Asian Journal of Andrology (2022) 24, 243–247
www.asiaandro.com; www.ajandrology.com

Open Access

INVITED ORIGINAL ARTICLE

Male Infertility

From azoospermia to macrozoospermia, a phenotypic continuum due to mutations in the *ZMYND15* gene

Zine-Eddine Kherraf[1,2], Caroline Cazin[1,2], Florence Lestrade[3], Jana Muronova[1], Charles Coutton[1,4], Christophe Arnoult[1], Nicolas Thierry-Mieg[5], Pierre F Ray[1,2]

Thanks to tremendous advances in sequencing technologies and in particular to whole exome sequencing (WES), many genes have now been linked to severe sperm defects. A precise genetic diagnosis is obtained for a minority of patients and only for the most severe defects like azoospermia or macrozoospermia which is very often due to defects in the aurora kinase C (*AURKC*) gene. Here, we studied a subject with a severe oligozoospermia and a phenotypic diagnosis of macrozoospermia. *AURKC* analysis did not reveal any deleterious variant. WES was then initiated which permitted to identify a homozygous loss of function variant in the zinc finger MYND-type containing 15 (*ZMYND15*) gene. ZMYND15 has been described to serve as a switch for haploid gene expression, and mice devoid of ZMYND15 were shown to be sterile due to nonobstructive azoospermia (NOA). In man, *ZMYND15* has been associated with NOA and severe oligozoospermia. We confirm here that the presence of a bi-allelic *ZMYND15* variant induces a severe oligozoospermia. In addition, we show that severe oligozoospermia can be associated macrozoospermia, and that a phenotypic misdiagnosis is possible, potentially delaying the genetic diagnosis. In conclusion, genetic defects in *ZMYND15* can induce complete NOA or severe oligozoospermia associated with a very severe teratozoospermia. In our experience, severe oligozoospermia is often associated with severe teratozoospermia and can sometimes be misinterpreted as macrozoospermia or globozoospermia. In these instances, specific *AURKC* or dpy-19 like 2 (*DPY19L2*) diagnosis is usually negative and we recommend the direct use of a pan-genomic techniques such as WES.

Asian Journal of Andrology (2022) **24**, 243–247; doi: 10.4103/aja202194; published online: 11 January 2022

Keywords: azoospermia; gene defect; macrozoospermia; male infertility

INTRODUCTION

For many years, the only genetic tests carried out to investigate the genetic causes of male infertility were, for patients with nonobstructive azoospermia (NOA), the realization of a karyotype and the search for microdeletions of the azoospermia factor (AZF) regions in the Y chromosome, and for subject with obstructive azoospermia, the search for mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. Then, genetic defects were identified in critical genes responsible for specific and severe forms of teratozoospemia like aurora kinase C (*AURKC*) in macrozoospermia, dpy-19 like 2 (*DPY19L2*) in globozoospermia or Sad1 and UNC84 domain containing 5 (*SUN5*) in acephalic spermatozoa syndrome. Macrozoospermia, also known as large-headed spermatozoa is a rare cause of male infertility characterized by the presence of a majority of large-headed multi-flagellar spermatozoa in the ejaculate.[1,2] Since its first description in 1977,[1] numerous studies were conducted to better describe its pathophysiology. In 1996, Yurov *et al*.[3] studied an infertile man affected by macrozoospermia using fluorescent *in situ* hybridization (FISH) and observed a high level of large headed spermatozoa presenting a diploid content in contrast to normal-sized sperm cells that had an apparent haploid content. Since this publication, other teams performed similar works and confirmed that large-headed spermatozoa had an abnormal chromosomal content and were aneuploid.[4–8] In 2007, Dieterich *et al*.[6] investigated by genome-wide microsatellite mapping a cohort of 14 North-African patients with macrozoospermia and identified a homozygous deletion of a single base pair in the *AURKC* gene (c.144delC; p.Leu49TrpfsTer23) in all studied subjects. Further studies from the same group demonstrated that all spermatozoa from patients with an *AURKC* gene defects were tetraploid, indicating that both meiotic divisions I and II were affected by incomplete segregation of homologous chromosomes during meiosis I and sister chromatids during meiosis II and associated with failure of nuclear cleavage and cytokinesis.[9] *AURKC* is by far the most frequent gene found to be associated with macrozoospermia, as 72 out of 87 men (83%) with macrozoospermia were found to carry bi-allelic AURKC variants,[10] and no other gene has so far been clearly associated with this phenotype in human. *AURCK* genetic diagnosis is one of the rare genetic diagnoses for male infertility with a clear-cut clinical relevance as it was shown

[1]Univ. Grenoble Alpes, INSERM U1209, CNRS UMR 5309, Institute for Advanced Biosciences, Team Genetics Epigenetics and Therapies of Infertility, Grenoble 38000, France; [2]CHU Grenoble Alpes, UM GI-DPI, Grenoble 38000, France; [3]CHR Metz Thionville Hospital, 1 allée Château, METZ 57000, France; [4]CHU Grenoble Alpes, UM of Chromosomic Genetics, Grenoble 38000, France; [5]Univ. Grenoble Alpes, CNRS, TIMC-IMAG, Grenoble F-38000, France.
Correspondence: Dr. PF Ray (pray@chu-grenoble.fr)
Received: 26 July 2021; Accepted: 18 October 2021

that all spermatozoa are tetraploid and no pregnancy has ever been reported following intracytoplasmic sperm injection (ICSI) for men with *AURKC* bi-allelic loss of function variant. Following a positive *AURKC* diagnosis, patients should thus be orientated towards sperm donation or adoption. For subjects with a pure phenotype of macrozoospermia, with a fair sperm concentration (>5 × 10^6 ml^{-1}), and with 100% abnormal spermatozoa with much enlarged heads and a high frequency of multiple flagella (often 4 flagella), we noticed that AURKC diagnosis efficiency was very high. On the contrary, diagnosis efficiency dropped for patients with severe (<1 × 10^6 ml^{-1}) oligozoospermia (SO) and mixed morphological anomalies.[10]

Here, the studied patient had some large-headed spermatozoa and was addressed to Grenoble molecular genetic laboratory for *AURKC* diagnosis. However, he did not present a pure phenotype of macrozoospermia as he also showed a severe oligozoospermia, with mixed morphological anomalies and a very high rate of necrospermia. Not surprisingly, *AURKC* diagnosis was negative and we pursued the genetic analysis by realizing whole exome sequencing (WES).

PARTICIPANT AND METHODS

Subject and initial investigations

A single subject of Turkish origin was addressed to Grenoble Hospital, France from the Metz Infertility Center, France, for the genetic investigation of macrozoospermia. Semen samples were obtained by masturbation after 2–7 days of sexual abstinence. Semen samples were incubated for liquefaction at 37°C for 30 min; ejaculate volume and pH together with sperm concentration, morphology and motility were evaluated according to the World Health Organization (WHO) guidelines.[11] Sperm vitality was assessed by eosin staining. Informed consent was obtained according to local protocols and the principles of the Declaration of Helsinki. The study was approved by Grenoble Ethics Committee, and samples were then stored in the Fertithèque collection declared to the French Ministry of Health (DC-2015-2580) and the French Data Protection Authority (DR-2016-392).

The whole *AURKC* gene was sequenced as described previously[6,10] but no deleterious variants were identified. Whole exome sequence was subsequently realized.

WES and variants filtering

Genomic DNA was isolated from blood samples and sequenced by Novogene (Cambridge, UK). Coding regions and intron/exon boundaries were sequenced after enrichment using SureSelect Human All Exon V6 (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 version 3.0 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,[12] reads are aligned with BWA-MEM 0.7.17,[13] duplicates are marked using samblaster 0.1.24,[14] and BAM files are sorted and indexed with samtools 1.9.[15] Single nucleotide variations (SNVs) and short indels are called from each BAM file using strelka 2.9.10[16] 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 <15% of reads supporting the ALT allele) are discarded. Variant Effect Predictor[17] 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 the study of Arafah *et al*.[18]

Sanger verification of the variant

The zinc finger MYND-type containing 15 (*ZMYND15*) *v*ariant 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: ZMYND15_F_1, TACCCGCTGACCGTGTACTA; ZMYND15_R_1, AAATGCTGCTCGTCGCTTTC.

RESULTS

Couple infertility investigation and treatment

The subject and his spouse had a long history of infertility. The couple suffered from primary infertility since 2000. An initial sperm investigation was carried out in 2002. Sperm volume was normal (3.2 ml) with a normal pH (7.6) and viscosity. Sperm analysis highlighted a severe oligozoospermia (0.6 × 10^6 ml^{-1}) with 100% teratozoospermia, with mainly large-headed spermatozoa, associated with 0% sperm motility correlated with a very high necrospermia (<10% vitality). A high number of round germ cells were detected (0.4 × 10^6 ml^{-1}) with few leukocytes (100 ml^{-1}). The first ICSI attempt was realized. The smaller spermatozoa (as normal as possible) were selected for injection but with no sign of motility. A total of 10 mature oocytes were injected but no embryos could be obtained. Sperm activation with Pentoxifylline did not permit to initiate any sperm movement. A testicular sperm extraction (TESE) was then carried out with the hope of obtaining viable, motile spermatozoa to achieve a successful ICSI. Unfortunately, no motile spermatozoa could be identified and the TESE was unsuccessful.

In 2010, the couple came back to initiate some genetic investigations. Due to high proportion of large-headed spermatozoa, a specific diagnosis of the *AURKC* gene was initially requested.

Identification of a homozygous frameshift variant in ZMYND15

All of *AURKC* coding exons were sequenced using the Sanger technique as previously described.[6] No deleterious variants were identified.

Exome sequencing was performed on Patient 1 (P1) using an Agilent V6 capture kit. Overall, 83.0% and 96.0% of all nucleotides from the targeted genes were sequenced with a sequencing depth of at least twenty and ten times, respectively. As most of the gene defects responsible for male infertility identified so far have a recessive transmission, we focused our search on hemizygous or bi-allelic variants. Furthermore, we eliminated the variants scored as "low" (synonymous variant and intronic variants not predicted to impact splicing). Overall, a total of 19 genes remained as potential candidates after default filtering (Participants and Methods) and exclusion of heterozygous and "low" variants (**Table 1**). A total of 7 genes had a homozygous variant and 13 carried two or three variants. For these last genes, as no familial study had been undertaken, it was not possible to know if the identified variants were allelic or bi-allelic (**Table 1**). Only

Table 1: List of the potential candidate gene variants

Gene	*Position*	*Reference*	*Altered sequence*	*Genotype*	*Impact*	*HGVSc*	*HGVSp*	*gnomAD_AF*	*Exp ratio*
ZMYND15	Chr17:4744208	AAAAC	A	HV	High	ENST00000269289.10:c.1520_1523del	ENSP00000269289.6:p.Lys507SerfsTer3	3.976e-06	16.18
AC026954.2	Chr17:7317527	T	C	HV	Moderate	ENST00000575474.1:c.694A>G	ENSP00000468772.1:p.Met232Val	0.005177	2.86
NEURL4	Chr17:7317527	T	C	HV	Moderate	ENST00000399464.7:c.4252A>G	ENSP00000382390.2:p.Met1418Val	0.005177	1.49
PFAS	Chr17:8268959	G	T	HV	Moderate	ENST00000314666.11:c.3712G>T	ENSP00000313490.6:p.Val1238Leu	0.001759	1.49
ZZEF1	Chr17:4114401	T	C	HV	Moderate	ENST00000381638.7:c.764A>G	ENSP00000371051.2:p.Tyr255Cys	0.005752	1.18
HCFC1	ChrX:153955425	C	T	HV	Moderate	ENST00000310441.12:c.2974G>A	ENSP00000309555.7:p.Ala992Thr	0.0007993	1.12
FAM47A	ChrX:34131786	A	C	HV	Moderate	ENST00000346193.4:c.493T>G	ENSP00000345029.3:p.Cys165Gly	2.762e-05	1
DNAH3	Chr16:20952510	C	T	HET	Moderate	ENST00000261383.3:c.11111G>A	ENSP00000261383.3:p.Arg3704Lys	0.005522	18.84
DNAH3	Chr16:21106509	C	T	HET	Moderate	ENST00000261383.3:c.2265G>A	ENSP00000261383.3:p.Met755Ile	7.965e-06	18.84
USP44	Chr12:95521132	G	A	HET	Moderate high	ENST00000258499.8:c.1804C>T	ENSP00000258499.3:p.Pro602Ser	0.002688	12.48
USP44	Chr12:95534028	C	T	HET	Moderate	ENST00000258499.8:c.229G>A	ENSP00000258499.3:p.Val77Ile	0.0002426	12.48
GOLGA8K	Chr15:32392862	G	T	HET	Moderate	ENST00000512626.2:c.1813C>A	ENSP00000426691.2:p.His605Asn	0.001174	7.03
GOLGA8K	Chr15:32393182	G	A	HET	Moderate	ENST00000512626.2:c.1577C>T	ENSP00000426691.2:p.Ala526Val	0.001	7.03
DLGAP2	Chr8:1565882	G	C	HET	Moderate	ENST00000637795.2:c.1430G>C	ENSP00000489774.1:p.Gly477Ala	4.287e-06	4.29
DLGAP2	Chr8:1668509	C	T	HET	Moderate	ENST00000637795.2:c.1991C>T	ENSP00000489774.1:p.Thr664Met	0.006155	4.29
AKAP1	Chr17:57114565	G	A	HET	Moderate high	ENST00000621116.4:c.2210G>A	ENSP00000478212.1:p.Arg737His	3.978e-06	3.98
AKAP1	Chr17:57118419	A	G	HET	Moderate	ENST00000621116.4:c.2539A>G	ENSP00000478212.1:p.Ser847Gly	0.001054	3.98
ADAMTS17	Chr15:99997488	A	G	HET	Moderate	ENST00000268070.9:c.2693T>C	ENSP00000268070.4:p.Val898Ala	0.0005479	3.84
ADAMTS17	Chr15:100133231	C	T	HET	Moderate	ENST00000268070.9:c.1558G>A	ENSP00000268070.4:p.Glu520Lys	2.256e-05	3.84
TMC3	Chr15:81332820	G	A	HET	Moderate	ENST00000359440.6:c.2902C>T	ENSP00000352413.5:p.Arg968Trp	0.002026	3.5
TMC3	Chr15:81344866	G	A	HET	Moderate	ENST00000359440.6:c.1418C>T	ENSP00000352413.5:p.Thr473Ile	0.0002891	3.5
ZNF100	Chr19:21765709	C	G	HET	Moderate	ENST00000358296.11:c.81G>C	ENSP00000351042.5:p.Gln27His	0.0001797	2.78
ZNF100	Chr19:21765711	G	A	HET	High	ENST00000358296.11:c.79C>T	ENSP00000351042.5:p.Gln27Ter	0.0001637	2.78
ZNF100	Chr19:21765722	C	T	HET	Moderate	ENST00000358296.11:c.68G>A	ENSP00000351042.5:p.Ser23Asn	0.0002354	2.78
ATP13A1	Chr19:19647741	T	C	HET	Moderate	ENST00000357324.11:c.2651A>G	ENSP00000349877.6:p.Asn884Ser	3.965e-05	1.52
ATP13A1	Chr19:19655438	C	T	HET	Moderate	ENST00000357324.11:c.1412G>A	ENSP00000349877.6:p.Ser471Asn		1.52
NLRC3	Chr16:3544282	C	T	HET	Moderate	ENST00000359128.10:c.2819G>A	ENSP00000352039.6:p.Arg940His	0.0001846	1.32
NLRC3	Chr16:3564398	C	T	HET	Moderate	ENST00000359128.10:c.539G>A	ENSP00000352039.6:p.Arg180Gln	5.767e-05	1.32
MUC4	Chr3:195784629	G	C	HET	Moderate	ENST00000463781.8:c.6951C>G	ENSP00000417498.3:p.His2317Gln	9.578e-05	1.31
MUC4	Chr3:195789532	G	A	HET	Moderate	ENST00000463781.8:c.2048C>T	ENSP00000417498.3:p.Pro683Leu	0.0001645	1.31
PRAMEF11	Chr1:12827574	A	G	HET	Moderate	ENST00000619922.1:c.550T>C	ENSP00000480027.2:p.Cys184Arg	0.0001809	1
PRAMEF11	Chr1:12827631	A	G	HET	Moderate	ENST00000619922.1:c.493T>C	ENSP00000480027.2:p.Tyr165His	6.827e-05	1

All potentially bi-allelic variants with a possible deleterious effect on the protein are indicated. The gene symbols (Column 1); the chromosomal position of the variant (position), the sequence of reference and the altered sequence (column 2–4); the genotype of the variant, whether it is homozygous or hemizygous (HV) or HET (column 5); the expected impact of the variant on the protein function (column 6); the reference of the affected transcripts and the impact of the variant referring to the transcript cDNA sequence (column 7, HGVSc); the reference of the affected protein and the impact of the variant referring to the protein sequence and indication of the amino-acid variations (column 8, HGVSp); the allelic frequency of the variant as indicated by gnomAD (https://gnomad.broadinstitute.org/; column 9, gnomAD); and last, the testis expression ratio (column 10, Exp ratio), calculated by dividing the level of testis expression by the averaged expression of all other organs. All expression values were obtained from GTEX (https//gtexportal.org/home/)

one gene, *ZMYND15*, carried a homozygous variant characterized as "high," predicted to code for a truncated and thus nonfunctional protein. All the other identified variants had a low probability of being deleterious, mainly because they were predicted to have only a moderate impact and/or because the concerned genes are not strongly expressed in the testis and/or because of the possibility that the identified variants within a same gene are allelic.

The presence of the homozygous *ZMYND15* variant (c.1520_1523delAACA; p.Lys507SerfsTer3; rs587777432) was confirmed by Sanger sequencing (**Figure 1**). The identified variant is a frameshift deletion of 4 nucleotides creating a stop codon in exon 9 of 14 of the *ZMYND15* gene (**Figure 2**). As the same variant has already been found in three brothers with azoospermia[19] and other *ZMYND15* homozygous loss of function variants have been identified in subjects with SO,[20] we are confident that the identified variant is responsible for P1's infertility.

DISCUSSION

Here, we studied a subject (P1) with long history of infertility presenting with SO, macrozoospermia and a complete absence of sperm motility (OATS). Most patients with a typical macrozoospermia with 100% sperm anomalies and multiple flagella carry AURKC variants. For these subjects, ICSI should be ruled out as all spermatozoa are tetraploid.[9] For P1, *AURKC* analysis was negative, ruling out a typical diagnosis

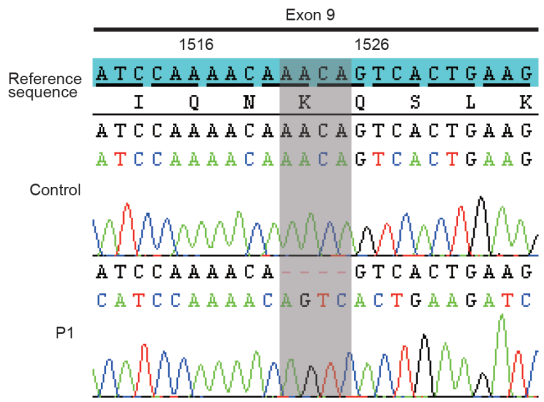

Figure 1: Sanger sequencing highlighting the deletion of nucleotides c.1520_1523 in *ZMYND15* exon 9. Electropherograms from Sanger sequencing indicating the homozygous state of the identified variant c.1520_1523del. The deleted nucleotides are highlighted in gray. *ZMYND15*: zinc finger MYND-type containing 15.

of macrozoospermia. Exome sequencing revealed a homozygous loss of function variant in *ZMYND15*, a gene already described in relation with azoospermia and SO. *Zmynd15* has been described in mice to code for a histone deacetylase-dependent transcriptional repressor essential for spermiogenesis and male fertility and was shown to control the temporal expression of haploid cell genes during spermiogenesis.[21] In mice, inactivation of *Zmynd15* results in early activation of transcription of important haploid genes including *Prm1, Tnp1, Spem1,* and *Catsper3* resulting in late spermatids loss and complete azoospermia.[21] The presence of *ZMYND15* loss of function homozygous variants has also been reported to induce male infertility in human. In fact, the same homozygous variant (c.1520_1523delAACA) has already been described in three azoospermic brothers of Turkish origin.[19] As the variant is extremely rare and P1 is also of Turkish origin, it is likely that these individuals have a common ancestor. Recently, a large cohort of 414 infertile Chinese patients composed of 219 subjects with SO and 195 subjects with NOA was screened by WES. Homozygous loss of function variants were identified in three unrelated patients, all three presenting with SO and not NOA.[20] The three identified variants are located in exons 6 (one subject) and 10 (two subjects), relatively close to the variant identified here in P1 present in exon 9 (**Figure 2**).

This is therefore the third report of homozygous loss of function variants in *ZMYND15* in infertile men. Surprisingly, in each publication, the described phenotype was different: the three Turkish brothers had azoospermia, whereas our patient with the same mutation had SO with macrozoospermia, and the three Chinese subjects with different variants were all described to have SO but with no precise description of their sperm count or the presence of morphological defects. Also, in addition to SO and macrozoospermia, none of the spermatozoa from P1 showed any motility due to a very high frequency of necrospermia. Testicular sperm were extracted which showed the same defects. In consequence, ICSI was unsuccessful and no pregnancy could be achieved. Again this is in contradiction with what was achieved for one of the Chinese subject, who had a successful ICSI and an ongoing pregnancy. We have frequently observed that SO is often associated with nonspecific teratozoospermia. We can conclude that a severely altered spermatogenesis will often produce few and morphologically abnormal spermatozoa. This is what was observed here, similarly to what we had observed in a patient with a defect in the serine peptidase inhibitor Kazal type 2 (*SPINK2*) gene.[22]

These observations highlight that even an identical genetic event can lead to a different phenotype (NOA, SO, and oligoasthenoteratozoospermia [OATS]). This confirms that there is a phenotypic continuum between these sperm defects. This is especially

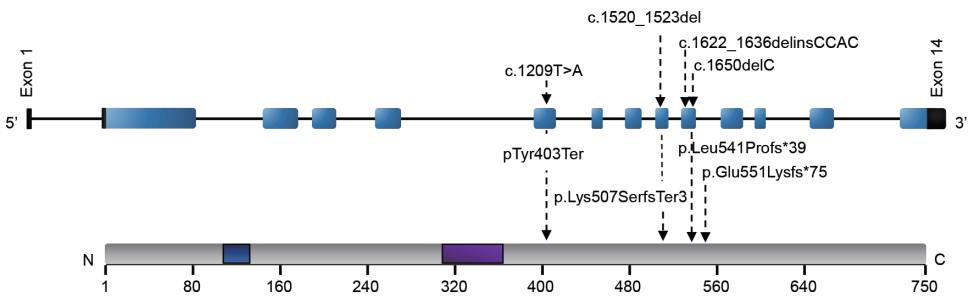

Figure 2: Location and impact of identified *ZMYND15* variant. Location of the variant within *ZMYND15* gene (transcript ENST00000269289.10) and protein ENSP00000269289.6. Coiled-coil domain (109–129 AA) is indicated by blue box; zinc finger, MYND-type domain (313–359 AA) is indicated in purple. *ZMYND15*: zinc finger MYND-type containing 15; AA: amino acid.

true for postmeiotic genes such as *ZMYND15* which can lead to NOA or SO, potentially depending on the patient's genetic background and/or environmental factors, including living habits. As expressed before,[20] the phenotype could also become more severe with age and SO/OATS could become NOA in an aging subject.

At present, treatment counseling for patients with *ZMYND15* defects cannot be formally established. Here, we observed a very high rate of necrospermia which was not compatible with a successful ICSI. This was not reported in the Chinese patients with SO, one of whom was reported to have a successful ICSI. Further cases should be reported to assess if ICSI or TESE followed by ICSI should be proposed to subjects harboring *ZMYND15* defects.

AUTHOR CONTRIBUTIONS

Z-EK, C Cazin, CA, and PFR analyzed the data and wrote the manuscript. Z-EK, C Cazin, C Coutton, JM and NT-M performed and analyzed the genetic data. FL provided clinical samples and data. Z-EK and PFR designed the study, supervised all molecular laboratory work, had full access to all of the data in the study, and took responsibility for the integrity of the data and its accuracy. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

ACKNOWLEDGMENTS

This work was financed in part by the French Research Agency: grant to PFR (FLAGEL-OME: ANR-19-CE17-0014).

REFERENCES

1 Nistal M, Paniagua R, Herruzo A. Multi-tailed spermatozoa in a case with asthenospermia and teratospermia. *Virchows Arch B Cell Pathol* 1977; 26: 111–8.
2 Ray PF, Toure A, Metzler-Guillemain C, Mitchell MJ, Arnoult C, *et al*. Genetic abnormalities leading to qualitative defects of sperm morphology or function. *Clin Gene* 2017; 91: 217–32.
3 Yurov YB, Saias MJ, Vorsanova SG, Erny R, Soloviev IV, *et al*. Rapid chromosomal analysis of germ-line cells by FISH: an investigation of an infertile male with large-headed spermatozoa. *Mol Hum Reprod* 1996; 2: 665–8.
4 Benzacken B, Gavelle FM, Martin-Pont B, Dupuy O, Lièvre N, *et al*. Familial sperm polyploidy induced by genetic spermatogenesis failure: case report. *Hum Reprod* 2001; 16: 2646–51.
5 Devillard F, Metzler-Guillemain C, Pelletier R, DeRobertis C, Bergues U, *et al*. Polyploidy in large-headed sperm: FISH study of three cases. *Hum Reprod* 2002; 17: 1292–8.
6 Dieterich K, Rifo RS, Faure AK, Hennebicq S, Amar BB, *et al*. Homozygous mutation of *AURKC* yields large-headed polyploid spermatozoa and causes male infertility. *Nat Genet* 2007; 39: 661–5.
7 Mateu E, Rodrigo L, Prados N, Gil-Salom M, Remohí J, *et al*. High incidence of chromosomal abnormalities in large-headed and multiple-tailed spermatozoa. *J Androl* 2006; 27: 6–10.
8 Perrin A, Morel F, Moy L, Colleu D, Amice V, *et al*. Study of aneuploidy in large-headed, multiple-tailed spermatozoa: case report and review of the literature. *Fertil Steril* 2008; 90: e13–7.
9 Dieterich K, Zouari R, Harbuz R, Vialard F, Martinez D, *et al*. The aurora kinase C c.144delC mutation causes meiosis I arrest in men and is frequent in the North African population. *Hum Mol Genet* 2009; 18: 1301–9.
10 Khelifa MB, Coutton C, Blum MG, Abada F, Harbuz R, *et al*. Identification of a new recurrent aurora kinase C mutation in both European and African men with macrozoospermia. *Hum Reprod* 2012; 27: 3337–46.
11 Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HW, *et al*. World Health Organization reference values for human semen characteristics. *Hum Reprod Update* 2010; 16: 231–45.
12 Chen S, Zhou Y, Chen Y, Gu J. Fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* 2018; 34: i884–90.
13 Li H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *arXiv:1303.3997 [q-bio]* 2013.
14 Faust GG, Hall IM. SAMBLASTER: fast duplicate marking and structural variant read extraction. *Bioinformatics* 2014; 30: 2503–5.
15 Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, *et al*. The sequence alignment/map format and SAMtools. *Bioinformatics* 2009; 25: 2078–9.
16 Kim S, Scheffler K, Halpern AL, Bekritsky MA, Noh E, *et al*. Strelka2: fast and accurate calling of germline and somatic variants. *Nat Methods* 2018; 15: 591–4.
17 McLaren W, Gil L, Hunt SE, Riat HS, Ritchie GR, *et al*. The ensembl variant effect predictor. *Genome Biol* 2016; 17: 122.
18 Arafah K, Lopez F, Cazin C, Kherraf ZE, Tassistro V, *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.
19 Ayhan Ö, Balkan M, Guven A, Hazan R, Atar M, *et al*. Truncating mutations in *TAF4B* and *ZMYND15* causing recessive azoospermia. *J Med Genet* 2014; 51: 239–44.
20 Hu TY, Zhang H, Meng LL, Yuan SM, Tu CF, *et al*. Novel homozygous truncating variants in *ZMYND15* causing severe oligozoospermia and their implications for male infertility. *Hum Mutat* 2021; 42: 31–6.
21 Yan W, Si Y, Slaymaker S, Li J, Zheng H, *et al*. *Zmynd15* encodes a histone deacetylase-dependent transcriptional repressor essential for spermiogenesis and male fertility. *J Biol Chem* 2010; 285: 31418–26.
22 Kherraf ZE, Christou-Kent M, Karaouzene T, Amiri-Yekta A, Martinez G, *et al*. SPINK2 deficiency causes infertility by inducing sperm defects in heterozygotes and azoospermia in homozygotes. *EMBO Mol Med* 2017; 9: 1132–49.22