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

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

Marine Paci [a,b], Razan Elkhatib [a], Guy Longepied [a], Sylviane Hennebicq [c], Julien Bessonat [c], Blandine Courbière [b], Patrice Bourgeois [a], Nicolas Levy [a], Michael J Mitchell [a], Catherine Metzler-Guillemain [a,b,*]

[a] Aix Marseille University, Inserm, GMGF, 13385 Marseille Cedex 5, France; [b] APHM Hôpital La Conception, Pôle Femmes-Parents-Enfants, Centre Clinico-Biologique d'Assistance Médicale à la Procréation-CECOS, 13385 Marseille Cedex 5, France; [c] CHU de Grenoble, Centre d'Assistance Médicale à la Procréation-CECOS, BP217, Grenoble Cedex 9, France

* Corresponding author. *E-mail address*: catherine.metzler-guillemain@univ-amu.fr (C Metzler-Guillemain).

## Abstract

The aim of this study was to characterize the nuclear lamina (NL) and lamin chromatin-partners in spermatozoa from four *DPY19L2*-deleted globozoospermic patients. We tested for spermatid transcripts encoding lamins and their chromatin-partners emerin, LAP2α, BAF and BAF-L, by reverse transcriptase-PCR using spermatozoa RNA. We also determined the localization of lamin B1, BAF and BAF-L by immunofluorescent analysis of spermatozoa from all patients. In RNA from globozoospermic and control spermatozoa we detected transcripts encoding lamin B1, lamin B3, emerin, LAP2α and BAF-L, but not A-type lamins. In contrast, BAF transcripts were detected in globozoospermic but not control spermatozoa. The NL was immature in human globozoospermic spermatozoa: lamin B1 signal was detected in the nuclei of globozoospermic spermatozoa in significantly higher proportions than the control ($P < 0.05$; 56–91% versus 40%) and was predominantly observed at the whole nuclear periphery, not polarized as in control spermatozoa. Conversely, BAF and BAF-L were detected in control, but not globozoospermic spermatozoa. Our results strongly emphasize the importance of the NL and associated proteins during human spermiogenesis. In globozoospermia, the lack of maturation of the NL, and the modifications in expression and location of chromatin-partners, could explain the chromatin defects observed in this rare phenotype.

*Keywords*: BAF, BAF-L, globozoospermia, lamin, nuclear lamina, spermiogenesis

## Key message

In spermatozoa from *DPY19L2*-deleted globozoospermic patients, the nuclear lamina (NL) is abnormally retained at the nuclear periphery, and the expression and location of BAF and BAF-L are modified. These

alterations may contribute to the chromatin defects observed in this rare phenotype. Our results support important roles for the NL, BAF and BAF-L during human spermiogenesis.

## Introduction

The nuclear lamina (NL), a component of the nuclear envelope, is a filamentous protein network situated between the inner nuclear membrane and the chromatin. The NL is mainly composed of lamins, intermediate filament proteins (type V) essential for the structure, shape, assembly and mechanical stability of the nucleus. The NL is involved in a wide spectrum of biochemical interactions between lamins, chromatin and a variety of partners: the lamin-binding proteins (Cau *et al.*, 2014). It has been shown that the NL is connected to the cytoskeleton and the centrosome (via SUN-domain proteins) and to the chromatin (via LEM domain proteins and the lamin B receptor). Moreover, it has been shown that LEM domain proteins connect the NL to the chromatin by binding a conserved metazoan chromatin protein called barrier to autointegration factor (BAF), encoded by the *BANF1* gene, which can bridge DNA and interacts with histones (Schirmer and Foisner, 2007; Schirmer and Gerace, 2005; Wilson and Foisner, 2010). In mammals, the NL is mainly composed of A-type or B-type lamins. A-type lamins are encoded by the LMNA gene, which produces four transcripts by alternative splicing including two widely expressed major A-type lamins: lamin A and lamin C, and two other isoforms with more restricted expression: A$\Delta$10 in tumoral cells and C2 in meiotic male germ cells (Furukawa *et al.*, 1994; Machiels *et al.*, 1996; Nakajima and Abe, 1995). There are two distinct genes that encode three B-type lamins: LMNB1 encodes lamin B1 while LMNB2 encodes lamin B2 and the spermatid-specific isoform lamin B3 (Dechat *et al.*, 2000; Elkhatib *et al.*, 2015; Furukawa and Hotta, 1993; Vester *et al.*, 1993).

During spermiogenesis, the post-meiotic and final phase of spermatogenesis, the spermatid nucleus is subjected to a unique remodelling process including an extreme compaction of the chromatin orchestrated by dynamic interactions between the NE and the manchette (Kierszenbaum and Tres, 2004). In humans, we recently showed that the human spermatid NL is devoid of A-type lamins and composed only of B-type lamins (Elkhatib *et al.*, 2015). Furthermore, the localization of lamin B1 and B2/B3 changes during spermiogenesis with a progressive polarization to the posterior pole of the elongating spermatids, suggesting that B-type lamins play a central role in the intense remodelling of the spermatid nucleus.

Human globozoospermia is a rare monomorphic teratozoospermia first described over 40 years ago which affects less than 0.1% of infertile men and is characterized by malformed round sperm heads without an acrosome (Baccetti *et al.*, 1977; Dam *et al.* 2007a; Holstein *et al.*, 1973; Pedersen and Rebbe, 1974; Perrin *et al.*, 2013; Schirren *et al.*, 1971; Singh, 1992). Recent studies have shown that most cases have an autosomal recessive genetic cause, and mutations in two genes have been independently linked to the occurrence of human globozoospermia. The

most frequent are homozygous deletions of *DPY19L2*, a gene encoding an uncharacterized protein localized to the inner nuclear membrane, which explain more than 80% of globozoospermia cases (Carson *et al.*, 2006; Harbuz *et al.*, 2011; Koscinski *et al.*, 2011). In the mouse, knockout of *Dpy19l2* leads to the destabilization of the NL, with persistence of lamin B1 throughout the nuclear periphery in round spermatids, and a failure of sperm nuclear shaping and loss of the unbound acrosomal vesicle (Pierre *et al.*, 2012), a phenotype identical to that found in men deleted for *DPY19L2*. Homozygosity for two different loss-of-function variants in SPATA16 (spermatogenesis-associated 16) have been found in three unrelated cases of globozoospermia (Dam *et al.*, 2007b; Elinati *et al.*, 2016; Lu *et al.*, 2006; Xu *et al.*, 2003).

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

## Materials and methods

### Patients

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

### Patients and semen

Semen was collected at our academic Center of Reproductive Medicine by masturbation after 2–6 days of sexual abstinence. After liquefaction, semen analysis and cryopreservation was performed as previously described (Elkhatib *et al.*, 2015). All patients and the control gave an informed consent for the conservation of the remnant sperm in the Germetheque biobank and their use in studies on human fertility in accordance with the Helsinki Declaration of 1975 on human experimentation. The Germetheque Scientific Committee approved the present study design (reference 2013-MRS01 approval on 5 February 2013). For all patients with globozoospermia from Marseille, DNA was

**Comment [SH1]:** Author: please give the full correct title of the centre here.

stored in the labelled Biological Resource Centre (CRB TAC), Department of Medical Genetics, Timone Hospital of Marseille and was sent to Grenoble for DPY19L2 genotyping (Harbuz *et al.*, 2011). All patients were DPY19L2-deleted, with three homozygous and one heterozygous deletion. Patient characteristics and sperm parameters according to WHO criteria (WHO, 2010) are reported in **Supplementary Table 1**. Intracytoplasmic sperm injection (ICSI) was performed for the four DPY19L2-deleted globozoospermic patients. Following eight stimulation cycles, pregnancy was achieved for two couples and resulted in the birth of a total of three healthy boys.

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

Following storage in liquid nitrogen, sample straws were thawed and prepared as previously reported (Elkhatib *et al.*, 2015). Ten million spermatozoa were used for each RNA extraction, leading to 880–3600 ng of RNA. For each patient and control, we checked for the presence of spermatid RNA, for the lack of contaminating round cell RNA, and for early testicular germ cells and epithelial cells (Lambard *et al.*, 2004). The positive control was total RNA extracted from human testis (636533 Clontech). Lamins and direct or indirect partners such as emerin, Lap2α, BAF and BAF-L transcripts were amplified with Q5 Taq polymerase (New England Biolabs), as recommended by the manufacturer. The primer pairs and their annealing temperature for each transcript are as follows: lamin A/C (123 bp): o4683/o4384 – 70°C, lamin C2 (100 bp): o4686/o4687 – 67°C, lamin B1 (110 bp): o4479/o4480 – 66°C, lamin B2 (500 bp): o4369/o4370 – 72°C, lamin B3 (300 bp): o3972/o4370 – 72°C, emerin (157 pdb): o5097/o5098 – 69°C, LAP2α (176 pdb): o4087/o4088 – 64°C, BAF (167 pdb): o4094/4095 – 69°C, BAF-L (160 pdb): o4096/o4097. The Q5 GC-enhancer additive was used for all PCRs except for lamin B1, LAP2α and BAF. (Sequences of primers are provided in **Supplementary Table 2**.)

**Comment [AH2]:** Author: What does 'pdb' stand for?

## Aniline blue

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

## Immunocytochemistry

Thawed spermatozoa were spread onto polylysine-coated slides by cytospin (Shandon) and fixed as previously reported (Elkhatib *et al.*, 2015). We used lectins (Lectin PNA Alexa Fluor 594 conjugates, L-21409 Molecular Probes) at a dilution of 1:600 to mark the acrosomes. The antibodies and the dilutions used were rabbit anti-lamin B1 (1:100, ab16048 Abcam), rabbit anti-BAF-L (1:100, HPA042635 Sigma), rabbit anti-BAF (1:600, ab129184 Abcam), rabbit anti-SPACA1 (1:100, HPA026744 Sigma) and anti-alpha-tubulin mouse monoclonal (1:200, T6074 Sigma). After two washes in PBS, detection was performed using

Alexa Fluor 488 goat anti-rabbit IgG (1:400, Invitrogen) or cyanine-3 fluor 596 goat anti-mouse IgG (1:2000 ab6946 Abcam) for 1 h at 37°C. For co-immunofluorescence slides were rinsed in PBS and incubated with the second primary antibody followed by a second detection step. After washes in PBS, slides were mounted with 25–75 ng/ml 4(6-diamidino-2-phenylindole) (DAPI) in Vectashield mounting medium for microscope analysis. The slides were analysed using the Zeiss ApoTome 2 microscope (Zeiss, Oberkochen, Germany) equipped with an AxioCam MRm camera. Images were captured and merged with the ZEN software, and were treated using ImageJ software. For each patient and control, 300 spermatozoa were analysed in duplicate or triplicate. All antibodies were tested in individual staining reactions for their specificity and performance. Controls without primary antibodies were all negative. We did not use pre-immune serum as a negative control, which represents a limitation of the study, as normal serum contains an antibody which reacts with the acrosome. However, our study is focused on globozoospermia that is characterized by the lack of acrosome, and the anti-lamin B1, anti-BAF and anti-BAF-L antibodies used in our study never label the acrosome on control spermatozoa.

## Results

### Transcripts for lamin B1, lamin B3, emerin, LAP2α, BAF and BAF-L are present in spermatozoa from patients with globozoospermia

Human spermatozoa can be used to identify transcripts present in spermatids (Sendler *et al.*, 2013). In order to study expression of the known human lamins during globozoospermic spermiogenesis, we performed reverse transcriptase-PCR on RNA extracted from purified spermatozoa from four men with globozoospermia and one normospermic fertile man. For all patients with globozoospermia, we detected a transcript for lamin B1 and lamin B3, but no transcript for lamin B2 or the A-type lamins (A/C and C2). In addition, we detected a transcript for emerin, LAP2α and BAF-L in spermatozoa RNA from each patient. The same results were obtained for the normospermic control, as previously reported for A-type and B-type lamins (Elkhatib *et al.*, 2015). In contrast, BAF transcripts were not detected in the fertile control but were clearly present in spermatozoa from the four globozoospermic patients (**Figure 1**).

### Abnormal localization of lamin B1 in spermatozoa from patients with globozoospermia

To determine the expression pattern and cellular localization of lamin B1 during human spermiogenesis in patients with globozoospermia, we performed co-immunofluorescence analysis using antibodies specific for lamin B1 in association with antibodies directed against alpha-tubulin (cytoskeletal component) to visualize the posterior pole of the nucleus in the absence of the acrosome. The percentages of spermatozoa with a lamin B1 signal ranged from 56% to 91% in patients, and was 40% in the normospermic control ($P < 0.05$). We previously described a lamin B1 signal in 33–47% of spermatozoa on fertile controls, present exclusively

at the posterior pole of the nucleus (Elkhatib *et al.*, 2015). We noted that in each patient, a variety of cellular localizations were observed. Indeed, the lamin B1 signal was present either around the whole nucleus without polarization (44–69% of labelled spermatozoa), at the posterior pole of the nuclei (15–38% of labelled spermatozoa), or as a discontinuous signal at the nuclear periphery (2–28% of labelled spermatozoa) (**Figures 2** and **3**). These results demonstrate that the remodelling of the spermatid NL that occurs during normal spermiogenesis is severely compromised in the globozoospermic patients.

## Localization of acrosomal component SPACA1 in spermatozoa from patients with globozoospermia

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

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

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

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

BAF (barrier to autointegration factor) is a chromatin protein that binds to lamin partners such as LEM domain proteins (e.g. LAP, emerin, Man1) localized predominantly at the inner nuclear membrane (Schirmer and Foisner, 2007). Lamins, LEM domain proteins and BAF contribute to nuclear structure, assembly and chromatin organization. BAF-L (barrier to autointegration factor-like), encoded by *BANF2*, is 40% identical to BAF in humans. BAF-L is a nuclear protein with a predominant expression in the testis (Tifft *et al.*, 2006). We have screened for sperm labelling with antibodies against LEM domain proteins, BAF and BAF-L and only detected a signal on normal sperm with antibodies against BAF and BAF-L in 32% and 80% of spermatozoa, respectively. BAF and BAF-L signals appear like a small cup at the posterior nuclear pole of ejaculated spermatozoa. In

patients, the BAF-L signal was visible at the posterior pole of the nucleus in less than 2% of spermatozoa, and BAF was never detected ($P < 0.05$ compared with control) (**Figure 6**).

## Discussion

Our study has focused on the potential involvement of lamins, and the proteins that may link the NL to the chromatin, in the nuclear abnormalities that characterize globozoospermic spermatozoa. To the best of our knowledge, the present study is the first of the NL and its associated proteins in this rare morphological sperm abnormality. We have recently shown that the NL of the human spermatid lacks A-type lamins, and is only composed of B-type lamins that are gradually removed from the nuclear envelope during normal spermiogenesis (Elkhatib *et al.*, 2015). Moreover, in this study we searched for the involvement of lamin partners and indirect chromatin-related proteins in globozoospermic spermatozoa compared with a normospermic control. The results obtained in our study illustrate three important points concerning globozoospermia.

The first point is that in globozoospermia linked to DPY19L2 deletion, the expression of A-type and B-type lamins is not modified compared with normospermic fertile men. In all patients, A-type lamin transcripts were not detectable, and lamin B1 and the spermatid-specific lamin B3 transcripts were detected. The presence of lamin B1 transcripts is in agreement with its important role in nuclear stability (Ji *et al.*, 2007). The lamin B2 transcript was not detected. Our results demonstrate that the nuclear abnormalities observed in globozoospermia are not the result of, or the cause of, a lack of expression of the lamin B1 or B3 transcripts, nor of an ectopic expression of lamin B2 transcripts during spermiogenesis.

The second point is that the expression profile of chromatin-related proteins is modified in globozoospermic spermatids. Our results show that the BAF transcript is readily detected in globozoospermic spermatozoa (four out of four cases), whereas BAF transcripts were absent from the spermatozoa of the normospermic patient even though the BAF protein was detected on control spermatozoa. This suggests that, during normal spermiogenesis, the BAF transcript is normally unstable or eliminated in round spermatids, and that it somehow becomes persistent in globozoospermic spermatids. The chromatin is known to be poorly condensed in globozoospermic spermatozoa (Carrell *et al.*, 1999; Vicari *et al.*, 2002; Vozdova *et al.*, 2014). Furthermore, in human and mouse spermatids lacking DPY19L2, chromatin compaction is abnormal, with defective transport of protamine into the nucleus and elevated retention of nuclear histones (Yassine *et al.*, 2015). One explanation for the presence of BAF transcripts in globozoospermic spermatozoa might therefore be the presence of immature histonylated chromatin that retains a transcriptional competence at later steps of spermiogenesis than normal. Thus the detection of BAF transcripts in spermatozoa may be an indicator of abnormal chromatin compaction. Furthermore, if the BAF transcript is normally selectively eliminated during spermiogenesis, then its persistence in spermatozoa could impact fertilization or post-fertilization

events, even though pregnancies have been obtained for two of the patients studied here.

Thirdly and finally, our results provide strong evidence that the NL is strongly modified in globozoospermic spermatozoa compared with normal spermatozoa, with potential consequences for the NL–chromatin protein network. The lamin B1 signal is significantly more frequently detected in globozoospermic spermatozoa compared with control and other normospermic samples tested in our previous study (Elkhatib *et al.*, 2015). Moreover. in patients, the lamin B1 signal is most frequently visible all around the nuclear periphery, without any polarization, which is radically different from its restricted localization in normal spermatozoa (Elkhatib *et al.*, 2015). This abnormal localization corresponds to that observed in round spermatids in the DPY19L2-KO mouse, and is probably related to the absence of acrosome anchoring at the anterior pole of the spermatid nucleus (Pierre *et al.*, 2012). We conclude that the normal process of spermatid NL remodelling is severely compromised during spermiogenesis in globozoospermic patients. In addition to lamin B1, our study shows that the localization of BAF and BAF-L is affected in globozoospermic spermatozoa. BAF and BAF-L were detected at the posterior pole of human normospermic control spermatozoa but were not detected in patients with globozoospermia. Similarly to lamin B1, BAF and BAF-L may therefore normally be located at the posterior pole of the spermatozoa nucleus, a site at which histonylated chromatin is concentrated (Li *et al.*, 2008; Zalensky *et al.*, 2002). This suggests that the positioning of these proteins at the posterior pole of the nucleus may depend on the replacement of histones by protamines throughout the nucleus during normal chromatin remodelling. The absence of BAF and BAF-L at the posterior pole in globozoospermic spermatozoa could be a consequence of the defective chromatin remodelling in this rare pathology. This should now be explored in a larger cohort of globozoospermic and fertile patients. It is noteworthy that in the context of assisted reproduction the severe disorganization of the spermatid nucleus linked to DPY19L2 deletion is not an absolute obstacle to fertilization and normal development, but it may contribute to the poor fertilization and pregnancy rates described for ICSI of globozoospermic spermatozoa. We anticipate that the same type of immature NL will be found in some patients with a high incidence of spermatozoa bearing an abnormal acrosome, particularly a small one, as it has been shown that altered chromatin condensation often accompanies anomalies of the acrosome (Perdrix *et al.*, 2011). It will now be of interest to characterize the organization of the spermatozoa NL in such cases.

In conclusion, our results strongly support an important role for the remodelling of the NL and its associated proteins in human spermiogenesis. In globozoospermia, we provide new evidence, on the one hand, for a severe lack of maturation of the NL, and on the other hand, for dramatic modifications in the location of chromatin-related NL partners in DPY19L2-deleted spermatozoa. We detected BAF transcripts in globozoospermic but not control spermatozoa, and we suggest that BAF may be a biomarker of abnormally elevated levels of immature histonylated chromatin in spermatozoa. Further experiments are now

necessary to investigate whether other direct or indirect lamin-associated proteins are involved in globozoospermia, and to what extent abnormal expression of NE proteins is responsible for human sperm abnormalities.

## Acknowledgements

We are grateful to the patients who gave their informed consent to the use of their samples for research. We thank C. Metton and M. J. Fays-Bernardin for technical assistance and Germetheque support. This work was supported by grants from the Agence de la Biomedicine, AOR 'AMP, diagnostic prenatal et diagnostic génétique' 2013, Inserm and Aix-Marseille Université. The Germetheque biobank was supported by grants from the ANR (Agence Nationale de la Recherche), the Agence de la Biomedicine, the Centre Hospitalier Universitaire of Toulouse and APHM (Assistance Publique Hôpitaux de Marseille).

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*Declaration: The authors report no financial or commercial conflicts of interest.*

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

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

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

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

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

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

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

Figure 1

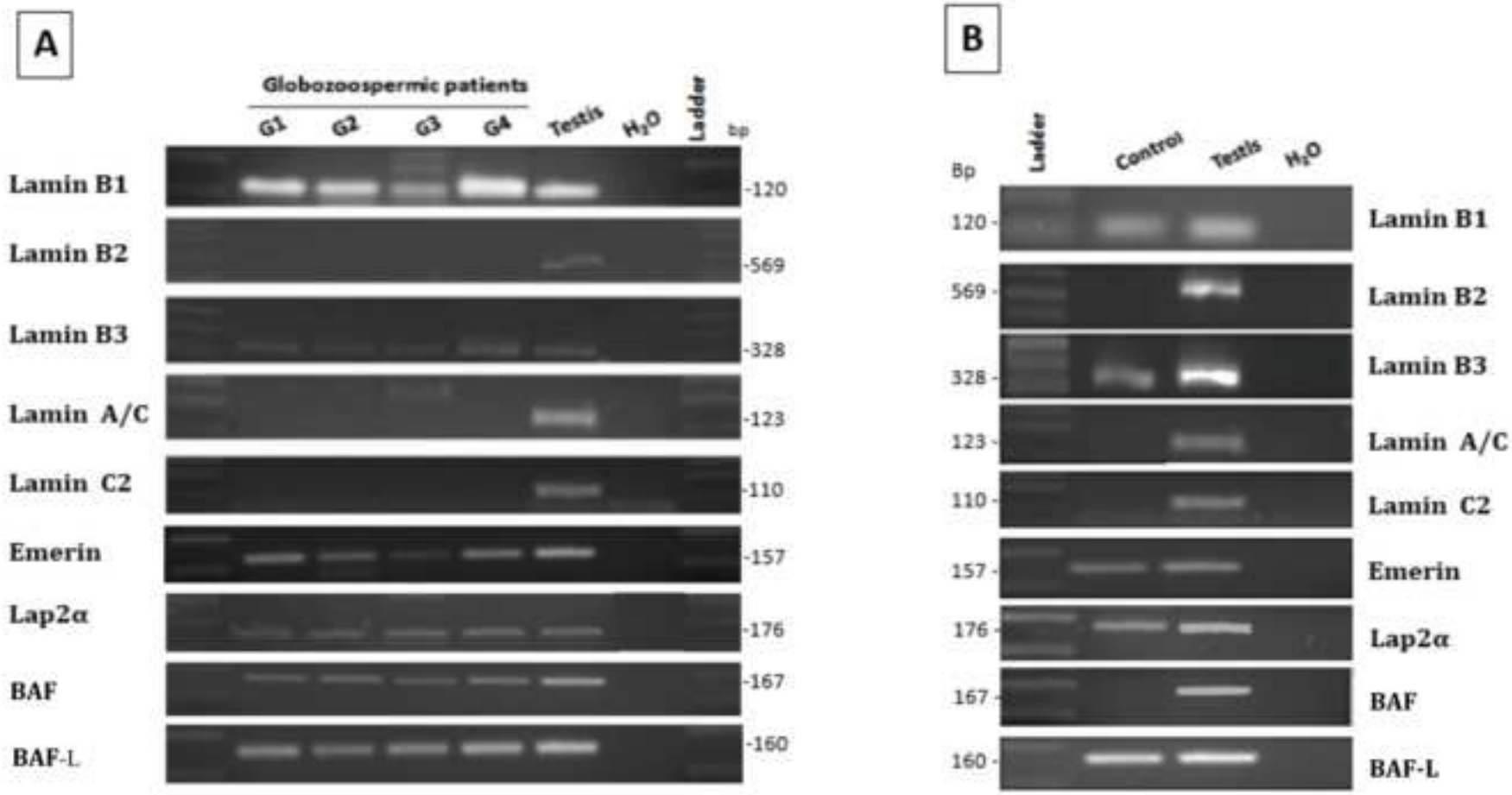
A
Globozoospermic patients
G1
G2
G3
G4
Testis
$H_2O$
Ladder
bp
Lamin B1
Lamin B2
Lamin B3
Lamin A/C
Lamin C2
Emerin
Lap2α
BAF
BAF-L
120
569
328
123
110
157
176
167
160
B
Ladder
Control
Testis
$H_2O$
Bp
120
569
328
123
110
157
176
167
160
Lamin B1
Lamin B2
Lamin B3
Lamin A/C
Lamin C2
Emerin
Lap2α
BAF
BAF-L

**Figure 2**

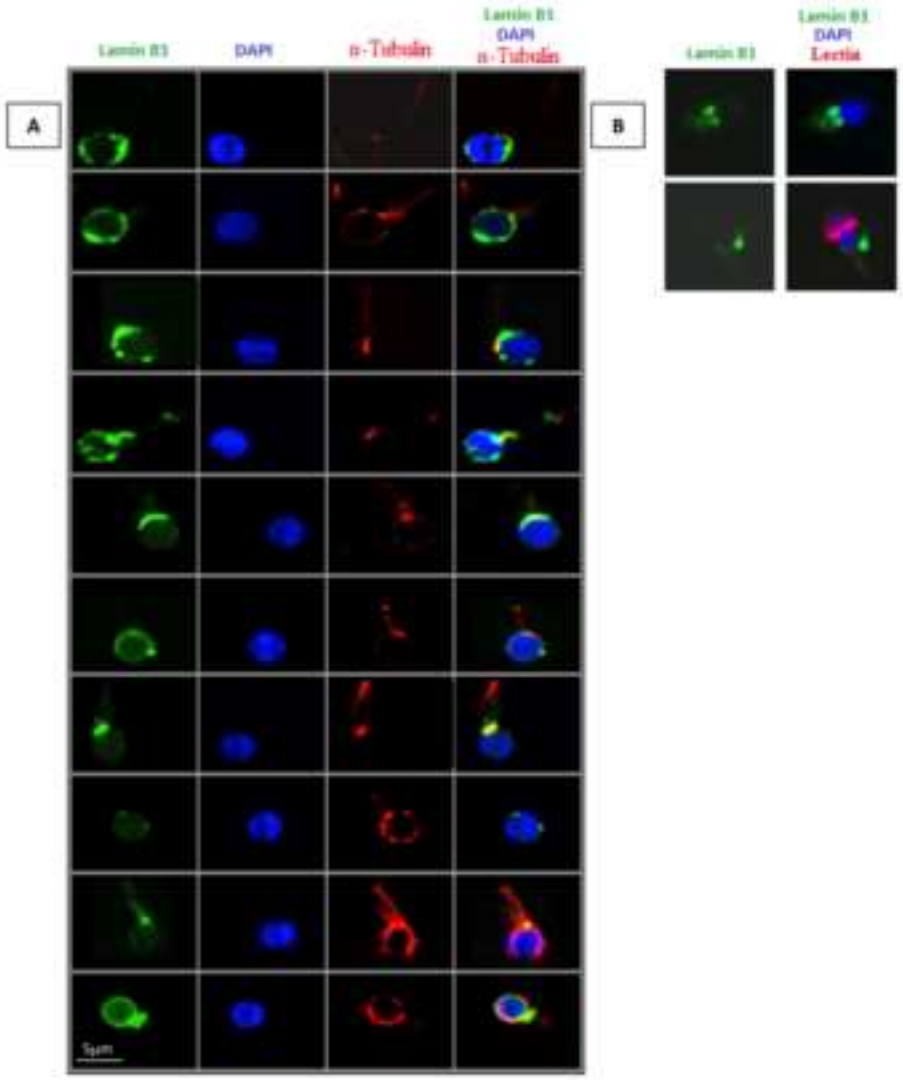
Lamin B1
DAPI
α-Tubulin
Lamin B1
DAPI
α-Tubulin
A
B
Lamin B1
DAPI
Lectin
5μm

**Figure 3**

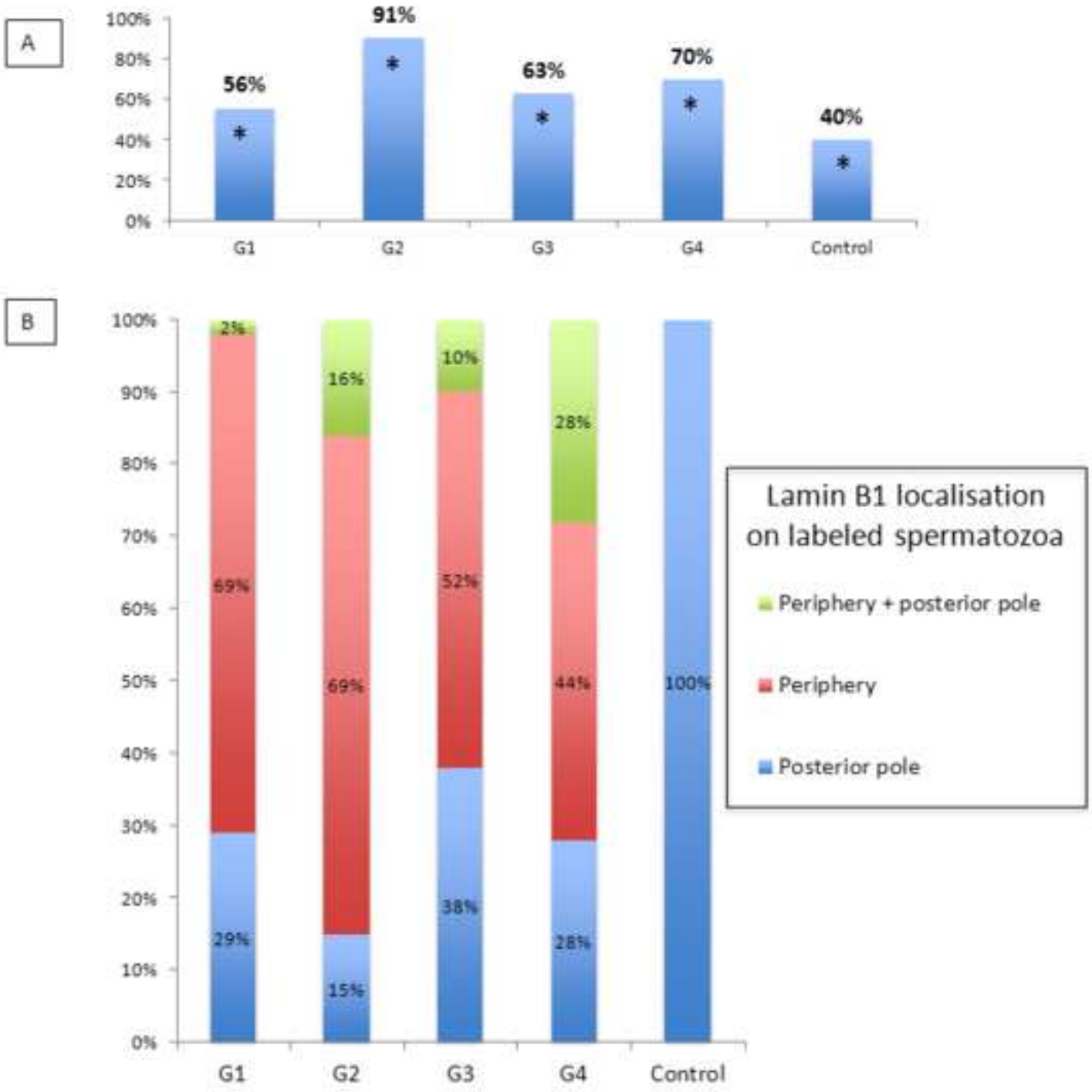
A
100%
80%
60%
40%
20%
0%
56%
91%
63%
70%
40%
*
G1
G2
G3
G4
Control
B
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
2%
69%
29%
16%
69%
15%
10%
52%
38%
28%
44%
28%
100%
G1
G2
G3
G4
Control
Lamin B1 localisation on labeled spermatozoa
Periphery + posterior pole
Periphery
Posterior pole

**Figure 4**

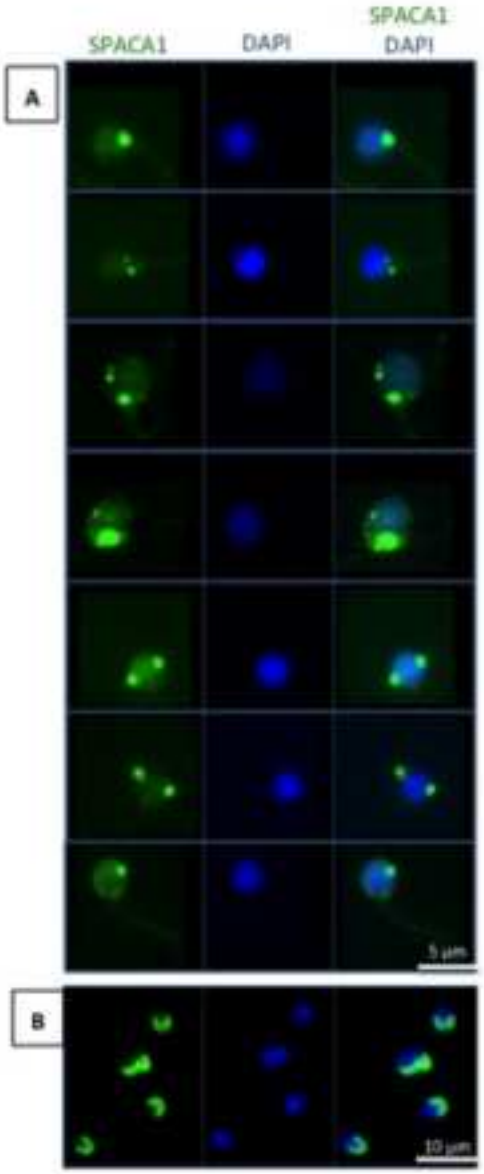

**Figure 5**

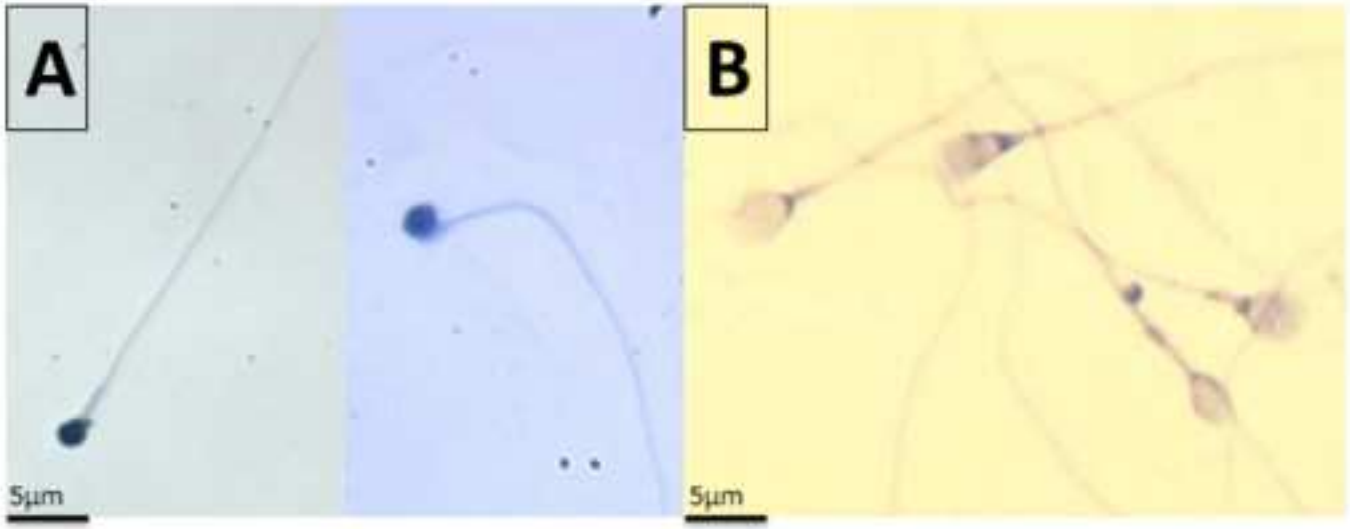

Figure6