

CASE REPORT

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Identification of a novel deletion mutation in *DPY19L2* from an infertile patient with globozoospermia: a case report



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Abstract

Background: Male infertility is an increasing medical concern worldwide. In most cases, genetic factors are considered as the main cause of the disease. Globozoospermia (MIM102530) (also known as round-headed sperm) is a rare and severe malformed spermatospermia caused by acrosome deficiency or severe malformation. A subset of genetic mutations, such as *DNAH6*, *SPATA16*, *DPY19L2*, *PICK1*, and *CCIN* related to globozoospermia, have been reported in the past few years. The *DPY19L2* mutation is commonly found in patients with globozoospermia. Herein, a 180-kbp homozygote deletion at 12q14.2 (g.63950001–64130000) was identified by copy number variation sequencing (CNVseq) in a patient with a globozoospermia, including the complete deletion of *DPY19L2*.

Case presentation: A 27-year-old patient at the First Affiliated Hospital of Xiamen University was diagnosed with infertility because, despite normal sexual activity for 4 years, his wife did not conceive. The patient was in good health with no obvious discomfort, no history of adverse chemical exposure, and no vices, such as smoking and drinking. The physical examination revealed normal genital development. However, semen tests showed a normal sperm count of 0% and the morphology was the round head. Sperm cytology showed that acrosomal enzyme was lower than normal. Reproductive hormones were in the normal range. B ultrasound did not show any abnormal seminal vesicle, prostate, bilateral testis, epididymis, and spermatic veins. The karyotype was normal, 46, XY, and no microdeletion of Y chromosome was detected. However, a homozygous deletion mutation was found in *DPY19L2*, which was further diagnosed as globozoospermia.

Conclusions: The present study reported a male infertility patient who was diagnosed with globozoospermia. The analysis of gene mutations revealed that *DPY19L2* had a homozygous mutation, which was the primary cause of globozoospermia.

Keywords: Case report, Globozoospermia, Gene mutations, CNV sequencing, *DPY19L2*

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Background

Infertility has gradually become a medical issue attracting worldwide attention. In most cases, the gene factors are considered to be a major cause of the disease [1, 2]. Globozoospermia (MIM102530) is a rare (incidence 0.1%) form of severe monomorphic teratozoospermia that leads to primary male infertility and is characterized by round-headed spermatozoa without acrosome, an abnormal nuclear membrane, and midpiece defects in the ejaculate. Thus, globozoospermia is a severe reproductive issue requiring urgent resolution [3–6].

Previous studies have suggested that gene mutations might be the pathology underlying human globozoospermia, and several genes, such as *DNAH6* [7] and *SPATA16* [8], cause globozoospermia. *DPY19L2* (DPY-19-like 2, MIM 613893) mutations affect sperm head elongation and acrosome formation and accounts for approximately 75% of the patients with globozoospermia [9–13]. *SPATA16* was the first gene reported to be involved in the pathogenesis of globozoospermia in humans [8]. *SPATA16* are located in Golgi body and anterior acrosome vesicles that are transported to the anterior segment to form acrosome during the formation of the spermatozoon, suggesting a key role of the protein in the formation of acrosome [8, 14]. *SPATA16* mutations have been identified in some acrosome absent cases that result in globozoospermia as the membrane is unable to bind the zona pellucida and fertilize the oocyte [8, 14, 15]. *PICK1* gene encoding a cytosolic protein is found in the proacrosomal vesicles of round spermatids; the mutations in this gene lead to the failure of the merge of proacrosomal vesicles, thereby resulting in globozoospermia [16–18]. *CCIN* encodes a major basic protein of the mammalian sperm head cytoskeleton, and its absence or altered arrangement of the calicin protein is related to globozoospermia [19, 20]. In mouse models, the knockout of *Gopc* [21], *Hrb* [22, 23], *Csnk2a2* [24], *Hsp90beta1* [25], *Vps54* [26], *Mfsd14a* [27], and *GMI30* [28] can replicate the round-headed sperm phenotype, but whether these gene mutations also cause this phenotype in humans needs to be investigated further. Although the mutations of *DPY19L2* account for up to 75% of globozoospermia, the genetic mutation spectrum is not yet completely fully elucidated, necessitating an in-depth investigation to deduce the involvement of *DPY19L2* in globozoospermia.

In the present study, we investigated an infertile patient with globozoospermia and identified a homozygous deletion mutation in *DPY19L2*. This study confirmed that *DPY19L2* mutation is the main cause of globozoospermia, which broadened the mutation spectrum of the gene.

Case presentation

Clinical presentation and family history

The proband (aged 27 years, II:1) and his family were recruited from the First Affiliated Hospital of Xiamen

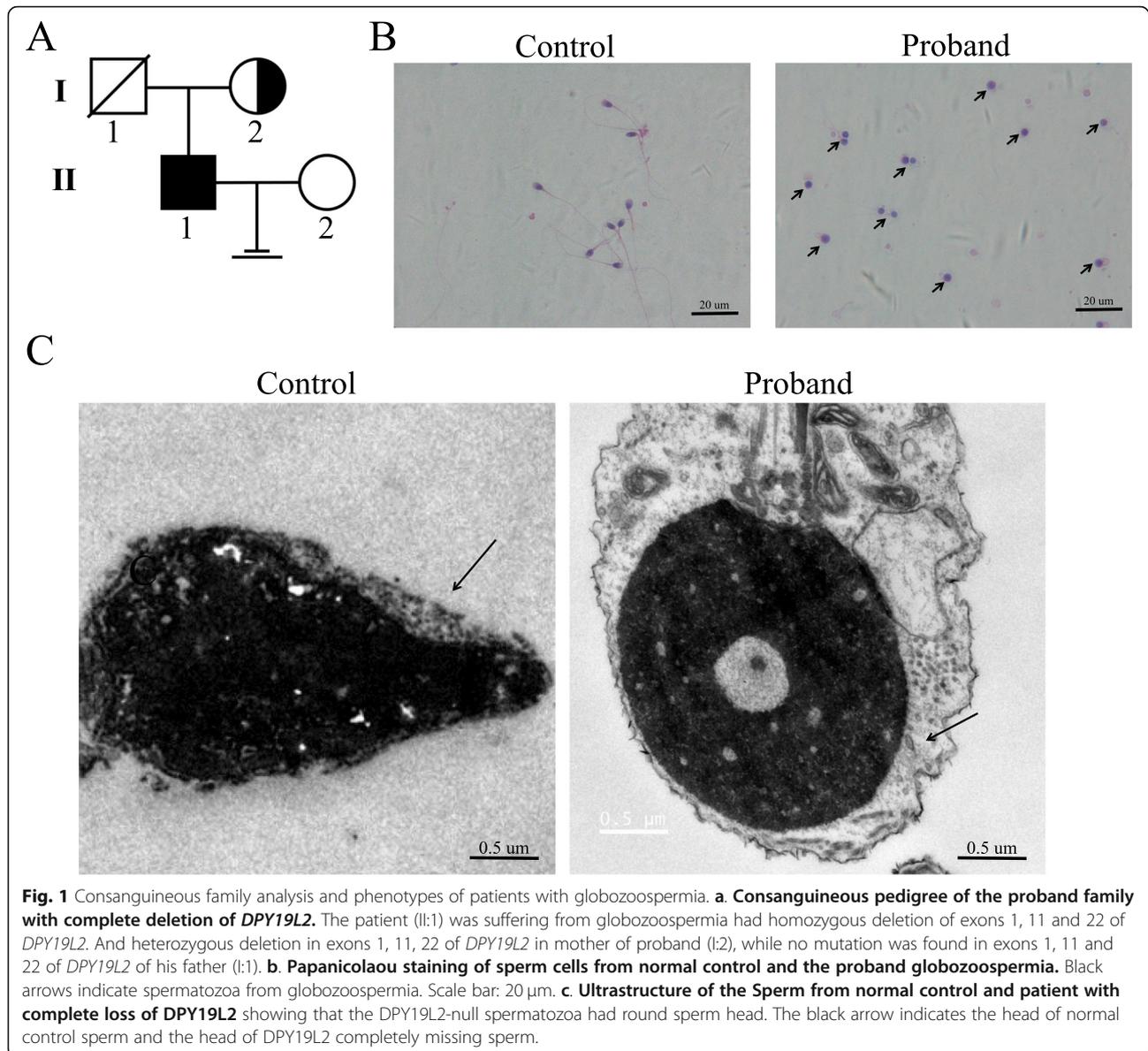
University. Pedigree analysis revealed recessive autosomal (AR) inheritance (Fig. 1a). He had sexual life 2–3 times/week with normal erection and ejaculation during the last 4 years after marriage, but his wife did not conceive. The patient did not have any bad chemical contact history or habits such as smoking and drinking. The patient was in good health with no obvious discomfort. Physical examination data were as follows: height, 174 cm; weight, 66 kg; external genital development, normal; bilateral testicular size and bilateral spermatic vein, normal. The semen was light yellow and could be liquefied within 30 min. The examination results from our hospital were as follows: semen volume, 5.5–6.0 mL; semen pH, 7.2; sperm density, $9.1\text{--}11.3 \times 10^6/\text{mL}$; pro-sad percentage motility, 15–18%; non-prosad percentage motility, 12–15%. Sperm morphology examined by Papanicolaou staining showed 0% normal morphology. The biochemical testing of seminal plasma showed that the content of sperm acrosomase was $32.6 \text{ uIU}/10 \times 6$; neutral glycosidase, 20.6 mU; fructose level, 17.5 μmol ; zinc level, 5.4 μmol . The reproductive hormones were within normal ranges (follicle-stimulating hormone (FSH) 4.56 mIU/mL, luteinizing hormone (LH) 5.87 mIU/mL, T 4.34 ng/mL, E2 33 pg/mL, PRL 12.62 ng/mL). B-ultrasound showed no abnormalities in the seminal vesicles, prostate, bilateral testes, epididymis, and spermatic veins. The chromosomal karyotype of the patient was normal, 46, XY, and no microdeletions were detected in the Y chromosome. The patient's family had yet to undergo follow-up treatment.

A volume of 5 mL peripheral blood was withdrawn from the patient, and each of his parents. The control subject was a healthy male, aged 28 years, with normal fertility. Written informed consent was obtained from each participant. This study was approved by the Ethics Committee of the First Affiliated Hospital of Xiamen University.

Mutations in *DPY19L2* in the patient with globozoospermia

In order to determine the genetic variants associated with globozoospermia, we performed copy number variation sequencing (CNVseq) on the DNA samples of the proband and his parents. The mutation was detected in the *DPY19L2* gene (Fig. 1). Approximately 180 kbp of homozygotic deletion was found on chromosome 12 locus 12q14.2 in the patient sample (g.63950001–64,130,000) (Fig. 2a). The functional gene in this segment included in the Decipher database was *DPY19L2*, which is related to spermatogenesis disorder. The main clinical phenotype is round head sperm, which eventually leads to male infertility.

Additionally, the verification by CNV confirmed the homozygous deletion of exons 1, 11, and 22 of *DPY19L2*



in the proband. A similar phenotype was detected in the mother's genome, while no such variation was found in the father's genome (Supplementary Table 1).

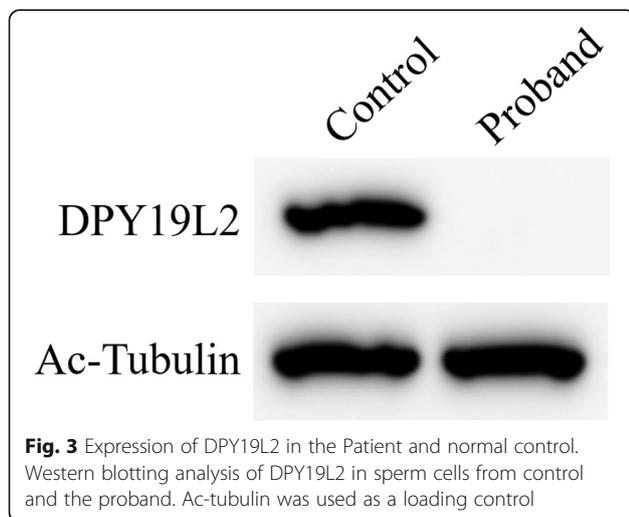
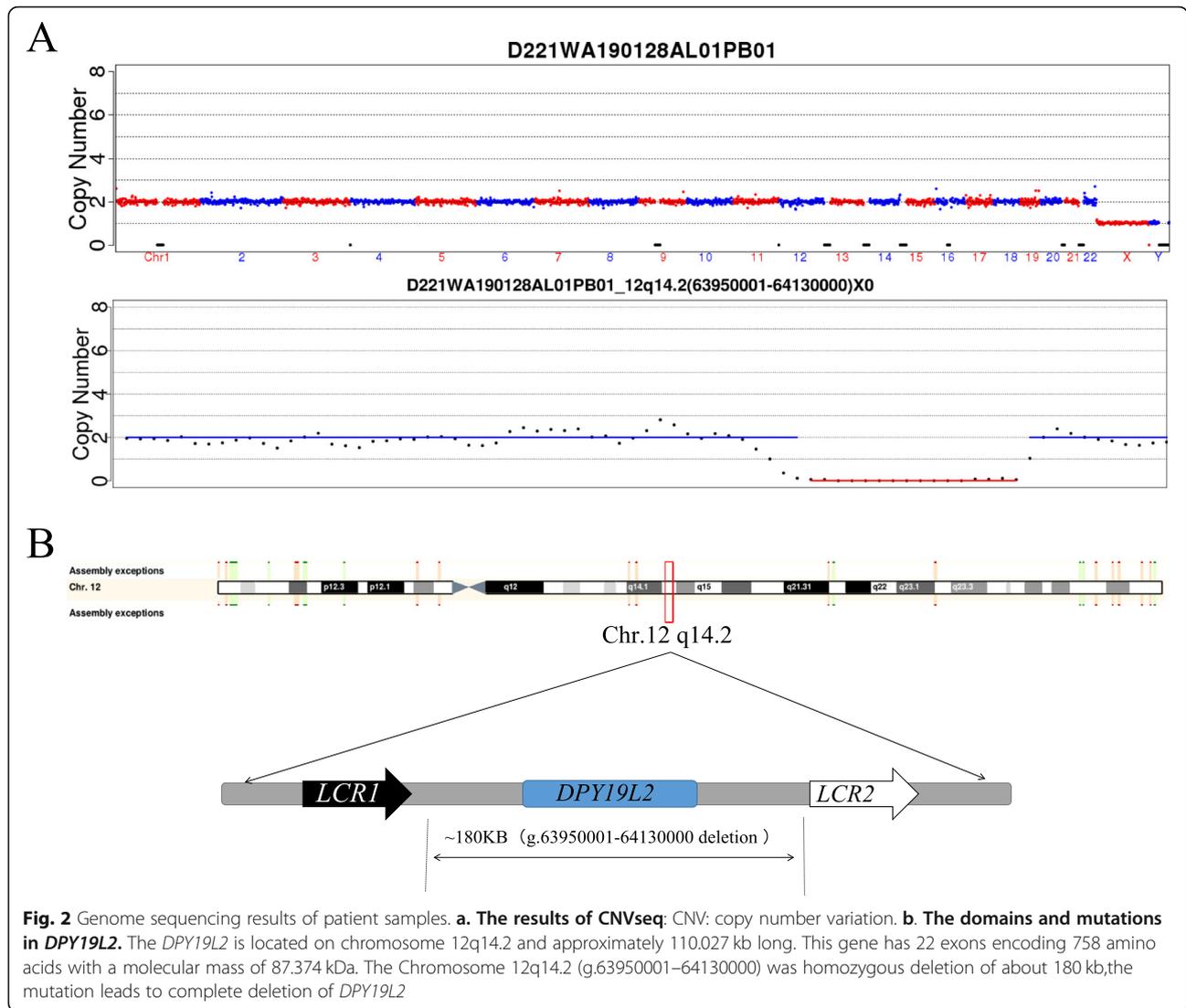
Papanicolaou staining showed that the patient's sperms had abnormal head development. As shown by the black arrow, the most common sperm defect in the patient was the appearance of the round head (Fig. 1b). Based on these results, the patient was diagnosed with globozoospermia (Fig. 1a, II:1).

Transmission electron microscopy (TEM) further confirmed these defects, and the sperm from the patient showed numerous ultrastructural defects in the head. The ultrastructure of the sperm in the control patient was normal, while that of the proband was round (Fig. 1c).

We determined the levels of *DYPL92* in the sperm using Western blotting and found that *DYP19L2* was not expressed in the sperm of the patient (Fig. 3).

Discussion and conclusion

Globozoospermia is a disease of sperm malformation characterized by round head sperm and lack of acrosome. These sperms cannot penetrate the zona pellucida of the oocyte, leading to failed fertilization and infertility [29]. When the sperms are injected into the cytoplasm, the fertilization rate tends to be low. Hitherto, the causes of the disorder remain unclear. *DPY19L2* is a testis-specific transmembrane protein that is highly expressed in the testes. It anchors the acrosomal membrane to the nuclear membrane and is required for sperm head



elongation and acrosome formation during spermatogenesis. However, mutations in this gene lead to instability and loss of acrosome [4, 5, 11, 30]. *DPY19L2* is a pathogenic gene underlying spermatogenic failure, which is an AR inheritance disorder; the homozygous variation might lead to morbidity [31]. In the current study, the data of Papanicolaou staining and electron microscopy provided sufficient evidence of this phenotype in the patient's sperm. To determine the genetic variation that leads to male infertility, genome-wide CNVseq was performed on the patient presenting globozoospermia. The results showed that *DPY19L2* was absent.

Previous studies have shown that *DPY19L2* mutation types exhibit variations in frequency distribution, which might be attributed to the limited number of patients recruited or analyzed in these studies. Also, the frequency distribution of *DPY19L2* mutation types may be different in pure Chinese patients with this AR genetic disease

due to the change in social and cultural factors such as blood relationships [4, 32].

Furthermore, reverse transcription-polymerase chain reaction (RT-PCR) detected the tiny variation of *DPY19L2* in the sample through sequencing; however, the large fragment variation of the gene could not be determined. Thus, other quantitative PCR methods such as MLPA could be utilized to detect the large fragment variation of the gene [4]. Nonetheless, due to the high homology between *DYP19L2* and its pseudogene, selecting a highly specific probe with a unique sequence matching the specific *DPY19L2* nucleotide to overcome the interference of *DPY19L2* pseudogene is essential [4, 32]. Moreover, any loss of heterozygotes shown in MLPA should be further confirmed by a long-range PCR [4].

Herein study, we found that 180-kbp homozygous deletions (g.63950001–64130000) at 12q14.2 on chromosome 12 in the sample of the patient subjected to CNVseq, including complete loss of *DYP19L2*. Complete gene loss leads to complete loss of *DYP19L2*, which might result in complete loss of *DYP19L2* function; thus, the sperm of the patient presents globozoospermia. Therefore, this study broadens the mutation spectra of *DYP19L2* mutations that cause globozoospermia.

In summary, our findings confirm that mutations in *DYP19L2* are the major causes of globozoospermia in humans. The homozygosity deletion of *DYP19L2* affects the normal development of sperm head, leading to the typical globozoospermia phenotype. Therefore, the current study provides researchers and clinicians with updated information about sperm with globozoospermia.

Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s13039-020-00495-1>.

Additional file 1: Supplementary Table 1. Results of exon mutation test on proband and his parents

Abbreviations

TEM: Transmission electron microscopy; AR: Autosomal recessive; RT-PCR: Reverse transcription-polymerase chain reaction; MLPA: Multiplex ligation-dependent probe amplification; CNVseq: Copy number variation sequencing

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Declarations

The authors declare no competing or financial interests.

Authors' contributions

YZ recruited the family, coordinated the research, and performed genetic analyses. RW and XZ performed molecular experiments and drafted the manuscript. WL and YY performed molecular experiments. ZL and NL designed the study and performed data analyses. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

The patient provided written consent before participation in the study. The Ethics Committee at the First Affiliated Hospital of Xiamen University approved the data for publication.

Consent for publication

The patient provided written consent for publication of their data.

Competing interests

The authors declare that they have no competing interests.

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References

- Krausz C. Male infertility: pathogenesis and clinical diagnosis. *Best Pract Res Clin Endocrinol Metab.* 2011;25(2):271–85.
- O'Flynn O'Brien KL, Varghese AC, Agarwal A. The genetic causes of male factor infertility: a review. *Fertil Steril.* 2010;93(1):1–12.
- Dam AH, Feenstra I, Westphal JR, Ramos L, van Golde RJ, Kremer JA. Globozoospermia revisited. *Hum Reprod Update.* 2007;13(1):63–75.
- Coutton C, Zouari R, Abada F, Ben Khelifa M, Merdassi G, Triki C, Escalier D, Hesters L, Mitchell V, Levy R, et al. MLPA and sequence analysis of *DPY19L2* reveals point mutations causing globozoospermia. *Hum Reprod.* 2012;27(8):2549–58.
- Harbuz R, Zouari R, Pierre V, Ben Khelifa M, Kharouf M, Coutton C, Merdassi G, Abada F, Escoffier J, Nikas Y, et al. A recurrent deletion of *DPY19L2* causes infertility in man by blocking sperm head elongation and acrosome formation. *Am J Hum Genet.* 2011;88(3):351–61.
- De Braekeleer M, Nguyen MH, Morel F, Perrin A. Genetic aspects of monomorphic teratozoospermia: a review. *J Assist Reprod Genet.* 2015;32(4):615–23.
- Li L, Sha YW, Xu X, Mei LB, Qiu PP, Ji ZY, Lin SB, Su ZY, Wang C, Yin C, et al. DNAH6 is a novel candidate gene associated with sperm head anomaly. *Andrologia.* 2018;50(4).
- Dam AH, Kosciński I, Kremer JA, Moutou C, Jaeger AS, Oudakker AR, Tournaye H, Charlet N, Lagier-Tourenne C, van Bokhoven H, et al. Homozygous mutation in *SPATA16* is associated with male infertility in human globozoospermia. *Am J Hum Genet.* 2007;81(4):813–20.
- Kosciński I, Elinati E, Fossard C, Redin C, Muller J, Velez de la Calle J, Schmitt F, Ben Khelifa M, Ray PF, Kilani Z, et al. *DPY19L2* deletion as a major cause of globozoospermia. *Am J Hum Genet.* 2011;88(3):344–50.
- Ellnati E, Kuentz P, Redin C, Jaber S, Vanden Meerschaut F, Makarian J, Kosciński I, Nasr-Esfahani MH, Demiroglu A, Gurgan T, et al. Globozoospermia is mainly due to *DPY19L2* deletion via non-allelic homologous recombination involving two recombination hotspots. *Hum Mol Genet.* 2012;21(16):3695–702.
- Zhu F, Gong F, Lin G, Lu G. *DPY19L2* gene mutations are a major cause of globozoospermia: identification of three novel point mutations. *Mol Hum Reprod.* 2013;19(6):395–404.

12. Coutton C, Abada F, Karaouzene T, Sanlaville D, Satre V, Lunardi J, Jouk PS, Arnoult C, Thierry-Mieg N, Ray PF. 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(3).
13. Ghazavi F, Peymani M, Hashemi MS, Ghaedi K, Nasr-Esfahani MH. Embryos derived from couples with consanguineous marriages with globozoospermia should be screened for gender or DPY19L2 deletion. *Andrologia.* 2019;51(4).
14. Ellnati E, Fossard C, Okutman O, Ghedir H, Ibalá-Romdhane S, Ray PF, Saad A, Hennebicq S, Viville S. A new mutation identified in SPATA16 in two globozoospermic patients. *J Assist Reprod Genet.* 2016;33(6):815–20.
15. Fujihara Y, Oji A, Larasati T, Kojima-Kita K, Ikawa M. Human Globozoospermia-Related Gene Spata16 Is Required for Sperm Formation Revealed by CRISPR/Cas9-Mediated Mouse Models. *Int J Mol Sci.* 2017; 18(10).
16. Xiao N, Kam C, Shen C, Jin WY, Wang JQ, Lee KM, Jiang LW, Xia J. PICK1 deficiency causes male infertility in mice by disrupting acrosome formation. *J Clin Invest.* 2009;119(4):802–12.
17. He J, Xia MY, Tsang WH, Chow KL, Xia J. ICA1L forms BAR-domain complexes with PICK1 and is crucial for acrosome formation in spermiogenesis. *J Cell Sci.* 2015;128(20):3822–36.
18. Li YH, Zhang N, Wang YN, Shen Y, Wang Y. Multiple faces of protein interacting with C kinase 1 (PICK1): structure, function, and diseases. *Neurochem Int.* 2016;98:115–21.
19. Hess H, Heid H, Franke WW. Molecular characterization of mammalian Cylicin, a basic-protein of the sperm head cytoskeleton. *J Cell Biol.* 1993; 122(5):1043–52.
20. Vonbulow M, Heid H, Hess H, Franke WW. Molecular nature of Calicin, a major basic-protein of the mammalian sperm head cytoskeleton. *Exp Cell Res.* 1995;219(2):407–13.
21. Bizkarguenaga M, Gomez-Santos L, Madrid JF, Saez FJ, Alonso E. Increase of germ cell nuclear factor expression in globozoospermic Gopc(–/–) knockout mice. *Andrology.* 2019;7(3):319–28.
22. Kang-Decker N, Mantchev GT, Juneja SC, McNiven MA, van Deursen JM. Lack of acrosome formation in Hrb-deficient mice. *Science.* 2001;294(5546): 1531–3.
23. Juneja SC, van Deursen JM. A mouse model of familial oligoasthenoteratozoospermia. *Hum Reprod.* 2005;20(4):881–93.
24. Xu X, Toselli PA, Russell LD, Seldin DC. Globozoospermia in mice lacking the casein kinase II alpha' catalytic subunit. *Nat Genet.* 1999;23(1):118–21.
25. Audouard C, Christians E. Hsp90beta1 knockout targeted to male germline: a mouse model for globozoospermia. *Fertil Steril.* 2011;95(4):1475–7 e1471–1474.
26. Paiardi C, Pasini ME, Gioria M, Berruti G. Failure of acrosome formation and globozoospermia in the wobbler mouse, a Vps54 spontaneous recessive mutant. *Spermatogenesis.* 2011;1(1):52–62.
27. Doran J, Walters C, Kyle V, Wooding P, Hammett-Burke R, Colledge WH. Mfsd14a (Hiat1) gene disruption causes globozoospermia and infertility in male mice. *Reproduction.* 2016;152(1):91–9.
28. Han F, Liu C, Zhang L, Chen M, Zhou Y, Qin Y, Wang Y, Chen M, Duo S, Cui X, et al. Globozoospermia and lack of acrosome formation in GM130-deficient mice. *Cell Death Dis.* 2017;8(1):e2532.
29. Vicari E, Perdichizzi A, De Palma A, Burrello N, D'Agata R, Calogero AE. Globozoospermia is associated with chromatin structure abnormalities: case report. *Hum Reprod.* 2002;17(8):2128–33.
30. Pierre V, Martinez G, Coutton C, Delaroché J, Yassine S, Novella C, Pernet-Gallay K, Hennebicq S, Ray PF, Arnoult C. Absence of Dpy19l2, a new inner nuclear membrane protein, causes globozoospermia in mice by preventing the anchoring of the acrosome to the nucleus. *Development.* 2012;139(16): 2955–65.
31. Noveski P, Madjunkova S, Maleva I, Sotirovska V, Petanovski Z, Plaseska-Karanfilska D. A homozygous deletion of the Dpy19l2 gene is a cause of Globozoospermia in men from the republic of Macedonia. *Balkan J Med Genet.* 2013;16(1):73–6.
32. Carson AR, Cheung J, Scherer SW. Duplication and relocation of the functional DPY19L2 gene within low copy repeats. *BMC Genomics.* 2006;7:45.

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