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Whole exome sequencing identifies a new *DPY19L2* variant c.1232_1233insA in Tunisian infertile patient with globozoospermia

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Abstract

Background Globozoospermia is a rare male infertility disorder characterized by the presence in the ejaculate of round-headed sperm cells lacking an acrosome, a crucial structure for fertilization. While genetic factors play a significant role in the etiology of this condition, the precise mechanisms remain elusive. Recent studies have identified mutations in the *DPY19L2* gene as a major cause of globozoospermia. This gene encodes a protein involved in the development and maturation of sperm cells, particularly the acrosome biogenesis. Mutations in *DPY19L2* disrupt this process, leading to the production of abnormal sperm cells. However, *DPY19L2* mutations account for only a subset of globozoospermia cases, suggesting the involvement of other genetic factors.

Patients and methods Eighteen infertile men with total and partial globozoospermia were investigated. For all patients, screening of *DPY19L2* total deletions was carried out by a qualitative PCR. When a positive diagnosis was not obtained (absence of a homozygous deletion), WES was conducted.

Results Total deletion of *DPY19L2* was found in 33% of all patients and in 60% of total globozoospermic ones. WES conducted in only two patients identified a novel *DPY19L2* frameshift mutation (c.1232_1233insA) p.Arg412GlufsTer3 in one patient with total globozoospermia. The pathogenesis of this new variant is supported by In Silico prediction tools and by its absence in control patients and different published databases.

Conclusions We confirm here the large implication of *DPY19L2* in Globozoospermia, and we enrich the gene spectral mutation. Identification of the genetic basis of globozoospermia is crucial for accurate diagnosis, genetic counseling, and the development of potential therapeutic interventions.

Keywords Male infertility, Globozoospermia, *DPY19L2*, Whole exome sequencing

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Background

Infertility is estimated to affect up to 15% of couples of reproductive age. In nearly half of cases, a male factor is believed to be present, often manifested by a quantitative and/or qualitative defect of sperm parameters [1]. Teratozoospermia is a qualitative defect defined by < 4% of the spermatozoa with normal morphology, according to the World Health Organization's 2021 publication (WHO, 2021) [2].

Globozoospermia syndrome (MIM #613958) is a rare type of monomorphic teratozoospermia (around 0.1%) [1] which is characterized by the production of round-headed spermatozoa without acrosomes [3]. It was first described in 1976 by Wolff Schill and Moritz and is classified by Singh (1992) into type I and type II according to the percentage of abnormal spermatozoa [4]. In type I, also known as total globozoospermia, 100% of the spermatozoa have a small, round, and acrosome-free head. In type II, called partial globozoospermia, 20–90% of spermatozoa have no acrosome or a reduced acrosome. In this situation, men have both normal and round-headed sperm cells with large cytoplasmic droplets, which impair motility [4].

Several genes (> 60) have been implicated in the different steps of acrosome biogenesis in knock-out mouse models and have been demonstrated to be responsible for globozoospermia [5]. In humans, the main gene involved was *DPY19L2* [6]. Less frequently, other genes playing a role in acrosome biogenesis have been reported, like *SPATA16* [7], *PICK1* [8], *ZPBP1* [9], *CCDC62, CCIN, GGN, C2CD6, DNAH17*, and *C7orf61* [10].

Nonetheless, globozoospermia represents a heterogeneous disorder, and determinants of the phenotype–genotype correlation remain unclear. In Tunisia, this severe form of teratozoospermia has been explored only in small cohorts of unrelated men.

The aim of this study was to assess the involvement of *DPY19L2* in globozoospermia in the Tunisian population.

Methods

Patients

We recruited 18 patients, all addressed to Pasteur Institute of Tunis between 2012 and 2024 for the genetic investigation of globozoospermia. All patients had a medical consultation for infertility and a sperm analysis revealing complete or partial globozoospermia in accordance with the recommendations of the World Health Organization 2010. Informed and written consent was obtained from all the patients participating in the study according to local protocols and the principles of the Declaration of Helsinki. The study was approved by local ethics committees.

Genomic DNA extraction

Genomic DNA was extracted from peripheral blood leukocytes on EDTA, using the FlexiGene ®DNA Kit (Qiagen,Germany) according to the manufacturer's instructions. DNA quantity and purity were evaluated using a NanoDrop™ spectrophotometer. DNA samples were coded to keep participants anonymous.

DPY19L2 deletion screening

To screen total deletion of *DPY19L2*, polymerase chain reaction was carried out using specific probes for exon 11 and exon 22, LCR1 and LCR2 according to protocols described previously by Harbouz et al. 2011 (6). PCR Mix was prepared with Applied Biosystems™ AmpliTaq Gold™ 360 Master Mix with a 25-µl of final product (10 µl Master mix, 1 µl primer F 10 µM, 1 µl primer R 10 µM, DNA (50 ng/ml) and QspH2O). PCR products were analyzed by electrophoresis on 1% agarose gels containing Gel RED DNA staining and visualized by exposure to ultraviolet light.

Whole exome sequencing and bioinformatics analysis

Due to limited financial resources, human whole exome was performed for 2 globozoospermic type I patients without a *DPY19L2* homozygous deletion. Exons and intron boundaries were captured using the Human Core Exome + Human RefSeq panel (Twist Bioscience, CA, USA). Sequencing was performed on Illumina NovaSeq 6000 (S4 flow cell) with an expected throughput of 5 GB of paired-end reads (2 × 150 bp).

The reads were analyzed using version v1.1.0 of our previously described [PMID: 35172124] bioinformatics pipeline, developed in-house. It consists of two modules, both distributed under the GNU General Public License v3.0.

Briefly, using the first module (https://github.com/ntm/grexome-TIMC-Primary), adapters were trimmed and low-quality reads filtered with fastp 0.23.4 [https://doi.org/10.1002/imt2.107], reads were aligned on GRCh38 with bwa-mem2 v2.2.1 [https://doi.org/10.1109/IPDPS.2019.00041], duplicates were marked using samblaster® v0.1.26 [https://doi.org/10.1093/bioinformatics/btu314], and BAM files were sorted and indexed with samtools® 1.20 [https://doi.org/10.1093/gigascience/giab008]. SNVs and short indels were called from each BAM file using strelka 2.9.10 [https://doi.org/10.1038/s41592-018-0051-x] and GATK 4.4 to produce individual GVCF files. These were finally merged with our entire collection of exomes (1050 as currently) to obtain a single multi-sample GVCF per caller. Using both variant callers independently provides an easy initial cross-validation procedure.

The second module (https://github.com/ntm/grexome-TIMC-Secondary) processed each merged GVCF and produced annotated analysis-ready TSV files. This was achieved by performing 17 streamlined tasks, including the following. Low-quality variant calls (DP < 10, GQX < 20, or less than 4 reads/15% of all reads supporting the ALT allele) were discarded. Variant Effect Predictor v111 [https://doi.org/10.1186/s13059-016-0974-4] was used to annotate the variants and predict their impact. Gene expression data from the Genotype-Tissue Expression project (GTEx v7) were added. Variants with a minor allele frequency greater than 1% in gnomAD v2.0 or 3% in the 1000 Genomes Project phase 3 were filtered.

Sanger sequencing

To confirm the candidate variant revealed by WES, Sanger sequencing was performed using an automated sequencer (ABI 3500; Applied Biosystems, Foster City, CA, United States) and a cycle sequencing reaction kit (Bigdye Terminator v3.1 kit, Applied Biosystems). Sequences were analyzed using BioEdit software version 7.2.5.

Results

Patient's and semen characteristics

Median age of patients was 44.25 years ± 5.96. Among 18 patients, four (22%) have consanguineous parents. Familial male infertility was reported in three (16%) cases.

Semen parameters revealed a normal sperm count (> 15 million/ml) in the majority of cases associated with a lower sperm motility. Ten (55%) patients showed a globozoospermia type I, while 8 (45%) presented a globozoospermia type II, with a percentage of round-headed spermatozoa ranging from 7 to 74% (Table 1).

The comparison of sperm parameters between the patients of the two groups does not show any significant differences despite the high percentage of abnormal forms and round heads spermatozoa (Table 2).

DPY19L2 deletion analysis

Molecular analysis, carried out by qualitative PCR, revealed a total deletion of the *DPY19L2* gene in 6 out of 18 patients (33.3%) which represents 60% of patients with total globozoospermia (Fig. 1).

All patients carrying a deletion in *DPY19L2* displayed complete globozoospermia. No patient with partial globozoospermia has a *DPY19L2* deletion. In addition to the high percentage of atypical and round-head spermatozoa, sperm motility in deleted patients is significantly decreased (Table 3).

Whole exome sequencing

Due to financial limitations, WES was performed in only 2 patients with total globozoospermia. The

Table 1 Semen parameters of 18 globozoospermic patients

Patient	Volume (ml)	Concentration (M/ml)	Total motility	Abnormal forms (%)	Round head spz (%)
P1	2,2	34,4	35	96	74
P2	2,5	33	40	93	60
P3	ND	ND	ND	ND	100
P4	1,4	108	23	100	100
P5	3,1	72	35	100	100
P6	1,4	81	9	99	26
P7	1	21,3	15	72	22
P8	ND	ND	ND	ND	7
P9	ND	ND	ND	ND	ND
P10	2	100	30	100	100
P11	3,8	4,7	15	45	90
P12	3,5	36,5	20	100	100
P13	4,8	304	63	53	18
P14	ND	ND	ND	ND	ND
P15	ND	ND	ND	ND	100
P16	3,7	16	8	100	96
P17	1,6	6	4	100	100
P18	4,6	44	55	100	100

Table 2 Mean ± SD, and significance of the sperm parameters between complete and partial form of globozoospermia (*t*-test). Significant *P* values are in bold

Type of globozoospermia	Volume (ml)	Sperm concentration (10^6/ml)	Total motility (%)	Abnormal forms (%)	Round head spz (%)
Type I	2.84 ± 1.20	54.64 ± 39.81	25 ± 17.24	100 ± 0.0	99.56 ± 1.33
Type II	2.38 ± 1.48	94.74 ± 119.19	32.4 ± 21.51	82.6 ± 19.65	34.5 ± 26.33
P value	0.63	0.36	0.49	**0.021**	**<0.001**

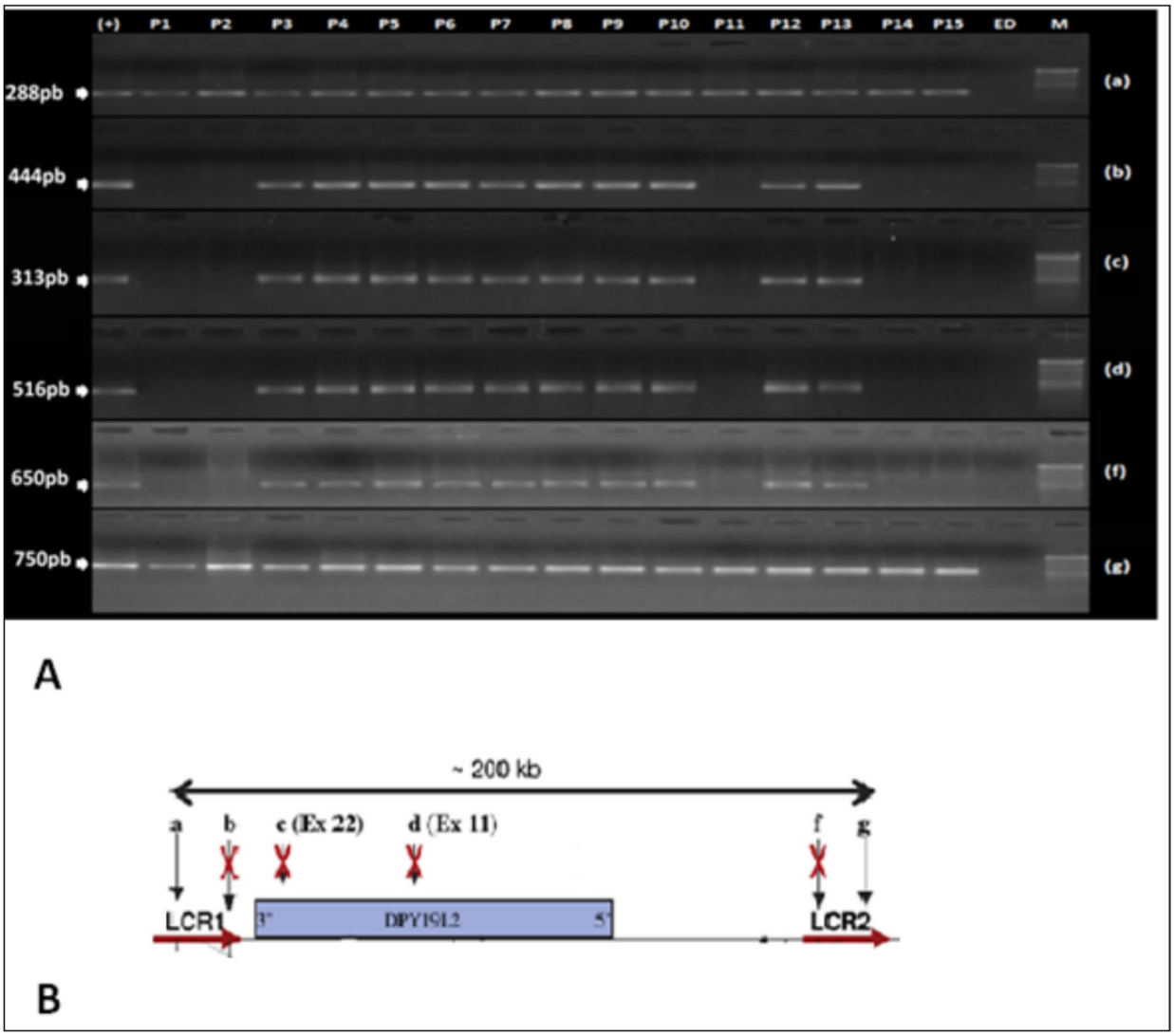

Fig. 1 Screening of *DPY19L2* deletion. **A** Results of PCRs in fifteen globozoospermic patients: (+) DNA from a fertile man, P1, P2, P11, P14, P15: globozoospermic patients with total deletion of *DPY19L2*, P3–P10, P12, and P13: No *DPY19L2* total deletion; (ED) blank.**B** Schematic representation of the region analyzed (in kb). **a** and **b** loci located approximately 25 kb and 9 kb from the 3′ region of the *DPY19L2* gene; **c** and **d** correspond to the position of exons 22 and 11 of the *DPY19L2* gene; **f** and **g** loci located approximately 62 and 77 kb from the 5′ region of the *DPY19L2* gene. LCRs 1 and 2 (the arrows in red) represent two duplicated sequences of 28 kb, presenting 97% sequence identities, located on either side of the *DPY19L2* gene. PCR results in**a** indicate that the absence of **b**, **c**, **d**, and **f** amplification confirmed the total deletion of *DPY19L2*

Table 3 Mean ± SD and significance of the sperm parameters between globozoospermic samples of men with and without *DPY19L2* deletions (*t*-test). Significant *P* values are in bold

DPY19L2 deletion	Volume (ml)	Sperm concentration (10^6/ml)	Total motility (%)	Abnormal forms (%)	Round head spz (%)
Present	2.67 ± 1.12	72.17 ± 35.75	26.00 ± 7.94	100 ± 0.0	100 ± 0.0
Absent	2.76 ± 1.36	64.44 ± 89.71	27.40 ± 20.61	85.80 ± 21.26	63.00 ± 37.77
P value	*0.38*	*0.37*	***0.04***	*0.28*	*0.07*

bioinformatics analysis (Methods) allowed focusing on rare variants severely impacting a protein-coding testis-expressed gene, homozygous in the patient and never homozygous in any control individual. This yielded a short-list of 1–5 candidate variants per patient for further evaluation.

Interestingly, a novel variant of *DPY19L2* was detected in one patient (P18). The variant: (c.1232_1233insA) p.Arg412GlufsTer3 is a homozygous frameshift mutation present in patient P18 and leading to a truncated protein with 347 amino acids by inserting an adenine and creating a stop codon, eliminating the C-terminal domain of the protein. The novel variant was confirmed by direct sequencing (Fig. 2). For the second patient, no pathogenic/likely pathogenic variant was found.

Discussion

Male infertility is a very heterogeneous condition affecting around half of the infertile couples [11]. Teratozoospermia is a heterogeneous group including a wide range of abnormal sperm phenotypes affecting, solely or simultaneously, the head, neck, midpiece, and tail [12]. Monomorphic teratozoospermia is a rare syndrome defined by the presence of a unique phenotype. Four syndromes are well identified: the globozoospermia, the macrocephaly, decapitated spermatozoa syndrome, and multiple morphologic anomalies of flagella [12]. Globozoospermia is a rare and severe disorder in male infertility. It is characterized by the presence in the ejaculate of a majority of round-headed spermatozoa devoid of acrosome [6]. The acrosome plays a crucial role during the fertilization process. The lack of acrosome structures leads to the disability of spermatozoa binding to the zona pellucida, so that no acrosome reaction can occur and the fertilization process fails [13]. Then, males with total globozoospermia are infertile; men with partial globozoospermia have decreased fertility or may even be infertile [14]. Globozoospermia is mainly due to the acrosome biogenesis failure and involves different steps: pro-acrosomal Golgi-derived vesicles formation, trafficking and fusion, and/or a disruption of the interaction between the acrosome and the sperm nuclear envelope through the acroplaxome junction [12]. More than 60 genes implicated in globozoospermia and involved in the acrosome biogenesis have been identified with the creation of different knock-out mouse models. But a few mutations have been identified in their human orthologs [12]. Among the globozoospermia-associated genes analyzed in the literature, *DPY19L2* is the most frequently mutated gene in affected patients [15]. *DPY19L2* gene is located on chromosome 12 (12q14.2) and contains 22 coding exons. *DPY19L2* belongs to a new family of transmembrane proteins of the nuclear envelope including four homologous proteins, *DPY19L1–DPY19L4* [16]. It is localized on the inner membrane of the sperm nuclear envelope (NE), facing the acrosome and with both its N- and C-terminuses embedded in the nucleoplasm. The inner NE-localized *DPY19L2* facilitates the attachment of the acrosome to the NE. In *Dpy19l2*-deficient mouse spermatids, the development of the acrosome has been found to be hampered as early as step 4 spermatids. Without *Dpy19l2*, the acrosome matrix is no longer bound tightly to the acroplaxome, and the acrosome extension along the nucleus stops. All these defects result in round-headed spermatozoa [17].

The first variant of *DPY19L2* reported in globozoospermia in humans was a homozygous deletion of 200 kb on chromosome 12, which contains the DPY19L2 locus. The large-scale deletion led to the complete loss of the *DPY19L2* gene, and the affected sperms were round-headed without acrosomes [6]. Later, several studies [18, 19] demonstrated that the homozygous *DPY19L2* deletion is the most common mutation reported in globozoospermia in humans. It was explained by a non-allelic homologous recombination (NAHR) between two highly similar 28 kb low copy repeats (LCRs) flanking the gene [6, 20]. In the present study, we evaluated the DPY19L2 deletion by a qualitative PCR targeting exon 11, exon 22, LCR1, and LCR2. Total deletion of *DPY19L2* was found in 33.3% of all patients and 60% of patients with total globozoospermia (P3, P4, P5, P12, P14, P15). All these deleted patients presented total globozoospermia. No deletion was found in patients with partial globozoospermia (with a percentage of round head spermatozoa less than 75%). Our findings agree with those in previous literature [6, 18, 21], suggesting that *DPY19L2* defects could contribute to this severe form of teratozoospermia and so,

cDNA YourSeq

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caAAACAAcT TTTAAAAGCA AGGCTGTATG GATATAGCAG AATGAAACAT  50
AAAGGAAGAG TGGCCTGAAA GTTAGTTGTG AAATATGTGA GCTAACTTCT 100
TGAAAATGAT TCTAGTCCTT GCTAAGGACT ATTTCCTTAC ATATCTATCT 150
ACCTTAGATA GTAACTATTT TTAAGACAGT AGCTATTTAT TAACTATAAA 200
TCATTAGCAT TTTAAAGCAT ATTAACTGTG TTCAGTTTAT AGAAATAAAA 250
AGTAGAATTT AAATAAACTG CATTAAAAAC ATTGAATCTT ACCCAAAAGT 300
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TGCCTAAAAT GCACATAAGG GAAAATGAAA CAATTCAATA CAATTAACAT 400
TTATATTTTG TATAAATGGT ACATTTCAAA AATTATATAG CCACCCTGTT 450
CCATCTCAGC TATCACCAGC CATTTTCCTC CTTAACTGTT GATTTTTCAA 500
ATTTTGGACA AATTTTGGCA AATTTCCTTG AGGTGAGAGT TAAATGGTTT 550
GTGTGTCAAA AAGTTGCCTC ATTATTTCCT CACTTAAATA CCCTGACCAT 600
TTTCTGCAGG CTAATaa
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Genomic chr12 :

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agttatgttg aagggaaggt taattgaagc tagagagaga aactcaagac  63608323
AAACAAaTTT TaAAAAGCAA GGCTGTATGG ATATAGCAGA ATGAAACATA  63608373
AAGGAAGAGT GGCCTGAAAG TTAGTTGTGA AATATGTGAG CTAACTTCTT  63608423
GAAAATGATT tCTAGTCCTT GCTAAGGACT ATTTCCTTAC ATATCTATCT  63608473
ACCTTAGATA GTAACTATTT TTAAGACAGT AGCTATTTAT TAACTATAAA  63608523
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AGTAGAATTT AAATAAACTG CATTAAAAAC ATTGAATCTT ACCCAAAAGT  63608623
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CATCTCAGCT ATCACCAGCC ATTTTCCTCC TTAACTGTTG ATTTTTCAAA  63608823
TTTTGGACAA ATTTTGGCAA ATTTCCTTGA GGTGAGAGTT AAATGGTTTG  63608873
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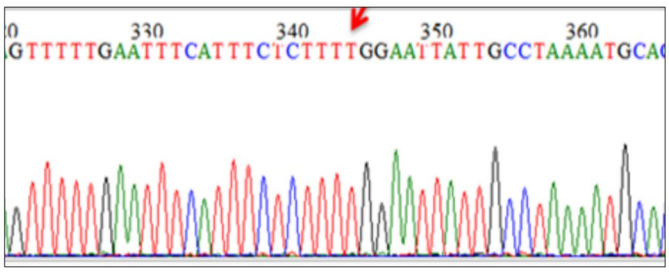

Fig. 2 Sanger confirmation of the c.1232_1233insA in P18 comparing with genome reference by BLAT

genetic investigation of *DPY19L2* could be achieved only in a complete form of globozoospermia.

In addition to the loss of *DPY19L2* protein, substitution of certain amino acids in the protein can also lead to globozoospermia. More than twenty point variants of *DPY19L2* have been described in globozoospermic patients, with *DPY19L2* variants. Reported *DPY19L2* variants include nonsense mutations, missense mutations, intronic mutations, frameshift mutation, splice site mutation, and deletions of certain nucleotides resulting in mutated or truncated *DPY19L2* proteins [1, 3, 22]. In this study, we identified in one patient with total globozoospermia a novel mutation: (c.1232_1233insA) p.Arg412GlufsTer3 in exon 12. It is a homozygous frameshift mutation leading to a truncated and inefficient protein (411 amino acids/758). This variant is absent in any other patient from our current base (1038 exomes), even in the HET state; and it is not present (even HET) in gnomAD v4.1.0: https://gnomad.broadinstitute.org/region/12-63608641-63608681?dataset=gnomad_r4 suggesting that c.1232_1233insA could be a novel *DPY19L2* mutation associated with total globozoospermia. Further functional investigations will be conducted to ensure its functional annotation.

The majority of studies investigating the genetic background of globozoospermia included North African

or Middle Eastern infertile men: Tunisian [1, 19], Algerian [6] and Iranian [21]. In these geographical areas, globozoospermia shows a greater incidence in contrast to Western countries. A possible higher rate of consanguineous marriages may increase the expression of this autosomal recessive trait. *DPY19L2* mutations were also described in Italian [23] and Chinese [19] globozoospermic patients.

Other genes involved in acrosome biogenesis were reported in globozoospermic patients. *SPATA16* (Spermatogenesis associated 16) located on 3q26.31 was the first gene associated with globozoospermia; it was found as a homozygous mutation (848G→A) in three affected brothers from an Ashkenazi Jewish family [7]. *SPATA16* is localized to the Golgi apparatus and to the proacrosomal vesicles, which fuse to form the acrosome during spermiogenesis, but it is also present in the acrosomes of mature spermatozoa. A few years later, a new mutation in *SPATA16* was found in heterozygous and homozygous patients with a deletion in exon 2 [24]. Interestingly, this new mutation led to a severe total globozoospermia and important DNA fragmentation [25]. *PICK1* is another gene discovered in patients with globozoospermia type I who were homozygous for a missense mutation (G198A) in exon 13 [9]. PICK1 or Protein interacting with C kinase 1 is a peripheral membrane protein involved in protein trafficking, a function that has been initially well characterized in neurons. In testis, PICK1 was highly expressed in round spermatids and localized to Golgi-derived proacrosomal granules [16]. However, the real contribution of this gene to the origin of globozoospermia is uncertain. In 2012, PICK1 was studied in 381 teratozoospermic and 204 control patients, and no statistically significant and functionally relevant mutations were discovered in the *PICK1* gene [8]. In addition, no mutation of *PICK1* was reported in infertile patients with globozoospermia in recent studies by Modarres et al., 2016 [21] and Faja et al., 2021 [5].

Coiled Coil Domain-Containing 62 (CCDC62) is another gene described firstly in mouse models (in 2017) with infertility and acrosome defects by Li et al., [23]. CCDC62 is likely present in the Golgi apparatus during the first stages of spermatogenesis and then in the acrosome during the elongation and maturation phases. Recently, Oud et al. identified two homozygous mutations in *CCDC62* in one patient with globozoospermia: one nonsense mutation c.442C>T; p.(Gln148Ter) and one missense mutation c.847C>T; p.(His283Tyr) [23]. In humans, *CCDC62* was shown to be co-expressed with *ZPBP.* ZPBP1 is located in the acrosomal membrane and likely interacts with multiple acrosomal matrix proteins and is involved in sperm-egg interaction during fertilization [16]. Homozygous and heterozygous missense and splicing mutations in *ZPBP1* were reported in *patients* with 64% to 98% of heads defects *by Yatsenko* et al*. 2012* [9]. A nonsense mutation, c.931C>T; p.(Gln311Ter), was also found in a total globozoospermic patient by Oud et al. [10]. Using whole exome sequencing, new candidate genes were identified in globozoospermia: *C2CD6* (also known as *ALS2CR11*), *CCIN*, *C7orf61*, *DHNA17*, and *GGN* [23]. However, their implications should be confirmed by functional investigations.

Conclusion

This study concludes that *DPY19L2* plays a central role in globozoospermia among Tunisian individuals. The high frequency of total *DPY19L2* deletions indicates that screening for this specific genetic alteration in Tunisian patients diagnosed with globozoospermia type I could be a achieved prior to undertaking WES.

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Authors' contributions
W.A and N.T analyzed the data and whrote the main manuscript, W.A performed genetic consultation and collected clinical data of patients; W.A and W.H and M.BK performed DNA extraction and PCRs , S.M, H. E and A.Z, performed semen analysis, A.C and F.Z performed clinical examination of infertiles couples, N.T performed data analysis of whole exome sequencing , P.R supervised bio informatic analysis, A.A supervised all molecular laboratory work. All authors reviewed the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The study was approved by the Bio-Medical Ethics committee of the Institute Pasteur of Tunis. The committee's reference number is 2022/23/I/V1. All patients included in this study were consented to participate and signed a written informed consent.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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