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Abnormal retention of nuclear lamina and disorganization of chromatin-related proteins in spermatozoa from DPY19L2-deleted globozoospermic patients

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HAL Authorization

1 Short title: Abnormal NL in spermatozoa from globozoospermic patients

2 3 4 Abnormal retention of nuclear lamina and 5 6 disorganization of chromatin-related 7 8 proteins in spermatozoa from *DPY19L2*- 9 10 deleted globozoospermic patients

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21

22 Abstract

23
24 The aim of this study was to characterize the nuclear lamina (NL) and
25 lamin chromatin-partners in spermatozoa from four *DPY19L2*-deleted
26 globozoospermic patients. We tested for spermatid transcripts encoding
27 lamins and their chromatin-partners emerin, LAP2 α , BAF and BAF-L, by
28 reverse transcriptase-PCR using spermatozoa RNA. We also determined
29 the localization of lamin B1, BAF and BAF-L by immunofluorescent analysis
30 of spermatozoa from all patients. In RNA from globozoospermic and
31 control spermatozoa we detected transcripts encoding lamin B1, lamin B3,
32 emerin, LAP2 α and BAF-L, but not A-type lamins. In contrast, BAF
33 transcripts were detected in globozoospermic but not control
34 spermatozoa. The NL was immature in human globozoospermic
35 spermatozoa: lamin B1 signal was detected in the nuclei of
36 globozoospermic spermatozoa in significantly higher proportions than the
37 control ($P < 0.05$; 56–91% versus 40%) and was predominantly observed
38 at the whole nuclear periphery, not polarized as in control spermatozoa.
39 Conversely, BAF and BAF-L were detected in control, but not
40 globozoospermic spermatozoa. Our results strongly emphasize the
41 importance of the NL and associated proteins during human
42 spermiogenesis. In globozoospermia, the lack of maturation of the NL, and
43 the modifications in expression and location of chromatin-partners, could
44 explain the chromatin defects observed in this rare phenotype.
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47 *Keywords*: BAF, BAF-L, globozoospermia, lamin, nuclear lamina, spermiogenesis
48

49 Key message

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51 In spermatozoa from *DPY19L2*-deleted globozoospermic patients, the
52 nuclear lamina (NL) is abnormally retained at the nuclear periphery, and
53 the expression and location of BAF and BAF-L are modified. These
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1 alterations may contribute to the chromatin defects observed in this rare
2 phenotype. Our results support important roles for the NL, BAF and BAF-L
3 during human spermiogenesis.
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5 Introduction

6
7 The nuclear lamina (NL), a component of the nuclear envelope, is a
8 filamentous protein network situated between the inner nuclear
9 membrane and the chromatin. The NL is mainly composed of lamins,
10 intermediate filament proteins (type V) essential for the structure, shape,
11 assembly and mechanical stability of the nucleus. The NL is involved in a
12 wide spectrum of biochemical interactions between lamins, chromatin and
13 a variety of partners: the lamin-binding proteins (Cau *et al.*, 2014). It has
14 been shown that the NL is connected to the cytoskeleton and the
15 centrosome (via SUN-domain proteins) and to the chromatin (via LEM
16 domain proteins and the lamin B receptor). Moreover, it has been shown
17 that LEM domain proteins connect the NL to the chromatin by binding a
18 conserved metazoan chromatin protein called barrier to autointegration
19 factor (BAF), encoded by the *BANF1* gene, which can bridge DNA and
20 interacts with histones (Schirmer and Foisner, 2007; Schirmer and
21 Gerace, 2005; Wilson and Foisner, 2010). In mammals, the NL is mainly
22 composed of A-type or B-type lamins. A-type lamins are encoded by the
23 LMNA gene, which produces four transcripts by alternative splicing
24 including two widely expressed major A-type lamins: lamin A and lamin C,
25 and two other isoforms with more restricted expression: A Δ 10 in tumoral
26 cells and C2 in meiotic male germ cells (Furukawa *et al.*, 1994; Machiels
27 *et al.*, 1996; Nakajima and Abe, 1995). There are two distinct genes that
28 encode three B-type lamins: LMNB1 encodes lamin B1 while LMNB2
29 encodes lamin B2 and the spermatid-specific isoform lamin B3 (Dechat *et al.*,
30 2000; Elkhatib *et al.*, 2015; Furukawa and Hotta, 1993; Vester *et al.*,
31 1993).
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34 During spermiogenesis, the post-meiotic and final phase of
35 spermatogenesis, the spermatid nucleus is subjected to a unique
36 remodelling process including an extreme compaction of the chromatin
37 orchestrated by dynamic interactions between the NE and the manchette
38 (Kierszenbaum and Tres, 2004). In humans, we recently showed that the
39 human spermatid NL is devoid of A-type lamins and composed only of B-
40 type lamins (Elkhatib *et al.*, 2015). Furthermore, the localization of lamin
41 B1 and B2/B3 changes during spermiogenesis with a progressive
42 polarization to the posterior pole of the elongating spermatids, suggesting
43 that B-type lamins play a central role in the intense remodelling of the
44 spermatid nucleus.
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46 Human globozoospermia is a rare monomorphic teratozoospermia first
47 described over 40 years ago which affects less than 0.1% of infertile men
48 and is characterized by malformed round sperm heads without an
49 acrosome (Baccetti *et al.*, 1977; Dam *et al.* 2007a; Holstein *et al.*, 1973;
50 Pedersen and Rebbe, 1974; Perrin *et al.*, 2013; Schirren *et al.*, 1971;
51 Singh, 1992). Recent studies have shown that most cases have an
52 autosomal recessive genetic cause, and mutations in two genes have been
53 independently linked to the occurrence of human globozoospermia. The
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1 most frequent are homozygous deletions of *DPY19L2*, a gene encoding an
2 uncharacterized protein localized to the inner nuclear membrane, which
3 explain more than 80% of globozoospermia cases (Carson *et al.*, 2006;
4 Harbuz *et al.*, 2011; Kosciński *et al.*, 2011). In the mouse, knockout of
5 *Dpy19l2* leads to the destabilization of the NL, with persistence of lamin
6 B1 throughout the nuclear periphery in round spermatids, and a failure of
7 sperm nuclear shaping and loss of the unbound acrosomal vesicle (Pierre
8 *et al.*, 2012), a phenotype identical to that found in men deleted for
9 *DPY19L2*. Homozygosity for two different loss-of-function variants in
10 SPATA16 (spermatogenesis-associated 16) have been found in three
11 unrelated cases of globozoospermia (Dam *et al.*, 2007b; Elinati *et al.*,
12 2016; Lu *et al.*, 2006; Xu *et al.*, 2003).

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15 In the present study, we set out to characterize the NL structure in
16 spermatozoa from four *DPY19L2*-globozoospermic patients, by
17 investigating the expression pattern of A-type and B-types lamins and
18 some indirect chromatin-partners, and their localization in spermatozoa.
19 We found that the pattern of transcript retention in globozoospermic and
20 control spermatozoa is completely different for the BAF transcripts.
21 Moreover, the structure of the NL, and the NL-associated proteins present,
22 are radically different in spermatozoa from patients with globozoospermia.
23 The potential links between NL modification and the chromatin defects
24 observed in globozoospermia are discussed.

25 26 **Materials and methods**

27 28 **Patients**

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30 Sperm samples were obtained from four patients with characteristic
31 globozoospermia diagnosed by semen analysis between December 2013
32 and May 2014 (Germetheque number G1: R011300036, G2: R111102725,
33 G3: R01140016, G4: R011400019). All patients consulted for primary
34 couple infertility ranging from 1 to 4 years. The age of patients ranged
35 from 30 to 40 years. Two patients were from Turkey with consanguinity in
36 the family (**Supplementary Figure 1**). Two other patients were from
37 Western Europe, with no known consanguinity. The control was a patient
38 with normal sperm analysis who consulted for secondary infertility
39 (Germetheque number R010900544). All experiments described here
40 were previously performed on many fertile controls (Elkhatib *et al.*, 2015).

41 42 **Patients and semen**

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44 Semen was collected at our academic **Center of Reproductive Medicine** by
45 masturbation after 2–6 days of sexual abstinence. After liquefaction,
46 semen analysis and cryopreservation was performed as previously
47 described (Elkhatib *et al.*, 2015). All patients and the control gave an
48 informed consent for the conservation of the remnant sperm in the
49 Germetheque biobank and their use in studies on human fertility in
50 accordance with the Helsinki Declaration of 1975 on human
51 experimentation. The Germetheque Scientific Committee approved the
52 present study design (reference 2013-MRS01 approval on 5 February
53 2013). For all patients with globozoospermia from Marseille, DNA was
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1 stored in the labelled Biological Resource Centre (CRB TAC), Department
2 of Medical Genetics, Timone Hospital of Marseille and was sent to
3 Grenoble for DPY19L2 genotyping (Harbuz *et al.*, 2011). All patients were
4 DPY19L2-deleted, with three homozygous and one heterozygous deletion.
5 Patient characteristics and sperm parameters according to WHO criteria
6 (WHO, 2010) are reported in **Supplementary Table 1**. Intracytoplasmic
7 sperm injection (ICSI) was performed for the four DPY19L2-deleted
8 globozoospermic patients. Following eight stimulation cycles, pregnancy
9 was achieved for two couples and resulted in the birth of a total of three
10 healthy boys.

11 Spermatozoa RNA extraction, RT-PCR and quality control of spermatozoa 12 RNA extracts

13 Following storage in liquid nitrogen, sample straws were thawed and
14 prepared as previously reported (Elkhatib *et al.*, 2015). Ten million
15 spermatozoa were used for each RNA extraction, leading to 880–3600 ng
16 of RNA. For each patient and control, we checked for the presence of
17 spermatid RNA, for the lack of contaminating round cell RNA, and for early
18 testicular germ cells and epithelial cells (Lambard *et al.*, 2004). The
19 positive control was total RNA extracted from human testis (636533
20 Clontech). Lamins and direct or indirect partners such as emerlin, Lap2 α ,
21 BAF and BAF-L transcripts were amplified with Q5 Taq polymerase (New
22 England Biolabs), as recommended by the manufacturer. The primer pairs
23 and their annealing temperature for each transcript are as follows: lamin
24 A/C (123 bp): o4683/o4384 – 70°C, lamin C2 (100 bp): o4686/o4687 –
25 67°C, lamin B1 (110 bp): o4479/o4480 – 66°C, lamin B2 (500 bp):
26 o4369/o4370 – 72°C, lamin B3 (300 bp): o3972/o4370 – 72°C, emerlin
27 (157 pdb): o5097/o5098 – 69°C, LAP2 α (176 pdb): o4087/o4088 – 64°C,
28 BAF (167 pdb): o4094/4095 – 69°C, BAF-L (160 pdb): o4096/o4097. The
29 Q5 GC-enhancer additive was used for all PCRs except for lamin B1,
30 LAP2 α and BAF. (Sequences of primers are provided in **Supplementary
31 Table 2**.)

32 Aniline blue

33 Semen samples were washed twice in PBS 1 \times , and prepared as previously
34 described (Yassine *et al.*, 2015). For each patient and control, 200
35 spermatozoa were then analysed using a transmitted light microscope and
36 a 100 \times objective with oil.

37 Immunocytochemistry

38 Thawed spermatozoa were spread onto polylysine-coated slides by
39 cytospin (Shandon) and fixed as previously reported (Elkhatib *et al.*,
40 2015). We used lectins (Lectin PNA Alexa Fluor 594 conjugates, L-21409
41 Molecular Probes) at a dilution of 1:600 to mark the acrosomes. The
42 antibodies and the dilutions used were rabbit anti-lamin B1 (1:100,
43 ab16048 Abcam), rabbit anti-BAF-L (1:100, HPA042635 Sigma), rabbit
44 anti-BAF (1:600, ab129184 Abcam), rabbit anti-SPACA1 (1:100,
45 HPA026744 Sigma) and anti-alpha-tubulin mouse monoclonal (1:200,
46 T6074 Sigma). After two washes in PBS, detection was performed using
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1 Alexa Fluor 488 goat anti-rabbit IgG (1:400, Invitrogen) or cyanine-3 fluor
2 596 goat anti-mouse IgG (1:2000 ab6946 Abcam) for 1 h at 37°C. For co-
3 immunofluorescence slides were rinsed in PBS and incubated with the
4 second primary antibody followed by a second detection step. After
5 washes in PBS, slides were mounted with 25–75 ng/ml 4(6-diamidino-2-
6 phenylindole) (DAPI) in Vectashield mounting medium for microscope
7 analysis. The slides were analysed using the Zeiss ApoTome 2 microscope
8 (Zeiss, Oberkochen, Germany) equipped with an AxioCam MRm camera.
9 Images were captured and merged with the ZEN software, and were
10 treated using ImageJ software. For each patient and control, 300
11 spermatozoa were analysed in duplicate or triplicate. All antibodies were
12 tested in individual staining reactions for their specificity and performance.
13 Controls without primary antibodies were all negative. We did not use pre-
14 immune serum as a negative control, which represents a limitation of the
15 study, as normal serum contains an antibody which reacts with the
16 acrosome. However, our study is focused on globozoospermia that is
17 characterized by the lack of acrosome, and the anti-lamin B1, anti-BAF
18 and anti-BAF-L antibodies used in our study never label the acrosome on
19 control spermatozoa.
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21 Results

22 Transcripts for lamin B1, lamin B3, emerin, LAP2 α , BAF and BAF-L are 23 present in spermatozoa from patients with globozoospermia

24 Human spermatozoa can be used to identify transcripts present in
25 spermatids (Sendler *et al.*, 2013). In order to study expression of the
26 known human lamins during globozoospermic spermiogenesis, we
27 performed reverse transcriptase-PCR on RNA extracted from purified
28 spermatozoa from four men with globozoospermia and one normospermic
29 fertile man. For all patients with globozoospermia, we detected a
30 transcript for lamin B1 and lamin B3, but no transcript for lamin B2 or the
31 A-type lamins (A/C and C2). In addition, we detected a transcript for
32 emerin, LAP2 α and BAF-L in spermatozoa RNA from each patient. The
33 same results were obtained for the normospermic control, as previously
34 reported for A-type and B-type lamins (Elkhatib *et al.*, 2015). In contrast,
35 BAF transcripts were not detected in the fertile control but were clearly
36 present in spermatozoa from the four globozoospermic patients (**Figure**
37 **1**).

38 Abnormal localization of lamin B1 in spermatozoa from patients with 39 globozoospermia

40 To determine the expression pattern and cellular localization of lamin B1
41 during human spermiogenesis in patients with globozoospermia, we
42 performed co-immunofluorescence analysis using antibodies specific for
43 lamin B1 in association with antibodies directed against alpha-tubulin
44 (cytoskeletal component) to visualize the posterior pole of the nucleus in
45 the absence of the acrosome. The percentages of spermatozoa with a
46 lamin B1 signal ranged from 56% to 91% in patients, and was 40% in the
47 normospermic control ($P < 0.05$). We previously described a lamin B1
48 signal in 33–47% of spermatozoa on fertile controls, present exclusively
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1 at the posterior pole of the nucleus (Elkhatib *et al.*, 2015). We noted that
2 in each patient, a variety of cellular localizations were observed. Indeed,
3 the lamin B1 signal was present either around the whole nucleus without
4 polarization (44–69% of labelled spermatozoa), at the posterior pole of
5 the nuclei (15–38% of labelled spermatozoa), or as a discontinuous signal
6 at the nuclear periphery (2–28% of labelled spermatozoa) (**Figures 2** and
7 **3**). These results demonstrate that the remodelling of the spermatid NL
8 that occurs during normal spermiogenesis is severely compromised in the
9 globozoospermic patients.

12 Localization of acrosomal component SPACA1 in spermatozoa from patients 13 with globozoospermia

14 One of the characteristic features of globozoospermia is the malformation
15 or loss of the acrosome (Pierre *et al.*, 2012). SPACA1 (sperm acrosome
16 associated 1) is a membrane protein that localizes to the equatorial
17 segment of spermatozoa in mammals and is reported to function in
18 sperm–egg fusion (Fujihara *et al.*, 2012). In order to visualize acrosomal
19 structures in globozoospermic spermatozoa, we used an antibody directed
20 against SPACA1. In patients, a SPACA1 signal was detected on 23–42% of
21 spermatozoa ($n = 200$): a small signal was visible on the sperm head
22 either at the anterior pole, or at the periphery, but sometimes on the
23 sperm neck where the flagellum meets the sperm head. We detected a
24 SPACA1 signal covering the anterior pole of the nucleus in 85% of
25 normospermic control spermatozoa (**Figure 4**).

28 DNA compaction abnormalities in *DPY19L2*-deleted globozoospermic sperm

29 An increased retention of histones in spermatozoa chromatin has been
30 shown to be a characteristic of *DPY19L2*-deleted globozoospermia
31 (Yassine *et al.*, 2015). We therefore evaluated DNA compaction in
32 ejaculated sperm by acidic aniline blue staining, which binds to
33 spermatozoa that have retained abnormally high levels of histones inside
34 the nucleus. In patients, 76–88% of spermatozoa (mean: 82%, $n = 500$)
35 were strongly stained by aniline blue (**Figure 5**). In the control only 30%
36 of spermatozoa were strongly stained by aniline blue ($P < 0.05$).

39 BAF and BAF-L proteins are not detected in spermatozoa from 40 globozoospermic patients

41 BAF (barrier to autointegration factor) is a chromatin protein that binds to
42 lamin partners such as LEM domain proteins (e.g. LAP, emerin, Man1)
43 localized predominantly at the inner nuclear membrane (Schirmer and
44 Foisner, 2007). Lamins, LEM domain proteins and BAF contribute to
45 nuclear structure, assembly and chromatin organization. BAF-L (barrier to
46 autointegration factor-like), encoded by *BANF2*, is 40% identical to BAF in
47 humans. BAF-L is a nuclear protein with a predominant expression in the
48 testis (Tifft *et al.*, 2006). We have screened for sperm labelling with
49 antibodies against LEM domain proteins, BAF and BAF-L and only detected
50 a signal on normal sperm with antibodies against BAF and BAF-L in 32%
51 and 80% of spermatozoa, respectively. BAF and BAF-L signals appear like
52 a small cup at the posterior nuclear pole of ejaculated spermatozoa. In
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1 patients, the BAF-L signal was visible at the posterior pole of the nucleus
2 in less than 2% of spermatozoa, and BAF was never detected ($P < 0.05$
3 compared with control) (**Figure 6**).
4

5 Discussion

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7 Our study has focused on the potential involvement of lamins, and the
8 proteins that may link the NL to the chromatin, in the nuclear
9 abnormalities that characterize globozoospermic spermatozoa. To the best
10 of our knowledge, the present study is the first of the NL and its
11 associated proteins in this rare morphological sperm abnormality. We
12 have recently shown that the NL of the human spermatid lacks A-type
13 lamins, and is only composed of B-type lamins that are gradually removed
14 from the nuclear envelope during normal spermiogenesis (Elkhatib *et al.*,
15 2015). Moreover, in this study we searched for the involvement of lamin
16 partners and indirect chromatin-related proteins in globozoospermic
17 spermatozoa compared with a normospermic control. The results obtained
18 in our study illustrate three important points concerning globozoospermia.
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21 The first point is that in globozoospermia linked to DPY19L2 deletion, the
22 expression of A-type and B-type lamins is not modified compared with
23 normospermic fertile men. In all patients, A-type lamin transcripts were
24 not detectable, and lamin B1 and the spermatid-specific lamin B3
25 transcripts were detected. The presence of lamin B1 transcripts is in
26 agreement with its important role in nuclear stability (Ji *et al.*, 2007). The
27 lamin B2 transcript was not detected. Our results demonstrate that the
28 nuclear abnormalities observed in globozoospermia are not the result of,
29 or the cause of, a lack of expression of the lamin B1 or B3 transcripts, nor
30 of an ectopic expression of lamin B2 transcripts during spermiogenesis.
31

32 The second point is that the expression profile of chromatin-related
33 proteins is modified in globozoospermic spermatids. Our results show that
34 the BAF transcript is readily detected in globozoospermic spermatozoa
35 (four out of four cases), whereas BAF transcripts were absent from the
36 spermatozoa of the normospermic patient even though the BAF protein
37 was detected on control spermatozoa. This suggests that, during normal
38 spermiogenesis, the BAF transcript is normally unstable or eliminated in
39 round spermatids, and that it somehow becomes persistent in
40 globozoospermic spermatids. The chromatin is known to be poorly
41 condensed in globozoospermic spermatozoa (Carrell *et al.*, 1999; Vicari *et*
42 *al.*, 2002; Vozdova *et al.*, 2014). Furthermore, in human and mouse
43 spermatids lacking DPY19L2, chromatin compaction is abnormal, with
44 defective transport of protamine into the nucleus and elevated retention of
45 nuclear histones (Yassine *et al.*, 2015). One explanation for the presence
46 of BAF transcripts in globozoospermic spermatozoa might therefore be the
47 presence of immature histonylated chromatin that retains a transcriptional
48 competence at later steps of spermiogenesis than normal. Thus the
49 detection of BAF transcripts in spermatozoa may be an indicator of
50 abnormal chromatin compaction. Furthermore, if the BAF transcript is
51 normally selectively eliminated during spermiogenesis, then its
52 persistence in spermatozoa could impact fertilization or post-fertilization
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1 events, even though pregnancies have been obtained for two of the
2 patients studied here.
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4 Thirdly and finally, our results provide strong evidence that the NL is
5 strongly modified in globozoospermic spermatozoa compared with normal
6 spermatozoa, with potential consequences for the NL–chromatin protein
7 network. The lamin B1 signal is significantly more frequently detected in
8 globozoospermic spermatozoa compared with control and other
9 normospermic samples tested in our previous study (Elkhatib *et al.*,
10 2015). Moreover, in patients, the lamin B1 signal is most frequently visible
11 all around the nuclear periphery, without any polarization, which is
12 radically different from its restricted localization in normal spermatozoa
13 (Elkhatib *et al.*, 2015). This abnormal localization corresponds to that
14 observed in round spermatids in the DPY19L2-KO mouse, and is probably
15 related to the absence of acrosome anchoring at the anterior pole of the
16 spermatid nucleus (Pierre *et al.*, 2012). We conclude that the normal
17 process of spermatid NL remodelling is severely compromised during
18 spermiogenesis in globozoospermic patients. In addition to lamin B1, our
19 study shows that the localization of BAF and BAF-L is affected in
20 globozoospermic spermatozoa. BAF and BAF-L were detected at the
21 posterior pole of human normospermic control spermatozoa but were not
22 detected in patients with globozoospermia. Similarly to lamin B1, BAF and
23 BAF-L may therefore normally be located at the posterior pole of the
24 spermatozoa nucleus, a site at which histonylated chromatin is
25 concentrated (Li *et al.*, 2008; Zalensky *et al.*, 2002). This suggests that
26 the positioning of these proteins at the posterior pole of the nucleus may
27 depend on the replacement of histones by protamines throughout the
28 nucleus during normal chromatin remodelling. The absence of BAF and
29 BAF-L at the posterior pole in globozoospermic spermatozoa could be a
30 consequence of the defective chromatin remodelling in this rare
31 pathology. This should now be explored in a larger cohort of
32 globozoospermic and fertile patients. It is noteworthy that in the context
33 of assisted reproduction the severe disorganization of the spermatid
34 nucleus linked to DPY19L2 deletion is not an absolute obstacle to
35 fertilization and normal development, but it may contribute to the poor
36 fertilization and pregnancy rates described for ICSI of globozoospermic
37 spermatozoa. We anticipate that the same type of immature NL will be
38 found in some patients with a high incidence of spermatozoa bearing an
39 abnormal acrosome, particularly a small one, as it has been shown that
40 altered chromatin condensation often accompanies anomalies of the
41 acrosome (Perdrix *et al.*, 2011). It will now be of interest to characterize
42 the organization of the spermatozoa NL in such cases.
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45 In conclusion, our results strongly support an important role for the
46 remodelling of the NL and its associated proteins in human
47 spermiogenesis. In globozoospermia, we provide new evidence, on the
48 one hand, for a severe lack of maturation of the NL, and on the other
49 hand, for dramatic modifications in the location of chromatin-related NL
50 partners in DPY19L2-deleted spermatozoa. We detected BAF transcripts in
51 globozoospermic but not control spermatozoa, and we suggest that BAF
52 may be a biomarker of abnormally elevated levels of immature
53 histonylated chromatin in spermatozoa. Further experiments are now
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1 necessary to investigate whether other direct or indirect lamin-associated
2 proteins are involved in globozoospermia, and to what extent abnormal
3 expression of NE proteins is responsible for human sperm abnormalities.
4

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16

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30 **Figure 1.** RT-PCR amplification transcripts for lamin B1, B2, B3, A/C, C2
31 and for lamin chromatin-partners emerin, Lap2 α , BAF and BAF-L from
32 total human spermatozoa RNA. Agarose gel (3%) migration of RT-PCR
33 products amplified from total human RNA from ejaculated
34 globozoospermic spermatozoa for four men (A) and from a fertile patient
35 used as a control (B).

36 **Figure 2.** A: Different immunolocalizations of lamin B1 (green) in human
37 globozoospermic spermatozoa relative to the posterior pole labelled using
38 an anti- α -tubulin antibody labelling the sperm tail (red). B:
39 Immunolocalization of lamin B1 (green) in human spermatozoa from a
40 fertile patient with the acrosome identified using lectin PNA (red). Nuclei
41 are counterstained with DAPI (blue).

42 **Figure 3.** A: Percentage of spermatozoa labelled with anti-lamin B1
43 antibody for the four globozoospermic patients and the fertile control; *%
44 of labelled spermatozoa significantly higher than control ($P < 0.05$). B:
45 Distribution of the different lamin B1 localizations (posterior pole,
46 periphery or both) on labelled spermatozoa from the four globozoospermic
47 patients (G1–G4) and the fertile control.

48 **Figure 4.** A: Different immunolocalizations of SPACA-1 (green) in human
49 globozoospermic spermatozoa. B: Immunolocalization of SPACA-1 in
50 human spermatozoa from the fertile control. Nuclei are counterstained
51 with DAPI (blue).

52 **Figure 5.** Aniline blue staining of spermatozoa: (A) globozoospermic
53 spermatozoa, (B) fertile control.

1 **Figure 6.** Immunolocalization of BAF and BAF-L (green) in human
2 spermatozoa from (A) DPY19L2-deleted globozoospermic patients, and (B)
3 fertile patient. BAF and BAF-L signals appear like a small cup at the
4 posterior nuclear pole of ejaculated spermatozoa in the control; this signal
5 was not detectable. Signals are not detectable in globozoospermic
6 spermatozoa. Nuclei are counterstained with DAPI (blue).
7

8 **Supplementary Figure 1.** Family tree of two DPY19L2-deleted patients
9 (black arrow) from Turkey, with consanguinity in the family (G1 and G3).
10 In the G1 family, two cousins consulted for primary infertility.
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Figure 1

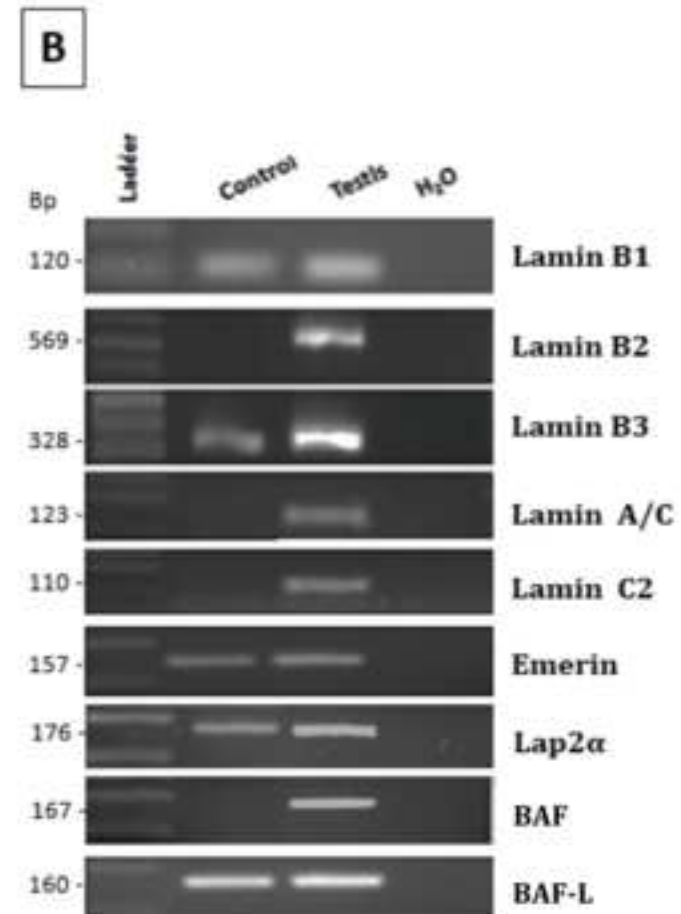
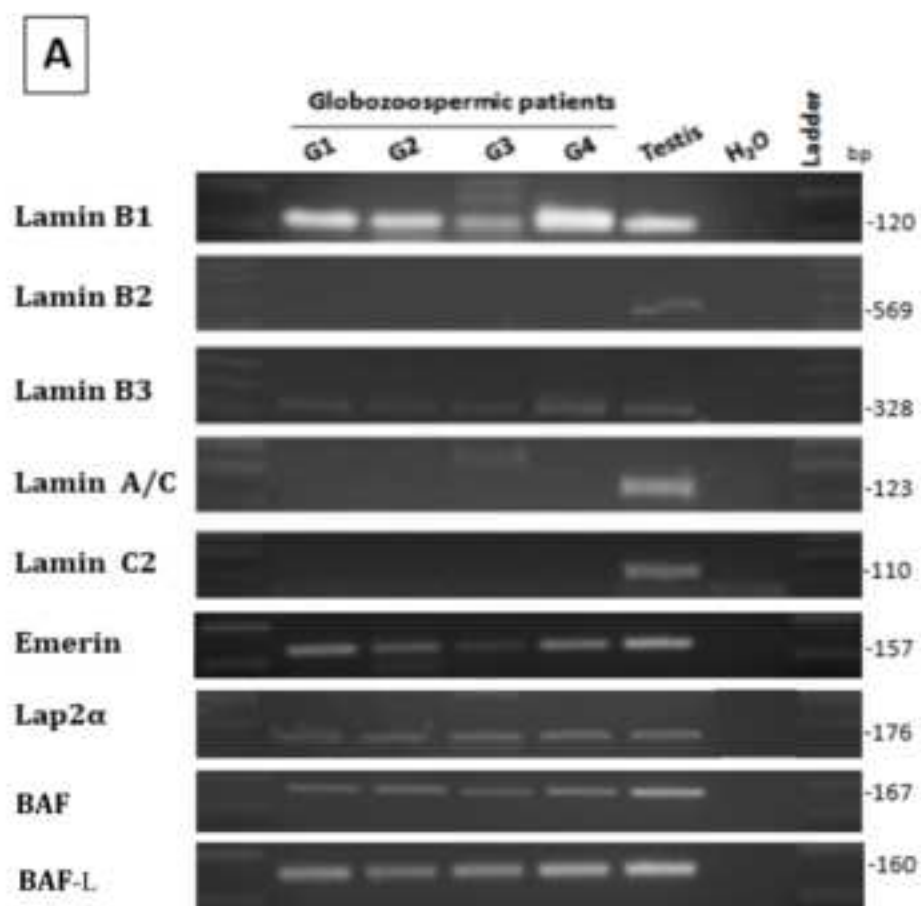


Figure 2

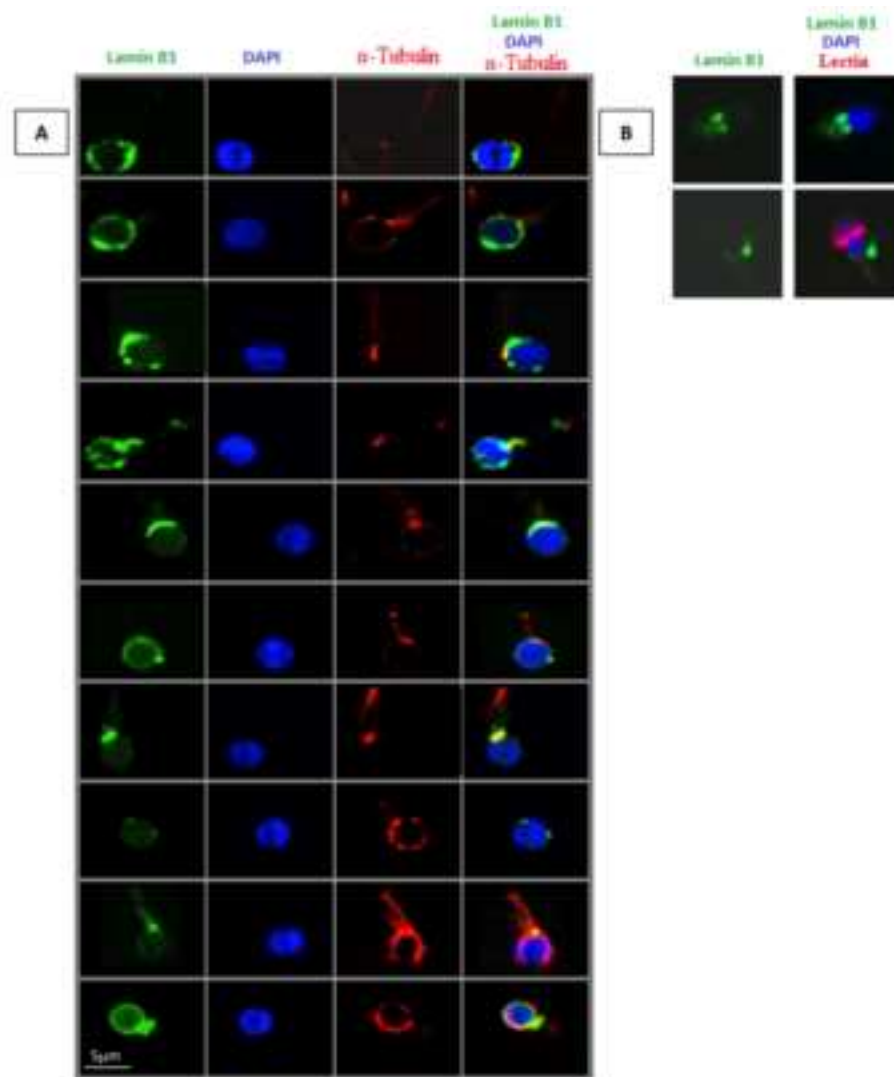


Figure 3

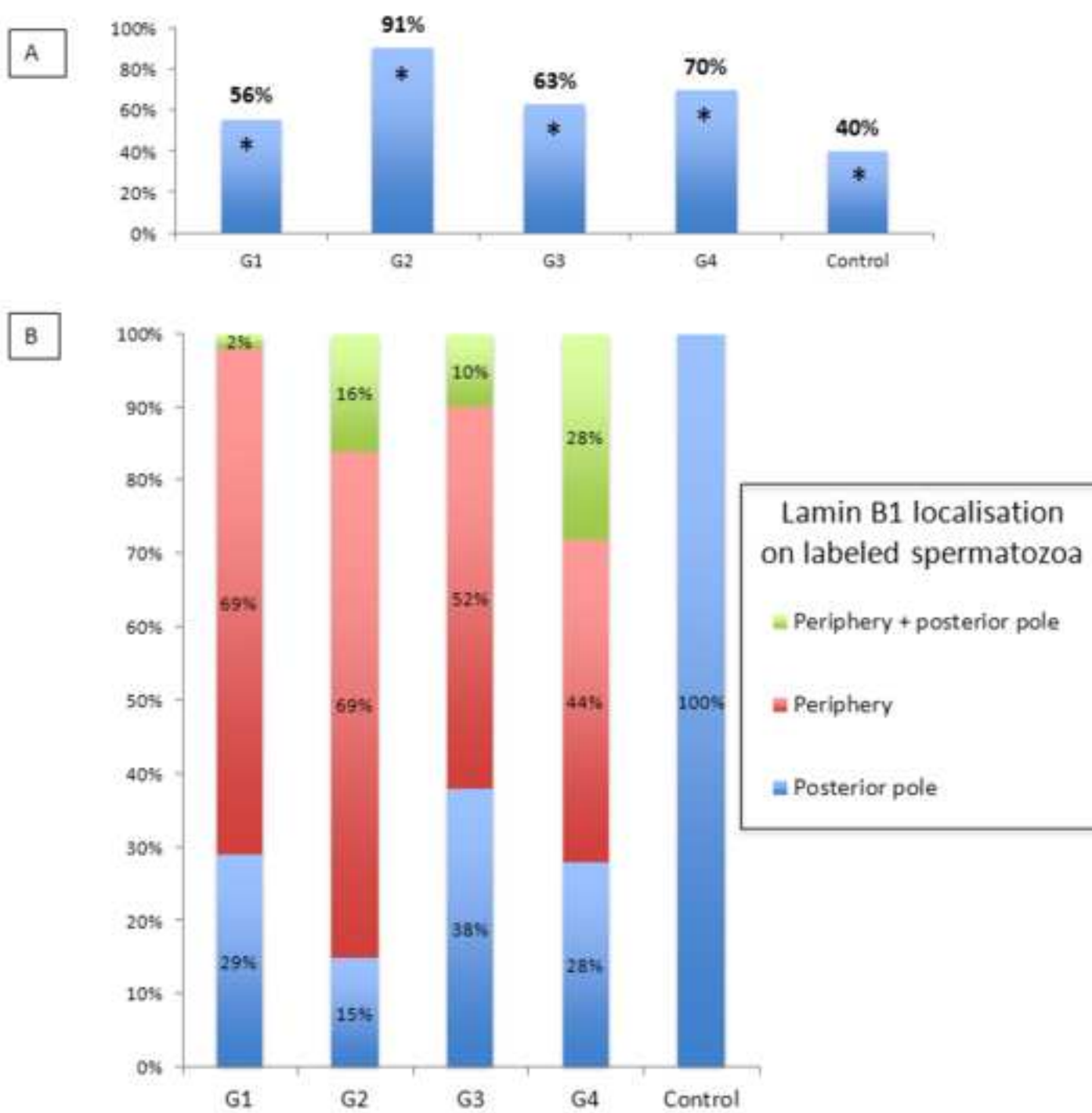


Figure 4

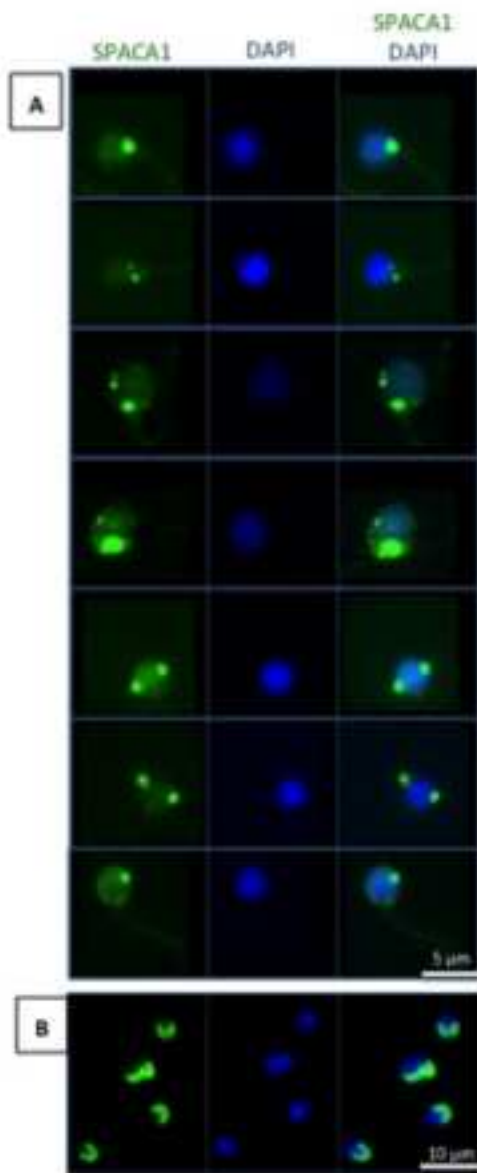


Figure 5

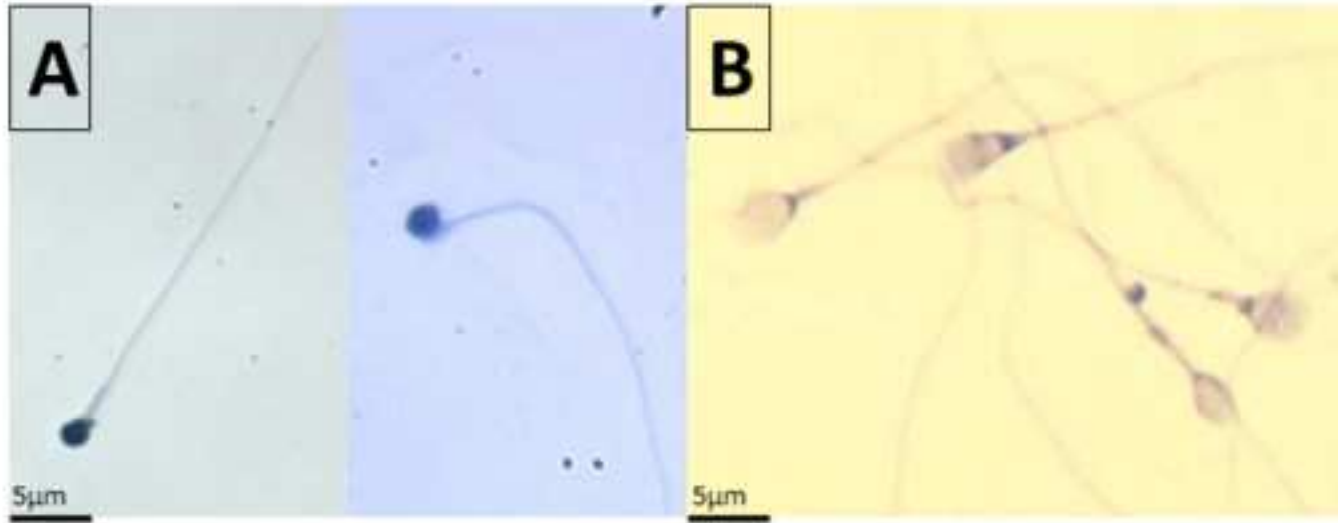


Figure6

