Genetic aspects of monomorphic teratozoospermia: a review

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Abstract Teratozoospermia is characterized by the presence of spermatozoa with abnormal morphology over 85 % in sperm. When all the spermatozoa display a unique abnormality, teratozoospermia is said to be monomorphic. Two forms of monomorphic teratozoospermia, representing less than 1 % of male infertility, are recognized: macrozoospermia (also called macrocephalic sperm head syndrome) and globozoospermia (also called round-headed sperm syndrome). Macrozoospermia is defined as the presence of a very high percentage of spermatozoa with enlarged head and multiple flagella. Meiotic segregation studies in 30 males revealed that over 90 % of spermatozoa were aneuploid, mainly diploid. Sperm DNA fragmentation studies performed in a few

patients showed an increase in DNA fragmentation index compared to fertile men. Four mutations in the *AURKC* gene, a key player in meiosis and more particularly in spermatogenesis, have been found to be responsible for macrozoospermia. Globozoospermia is characterized by round-headed spermatozoa with an absent acrosome, an aberrant nuclear membrane and midpiece defects. The rate of aneuploidy of various chromosomes in spermatozoa from 26 globozoospermic men was slightly increased compared to fertile men. However, this increase was of the same order as that commonly found in infertile men with altered sperm parameters. The majority of the studies found that globozoospermic males had a sperm DNA fragmentation index higher than in fertile men. Mutations or deletions in three genes, *SPATA16*, *PICK1* and *DPY19L2*, have been shown to be responsible for globozoospermia. Identification of the genetic causes of macrozoospermia and globozoospermia should help refine diagnosis and treatment of these patients, avoiding long and painful treatments. Elucidating the molecular causes of these defects is of utmost importance as intracytoplasmic sperm injection (ICSI) is very disappointing in these two pathologies.

Capsule Two forms of monomorphic teratozoospermia are recognized: macrozoospermia (or macrocephalic sperm head syndrome) and globozoospermia (or round-headed sperm syndrome). Both pathologies are characterized by a high rate of sperm aneuploidy and DNA fragmentation index compared to fertile males. Identification of their genetic causes should help refine diagnosis and treatment of these patients, avoiding long and painful treatments. Elucidating the molecular causes of these defects is of utmost importance as intracytoplasmic sperm injection is very disappointing in these two pathologies.

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Introduction

Infertility is defined as the inability for a couple to conceive a child following two years of unprotected sexual intercourse [1]. Infertility is estimated to affect up to 15 % of couples of reproductive age [2]. The causes of infertility are variable; they can be of male (20 %), female (34 %), mixed (38 %) or even idiopathic (8 %) origins [3].

Causes of infertility of male origin are numerous and multifactorial. Among them, teratozoospermia is characterized by the presence of spermatozoa with abnormal morphology over 85 % in sperm. To be considered as morphologically normal, a spermatozoon should have a normal acrosome, an oval head between 5 and 6 μm long and 2.5 and 3.5 μm width, a midpiece of 4.0 to 5.0 μm and a tail or flagellum about 50 μm long [4].

Teratozoospermia can be subdivided into 2 categories. An ejaculate presenting an excess of spermatozoa with more than one type of abnormality is considered polymorphic teratozoospermia. When all the spermatozoa display a unique abnormality, teratozoospermia is said to be monomorphic. Two forms of monomorphic teratozoospermia are recognized: macrozoospermia (also called macrocephalic sperm head syndrome) and globozoospermia (also called round-headed sperm syndrome) [5, 6]. They are the subject of this review.

Macrozoospermia

Macrozoospermia is a very rare morphologic disorder of spermatozoa observed in less than 1 % of infertile men. It can be defined as the presence of a very high percentage of spermatozoa with enlarged head, an irregular head shape, and multiple flagella (Fig. 1a) [7].

Meiotic segregation studies

Using fluorescent in situ hybridization (FISH), several investigators found no association between the frequency of morphologically and chromosomally abnormal sperm [8, 9], except in macrozoospermia [5]. In 1996, Yurov et al. found in an

infertile man that 40 % of his spermatozoa were large headed. They found that the majority of these macrocephalic spermatozoa contained a diploid chromosomal content, whereas the majority of normal-sized spermatozoa had a haploid content [10]. Since that publication, detailed meiotic segregation analysis using FISH has been reported in 30 males with large-headed, multiple-tailed sperm (Table 1). A strong correlation was found between the rate of sperm macrocephalic forms and the rate of aneuploidy ($R=0.88$, $p<0.001$).

Dieterich et al. (2007) performed FISH for chromosomes X,Y and 18 on a total of 3689 spermatozoa from 5 patients. Only 8 % of the analyzed spermatozoa were haploid while some 25 % were diploid [11]. Brahem et al. (2011) studied the semen samples of 12 males with macrocephalic sperm head syndrome. Triple color FISH analysis showed a mean total sperm aneuploidy rate of 98.33 % in the macrozoospermia group, including a mean rate of 22.7, 25.3 and 18.3 % of sperm nuclei with diploidy, triploidy and tetraploidy, respectively [12]. Altogether, the meiotic segregation analysis shows that more than 90 % of the sperm heads have an aneuploid composition (Fig. 1b).

Guthausen et al. (2006) found that only very low proportions of normal headed-size spermatozoa had a normal chromosomal content (X18 or Y18). Therefore, low fertilization and pregnancy rates may be due to the high incidence of aneuploidy in the apparently normal-sized spermatozoa that may be used for intracytoplasmic sperm injection (ICSI) [13].

Sperm DNA fragmentation studies

Two studies evaluated the presence of apoptosis-related DNA strand breaks in spermatozoa using the terminal deoxynucleotidyl transferase-mediated deoxyuridine

Fig. 1 Macrozoospermia. **a** May Grünwald-Giemsa staining showing spermatozoa with enlarged head and multiple flagella. **b** Fluorescent in situ hybridization (FISH) showing a triploid sperm (probes: 13q14 in green, 21q22 in red) and a tetraploid sperm (probes: CEPX in green, CEPY in red, 18q11.1-2 in aqua). **c** TUNEL assay showing a sperm head with fragmented DNA (left side) and a normal sperm head (right side)

Table 1 Meiotic segregation analysis using FISH in 30 males with macrozoospermia

Reference	Nr sperm	Macrocephalic forms	Multiple flagella	Abnormal acrosome	Diploidy	Triploidy	Tetraploidy	Aneuploidy
[14]	>500	78	51	94	29.6	24.7	21.9	94
[14]	>500	74	30	84	16.8	19.1	16.7	93.7
[14]	>500	87	47	96	18.5	24.5	21	95.2
[14]	>500	60	37	65	21	10.7	11.8	91.7
[14]	>500	95	70	96	22.1	24.3	15.1	98.7
[14]	>500	79	50	85	15.4	8.9	11.1	92.7
[14]	>500	98	30	98	20.2	25.4	17.8	99.3
[14]	>500	98	64	99	30	27	19.3	100
[54]	68	76	76	77	32.5	26.5	3	100
[54]	50	60	49	72	4.5	2	1	85.5
[54]	200	54	50	80	18	12	4	76
[13]	130	91	NA	NA	50	NA	NA	100
[13]	314	82	NA	NA	23	NA	NA	100
[55]	500	100	NA	NA	40	24	5.1	98.4
[56]	1148	50	72	63	21.6	62.4	13.3	NA
[57]	2948	100	30	NA	80.4	14.8	4.4	99.6
[57]	1645	>95	NA	>95	71.3	16	12.7	100
[57]	473	100	NA	NA	58.8	23.2	17.8	99.8
[58]	102	100	100	NA	9.8	16.7	17.6	91.2
[59]	1430	29.5	NA	NA	17.1	NA	3.0	51.4
[59]	2811	22	NA	NA	10.2	NA	1.9	43.6
[59]	1955	49.7	NA	NA	18.1	NA	12.7	71.7
[59]	3981	19	NA	NA	16.5	NA	0.07	25.6
[60]	500	70	NA	NA	19.4	10.2	NA	99.2
[8]	1656	64	28	52	25.1	2.2	NA	89.2
[5]	1124	100	100	NA	18.42	6.14	33.99	99.29
[15]	1010	78	30	NA	0.99	18.12	44.55	99.9
[15]	2522	83	27	NA	4.52	9.99	35.65	99.92
[15]	576	72	10	NA	3.47	18.23	43.23	100
[15]	1103	80	47	NA	2.9	11.97	71.08	100

Some frequencies were recalculated from the raw data

triphosphate nick-end labelling (TUNEL) assay. Brahem et al. (2011) calculated the DNA fragmentation index to be included between 33 and 75 % in 8 males with macrozoospermia while it was less than 10 % in controls [14]. In a study published by Perrin et al. (2011) on three patients, the proportion of spermatozoa with DNA fragmentation varied from 4.4 to 28 %, the mean DNA fragmentation percentage among the control men being 1.20 ± 0.95 % (Fig. 1c) [15]. In all patients, the rate of DNA fragmentation was significantly higher than in their respective control groups.

Molecular genetic studies

Dieterich et al. (2007) carried out a genome-wide microsatellite scan of ten infertile men (4 French citizens of North African descent and 6 from the Rabat region in Morocco) with a

large-headed sperm phenotype. Subsequently, they added 4 more males from Rabat [11]. They identified a small region of homozygosity at 19qter. As the aurora kinase C (*AURKC*) gene is located close to that region, they sequenced the coding sequence and detected a homozygous cytosine deletion in exon 3 (c.144delC) in all 14 individuals (Fig. 2). This mutation leads to the premature insertion of a stop codon, inducing the production of a non functional and truncated protein lacking its kinase domain [11].

Harbuz et al. (2009) analyzed 34 patients with macrozoospermia. The *AURKC* c.144delC mutation was found in a homozygous state in 32 patients. Two brothers had a compound genotype made of the c.144delC mutation and a new mutation in exon 6, c.686G.A (p.C229Y). They estimated the heterozygote rate to be 1 in 50 in the Maghreban population [16, 17].

Fig. 2 Schematic diagram of the *AURKC* and *DPY19L2* genes and localization of the mutations thus far identified. *Double-headed arrows* represent intragenic deletions; *yellow boxes* represent missense mutations; *blue boxes* represent nonsense mutations; *red boxes* represent splice-site mutations

Ben Khelifa et al. (2011) described a new mutation in *AURKC* in two brothers of Tunisian descent. They were compound heterozygotes for the c.144delC mutation and the c.436-2A.G mutation. This later mutation is located in the acceptor consensus splice site of exon 5 and leads to the skipping of exon 5. As a consequence, the mutant protein lacks the 50 amino acids coded by exon 5 (amino acids 146–195). These amino acids are localized in the middle of the catalytic domain and their absence is therefore very likely to severely hamper the functionality of the protein [18].

Ben Khelifa et al. (2012) genotyped 44 macrozoospermic males of European and North African origins. They identified a new nonsense mutation (c.744C.G, p.Y248X) in 11 males (9 homozygotes and 2 compound heterozygotes). Altogether, this group analyzed 83 probands. A mutation was identified in 68 out of these probands (82 %). The c.144delC and c.744C.G mutations represented 85.5 and 13 % of the mutated alleles respectively [19].

Eloualid et al. (2014) screened 326 infertile Moroccan patients for the presence of the *AURKC* c.144delC mutation. This mutation was detected in homozygous (4/326–1.23 %) and heterozygous (6/326–1.84 %) states, with frequencies of 1.23 and 1.84 %, respectively. Two homozygotes had azoospermia and the other 2 macrocephalic and multiflagellar spermatozoa. Eight heterozygous individuals carrying the c.144delC mutation were identified among 459 control fertile individuals (1.74 %) [20]. All 11 Moroccan males with a rate of macrocephalic spermatozoa representing at least 70 % of the total sperm concentration reported by El Kerch et al. (2011) were homozygous for the c.144delC mutation [21].

The *AURKC* gene, located at 19q13.43 has 7 coding exons and encodes a 309 amino acids protein that is a member of the Aurora subfamily of serine/threonine protein kinases [22]. It is a component of the chromosomal passenger complex (CPC), a complex that acts as a key regulator of mitosis. The CPC

complex has essential functions at the centromere in ensuring correct chromosome alignment and segregation and is required for chromatin-induced microtubule stabilization and spindle assembly. It plays also a role in meiosis and more particularly in spermatogenesis [23–25].

Globozoospermia

Globozoospermia is also a very rare condition, observed in less than 0.1 % of infertile males. It is characterized by round-headed spermatozoa with an absent acrosome, an aberrant nuclear membrane and midpiece defects [26]. Lack of acrosome, which production is a postmeiotic event in spermatogenesis, and round sperm head are its main characteristics [27]. The acrosomeless spermatozoon is unable to go through the zona pellucida and fuse with the oolemma of the oocyte and fertilization failures have been attributed to a deficiency in oocyte activation capacity, even when ICSI is attempted [28–30].

Meiotic segregation studies

Several workers have investigated the rate of aneuploidy of various chromosomes in spermatozoa from 26 globozoospermic men using FISH [6] (Table 2). The slightly increased aneuploidy rate observed in some patients could reflect disturbances in spermatogenesis, as commonly observed in patients with oligoastheno-teratozoospermia.

Brahem et al. (2011) compared the sperm aneuploid rate between patients with polymorphic teratozoospermia, globozoospermia, macrozoospermia and healthy fertile men [12]. They found a statistically significant increase in the rate of aneuploidy between all three teratozoospermic groups compared to the fertile group ($P < 0.001$). However, no significant

Table 2 Sperm aneuploidy rates in globozoospermic males reported in the literature

References	Patient	Disomy (%)														Diploidy (%)	Nr spz
		1	7	8	9	12	13	15	16	17	18	21	X	Y	XY		
[35]	Sib 1						0.7				0	0.6	0.6	0	12.1 *		±5000
[35]	Sib 2						0.3				0.2	3.0 *	0.4	0	0		±5000
[8]		0											0.5	0.2	0.4	0.1	3716
[61]	Sib 1						0.4	4.03 *			0.74	0.4	0.43	0.52			10,000
[61]	Sib 2						0.32	0.58			0.74	0.14	0.58	0.6			10,000
[62]			0			0					0		0.13	0	0		3885
[63]		2.0 for chromosomes 13, 18, 21, X and Y															1000
[64]		0.09						0.13				0.19	0.12	0.07	0.18 *	0.21	30,145
[65]	Pat 1		0.26		0.2		0.39				0.02	0.39	0	0.04	0.15	0.88 *	10,719
[65]	Pat 2		0.1		0.14		0.08				0.04	0.06	0.02	0.08	0.08	0.3	15,152
[66]							4.0 *		5.0 *			0.1 *				1.0	100
[67]	Pat 1										0.052			0.364 *		0.599 *	7388
[67]	Pat 2										0.078			0.364 *		0.494 *	
[68]							NS				NS	NS		0.66 *		NS	NA
[69]		Aneuploidy rate not higher in globozoospermic cells															NA
[70]								2.3	1.3	1.3			0.5	0.5			400
[71]	Pat 1			1.2 *		0.09					0.12		0.2	1.8 *	1.2 *	0.15	±500
[71]	Pat 2			2.0 *		0.15					0.15		0	2.75 *	3.0 *	2.0 *	±500
[15]							0.08				0.12	1.11 *	0.27 *	0.23 *	0.35 *	0.13	10,165
[72]		<1.0 for chromosomes 13, 18, 21, X and Y															500
[73]	Pat 1	1.06 for chromosomes 18, X and Y (NS)															658
[73]	Pat 2	1.18 for chromosomes 18, X and Y (NS)															1019
[73]	Pat 3	1.90 for chromosomes 18, X and Y (NS)															948
[73]	Pat 4	0.9 for chromosomes 18, X and Y (NS)															1000
[73]	Pat 5	0.8 for chromosomes 18, X and Y (NS)															963
[73]	Pat 6	0.9 for chromosomes 18, X and Y (NS)															950

* $p < 0.05$ when compared to controlsNS not significant ($p > 0.05$)

difference in sperm aneuploidy was found between globozoospermia and polymorphic teratozoospermia ($p > 0.05$).

In fact, variations found in the studies presented here are of the same order as those that are commonly found in the literature about infertile men with a normal karyotype but altered sperm parameters [15, 31].

Sperm DNA fragmentation studies

Several techniques are currently used to measure the sperm DNA integrity in the investigation of male fertility [32]. However, the most widely used technique is the TUNEL assay [32]. The DNA fragmentation index (DFI) was calculated in a few globozoospermic patients [6] (Table 3). The majority of the studies, although in a limited number, found that globozoospermic males had a sperm DNA fragmentation index statistically significantly higher than in fertile men.

Brahem et al. (2011) compared the rate of sperm DNA fragmentation between patients with polymorphic teratozoospermia, globozoospermia and healthy fertile men [12]. They found a statistically significant increase in the rate of sperm DNA fragmentation between the globozoospermia and the fertile groups (40.00 %±3.55 % versus 8.90 %±1.3 %) ($P < 0.01$). However, no significant difference in sperm DNA fragmentation was found between globozoospermia and polymorphic teratozoospermia (40.00 %±3.55 % versus 45.33 %±10.02 %) ($p > 0.05$).

These results suggested that sperm of globozoospermic males could carry abnormal remodeled chromatin, which could be a possible source of DNA fragmentation. Indeed, some workers reported abnormal chromatin condensation in globozoospermia, with a high heterogeneity in the degree of maturity [33], due to an altered replacement of histones by protamines [34, 35].

Table 3 Sperm DNA fragmentation rates in globozoospermic males reported in the literature

References	Number of patients	Number of spermatozoa	DNA fragmentation index		P value
			Patient	Control	
[74]	NA	NA	10 %	0.1 %	
[75]	1	NA	13 % ^a		NS
[76]	1	NA	37 %	22.5 %	$P<0.005$
[68]	1	NA	45.7 % ^b	30 %	
[77]	1	NA	97.1 % ^c	41.3 %	$P<0.05$
[70]	1	200	80 %	27±13 %	
[71]	1	500	40 %	12±2.12 %	$P<0.001$
[71]	1	500	80 %	12±2.12 %	$P<0.001$
[72]	1	500	6 %		NS
[15]	1	500	9.6 %	1.2 %	$P<0.001$
NA not available	[73]	700	18 %	^d	
^a SCSA and COMET assays	[73]	1000	29 %	^d	
^b sperm chromatin disperse test (Halosperm technique)	[73]	950	19 %	^d	
^c acridine orange test	[73]	1000	10 %	^d	
^d Normal values<13 %	[73]	960	2 %	^d	
NS not significant ($p>0.05$)	[73]	950	15 %	^d	

Molecular genetic studies

The presence of consanguineous marriages in families affected with globozoospermia and reports of two or more sibs in several families suggested a genetic contribution to globozoospermia in humans with an autosomal recessive mode of inheritance [6, 35–40]. Three genes segregating on an autosomal recessive mode have now been identified to be associated with globozoospermia in humans.

Dam et al. (2007) identified a homozygous mutation (c.848G.A) in exon 4 of the *SPATA16* (spermatogenesis-associated protein 16) gene (located at chromosome band 3q26.32), leading to the substitution of an arginine to a glutamine at residue 283 (R283Q) in the protein, in three brothers affected with globozoospermia from a consanguineous Ashkenazi Jewish family [39].

Liu et al. (2010) identified a homozygous G→A transition at nucleotide 1567 in exon 13 of the *PICK1* (protein interacting with PRKCA 1) gene (located at chromosome band 22q12.3-q13.2), generating a missense substitution (G393R) in the protein, in one patient [40].

These two genes could be involved in the same process of spermiogenesis. Indeed, the *SPATA16* protein localizes to the Golgi apparatus and the proacrosomal granules that are transported in the acrosome in round and elongated spermataids [41]. *Pick1* localizes to Golgi-derived proacrosomal granules and is involved in vesicle trafficking from the Golgi to the acrosome and participates in acrosome biogenesis [42].

In 2011, Kosciński et al. identified a homologous deletion of the *DPY19L2* (dpy-19-like 2) gene located at 12q14.2 in 4

globozoospermic brothers of a Jordanian consanguineous family and in three additional unrelated patients [43]. Subsequently, a whole genome SNP scan on 20 patients presenting with total globozoospermia allowed the identification of a 200 kb homozygous deletion encompassing only *DPY19L2* in 15 patients of different ethnic background [44]. More patients with globozoospermia associated with a homozygous deletion of the whole *DPY19L2* were reported [45–48]. The mechanism underlying this deletion is due to a non-allelic homologous recombination (NAHR) between two low copy repeats that share 96.5 % identity and flank the *DPY19L2* locus [43].

However, it appears very quickly that other genetic defects in the *DPY19L2* gene could be responsible for globozoospermia (Fig. 2). At present, three intragenic deletions have been described [45]. One patient had a deletion of exons 5, 6 and 7 inducing an aberrant splicing between exons 4 and 8 that would give rise to a protein with a deletion of 91 amino acids. Two other patients had a deletion of exons 5 and 6 inducing an aberrant splicing between exons 4 and 7 that would give rise to a frame shift, introducing a premature stop codon; it should be noted that the breakpoints location in these two patients was different.

The other genetic defects consisted in mutations [45, 47, 48]. A donor splice-site mutation in intron 11 (c.1218+1G.A) was identified in one patient. Six mutations, located in exons 9, 11, 15, 18 and 21, introduced a premature stop codon; two of these mutations (c.1183delT and c.1532delA) were identified in two unrelated patients each. Five point mutations, including one (c.869G.A) found in three unrelated males, lead to

an amino acid change; they were distributed in exons 8, 9, 10 and 15. Altogether, 10 of the 12 mutations thus far identified clustered between exons 5 and 15 (Fig. 2).

The *DPY19L2* gene belongs to a family of genes derived from the *DPY-19* gene, present in *Caenorhabditis elegans*, that is involved in the establishment of cell polarity in the worm. This family of proteins has no obvious homology with any other membrane proteins and represents a new family of integral membrane proteins [49]. The *DPY19L2* gene has 22 coding exons [50] and the resulting protein 758 amino acids. It might be involved in indicating the anterior pole of the spermatozoon and in the acroplaxome positioning, a subacrosomal cytoskeletal plate toward which Golgi-derived vesicles fuse [51].

The DPY19L2 protein is thought to be a transmembrane protein with 10 putative transmembrane segments with the N- and C-terminal domains located in the nucleoplasm. It is expressed specifically in spermatids and localized only in the inner nuclear membrane of the nucleus facing the acrosome. Dpy19l2 participates in the anchoring of the acrosome to the nucleus, bridging the nuclear envelope to both the nuclear dense lamina and the acroplaxome [44].

Conclusions

It is thought that between 1500 and 2000 genes are involved in the control of spermatogenesis. Therefore, it is expected that genetic alterations in these genes would disturb male fertility [52]. Furthermore, a novel mechanism of posttranscriptional control mediated by microRNAs has lately emerged as an important regulator of spermatogenesis [53]. Development of next generation sequencing is likely to identify new genes involved in male infertility.

Although monomorphic teratozoospermia is very rare, macrozoospermia and globozoospermia represent a brand new avenue for the search of genes involved in spermatogenesis. Identification of the genetic causes should help refine diagnosis and treatment of these patients, avoiding long and painful treatments. Elucidating the molecular causes of these defects is of utmost importance as ICSI is very disappointing in these two pathologies.

Conflict of interest The authors declare that they have no conflict of interest

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