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Corinne Loeuillet, Magali Dhellemmes, Caroline Cazin, Zine-eddine Kherraf, Selima Fourati Ben Mustapha, et al.. A recurrent ZP1 variant is responsible for oocyte maturation defect with degenerated oocytes in infertile females. *Clinical Genetics*, 2022, 102 (1), pp.22-29. 10.1111/cge.14144 . hal-03720194

**HAL Id: hal-03720194**





**<https://cnrs.hal.science/hal-03720194v1>**

Submitted on 11 Jul 2022

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

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## Funding information

Agence Nationale de la Recherche, Grant/Award Numbers: MASFLAGELLA (ANR-19-CE17-0014), FLAGEL-OME (ANR-19-CE17-0014); Inserm-Bettencourt Foundation; INSERM (Institut National de la Santé et de la Recherche Médicale)

## 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).<sup>1</sup> 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,

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arrested in the metaphase stage of meiosis II (MII). Fertilization leads to a second resumption of meiosis and extrusion of the second polar body.<sup>2</sup> 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.<sup>3</sup> *TUBB8* codes for a primate specific isoform of  $\beta$  tubulin and is necessary for the spindle formation in primate. Most of the oocytes were blocked at the MI stage, they could be fertilized but failed to develop beyond to the 4- or 8-cell stage. In 2017 and 2018, truncating homozygote mutations in *PATL2* were described as responsible for a GV block.<sup>4-6</sup> *PATL2* codes for a RNA binding protein and controls protein translation necessary for oocyte growth and GVBD.<sup>7</sup> In the mouse model, the oocytes were most often 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*<sup>8</sup> 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,<sup>5</sup> 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.<sup>9</sup> 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.<sup>5</sup> 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/bwa>). 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,<sup>10</sup> reads are aligned with BWA-MEM 0.7.17,<sup>11</sup> duplicates are marked using *samblaster* 0.1.24,<sup>12</sup> and BAM files are sorted and indexed with *samtools* 1.9.<sup>13</sup> SNVs and short indels are called from each BAM file using *strelka* 2.9.10<sup>14</sup> 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<sup>15</sup> 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.<sup>16</sup>

### 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).<sup>5</sup> Here we recruited and performed WES on an additional four subjects and reanalyzed WES data from all tested individuals.

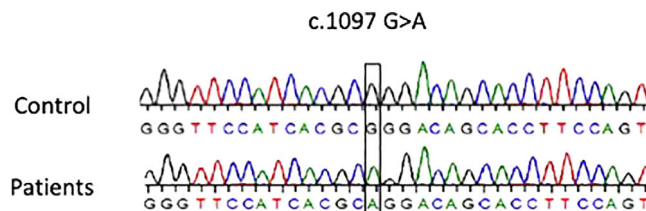
Overall, out of 28 subjects, WES was performed for 27 and we reanalysis the cumulated variants from all 27 subjects. Here we only focused on homozygous variants. After filtering the heterozygous variants and those classified as benign or of unknown significance, we obtained a total of 49 likely deleterious 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.<sup>9</sup> 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://varsome.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 (6 atretic oocytes recovered during the sister's IVF cycle). Her menstrual cycles were regulars. During the IVF cycle in 2016, 5 atretic oocytes were harvested.



**FIGURE 1** *ZP1* mutations identified in the OMD patients. Electropherogram of Sanger sequencing for the *ZP1*-mutated patients compared to the reference sequence obtained from control individuals. OMD, oocyte maturation defect [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

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 *ZP1* 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 MII oocytes (Table 1).

### 3.3 | Relatedness of the *ZP1*-associated patients

All *ZP1*-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

**TABLE 1** Clinical characteristics of the affected patients

Patients	Origin, and number of siblings (n)	Age	Empty follicle	GV	MI	MII	Atretic	Total
Patients with <i>ZP1</i> mutation (n = 5)								
P1	Algeria (4)	33	3	0	0	0	0	0
P2	Algeria (3)	39	0	0	0	0	5	5
P3	Tunisia (13)	31	0	0	0	0	12	12
		32	0	0	0	0	20	20
P4	Saudi Arabia (Not avail.)	38	0	0	0	0	4	4
P5	Tunisia (4)	26	0	0	0	0	7	7
Average		33.16	0.5	0	0	0	8	8
Patient with <i>TUBB8</i> variant		33	0	0	5	2	2	9
Patients with <i>PATL2</i> mutation (n = 6)								
Average		34	0	6	1.38	0	3.89	10
Patients without <i>PATL2</i> mutation (n = 16)								
Average		30	0	0.52	3.47	3.64	7.29	8

Note: The patients P1, P2, and P3 correspond to the newly recruited OMD women, P4 and P5 correspond to patients P14 and P15 patients already described in our previous study.<sup>5</sup>

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.<sup>17</sup>

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.<sup>18</sup> Thus the probability that the  $2n$  haplotypes carried by  $n$  unrelated individuals is larger than  $c$  is  $(1 - c)^{2ng}$ , which can be approximated by  $e^{-2ngc}$ . Therefore, the length of the shared ancestral haplotype can be approximated by an exponential distribution of rate  $2ng$ , leading to  $g \approx 1/(2nc)$ . 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.<sup>19</sup> 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  $(-1/r)\ln(c \cdot e^r / (e^r - 1))$  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,<sup>20</sup> 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

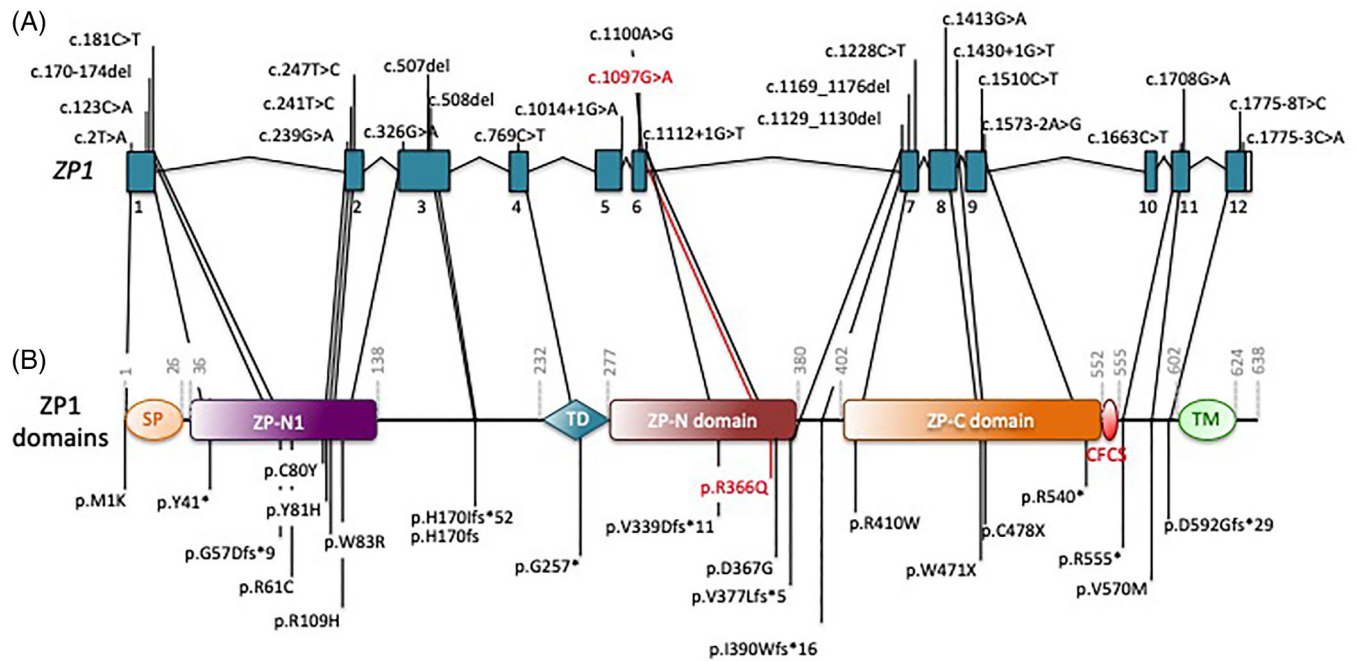
formally conclude that a missense variant is deleterious. Here, we have three strong arguments that permit to conclude without any doubt that the identified variant is responsible for the patient's infertility: (i) several publications have linked *ZP1* defects with OMD and several missense variants were postulated to be deleterious<sup>21</sup> (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 macrospermia and the c.144delC variant in *AURKC*,<sup>22</sup> (2) with globozoospermia and a whole *DPY19L2* deletion,<sup>23</sup> (3) with multiple morphological anomalies of the sperm flagella (MMAF) and a deletion of *WDR66* exons 20 and 21<sup>24</sup> and (4) with ovarian maturation defect carrying the *PATL2* p.Arg160Ter variant.<sup>5</sup> 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.<sup>9</sup> 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.<sup>9</sup> During oocyte maturation, the ZP is essential for the connection between the oocyte and the cumulus cells.<sup>25</sup> The ZP is composed of several glycoproteins: 3 and 4 ZP proteins are described in mouse and Humans, respectively.<sup>26</sup> The human ZP proteins *ZP1*, *ZP2*, and *ZP3* share 33%, 57%, and 67% identity with their respective mouse proteins.<sup>27</sup>

Numerous ZP mutations have been identified in infertile women with diverse types of infertilities such as PCOS (*ZP4*,<sup>28</sup>), endometriosis (*ZP4*,<sup>29</sup>), unexplained IVF attempts failures (*ZP1*<sup>30–32</sup>; *ZP2*<sup>31,33</sup>;

TABLE 2 ZP1 mutations described in the literature and the associated oocytes phenotypes

Clinic	Location	Sequence variation	Amino acid change	Variant type	Zygosity	Oocytes phenotype	Ref.	
1	IVF failure	Ex2	c.247T>C	p.Trp83Arg	Missense	Het	Degenerated	[31]
2	IVF failure	Ex3-ZP-N1	c.326G>A	p.Arg109His	Missense	Het	No ZP	[32]
3	IVF failure	Ex6-ZPD	c.1120A>G	p.Asp367Gly	Missense	Comp Het <sup>5</sup>	No ZP	[46]
4	IVF failure	Ex7-ZPD	c.1169_1176del	p.Ile390Thrfs*16	Frameshift	Hom	No ZP	[22,23,34,35]
5	IFS				Comp Het <sup>9</sup>	Smaller degenerated		
6	IFS				Comp Het <sup>10</sup>			
5	IVF failure	Ex7-ZPD	c.1215delG	p.Leu406Glyfs*24	Frameshift	Comp Het <sup>3</sup>	No ZP	[46]
6	IVF failure	Ex8-ZPD	c.1413G>A	p.Trp471*	Stop gain	Het	Cracked	[31]
7	EFS	Ex1-SP	c.2T>A	p.Met1Lys	Missense	Comp Het <sup>18</sup>	No oocytes	[49]
8	EFS	Ex1	c.123C>A	p.Tyr41*	Stop gain	Comp Het <sup>24</sup>	No oocytes	[33]
9	EFS	Ex1	c.170-174del	p.Gly57Aspfs*9	Stop gain	Comp Het <sup>4</sup>	Smaller, degenerated	[35]
10	EFS	Ex1	c.181C>T	p.Arg61Cys	Missense	Comp Het <sup>4</sup>	Degenerated	[36]
11	EFS	Ex2-TD	c.239G>A	p.Cys80Tyr	Missense	Comp Het <sup>12,13</sup>	No oocytes, no/abnormal ZP	[38]
12	EFS	Ex2-TD	c.241T>C	p.Tyr81His	Missense	Comp Het <sup>11,13</sup>	No oocytes, no/abnormal ZP	[38]
13	EFS	Ex3	c.507del	p.His170fs	Frameshift & stop gain	Comp Het <sup>11,12</sup>	No oocytes, no/abnormal ZP	[38]
14					Hom	Hom	No oocytes	[29]
15	EFS	Ex3	c.508del	p.His170Ilefs*52	Frameshift & stop gain	Comp Het <sup>23</sup>	No oocytes	[33]
16	EFS	Ex4-TD	c.769C>T	p.Gln257*	Stop gain	Hom	Degenerated	[37]
17	EFS	Intron 5	c.1014+1G>A	/	Splicing	Hom	No oocytes	[33]
18	EFS	Intron 6	c.1112+1G>T	p.Val339Aspfs*11	Splicing	Comp Het <sup>7</sup>	No oocytes	[49]
19	EFS	Ex7-ZPD	c.1129_1130del	p.Val377Leufs*5	Frameshift & stop gain	Hom	No oocytes	[33]
20	EFS	Ex7-ZPD	c.1228C>T	p.Arg410Trp	Missense	Hom	Degenerated	[29]
21	EFS	Intron 8	c.1430+1G>T	p.Cys478*	Splicing	Comp Het	No ZP	[29]
22	EFS	Ex9-ZPD	c.1510C>T	p.Arg504*	Stop gain	Hom	No oocytes	[33]
23	EFS	Intron 9	c.1573-2A>G	/	Splicing	Comp Het <sup>15</sup>	No oocytes	[33]
24	EFS	Ex11-CFCS	c.1663C>T	p.Arg555*	Stop gain	Comp Het <sup>8</sup>	No oocytes	[33]
25	EFS	Ex11	c.1708G>A	p.Val570Met	Missense	Hom	No oocytes or no ZP	[29]
26	EFS	Intron 11	c.1775-8T>C	p.Asp592Glyfs*29	Splicing	Comp Het	No ZP	[29]
27	OMD	Intron 11	c.1775-3C>A	/	Splicing	Hom	No ZP, immature (uncomplete penetrance)	[21]



**FIGURE 2** Overview of the ZP1 mutations reported for infertile women. (A) Schematic representation of ZP1 gene and (B) domains architecture of wild-type ZP1 with their boundaries (in gray). The mutations (A) and the corresponding amino acid consequence (B) identified so far are indicated in black (see details in Table 2). The c.1097G>A mutation and the p.R366Q amino acid change identified in our cohort are written in red. CFCS, consensus furin cleavage site; SP, signal peptide; TD, trefoil domain; TM, transmembrane domain. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

ZP3<sup>32,34</sup>), OMD (ZP1<sup>21</sup>) and mainly with the Empty Follicle Syndrome (EFS) pathology (ZP1<sup>29,33,35–39</sup>; ZP2<sup>29,38</sup> and ZP3<sup>29,40,41</sup>). 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.<sup>25,42</sup> Most mutations (20/27) were associated with an EFS diagnosis. EFS has also been related to mutations in the LH/choriogonadotropin receptor (*LHCGR*) gene<sup>43,44</sup> 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,<sup>45</sup>). Some rare variants were also reported to be associated with zona free oocytes<sup>29,30,32,38,46</sup> and OMD.<sup>21</sup>

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.<sup>9</sup> 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.<sup>46</sup> 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 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.<sup>47</sup> 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  $\alpha$ -tectorin which results in defective tectoral membrane assembly.<sup>48</sup> 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 *PATL2* 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 MII 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.

## ACKNOWLEDGEMENTS

This work was funded by the French National Research Agency (ANR), projects MASFLAGELLA (ANR-19-CE17-0014), FLAGEL-OME (ANR-19-CE17-0014) and the INSERM (Institut National de la Santé et de la Recherche Médicale) and Bettencourt Foundation.

## CONFLICT OF INTEREST

The authors declared no conflict of interest.

## PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/cge.14144>.

## 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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**How to cite this article:** Loeuillet C, Dhellemmes M, Cazin C, et al. A recurrent ZP1 variant is responsible for oocyte maturation defect with degenerated oocytes in infertile females. *Clinical Genetics.* 2022;102(1):22-29. doi:[10.1111/cge.14144](https://doi.org/10.1111/cge.14144)