obtained from Novogene sequencing center. Coding regions and intron/exon boundaries were sequenced after enrichment using SureSelect Human All Exon V6—from Agilent.

An alignment-ready GRCh38 reference genome (including ALT, decoy and HLA) was produced using “run-gen-ref hs38DH” from Heng Li’s bwakit package (https://github.com/lh3/bwakit). The exomes were analyzed using a bioinformatics pipeline developed in-house. The pipeline consists of two modules, both distributed under the GNU General Public License v3.0 and available on github.

The first module (https://github.com/ntm/grexome-TIMC-Primary) takes FASTQ files as input and produces a single merged GVCF file, as follows. Adaptors are trimmed and low-quality reads filtered with fastp 0.20.0,\(^10\) reads are aligned with BWA-MEM 0.7.17,\(^11\) duplicates are marked using samblaster 0.1.24,\(^12\) and BAM files are sorted and indexed with samtools 1.9.\(^13\) SNVs and short indels are called from each BAM file using strelka 2.9.10\(^14\) to produce individual GVCF files. These are finally merged with mergeGVCFs_strelka.pl to obtain a single multi-sample GVCF, which combines all exomes available in our laboratory.

The second module (https://github.com/ntm/grexome-TIMC-Secondary) takes each merged GVCF as input and produces annotated analysis-ready TSV files. This is achieved by performing up to 15 streamlined tasks, including the following. Low-quality variant calls (DP < 10, GQ < 20, or less than 15% of reads supporting the ALT allele) are discarded. Variant Effect Predictor v104\(^15\) is used to annotate the variants and predict their impact, allowing to filter low-impact (MODIFIER) variants and/or prioritize high-impact ones (e.g., stop-gain or frameshift variants). Variants with a minor allele frequency greater than 1% in gnomAD v2.0 or 3% in 1000 Genomes Project phase 3 are filtered. Additional information can be found in.\(^16\)

#### 2.3 | Sanger verification of the variant

The ZP1 variant identified by WES was subjected to a Sanger verification using an ABI 3500XL (Applied Biosystems). Analyses were performed using SeqScape software (Applied Biosystems). PCR amplification was achieved using the following primers (ZP1-F-Ex6 CACAGCACACGGAAGCTTTC; ZP1-R-Ex6 AGGACTCCAGCTC TCTCAGG).

### 3 | RESULTS

#### 3.1 | Identification of the mutation

In our initial study of a cohort of 23 OMD subjects, targeted PATL2 sequencing had been carried out on eight patients permitting to detect one patient with a homozygous PATL2 stop-gain variant (c.478C>T p.Arg160Ter). Twenty-two subjects were subsequently analyzed by WES (seven subjects negative for PATL2 and the remaining 15 which had not been analyzed). Five patients were homozygous for the initially identified PATL2 variant and one had a homozygous TUBB8 variant (c.363_366del, p.Lys122ArgfsTer13).\(^5\) Here we recruited and performed WES on an additional four subjects and reanalyzed WES data from all tested individuals.
Overall, out of 28 subjects, WES was performed for 27 and we reanalysis the cumulated variants from all 27 subjects. Here we only focused on homozygous variants. After filtering the heterozygous variants and those classified as benign or of unknown significance, we obtained a total of 49 likely deleterious variants distributed in the 27 analyzed subjects (Table S1). Two of the variants were described before: the PATL2 variant found in five subjects and the TUBB8 variant found in a single individual (Table S1). Forty-six variants concerned a single gene and were found in a single patient. Little information is available for most of these genes. Additional work that falls outside the scope of this manuscript is necessary to confirm or infirm the implication of some of these variants in OMD. Interestingly, the same homozygous missense variant in the ZP1 gene (for zona pellucida glycoprotein 1, also known as HRL163, OOMD, OOMD1, and HEL163) was found in five individuals. The variant (NM_207341.3: c.1097G>A; NP_997224.2:p.Arg366Gln; rs759371225), is positioned in ZP1 exon 6 (Figure 1). Surprisingly, 3 of the 4 newly recruited patients were homozygous for the ZP1 variant (and 2 of the 20 re-analyzed undiagnosed subjects). This gene is localized in chromosome 11q12.2 and contains 12 exons encoding a predicted 638 amino acid protein and was previously described to be linked with OMD. In a control population, the mutation is found only at a heterozygous state at a low frequency of 5.3E-5 (gnomAD), compatible with a recessive transmission. The variant is predicted to be pathogenic/damaging by 20 prediction software and tolerated or uncertain by 5 (https://vardis.vamosi.com/variant/hg19/rs759371225?annotation-mode=germline). Most importantly, the difference in the variant frequency between the patients’ group (10 variants out of 56 alleles) and a control population (GnomAD: 11 out of 251 030 alleles) is most highly significant (p-value: 2E−31; Fisher’s Exact Test for Count Data: Alternative: two.sided). Overall, out of the 27 OMD subjects analyzed, a clinical diagnosis was obtained for 12 (44.4%): 6 subjects (21.4%) carried the same homozygous PATL2 variant, 5, the same homozygous ZP1 variant (17.9%) and one carried a homozygous TUBB8 variant (3.6%).

3.2 | Characteristics of the ZP1 affected individuals and IVF results (Table 1)

We focused on the clinical characteristics observed for the five affected individuals during their IVF cycles. Unfortunately, at the time of the study, the IVF laboratory did not have any cameras linked to their microscopes and we do not have any photos of the abnormal oocytes. The values reported in Table 1 come from the careful examination of the embryos by the embryologists.

P1 had 9 sisters, 2 of whom were also infertile. On day 15 post-hormonal stimulation, 12 follicles (10–19 mm of diameter) were observed. However, on the day of oocyte pick up, only three empty cumulus oocyte complexes (COCs) were retrieved (Table 1).

P2 had a primary infertility since 1998. She had 3 sisters, one of whom was also infertile (5 atretic oocytes recovered during the sister’s IVF cycle). Her menstrual cycles were regular. During the IVF cycle in 2016, 5 atretic oocytes were harvested.

P3 presented a primary infertility since 2012. She had 4 sisters (1 single, 3 fertiles). Her menstrual cycles were normal and no hormonal defects were observed. Two IVF cycles were attempted leading to the retrieval of 12 and 20 atretic oocytes in 2015 and 2016, respectively.

P4 presented a primary infertility since 2006. Her parents were first cousins, she had 2 single sisters and 2 female cousins who were infertile. During the IVF cycle in 2012, 15 days post-hormonal stimulation, the follicles diameters were comprised between 13 and 19 mm of diameter. The day of puncture, 7 atretic oocytes were recovered.

P5 had normal hormonal levels and presented a primary infertility since 2004. In 2012, one IVF cycle was stopped at day 10 of hormonal stimulation due to the poor follicle development. In 2013, 4 atretic oocytes were retrieved.

It is interesting to note P1-3 and P5 had at least three siblings (Table 1). In addition, patient 3 indicated to have 13 brothers and sisters. This clearly suggests that (their most likely heterozygous mothers) did not present any subfertility, confirming a strictly recessive inheritance.

Overall, all five patients with the ZPI variant presented only atretic oocytes (8 on average) and no non-degenerated GV or embryos arrested in the first or second meiotic division (Table 1). One patient (P1) had only empty follicles (n = 3). We note that the number of empty follicle is likely underestimated, as this occurrence might not always be reported. In our previous study, we noted that the patients harboring the PATL2 truncation present mainly oocytes arrested at the GV stage (6 on average) but also some atretic oocytes (2 on average) or oocytes arrested at the M1 stage (1.38 on average).

The remaining undiagnosed patients present few arrests at the GV stage (0.58 on average) but also have a high number of atretic oocytes (7.29 on average). Overall their defect is less severe as they present on average 3.6 mature MI oocytes (Table 1).

3.3 | Relatedness of the ZP1-associated patients

All ZPI-mutated patients carried the same mutation and originated from North Africa or the Middle East. It is therefore likely that they all have a common ancestor and share a common haplotype. To check this hypothesis, we analyzed all the variants present around the ZP1 gene to verify if a common haplotype was present, and if yes, the
extend of this shared haplotype. This was in fact the case and we observed that all five subjects had a minimal common region of 692 kb (Table S2) which corresponds to 0.9 cM assuming a recombination rate of 1.30 cM/Mb.17

Using this value, we then aimed to estimate the period when the five individuals had a common ancestor or when the mutation arose. The probability of observing a single ancestral haplotype of size c (measured in morgans) or larger is equal to (1 − c/)g, where g is the age of the SNV measured in generations.18 Thus the probability that the 2n haplotypes carried by n unrelated individuals is larger than c is (1 − c)2n, which can be approximated by e−ngc. Therefore, the length of the shared ancestral haplotype can be approximated by an exponential distribution of rate 2ng, leading to g ≈ 1/(2ng). Here we have n = 5 individuals and c = 0.009 M, which provides an estimate of g = 11 generations for the ZP1 variant.

However, Labuda et al.19 have shown that dating arising from genetic clock methods is biased downward because these methods do not account for population expansion. To account for an exponential expansion of rate r per generation, the mathematical expression (−1/ln(c)/c − 1)) should be added to the estimates provided by genetic clock methods. If we assume that an upper bound for the expansion rate is given by the expansion r = 0.4, as was calculated for the Ashkenazi Jewish populations,20 the age of the mutation is in the range of 11–20 generations. Given 25 years for the generation time, the SNV would have appeared 275–500 years ago or this is the estimated period when the five subjects’ common ancestor lived.

4 | DISCUSSION

In the present study, we identified a homozygous missense variant in the ZP1 gene (p.Arg366Gln) in 5/27 patients of a North African cohort with OMD. Without strong functional data, it is always difficult to formally conclude that a missense variant is deleterious. Here, we have three strong arguments that permit to conclude without any doubt that the identified variant is responsible for the patient’s infertility: (i) several publications have linked ZP1 defects with OMD and several missense variants were postulated to be deleterious (Table 2); (ii) the identified variant is predicted by 20 out of 25 prediction softwares to be deleterious and (iii) this very rare variant is found in five affected subjects and the difference in the variant frequency between the patients’ group and a control population is most highly significant (p. value = 2E−31). As all patients had the same mutation and all came from the same geographical region, we can expect them to have a common ancestor. We made such an observation for several variants frequently found in patients (1) with macroosperma and the c.144delC variant in AURKC,22 (2) with globoosperma and a whole DPY19L2 deletion,23 (3) with multiple morphological anomalies of the sperm flagella (MMAF) and a deletion of WDR66 exons 20 and 2124 and (4) with ovarian maturation defect carrying the PATL2 p.Arg160Ter variant.5 Here again we clearly observed that all five patients carrying the ZP1 variant had a common haplotype suggesting that they had a common ancestor living 275–500 years ago.

The ZP1 protein is a component of the ZP which is a thick, elastic extracellular coat surrounding all mammalian oocytes, ovulated eggs and pre-implantation embryos.9 The ZP is secreted by growing oocytes and plays essential roles during mammalian reproduction in mechanisms such as acrosomal reaction, polyspermy prevention and embryo protection.9 During oocyte maturation, the ZP is essential for the connection between the oocyte and the cumulus cells.25 The ZP is composed of several glycoproteins: 3 and 4 ZP proteins are described in mouse and Humans, respectively.26 The human ZP proteins ZP1, ZP2, and ZP3 share 33%, 57%, and 67% identity with their respective mouse proteins.27

Numerous ZP mutations have been identified in infertile women with diverse types of infertilities such as PCOS (ZP4,28), endometriosis (ZP4,29), unexplained IVF attempts failures (ZP130–32; ZP231,33,34)}
| Clinic | Location | Sequence variation | Amino acid change | Variant type | Zygosity | Oocytes phenotype | Ref. |
|--------|----------|--------------------|------------------|--------------|----------|-------------------|------|
| IVF failure | Ex2 | c.247T>C | p.Trp83Arg | Missense | Het | Degenerated | [31] |
| IVF failure | Ex3-ZP-N1 | c.326G>A | p.Arg109His | Missense | Het | No ZP | [32] |
| IVF failure | Ex6-ZPD | c.1120A>G | p.Asp367Gly | Missense | Comp Het | No ZP | [46] |
| IVF failure | Ex7-ZPD | c.1169_1176del | p.Ile390Thrfs*16 | Frameshift | Hom | No ZP | [22,23,34,35] |
| IVF failure | Ex7-ZPD | c.1215delG | p.Leu406Glyfs*24 | Frameshift | Comp Het | No ZP | [46] |
| EFS | Ex1-SP | c.1413G>A | p.Trp471* | Stop gain | Het | Cracked | [31] |
| EFS | Ex1 | c.170-174del | p.Gly57Aspfs*9 | Stop gain | Comp Het | Smaller, degenerated | [35] |
| EFS | Ex1 | c.181C>T | p.Arg61Cys | Missense | Comp Het | Degenerated | [36] |
| EFS | Ex2-TD | c.239G>A | p.Cys807Tyr | Missense | Comp Het | No oocytes, no/abnormal ZP | [38] |
| EFS | Ex2-TD | c.241T>C | p.Tyr81His | Missense | Comp Het | No oocytes, no/abnormal ZP | [38] |
| EFS | Ex3 | c.507del | p.His170fs | Frameshift & stop gain | Comp Het | No oocytes, no/abnormal ZP | [38] |
| EFS | Ex4-TD | c.769C>T | p.Gln257* | Stop gain | Hom | Degenerated | [37] |
| EFS | Intron 5 | c.1014+1G>A | / | Splicing | Hom | No oocytes | [33] |
| EFS | Intron 6 | c.1112+1G>T | p.Val339Aspfs*11 | Splicing | Comp Het | No oocytes | [49] |
| EFS | Ex7-ZPD | c.1129_1130del | p.Val377Leufs*5 | Frameshift & stop gain | Hom | No oocytes | [33] |
| EFS | Ex7-ZPD | c.1228C>T | p.Arg410Trp | Missense | Hom | Degenerated | [29] |
| EFS | Intron 8 | c.1430+1G>T | p.Cys478* | Splicing | Comp Het | No ZP | [29] |
| EFS | Ex9-ZPD | c.1510C>T | p.Arg504* | Stop gain | Hom | No oocytes | [33] |
| EFS | Intron 9 | c.1573-2A>G | / | Splicing | Comp Het | No oocytes | [33] |
| EFS | Ex11-CFCS | c.1663C>T | p.Arg555* | Stop gain | Comp Het | No oocytes | [33] |
| EFS | Ex11 | c.1708G>A | p.Val570Met | Missense | Hom | No oocytes or no ZP | [29] |
| EFS | Intron 11 | c.1775-8T>C | p.Asp592Glyfs*29 | Splicing | Comp Het | No ZP | [29] |
| OMD | Intron 11 | c.1775-3C>A | / | Splicing | Hom | No ZP, immature (uncomplete penetrance) | [21] |
ZP3\textsuperscript{29,34}, OMD (ZP1\textsuperscript{21}) and mainly with the Empty Follicle Syndrome (EFS) pathology (ZP1\textsuperscript{29,33,35–39}, ZP2\textsuperscript{29,38} and ZP3\textsuperscript{29,40,41}). Interestingly, the ZP1 gene is the most mutated ZP gene and an overview of the 27 ZP1 mutations according to the patients’ clinical manifestations and to their position on the gene sequence and on the protein is shown in Table 2 and Figure 2. This high number of mutations found for the ZP1 gene is surprising as it is inversely correlated to its importance in the mouse. Indeed, the corresponding Zp1 KO mice are subfertile whereas the deletion of Zp2 or Zp3, which are less frequently found mutated in Humans, induced a female sterility in mice.\textsuperscript{25,42} Most mutations (20/27) were associated with an EFS diagnosis. EFS has also been related to mutations in the LH/choriogonadotropin receptor (LHCGR) gene\textsuperscript{43,44} and is characterized by the inability of the operator to find any oocyte after follicular aspiration after hormonal stimulation with apparently normal follicular growth and steroidogenesis (for review see,\textsuperscript{45}) Some rare variants were also reported to be associated with zona-free oocytes\textsuperscript{29,30,32,38,46} and OMD.\textsuperscript{21}

We have analyzed the position of the described ZP1 mutations in the protein sequence (Figure 2). ZP1 is a glycoprotein of 638 amino acids and is composed of several domains with a N-terminal signal peptide (SP), a cysteine rich P-type trefoil domain (TD), three ZP domains (ZP-N1, ZP-N, and ZP-C), a consensus furin cleavage site (CFCS) and a transmembrane-like domain (TM). The mutations are positioned all along the protein with 12 located in the ZP domains. It seems impossible to give a phenotypic consensus in function of the impacted domain as independently of their localization, these mutations result either in lack of oocytes, degenerated oocytes, oocytes without ZP.

The identified mutation c.1097G>A:p.Arg366Gln affects the ZP-N domain of the protein.\textsuperscript{9} Another mutation, affecting the adjacent amino acid (p.D367G), was detected in a compound heterozygous patient carrying a truncating variant as the second mutation (c.1215delG:p.L405fs). This patient was described to present zona-free oocytes.\textsuperscript{46} The authors showed that the mutation is responsible for the retention of the protein in the cytoplasm of transfected HeLa cells, hypothesizing that this intracellular sequestration might be responsible for the observed fertilization failure. Our mutation leads to an amino acid change p.Arg366Gln and affects a residue within the \(\text{fg}\) loop of the ZP-N domain corresponding to the R142 of the chicken Zp3 according to the described crystal structure, whose mutation is known to abolish its secretion.\textsuperscript{47} Thus, one hypothesis is that our mutation could have same effect, preventing ZP1 secretion and inducing the production of oocytes with a defective ZP. This does not seem to be the case as the atretic oocytes observed did seem to have some kind of left over ZP. Most unfortunately, at the time of the study, the collaborating IVF laboratory did not have any cameras linked to their microscopes and we do not have any photos of the abnormal oocytes.

The mutation may also result in the production of an abnormal protein conformation, rendering the assembly of the ZP impossible. Indeed, the mutation is present in the ZP-D domain of the protein and it has been described that mutations in such domains are responsible for the perturbation of fibrils interconnection. This is, for example, the case for mutation found in the \(\text{a-tectorin}\) which results in defective tectoral membrane assembly.\textsuperscript{48} Thus, altered ZP1 protein might result in the defective assembly of the ZP2 and ZP3 heterodimers filaments and consequently the absence of correct ZP.
Here, some highly abnormal, dark and degenerated oocytes could be collected for most patients with the identified ZP1 variant, except for one who had no oocyte. We postulate that following the abnormal formation of the ZP, the exchanges necessary for oocyte maturation are altered leading to an early arrest of oocyte maturation. The arrested oocytes might then quickly degenerate and disaggregate, leading to EFS or undergo a necrosis-like mechanism and appear as atretic. It is also possible that in some instances, the highly degenerated oocyte were not recognized as oocytes. Overall, the presence of atretic/degenerated oocytes and/or empty follicles can be considered to be the hallmark of ZP1 mutated patients.

We studied the largest cohort patients of North African origin with OMD. The WES analyses allow us to clearly associate: (i) the ZP1 gene with maturation blockage at the GV state, and (ii) the ZP1 gene to the production of atretic/degenerated oocytes or to the absence of oocyte, confirming the previous published data (Table 2). Until now, the ZP1 gene was mainly correlated with EFS. Here, we confirm a relative heterogeneity in the manifestation of the ZP1 genetic defects and believe that all the described anomalies might fall under the broad term of OMD. We suggest that OMD is a heterogeneous condition with (so far) four main genes giving distinct anomalies: TUBB8 mainly responsible for meiotic arrest, PATL2 mainly associated to GV arrest, WEE2 linked to a Mil blockage and ZP1 coupled to the production of empty follicles and/or atretic oocytes. As numerous patients remain undiagnosed (>50%), several other OMD genes likely remain to be discovered.

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CONFLICT OF INTEREST
The authors declared no conflict of interest.

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DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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REFERENCES
1. Yatsenko SA, Rajkovic A. Genetics of human female infertility. Biol Reprod. 2019;101:549-566.
2. Coticchio G, Dal Canto M, Mignini Renzini M, et al. Oocyte maturation: gamete-somatic cells interactions, meiotic resumption, cytoskeletal dynamics and cytoplasmic reorganization. Hum Reprod Update. 2015;21:427-454.
3. Feng R, Sang Q, Kuang Y, et al. Mutations in TUBB8 and human oocyte meiotic arrest. N Engl J Med. 2016;374:223-232.
4. Chen B, Zhang Z, Sun X, et al. Biallelic mutations in PATL2 cause female infertility characterized by oocyte maturation arrest. Am J Hum Genet. 2017;101:609-615.
5. Christou-Kent M, Kherraft Z, Amiri-Yekta A, et al. PATL2 is a key actor of oocyte maturation whose invalidation causes infertility in women and mice. EMBO Mol Med. 2018;10:e8515.
6. Huang L, Tong X, Wang F, et al. Novel mutations in PATL2 cause female infertility with oocyte germinal vesicle arrest. Hum Reprod. 2018;33:1183-1190.
7. Christou-Kent M, Dhellemmes M, Lambert E, Ray PF, Arnoult C. Diversity of RNA-binding proteins modulating post-transcriptional regulation of protein expression in the maturing mammalian oocyte. Cell. 2020;9:662.
8. Sang Q, Li B, Kuang Y, et al. Homozygous mutations in WEE2 cause fertilization failure and female infertility. Am J Hum Genet. 2018;102:649-657.
9. Wassarman PM, Litscher ES. Influence of the zona pellucida of the mouse egg on folliculogenesis and fertility. Int J Dev Biol. 2012;56:833-839.
10. Chen S, Zhou Y, Chen Y, Gu J. Fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics. 2018;34:1884-1890.
11. Li H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv 2013, g-bio:1303.3997.
12. Faust GG, Hall IM. SAMBLASTER: fast duplicate marking and structural variant read extraction. Bioinformatics. 2014;30:2503-2505.
13. Li H, Handsaker B, Wysoker A, et al. 1000 genome project data processing subgroup: the sequence alignment/map format and SAMTools. Bioinformatics. 2009;25:2078-2079.
14. Kim S, Schreiffer K, Halpern AL, et al. Strelka2: fast and accurate calling of germline and somatic variants. Nat Methods. 2018;15:591-594.
15. McLaren W, Gil L, Hunt SE, et al. The Ensembl variant effect predictor. Genome Biol. 2016;17:122.
16. Arafa K, Lopez F, Cazin C, et al. Defect in the nuclear pore membrane glycoprotein 210-like gene is associated with extreme uncondensed sperm nuclear chromatin and male infertility: a case report. Hum Reprod. 2021;36:693-701.
17. Jensen-Seaman MI, Furey TS, Payseur BA, et al. Comparative recombination rates in the rat, mouse, and human genomes. Genome Res. 2004;14:528-538.
18. Slatkin M, Rannala B. Estimating allele age. Ann Rev Genomics Hum Genet. 2000;1:225-249.
19. Labuda M, Labuda D, Korab-Laskowska M, et al. Linkage disequilibrium analysis in young populations: pseudo-vitamin D-deficiency rickets and the founder effect in French Canadians. Hum Genet. 1996;59:633-643.
20. Risch N, de Leon D, Ozelius L, et al. Genetic analysis of idiopathic torsion dystonia in Ashkenazi Jews and their recent descent from a small founder population. Nat Genet. 1995;9:152-159.
21. Okutman O, Demirel C, Tülek F, et al. Homozygous splice site mutation in ZP1 causes familial oocyte maturation defect. Genes. 2020;11(4):382.
22. Dieterich K, Soto Rifo R, Faure AK, et al. Homozygous mutation of AURKC yields large-headed polyploid spermatozoa and causes male infertility. Nat Genet. 2007;39:661-665.
23. Couton C, Abada F, Karaouzene T, et al. Fine characterisation of a recombination hotspot at the DPY19L2 locus and resolution of the paradoxical excess of duplications over deletions in the general population. PLoS Genet. 2013;9:e1003363.
24. Kherraf Z-E, Amiri-Yekta A, Dacheux D, et al. A homozygous ancestral SVA-insertion-mediated deletion in WDR66 induces multiple
morphological abnormalities of the sperm flagellum and male infertility. Am J Hum Genet. 2018;103:400-412.

25. Rankin T, Dean J. The molecular genetics of the zona pellucida: mouse mutations and infertility. Mol Hum Reprod. 1996;2:889-894.

26. Wassarman PM. Zona pellucida glycoproteins. J Biol Chem. 2008;283:24285-24289.

27. Hughes DC, Barratt CL. Identification of the true human orthologue of the mouse Zp1 gene: evidence for greater complexity in the mammalian zona pellucida? Biochim Biophys Acta. 1999;1447:303-306.

28. Wei X, Li Y, Liu Q, et al. Mutations in ZP4 are associated with abnormal zona pellucida and female infertility. J Clin Pathol. 2022;75(3):201-204.

29. Zou Y, Zhou J-Y, Guo J-B, et al. Mutation analysis of ZP1, ZP2, ZP3 and ZP4 genes in 152 Han Chinese samples with ovarian endometriosis. Mutat Res. 2019;813:46-50.

30. Sun L, Fang X, Chen Z, et al. Compound heterozygous ZP1 mutations identified in patients with oocyte anomalies. Fertil Steril. 2017;107:1364-1369.

31. Sun L, Fang X, Chen Z, et al. Compound heterozygous ZP1 mutations cause empty follicle syndrome in infertile sisters. Hum Mutat. 2019;40:2001-2006.

32. Cao Q, Zhao C, Zhang X, et al. Heterozygous mutations in ZP1 and ZP3 cause formation disorder of ZP and female infertility in human. J Cell Mol Med. 2020;24:8557-8566.

33. Chu K, He Y, Wang L, et al. Novel pathogenic variants identified in ZP1 in an infertile patient and a successful live birth following ICSI treatment. J Assist Reprod Genet. 2020;37:2853-2860.

34. Liu M, Shen Y, Zhang X, Wang X, Li D, Wang Y. Novel biallelic loss-of-function variants in ZP1 identified in an infertile female with empty follicle syndrome. J Assist Reprod Genet. 2020;37:2151-2157.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher’s website.

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