

Article

Molecular Analysis of *DPY19L2*, *PICK1* and *SPATA16* in Italian Unrelated Globozoospermic Men

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Abstract: This study aims to evaluate genetic contribution and sperm DNA fragmentation (SDF) in a cohort of 18 unrelated globozoospermic Italian men (Group G). Semen samples were assessed according to the WHO 2010 Laboratory Manual and compared with 31 fertile controls. We focused our genetic analysis on the exons of the main globozoospermia-associated genes, performing qualitative PCR to assess deletion of *DPY19L2* and sequencing to detect mutations of *SPATA16* and *PICK1*. SDF was evaluated using the TUNEL assay. In Group G, 10 patients had a complete form of globozoospermia, whereas 8 patients had a partial form. Molecular analysis revealed deletion of *DPY19L2* in six of the patients, all of them with complete globozoospermia, while no mutations were found in the examined exons of *PICK1* and *SPATA16*. TUNEL analysis showed a higher SDF% in Group G. Our findings confirm *DPY19L2* defects as the most frequent genetic alteration in Italian patients contributing to globozoospermic phenotypes. Furthermore, spermatozoa with acrosomal defects could also display high levels of SDF as a possible consequence of abnormally remodeled chromatin. The possible effect on offspring of chromatin structure abnormalities and altered DNA integrity should be carefully evaluated by clinicians, especially regarding the feasibility and safety of artificial reproductive techniques, which represent the only treatment that allows these patients to conceive.

Keywords: globozoospermia; acrosome; male infertility; genetics; sequencing; sperm DNA fragmentation

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Supplementary Materials:

Table S1. Primers used to amplify the main globozoospermia-associated genes.

Gene	Exons Examined	Primers	Reference
<i>SPATA16</i>	exon 4	F 5'-CCTGTGATTCATTCCATT-3' R 5'-TGTTATGCTATTACCAGAA-3'	[53]
<i>PICK1</i>	exon 13	F 5'-CTCCTGCGTTCCTGAACTG-3' R 5'-CCTCGTGTATCCCTGGACGG-3'	[17]
<i>DPY19L2</i>	exon 1	F 5'-GGCCAACTTCTTTCTACTCGGAC-3' R 5'-ATTTACAGTCGCCATGACG-3'	[21]
	exon 10	F 5'-CCAAAGAGGAGGTACCGTATAA-3' R 5'-GCCATCCATCTTTTTAATTCTG-3'	[21]
	exon 11	F 5'-AACCTCCTCAAGTGACTTAG-3' R 5'-TTGGCCAAGAGTCATT-3'	[21]
	exon 12	F 5'-GAAGGTTAATTGAAGCTAGA-3' R 5'-ATTAGCCTGCAGAAAATGGT-3'	[21]
	exon 20	F 5'-CAGAGGCAACAGGTACGTAT-3' R 5'-ACCCTTAGAACTGTGAAGATTA-3'	[21]
	exon 22	F 5'-CTTTATTATTAGGATATGTCTTTCCC-3' R 5'-TTACCTTTTAGTAATCAGAAAAATTTC-3'	[49]

Table S2. Sperm parameters of globozoospermic patients.

	Semen volume (ml)	Sperm Concentration (10 ⁶ /ml)	Total Sperm Number (10 ⁶ /ejaculate)	Progressive Motility (%)	Abnormal Forms (%)
Globo 1	1.8	55.0	99.0	55	90
Globo 2	3.5	28.0	98.0	35	100
Globo 3	0.9	25.0	22.5	15	100
Globo 4	3.0	58.0	174.0	40	100
Globo 5	2.8	90.0	252.0	40	100
Globo 6	2.5	60.0	150.0	50	80
Globo 7	0.5	140.0	70.0	40	100
Globo 8	3.2	66.0	211.2	40	100
Globo 9	5.0	18.0	90.0	30	90
Globo 10	1.5	40.0	60.0	45	90
Globo 11	2.2	120.0	264.0	45	100
Globo 12	1.0	310.0	310.0	35	100
Globo 13	1.2	20.0	24.0	15	95
Globo 14	3.0	75.0	225.0	35	100
Globo 15	4.2	12.0	50.4	20	100
Globo 16	4.5	120.0	540.0	40	80
Globo 17	2.5	80.0	200.0	35	90
Globo 18	2.5	35.0	87.5	40	88

Table S3. Mean \pm SD, median (in brackets) and significance of the sperm parameters between complete and partial form of globozoospermia (Mann–Whitney U test). Significant *P* values are in bold.

Form of Globozoospermia	Semen Volume (ml)	Sperm Concentration (10 ⁶ /ml)	Total Sperm Number (10 ⁶ /ejaculate)	Progressive Motility (%)	Abnormal Forms (%)
Complete	2.4 \pm 1.2 (2.9)	92.4 \pm 86.7 (70.5)	167.7 \pm 100.6 (192.6)	34.5 \pm 9.6 (37.5)	100.0 \pm 0.0 (100.0)
Partial	2.7 \pm 1.4 (2.5)	53.5 \pm 34.0 (47.5)	156.3 \pm 164.0 (94.5)	38.8 \pm 12.5 (40.0)	87.9 \pm 5.2 (90.0)
<i>P</i> value	0.965	0.315	0.515	0.408	< 0.001

Table S4. Mean \pm SD, median (in brackets) and significance of the sperm parameters between globozoospermic samples of men with and without *DPY19L2* deletions (Mann–Whitney U test). Significant *P* values are in bold.

<i>DPY19L2</i> Deletions	Semen Volume (ml)	Sperm Concentration (10 ⁶ /ml)	Total Sperm Number (10 ⁶ /ejaculate)	Progressive Motility (%)	Abnormal Forms (%)
Present	2.4 \pm 1.5 (2.6)	66.7 \pm 53.9 (51.5)	121.7 \pm 99.1 (84.0)	31.7 \pm 11.7 (35.0)	100.0 \pm 0.0 (100.0)
Absent	2.6 \pm 1.2 (2.5)	79.3 \pm 78.3 (59.0)	183.1 \pm 140.1 (162.0)	38.8 \pm 10.0 (40.0)	91.9 \pm 7.3 (90.0)
<i>P</i> value	0.750	0.820	0.385	0.250	0.024