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► **To cite this version:**

Julie Beurois, Caroline Cazin, Zine-Eddine Kherraf, Guillaume Martinez, Tristan Celse, et al.. Genetics of teratozoospermia: Back to the head. Best Practice and Research: Clinical Endocrinology and Metabolism, 2020, 34 (6), pp.101473. 10.1016/j.beem.2020.101473 . hal-03004973

**HAL Id: hal-03004973**

**<https://cnrs.hal.science/hal-03004973v1>**

Submitted on 10 Mar 2023

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# Genetics of teratozoospermia: back to the head

Julie Beurois (MSc, PhD student in Genetics)<sup>1</sup>, Caroline Cazin (MSc, PhD student in Genetics)<sup>1</sup>, Zine-Eddine Kherraf (MD, PhD, Assistant Professor in Reproductive Medicine)<sup>1,2</sup>, Guillaume Martinez (PhD, Assistant Professor in Genetics)<sup>1,2,3</sup>, Tristan Celse (MD, Consultant Geneticist)<sup>1,2,3</sup>, Aminata Touré (PhD, Director of Research)<sup>1</sup>, Christophe Arnoult (DVM, PhD, Director of Research)<sup>1</sup>, Pierre F. Ray (PhD, Professor of Reproductive Medicine)<sup>1,2</sup>, and Charles Coutton (PharmD, PhD, Professor of Genetics)<sup>1,2,3</sup>

<sup>1</sup> Université Grenoble Alpes, INSERM U1209, CNRS UMR 5309, Institute for Advanced Biosciences, Team Genetics Epigenetics and Therapies of Infertility, 38000 Grenoble, France

<sup>2</sup> CHU de Grenoble, UM GI-DPI, Grenoble, F-38000, France

<sup>3</sup> CHU Grenoble Alpes, UM de Génétique Chromosomique, Grenoble, France

\* Corresponding author: Prof. Charles Coutton, Laboratoire de Génétique Chromosomique, Hôpital Couple Enfant, CHU Grenoble Alpes, 38043 Grenoble cedex 9, France

Tel: +33-4-76-76-54-82; Email: CCoutton@chu-grenoble.fr

## **Abstract**

Spermatozoa are polarized cells with a head and a flagellum joined by the connecting piece. Head integrity is critical for normal sperm function, and head defects consistently lead to male infertility. Abnormalities of the sperm head are among the most severe and characteristic sperm defects. Patients presenting with a monomorphic head sperm defects such as globozoospermia or macrozoospermia were analyzed permitting to identify several key genes for spermatogenesis such as *AURKC* and *DPY19L2*. The study of patients with other specific sperm head defects such as acephalic spermatozoa have also enabled the identification of new infertility genes such as *SUN5*. Here, we review the genetic causes leading to morphological defects of sperm head. Advances in the genetics of male infertility are necessary to improve the management of infertility and will pave the road towards future strategies of treatments, especially for patients with the most severe phenotype as sperm head defects.

**Key words:** male infertility, teratozoospermia, gene defects, genetic diagnosis, sperm head, spermatogenesis, acrosome, globozoospermia, large headed spermatozoa, acephalic spermatozoa, intracytoplasmic sperm injection (ICSI)

## **Introduction**

Infertility is a major health concern, affecting approximately 50 million couples worldwide [1], or 12.5% of women and 10% of men [2]. It is defined by the World Health Organization (WHO) as the “failure to achieve a pregnancy after 12 months or more of regular unprotected sexual intercourse.” In half of these cases, spermatograms evidence a reduced sperm quantity or quality, demonstrating that a male factor is frequently found, alone, or in conjunction with a female cause. The diagnosis of male infertility is however most often only descriptive, the etiology of sperm defects remaining idiopathic in 30%–50% of cases [3]. One of the reasons for this lack of fundamental understanding is the heterogeneity of causal factors leading to male infertility. Moreover, precisely identifying and knowing the etiology of specific sperm defects will enable a better understanding of spermatogenesis.

The characterization and identification of the molecular basis of male infertility represents a significant challenge as over 4000 genes are thought to be involved in sperm production [4]. Defects in any of these genes can hamper spermatogenesis and induce one of many established sperm phenotypes. Mutant animal models have shown a variety of sperm phenotypes which have provided important clues to our understanding of the etiology of infertility. More recently, the emergence of next-generation sequencing (NGS), a rapidly evolving technology which has allowed major advances in human genetics, has greatly

facilitated the identification of genetic defects in human. This game-changing technology has shown itself to be a highly powerful research and diagnostic tool when applied to male infertility[5]. This is especially true for qualitative defects of spermatogenesis leading to morphological sperm abnormalities grouped under the term “teratozoospermia” [6,7]. This heterogeneous group of abnormalities includes a wide range of sperm phenotypes affecting several structures of the spermatozoa.

Spermatozoa are polarized cells with a head and a flagellum joined together by the connecting piece (neck) (Fig. 1A). The sperm head is completely surrounded by the plasma membrane anchored to the nuclear envelope by the posterior ring. Basically, the sperm head contains two main structures: the nucleus capped by the acrosome, which covers the anterior half of the nucleus and which is delimited by the outer and inner acrosomal membrane, facing the plasma membrane and the nuclear envelope, respectively (Fig. 1B). The acrosomal region is further subdivided into two regions: the anterior acrosomal region, which is involved in the acrosome reaction, and the posterior acrosomal region (equatorial segment), in which the outer acrosomal membrane does not fuse with the plasma membrane. [8]. The acrosome is a highly specialized organelle containing a variety of proteins, including hydrolytic enzymes and matrix molecules. The formation and organization of a mammalian sperm head occurs through different and numerous molecular and cellular processes during spermiogenesis. The formation of the acrosome starts in the early round spermatid, thanks to the fusion of vesicles from the Golgi apparatus and a flat acrosomal vesicle covers one-third of the nuclear apical surface at the last stages of the round spermatid (stage #8 in mouse) [9]. In the meantime, a complex process of histone exchange occurs in order to compact DNA. Canonical histones are first exchanged with testicular histone variants, which by destabilizing the nucleosome allow the insertion of protamine. This process of compaction is associated with nuclear shaping, making the head of the spermatozoon its characteristic shape. Head integrity is

critical for normal sperm function, and head defects consistently lead to male infertility. Abnormalities of the sperm head are among the most severe and characteristic sperm defects caused in most cases by single gene defects. These defects are particularly interesting as they constitute a precious source to decipher some of the mechanisms required for head biogenesis and function. Moreover, a better understanding of the physiopathology of head defects is a prerequisite for improving patient management and genetic counseling and should provide a basis for the development of therapeutic solutions tailored to the individual defects.

In this review, we discuss the gene defects demonstrated to be responsible for the different sperm phenotypes associated with sperm head defects in human (i.e., globozoospermia, macrozoospermia, and acephalic spermatozoa), summarize their molecular physiopathological etiologies, and detail the clinical implications arising from these data. The genetic causes of other sperm morphological defects such as multiple malformations of the sperm flagellum (MMAF phenotype) will not be discussed here given that they were recently thoroughly reviewed [10]. This review focuses on monogenic defects and will not describe the association studies which have not provided strong genetic evidence of a single monogenic contribution. Furthermore, we do not address syndromic infertility, in which infertility is associated with other clinical features.

### **Globozoospermia**

First described in humans in 1971, globozoospermia [MIM 613958, Spermatogenic Failure 9 (SPGF9)] is a rare (incidence 0.1%) and severe form of teratozoospermia characterized by the presence in the ejaculate of a large majority of round spermatozoa lacking acrosome [11,12] (Fig. 1C). Globozoospermic sperm are unable to adhere to or penetrate the zona pellucida, causing primary infertility. This phenotype exhibits two forms: “total” globozoospermia, referring to patients with a homogeneous phenotype with ~100% round-headed sperm, and “partial” globozoospermia, characterized by a mosaic of normal and

round-headed sperm in the ejaculate even if no precise cut-off threshold was clearly established. However, considering that globozoospermic sperm cells can be found with a proportion up to 9% in the general fertile population [13], globozoospermia is in practice diagnosed by semen analysis when a minimum of 20% to 50% of round-headed sperm cells is found in the ejaculate [14–16]. A rarer subtype of globozoospermia, also known as pseudo-globozoospermia or acrosome hypoplasia, has been described as the presence of an atrophied acrosome and almost systematically detached from the sperm head [17]. Although some contradictory results have been reported in the literature, an increased aneuploidy rate was regularly described in round-headed sperm as well as DNA damage related to defective chromatin condensation and DNA fragmentation compared to normal sperm cells [6,18].

The physiopathological mechanism leading to globozoospermia has been widely explored and, even if some uncertainty persists, it has been demonstrated that this phenotype mainly arises from a failure of proacrosomal Golgi-derived vesicle formation, trafficking, and fusion and/or a disruption of the interaction between the acrosome and the sperm nuclear envelope through the acroplaxome junction [6]. From a functional point of view, this abnormal acrosome formation results in a total depletion of hydrolytic and proteolytic acrosomal enzymes indispensable for the sperm-egg adhesion and penetration as well as for the oocyte activation with the phospholipase C zeta (PLC $\zeta$ ) which is a sperm-borne specific phospholipase located along the inner acrosomal membrane and in the perinuclear theca [19,20].

Genetic exploration of the globozoospermia phenotype began first with the creation of different knock-out mouse models allowing the identification of several genes involved in the different steps of the acrosome biogenesis [6,21]. To date, more than 60 genetically modified mice reported in the Mouse Genome Informatics database (<http://www.informatics.jax.org>) contain in their phenotypic description a term related to “globozoospermia.” This finding is

not surprising in view of the complexity and the multitude of proteins involved in acrosome biogenesis [22]. Despite this large list of candidate genes, only a few mutations have been identified in their human orthologs. In fact, currently, only five genes are confidently associated with globozoospermia in human: *SPATA16*, *DPY19L2*, *PICK1*, *ZBP1*, and *CCDC62*.

### *SPATA16*

Spermatogenesis-associated protein 16 (*SPATA16*; previously named NYD-SP12) is a protein highly expressed in human testis [23], which is located in the Golgi apparatus and in pro-acrosomal vesicles that fuse to form the acrosome during spermiogenesis [23,24] and whose function is hypothetically to be involved in maintaining the integrity of the sperm acrosome (Fig. 2). It was the first gene found to be responsible of human globozoospermia in 2007. A homozygous mutation (c.848G>A, p.R283Q) in *SPATA16* was identified in three affected brothers from a consanguineous Ashkenazi Jewish family [23] (Table 1). This variant induces the fourth exon skipping which causes a disruption in the C-terminal tetratricopeptide repeat domain (TPR domain) of *SPATA16* (Fig. 3 and Table 1). It is described as a protein–protein interaction module, highly conserved in mammals, which probably interact with GOPC and HRB proteins [23]. Moreover, in 2016 [25], two out of nineteen globozoospermic patients showed a new *SPATA16* homozygous deletion affecting exon 2 (Fig. 3 and Table 1). Interestingly, in addition to total globozoospermia, this new mutation led to 39% of spermatozoa with double or multiple heads, 26% of spermatozoa with multiple flagella (two or more), a high rate of aneuploidy (6% in *SPATA16* mutated patients against 2.38% in controls), and also increased DNA fragmentation (44% in *SPATA16* mutated patients against 10.25% in controls) [26]. *SPATA16* mutations may thus disturb the TPR domain, disorganize numerous protein–protein interactions, and lead to abnormal meiosis, which could explain this



particular phenotype of total globozoospermia. Overall, *SPATA16* mutations were found in 0.77% of cases of globozoospermia [23,25] (Fig. 4) in Europe and North Africa suggesting that *SPATA16* mutations are recurrent but rare cause of globozoospermia in humans.

### *DPY19L2*

In 2011, a recurrent whole-gene deletion of *DPY19L2* was reported in 15 out of 20 globozoospermic patients [27]. In spermatozoa, the DPY19L2 protein is located in the inner nuclear membrane and anchors the developing acrosome to the nuclear envelope through a cytoskeletal plate designated acroploxome [28](Fig. 2). In fact, in spermatozoa from *DPY19L2* mutated patients, the inner nuclear membrane is destabilized and then separated from the outer nuclear membrane, leading to the entire detachment of the acrosome. Furthermore, it has been shown that other nuclear proteins, such as SUN proteins, expressed during spermatogenesis, were involved in sperm head formation. Once stabilized in the inner nuclear membrane of spermatozoa, SUN proteins enroll KASH (Klarsicht, ANC-1, Syne Homology) proteins [29], located in the outer nuclear membrane, thus forming the LINC (Linker of Nucleoskeleton and Cytoskeleton) complex binding the two inner and outer nuclear envelopes, ensuring the maintenance of the nuclear membrane rigidity and anchoring the nucleus to cytoplasmic structure through the cytoplasmic domain of KASH proteins. It was then hypothesized that DPY19L2, SUN, and KASH proteins may be potential interacting partners [30] and that DPY19L2 may stabilize the LINC complex. DPY19L2 has also a large N-terminus intra-nuclear domain, which may bind at the same time the nuclear dense lamina. The super structure comprising the DPY19L2 and LINC complex may therefore link the luminal component of the nucleus to the acroploxome. A recent study using three-dimensional fluorescence in situ hybridization (FISH) demonstrated that DPY19L2-deficient globozoospermic patients display impaired sperm chromocenter organization and altered

spatial organization of chromosome 7, 18, and X [31]. This study further strengthens the hypothesis that DPY19L2 might be considered as a LINC-like protein having a crucial role in the organization of nuclear chromatin in the sperm nucleus through its interaction with the nuclear lamina. Therefore, after a first step of acroplaxome-acrosome complex docking on the outer nuclear membrane during spermatogenesis, a second step of partnership between DPY19L2, SUN proteins, and KASH proteins would allow and stabilize the establishment and spreading of the acrosome [32]. This hypothesis has however not been formally confirmed and the molecular partners of DPY19L2 remain to be characterized. Comparative testicular transcriptome studies of wild type (WT) and globozoospermic *Dpy19l2* KO mice were also realized to attempt to identify *Dpy19l2* molecular partners but no conclusive result was obtained [33]. More recent proteomic sperm analyses of globozoospermic patients carrying a *DPY19L2* homozygous deletion showed that 491 sperm proteins differentially expressed between patients and controls [34] including proteins involved in acrosome biogenesis but also in chromatin composition, sperm-egg binding and egg activation. In particular, authors identified IZUMO family proteins (IZUMO1-4) among the most downregulated [34]. In addition to its structural function during acrosome biogenesis, the C-mannosyltransferase function of its ancestral protein DPY-19 led to the hypothesis that DPY19L2 may also have a function in glycosylation of sperm proteins, but this remains to be demonstrated [35].

Concerning the genetic alterations of *DPY19L2* causing globozoospermia, the recurrent 200-kb homozygous deletion is the most frequent event reported in variable proportions ranging from an average of 19% to 75% of globozoospermic patients [7]. This high heterogeneity may be explained in part by the geographical origins of the studied cohorts of patients, the consanguinity degrees in studied families and by the inclusion of variable proportion of patients with partial globozoospermia [7]. The molecular mechanism leading to

the deletion is however consensual and was described to be mediated by Non-Allelic Homozygous Recombination (NAHR) occurring between two highly homologous 28-kb low copy repeats located on each side of the gene [36]. Several punctual mutations and small deletions have been subsequently described as causal [16,37–44]. Overall a total of 30 deleterious variants have been described including six splice variants, 11 loss of function variants, a deletion of three nucleotides, and 12 missense variants (Table 1 and Fig. 3). All of them represent approximately 20% of the pathological alleles (Fig. 4). Based on these data, the consensual diagnosis strategy for patients presenting a total or partial globozoospermia was to first screen for the presence of the *DPY19L2* gene deletion before searching for *DPY19L2* punctual mutations and then defects in other candidate genes. In our very recent study in which we analyzed a large cohort of 69 new globozoospermic patients, Sanger sequencing and whole-exome sequencing (WES) identified 25 patients with a homozygous *DPY19L2* deletion (36.2%) and 14 carrying other *DPY19L2* alterations (20.3%). Overall, 11 deleterious single nucleotide variants were identified including eight novel and three already published mutations [45]. Among these punctual mutations, four out of the 11 variants were observed in exon 8 (Table 1 and Fig. 3), which seems to be a mutational hotspot since the proportion of exon 8 missense mutation carriers is around 56.5% of all published patients, with a causal missense mutation [16,37–43,45]. Among these mutations, mutations affecting the arginine at position 290 and 298 of the DPY19L2 protein were already and regularly reported in the literature [7]. These two arginine locations were described to be essential for the C-mannosyltransferase activity of DPY-19 [35], the *DPY19L2* ortholog in *Caenorhabditis elegans*, suggesting that the central domain of DPY19L2 may support some critical functions which remains to be elucidated [6]. Interestingly, patients with a higher rate of round-headed spermatozoa were more often diagnosed and had a higher proportion of loss of function anomalies, highlighting a good genotype–phenotype correlation. In accordance, no gene

defects were identified in patients carrying less than 50% of round-headed spermatozoa while diagnosis efficiency rose to 73,5% for patients with > 50% of globozoospermia [45]. Therefore, Celse et al. proposed a novel diagnostic strategy focusing only on patients with at least 50% of globozoospermia and based on the realization of a classical qualitative PCR to detect the homozygous deletion and, if negative, followed by whole exome sequencing which is faster, more economical and more exhaustive than performing Sanger sequencing on the 22 exons of *DPY19L2* [45].

### *PICK1*

PICK1, or Protein interacting with C kinase 1, is a peripheral membrane protein involved in protein trafficking, a function that has been initially well characterized in neurons. PICK1 is ubiquitously expressed but mostly abundant in the brain and testis and remarkably contains both PSD-95/DlgA/ZO-1 (PDZ) domain and Bin-Amphiphysin-Rvs (BAR) domain through which the protein binds to a large number of membrane proteins and lipid molecules [46]. In testis, PICK1 was highly expressed in round spermatids and localized to Golgi-derived proacrosomal granules. Xiao et al. first observed that PICK1 interacted with GOPC and the primary catalytic subunit of protein kinase 2 (*CK2 $\alpha$ '*), proteins whose deficiencies led to globozoospermia in mice, and thus suggested that PICK1 is involved in vesicle trafficking from the Golgi apparatus to the acrosome [47] (Fig. 2). More recently, ICA1L, a protein which is also critical for acrosome formation at spermiogenesis, was identified as a new binding partner of PICK1 [48]. In humans, only one mutation in *PICK1* gene was reported in 2010 [49] in a Chinese total globozoospermic patient with homozygous missense mutation (c.198G>A) in exon 13 (p.G393R) (Table 1 and Fig. 3). The authors suggested that the altered amino acid G393R in the C-terminal domain may influence the negative regulation with the BAR domain and affect the formation of the acrosome because of the perturbation of lipid-

BAR domain binding. Although *PICK1* is involved in numerous biological functions, only isolated infertility with no other clinical features were reported for this patient, suggesting that the altered amino acid G393R only may induce alteration of germinal functions of *PICK1* [49]. In 2012, *PICK1* was studied by Yatsenko et al. in 381 teratozoospermic and 204 control patients. No statistically significant and functionally relevant mutations were discovered in the *PICK1* gene [50]. Therefore, until now, the homozygous missense mutation (c.198G>A) in exon 13 of *PICK1* was only found in a sporadic and isolated case of globozoospermic patient representing 0.26% of globozoospermia cases (Fig. 4).

### *ZPBP1*

The mouse ortholog *ZPBP1* (sp38) was first shown to be required for sperm acrosome formation, compaction and sperm-egg binding [51]. The lack of this protein in KO mice led to acrosome fragmentation, disrupted Sertoli–spermatid junctions and a defective sperm head morphology with characteristics reminiscent of globozoospermia in humans. In humans, *ZPBP1* is involved in sperm–egg interaction (binding and penetration) during fertilization and is located in the acrosomal membrane and likely interacts with multiple acrosomal matrix proteins [51] (Fig. 2). In 2012, Yatsenko et al. identified homozygous and heterozygous missense and splicing mutations in *ZPBP1* in 15 out of the 381 men with abnormal sperm head morphology (Table 1 and Fig. 3). These mutations were not observed in 240 matched controls [50]. They hypothesized that most of the missense mutations exert a dominant-negative effect due to altered *ZPBP1* protein folding and protein-protein interactions in the acrosome. All the identified mutations were found in patients with 64% to 98% of heads defects resembling the abnormal head morphology phenotype of *Zpbp1* mouse knockout model [50]. More recently Oud et al. identified a nonsense mutation, c.931C>T; p.(Gln311Ter), in a total globozoospermic patient (Table 1 and Fig.3). This variant is absent among the general population and is located in a homozygosity region of approximately 24

Mb on chromosome 7. This mutation may lead to a truncated protein lacking the 40 last amino acids of the conserved SP38 domain of the protein.

### *CCDC62*

Coiled Coil Domain-Containing 62 (CCDC62) was first described in 2017 in infertile male mice presenting spermatozoa with low motility (<5% motile spermatozoa), and middle piece, head, and acrosome defects [52]. CCDC62 is likely present in the Golgi apparatus during the first stages of spermatogenesis and then in the acrosome during the elongation and maturation phases (Fig. 2). It was shown that CCDC62 was required for cytoplasm removal and acrosome formation during spermiogenesis. CCDC62 shares similar coiled-coil domains with GOPC and PICK1, proteins whose deficiencies lead to globozoospermia in mice, but may interact only with GOPC [53]. Interestingly, in humans, *CCDC62* was also shown to be co-expressed with *ZBP1*. Recently, Oud et al. identified two homozygous mutations in *CCDC62* in one patient with globozoospermia: one nonsense mutation c.442C > T; p.(Gln148Ter) and one missense mutation c.847C > T; p.(His283Tyr) (Table 1 and Fig.3). A premature stop codon is located before the missense variant and is predicted to cause nonsense-mediated decay resulting in an absence of protein.

### *New candidate genes*

Using WES, a recent study also identified five interesting candidate genes to explain the globozoospermia in a cohort of 15 patients [16]. They identified rare missense mutations in *C2CD6* (also known as *ALS2CR11*), *CCIN*, *C7orf61*, and *DHNA17* in one patient each and a frameshift mutation in *GGN* in two patients. All these genes may have putative location or function in acrosomes, but these assumptions are not clearly supported by the mutant model when available. Moreover, these missense mutations were not validated with functional work

and should be interpreted with caution. As example, missense and truncating mutations in *DNAH17* were formally associated with other forms of teratozoospermia leading to severe flagellum malformations known as MMAF phenotype in human and mouse in three recent and independent publications [10].

Overall, *DPY19L2* remains the most frequently mutated gene in globozoospermic patients (56.19%) (Fig. 4), while mutations in *SPATA16*, *PICK1*, *ZBP1*, and *CCDC62* are only reported in a few or isolated human cases. However, the link between these minor genes and globozoospermia is appropriately documented and supported by functional data or animal model to believe in their involvement in this rare sperm phenotype. Investigation of other candidate genes research should be strengthened because about 38.40% (Fig. 4) of globozoospermic patients have no diagnosis. This effort could be further supported by all the data available including the numerous KO models or the recent proteomics studies and will finally allow a better understanding of the molecular and cellular processes involved in globozoospermia.

#### *Clinical management of globozoospermic patients*

The question of the clinical management of globozoospermic patients is still debated in the literature. For several years, failed fertilization after intracytoplasmic sperm injection (ICSI) was commonly reported in patients with globozoospermia [54]. A first hypothesis provided to explain these low success rates of ICSI was the lack of the phospholipase C zeta (PLC $\zeta$ ) associated with globozoospermia [20]. PLC $\zeta$  is a testis-specific phospholipase required to trigger calcium oscillations during fertilization which is a critical step for oocyte activation [19]. Therefore, to overcome this low success rate, ICSI combined with artificial oocyte activation using Ca<sup>2+</sup> ionophore were performed and demonstrated to be able to significantly improve the pregnancy rate of ICSI with spermatozoa from globozoospermic

patients regardless of the genotype [55,56]. In addition, a newly proposed assisted gamete treatment using calcium ionophore based on PLC $\zeta$  assay, mouse oocyte activation test, and the assessment of gene mutations provided higher fertilization (42.1%) and clinical pregnancy rates (36% vs. 0%) for couples with a sperm-related oocyte activation deficiency, including patients with *PCLZ1*, *DPY19L2*, *PICK1*, or *SPATA16* gene alterations [57]. However, some studies reported that the global live birth rate per transfer remains lower in globozoospermic patients than in other infertile patients in the same age group [58,59]. Moreover, other studies have expressed concerns regarding the possible epigenetic effects of such approaches on future generations [60]. In fact, this procedure could be questionable in terms of the dramatic sperm DNA damage related to defective chromatin condensation and high DNA fragmentation described in globozoospermic sperm cells [18,26].

### **Acephalic Spermatozoa**

Infertile men affected by acephalic spermatozoa syndrome (ASS) display ejaculate containing predominantly headless spermatozoa associated with few spermatozoa without flagella. ASS was previously known as pinhead spermatozoa syndrome because the retained cytoplasmic droplet was confused with a small-sized head [61]. This syndrome was also known as decapitated and decaudated sperm defect [62].

### *SUN5*

In 1999, Chemes et al. assumed that this phenotype could be related to a genetic cause [63] because of the familial incidence (two of their patients were brothers) and because of the fact that acephalic spermatozoa arise in the testis, during spermiogenesis, due to abnormal connecting piece development. Very recently, a genetic screening strategy that consisted of combining WES and targeted Sanger sequencing [64–69] allowed the identification of 17 bi-



allelic mutations in *SUN5* (Sad1 and UNC84 domain-containing 5) found in 22 patients among the merged cohort of 60 patients (37%) (Table 2, Fig. 5 and 6).

*SUN5*, also known as *SPAG4L/TSARG4*, is a member of SUN domains proteins located on chromosome 20 and is specifically expressed in developing male germ cells [70]. The encoded protein is located to the inner nuclear membrane at the junction between the sperm head and the flagella in elongating spermatids and mature spermatozoa [71]. As described above, SUN proteins are transmembrane proteins localized to the inner nuclear membrane and interact directly with KASH domains proteins to form LINC complexes between the outer and the inner nuclear membrane in order to link the nucleus to the cell cytoskeleton [72]. These complexes are implicated in numerous cell functions such as nuclear positioning and chromosomal segregation during meiosis. KO male mice, created by CRISPR-Cas9, are totally sterile. Although the flagella are well released in the lumen, the sperm heads are phagocytosed by Sertoli cells and therefore not retrieved in the epididymis [73]. Thus, *SUN5* was described to be necessary to connect the sperm head to the midpiece, explaining the acephalic phenotype. Most of the identified mutations were localized to the SUN domain and the conserved coiled coil domain (Fig. 5) inducing a premature stop codon in the mRNA and truncated the *SUN5* protein, thus disturbing the interaction with its partners. Indeed, studies have suggested that *SUN5* interacts with other proteins like the outer dense fiber 1 (ODF1) [67] because the truncation of *SUN5* led to a decreased expression and altered distribution of ODF1, a protein known to be essential for the structure of the head tail junction and the arrangement of the mitochondrial sheath. *SUN5* also interacts with *DNAJB13*, a coupling apparatus protein, during spermatogenesis [68]. *SUN5* and *DNAJB13* co-localized to the neck region of spermatids since the beginning of spermatid elongation. *DNAJB13* plays an essential role in spermiogenesis in the formation and function of flagellar axonemes [74] and its interaction with *SUN5* seems to be crucial for the head tail junction.

### *PMFBP1*

Three other studies using WES investigated infertile patients with acephalic spermatozoa and identified 10 bi-allelic mutations in the testis-specific gene *PMFBP1* (polyamine modulated factor 1 binding protein 1) in eight individuals [69,75,76] (Table 2 and Fig. 5). All those variants generate protein truncation by creation of a premature stop codon due to substitution mutation or unusual splicing of mRNA. These studies highlighted the role of *PMFBP1* in the attachment of the connecting piece to the nuclear envelop and thus maintaining the sperm head and tail integrity. Indeed, *PMFBP1* is localized at the head-tail connecting piece of sperm and interacts secondarily with *SUN5* and *SPATA6* in order to make the liaison of the sperm head to the flagella [75]. *PMFBP1* mutations also altered the expression of *TSAG10*. Production of male KO mice using CRISPR/Cas9 confirmed the infertility phenotype with the production of acephalic spermatozoa with a disorganized mitochondrial sheath and abnormal microtubules of the axoneme [75].

### *TSGA10*

WES studies also allowed the identification of three homozygous mutations in *TSGA10* (Testis Specific Gene Antigen 10) that might be causative for ASS (Table 2 and Fig. 5) [69,77,78]. The c.211delG variant results in a truncation of the protein leading to the loss of the entire C terminus part while the c.1739A>C variant, a variant predicted to impact protein function, damaged the midpiece structure thus leading to ASS. The c.545dupT variant in exon 8 resulted in a degraded transcript due to nonsense mediated decay leading to misarranged mitochondrial sheath and abnormal flagellum. Those studies should be considered with precaution since no functional validation with animal model were performed and that the variants were found only once for each.

Association of *TSGA10* with human infertility was first suggested because *TSGA10* mRNA could not be detected in infertile patients [79]. *TSGA10* is a testis-specific protein that is localized in the midpiece of the spermatozoa and associated with the centrosome by interacting with ODF2 protein [80], a component of the outer dense fibers surrounding the sperm tail axoneme. The N terminus part of the protein is localized in the principal piece and the C terminus part is localized in the midpiece of sperm to the centrosome and basal body and is involved in centriole assembly and function. Ultrastructural studies of acephalic spermatozoa showed that some cases had defects in the formation of the connecting piece during late spermiogenesis that led to separation of head from tail at the level of the proximal centriole [81], which was concordant with the observed breakage at the midpiece of the sperm [77].

#### *DNAH6*

Recently, a compound heterozygous missense variant (c.7706G>A; p.Arg2569His and c.2454A>T; p.Glu818Asp) (Table 2 and Fig. 5) was identified in *DNAH6* (dynein axonemal heavy chain 6) in a Chinese patient with ASS [82]. Li et al. showed the complete absence of *DNAH6* mRNA in the patient's spermatozoa and suggested that this compound heterozygous variant may induce non-sense mRNA decay. Semen examination showed sperm head defects with 30% of acephalic spermatozoa but also 69% of round or small headed spermatozoa and 1% of double tail or double head spermatozoa [82]. Two ICSI cycles were performed on 10 oocytes leading to two embryo transfers, but no pregnancy was achieved. *DNAH6* is an axonemal dynein heavy chain located in the neck region of normal spermatozoa and may be associated with centriolar proteins which are critical for sperm head–neck connection [82]. The formal involvement of *DNAH6* in ASS needs to be further investigated because *DNAH6* mutations were also associated with primary ciliary dyskinesia [83], azoospermia [84], and MMAF [85].

## *BRDT*

In 2017, Li et al. found a homozygous *BRDT* (bromodomain testis association) variant using WES (c.G2783A; p.Gly928Asp, exon 19) (Table 2 and Fig.5) in a patient with acephalic spermatozoa from a consanguineous family [86]. *BRDT* is a testis specific gene expressed in spermatocytes, round spermatids, elongated sperm, and mature sperm in humans. It contains two bromodomains involved in chromatin remodeling through a structural role in the condensation of acetylated chromatin [87]. The Gly928Asp mutation is in the P-TEFb binding domain, which mediates the interaction with transcription elongation factor and might affect the transcriptional activities of downstream genes. Using RNA-sequencing analysis of cells expressing the *BRDT* mutation, the authors found the p.Gly928Asp mutation protein causes mis-regulation of 899 genes compared with *BRDT* WT cells and that most of the upregulated genes in p.G928D cells were enriched in the processes of intracellular transport, RNA splicing, cell cycle and DNA metabolic process [86]. However, the role of *BRDT* in the physiopathological mechanism of the ASS remains to be fully clarified especially as *BRDT* was also reported to be associated in non-obstructive azoospermia [88].

## *CEP112*

Sha et al. recruited two infertile patients with an acephalic spermatozoa phenotype and performed WES and Sanger sequencing [89]. They found a homozygous variant in *CEP112* (Centrosomal Protein 112) in both patients (Table 2 and Fig. 5). These variants impacted the coiled-coil domain of *CEP112*, which likely hampers the protein function. *CEP112* is highly expressed in the testis and encodes a centrosome-associated protein, although its biological function in male reproduction has not been described in the literature. However, *CEP112* was considered by the authors to be a credible candidate gene because it plays a central role in anchoring the sperm head to the tail during the biogenesis of the centriole and establishes normal sperm head morphology [17].

### *Candidate genes and animal models*

Mouse models for acephalic spermatozoa were also reported in the literature. In 2015 Yuan et al. studied *Spata6* (Spermatogenesis Associated 6), a testis specific gene, in KO mice [90]. *Spata6* is required for the formation of the sperm connecting piece during spermiogenesis and is thought to be implicated in myosin-based microfilament transport via interaction with myosin subunits. Homozygous KO mice did not perfectly mimic the human phenotype by producing acephalic spermatozoa with a bent connecting piece. A similar phenotype has been described in knockout animals for *Oaz3* [91]. *Oaz3* (also known as *Oaz-t*) (Ornithine decarboxylase antizymes) is expressed specifically in germline cells during spermiogenesis and plays a role in cell growth and proliferation. KO male mice were infertile and the heads and tails of the sperm cells were separated during incubation which showed that *Oaz3* may be a factor in the formation of a rigid head–tail junction during late spermatogenesis. Furthermore, fertilization was successful after intracytoplasmic sperm injection using these deficient spermatozoa. Another study demonstrated that KO mice for *Hook1* have abnormal sperm head shape and fragile head–tail junction [92]. Indeed, *Hook1* is required for spermatid differentiation and is involved in the positioning of the microtubules of the manchette and the flagellum, thus it facilitates the attachment of intracellular organelles and the elongation of the manchette in late spermiogenesis. In addition, a missense heterozygous variant (c.848T>C; p.Gln286Arg) was found in the coiled-coil domain of *HOOK1* (Hook microtubule-tethering protein 1) [93] and was thought to be associated with ASS in three patients. Further studies are required to further validate the association. Finally, *Odf1* (outer dense fibre 1), which encodes the main protein of the sperm tail outer dense fibers that surround the axoneme, was inactivated in mice. KO male mice were infertile due to detachment of the sperm head and disorganization of the mitochondrial sheath and outer dense fibers, as observed using transmission electron microscopy [94]. Furthermore,

heterozygous *Odf2* mutant male mice were reported in another study as infertile. Indeed, *Odf2* haploinsufficiency induced sperm neck–midpiece separation. These cells were immotile, but capable of producing offspring by ICSI [95].

#### *Diagnosis strategy and ICSI prognosis*

Overall, mutations in *SUN5* have emerged as the primary underlying gene for the ASS phenotype (Fig. 6). Thus, for patients with acephalic spermatozoa, the genetic diagnosis should be first based on a targeted sequencing of the *SUN5* gene and subsequently, if no mutations are found, WES analysis may be useful. Concerning the prognosis of ICSI using the few residual sperm cells with a head and tail (ranging from 0.9 to 4.4% including spermatozoa with abnormal junction of head–tail) from some ASS patients, most studies reported a positive pregnancy and live births for both patients with *SUN5* [66,73] or *PMFBP1* [75,76] mutations. For these patients, ICSI seems to be a valuable therapeutic option with a good prognosis. Nevertheless, ICSI failure was reported for a couple with ASS patient carrying a *TSGA10* variant [80]. Thus, ICSI could be an optimal therapeutic approach to overcome infertility in ASS; however, due to the limited number of studies, it remains difficult to definitively conclude [96].

#### **Macrozoospermia**

Macrozoospermia, also known as large-headed spermatozoa, is a rare cause of male infertility observed in <1% of cases [97]. This phenotype is characterized by the presence of a majority of large-headed multiflagellar spermatozoa in the ejaculate. These morphological anomalies are often associated with severe oligo-asthenozoospermia [7]. Since its first description in 1977, numerous studies have been conducted to better describe its pathophysiology and showed that large-headed spermatozoa had an abnormal genomic DNA

content, and were either diploid, triploid, or tetraploid [98]. These findings suggested that both meiotic divisions were likely affected by incomplete segregation of homologous chromosomes during meiosis I and sister chromatids during meiosis II. This impaired segregation is followed by failure of nuclear cleavage and cytokinesis. Furthermore, it was shown that the very few normal sized-head spermatozoa found in ejaculate from some macrozoospermic patients also present a high rate of aneuploidy, demonstrating that the sperm head morphology was not correlated with the chromosomal content in macrozoospermia [99]. In addition, sperm DNA fragmentation studies using the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labelling (TUNEL) assay revealed a high level of apoptotic sperm cells in macrozoospermic men compared with that of controls [100].

A description of familial cases with consanguineous parents was suggestive of a genetic cause with an autosomal recessive inheritance. In 2007, Dieterich et al. used a genome-wide microsatellite scan to investigate a cohort of 14 macrozoospermic individuals who originated from North Africa and identified a homozygous deletion of a single base pair in the *AURKC* gene (c.144delC; p.Leu49TrpfsTer23) in all studied cases [101] (Fig.7) . The encoded protein is a member of the Aurora subfamily of serine/threonine protein kinases. The human genome contains three aurora kinases: *AURKA*, *AURKB*, and *AURKC* that share a similar structure and kinase activity, but exhibit a distinct cellular expression profile and subcellular localization [102]. *AURKA* operates at centrosomes and on spindle microtubules in mitotic cells [103]. *AURKB* and *AURKC* both are components of the chromosomal passenger complex (CPC) [104]. The CPC is involved in chromosome binding to kinetochores and thus plays an important role during cell division to ensure correct chromosome alignment and segregation and activation of the spindle assembly checkpoint [105]. Unlike mitotic cells that require only *AURKA* and *AURKB*, germ cells also need the

AURKC to ensure efficient meiosis [106]. Perturbation of the CPC in cultured cells gives rise to chromosome segregation errors and cytokinesis failure. Therefore, loss-of-function mutations of AURKC, the catalytic component of the CPC in meiotic germ cells, could lead to impaired meiosis and macrozoospermia [107].

*AURKC* is located on 19q13.3-qter. It has seven exons and is highly expressed in male germ cells. The c.144delC variant introduces a frameshift, leading to a premature stop codon and the production of a truncated protein that lacks the kinase domain. Another variant (c.744C>A; p.Y248\*) was further identified in the European and American populations in the homozygous state or was compound heterozygous with the c.144delC [108,109] (Fig. 7). The impact of these two variants was investigated through functional tests using a vector system that expresses the mutated mRNAs and proteins in oocytes from *Aurkc*<sup>-/-</sup> mice. Results confirmed the loss-of-function of c.144delC AURKC and showed that both mutations perturb the normal localization of the CPC [110]. Interestingly, women affected by AURKC mutations in the homozygous state are fertile thus excluding a fundamental role of *AURKC* in female meiosis and highlighting that molecular pathways involved in meiosis could be different between males and females [111].

Kimmins et al. generated *Aurkc* KO mice by homologous recombination and found that homozygous KO males had reduced fertility, producing smaller litters compared to WT animals or failed to produce pups [112]. At the cellular level, KO mice displayed abnormal sperm, characterized by defective chromatin condensation and acrosome malformation, suggesting a critical role of AURKC in the epigenetic program of the developing male germ cells in mice. Interestingly, the phenotype observed in mice suggests a role for *Aurkc* during spermiogenesis, which is not observed in human. This variable phenotypic expression between these two species could be explained by a plausible compensation of the AURKC



activity by an enhanced activity of another member of the aurora kinase family through its overexpression [112].

To date, the only causative gene for macrozoospermia in human is *AURKC*, and both recurrent mutations c.114delC and p.Y248\* account for almost all identified *AURKC* mutations [7]. Other rarer *AURKC* mutations have been more recently identified, such a missense variant (c.686G>A; p.C229Y) in exon 6 (Fig. 7). This substitution causes a cysteine to tyrosine change at amino acid 229, a highly conserved residue located within the kinase domain. This change leads to the production of a hypomorphic protein that cannot fully support cell cycle progression [110]. Intronic variants affecting splicing were also investigated. A splice acceptor variant c.436-2A>G located at the 3' end of intron 4-5 were identified as compound heterozygous with c.144delC in two Tunisian brothers [113] (Fig. 7). This variant causes the skipping of exon 5 in mature mRNA leading to the production of a truncated protein. Recently, a new homozygous missense variant (c.269G>A; p.Arg90Gln) in exon 3 of the *AURKC* gene was reported in a Chinese family with macrozoospermia leading to significantly decreased levels of *AURKC* mRNA in whole blood [114]. Expression studies in testis would have been more relevant to confirm the deleterious effect of the variant and to exclude the presence of putative tissue-specific alternative transcripts, but testicular biopsy samples were not available. Among *AURKC* mutations, the c.144delC is the most frequent and account for approximately 90% of the *AURKC* mutations [7]. In North Africa, the high frequency of this mutation in the homozygous state makes *AURKC* infertility among the most frequent single-gene defect in this population [115]. In European population the nonsense variant (c.744C>A; p.Y248\*) seems to be the main alteration observed in the *AURKC* gene. With regards to this divergence and the presence of two main founder effects related to the *AURKC*, molecular analysis of this gene in clinical practice should begin with screening of c.144delC in exon 3 and c.744C>A in exon 6. In case of an absence of negative screening or

the presence of a heterozygous variant, investigation of all exons 1–7 of *AURKC* with the flanking splicing regions is recommended.

The high rate of aneuploidy and DNA fragmentation, in addition to other anomalies of sperm parameters from macrozoospermic patients are the principal causes for infertility. Recurrent miscarriages or assisted reproduction failure using ICSI were invariably reported in macrozoospermic patients carrying *AURKC* mutations [7,114,116]. Therefore, ICSI is formally contraindicated in these cases even following deep morphological characterization and selection of sperm cells. For patients who do not carry *AURKC* mutations and display a lower percentage of macrocephalic spermatozoa in their ejaculate, ICSI should be discussed. In this case, sperm FISH analysis is useful to estimate the rate of aneuploidy in order to assess the feasibility and the success of ICSI [7].

### **To the top and beyond!**

Genetic investigation of monomorphic sperm phenotypes with head defects has allowed the identification of two major genes, *AURKC* and *DPY19L2*, in which mutations are the main genetic causes of macrozoospermia and globozoosmermia, respectively. To a lesser degree, mutations in the single gene *SUN5* seem to account for most cases (37%) observed for acephalic spermatozoa.

Interestingly, since the identification of *AURKC*, *DPY19L2*, and *SPATA16* a decade ago using homozygous mapping, few new genes have been identified despite the advent of NGS. No new genetic causes with clinical significance in macrozoospermia were formally identified in humans. For globozoospermia, other genes have only recently been suggested to be involved in this phenotype, but only a handful of patients carrying variants of these genes have been described, and the involvement of most of these genes in globozoospermia has not

been clearly demonstrated at this time [16]. One explanation may be that these two specific phenotypes are extremely rare and are only seen very occasionally in routine clinical practice whereas a very large cohort of patients with the same phenotype is needed to identify mutations in a minor gene with a relative lower prevalence. Therefore, in-depth bioinformatic analyses of NGS data together with the inclusion of additional patients will likely improve the diagnosis yield and bring to light other rare genetic defects inducing these phenotypes. Furthermore, while we can expect that a single gene defect will be found in patients with the most severe and pure phenotype, it is unlikely to find a monogenic cause in the remaining patients of the cohort with milder or more heterogeneous sperm phenotype. Indeed, in such patients, the causes of infertility may be often multifactorial involving variations in multiple genes (oligogenic or polygenic factors) coupled with environmental causes that are more challenging to explore. This hypothesis is supported by our recent study in a large cohort of globozoospermic patient in which a *DPY19L2* variant is only found in patients with an abnormal rate of sperm cells above 50% [45]. Finally, this may also be explained in part by the fact that some pathogenic variants may be not detected by WES, the most currently used approach for gene identification (e.g., deep intronic variants, complex copy number variations, structural variations). To improve the global diagnosis yield and detect new causative genes, more powerful techniques such as whole-genome sequencing may now be envisaged for the genetic exploration of teratozoospermic patients with WES negative results [117]. Conversely, WES enabled the identification of six different genes (*SUN5*, *PMFBP1*, *TSGA10*, *DNAH6*, *BRDT*, and *CEP112*) involved in acephalic spermatozoa, confirming that this phenotype is genetically heterogeneous as it is observed in the MMAF phenotype [10]. Despite this, a large part of the studied patients with acephalic spermatozoa remains without a diagnosis (~57%) (Fig. 6). Overall, numerous other genes are therefore likely to be involved in these three phenotypes and it is critical to pursue studies to improve our genetic

understanding of the molecular pathogenesis of sperm head defects. However, beyond the simple identification of candidate genes linked to a specific phenotype, it is important to decipher the protein function encoded by the genes identified and the physiopathological mechanisms leading to the phenotype by performing functional work and/or phenotypic characterization using animal models [118].

Currently assisted reproductive technologies can circumvent infertility, and in vitro fertilization using ICSI is possible for most patients regardless of the etiology of the defects. Unfortunately, the availability of these technologies does not guarantee success and almost half of all couples who seek reproductive assistance fail to achieve a pregnancy. The improvement of ART procedures is therefore particularly relevant, and this strongly relies on a better understanding of the molecular physiopathology of infertility. Some questions remain concerning the prognosis of ICSI using sperm with head abnormalities. Such prognoses are already available for *AURKC* patients for whom ICSI is contraindicated to patients with a homozygous *AURKC* pathogenic variant. However, the interest of ICSI in globozoospermia is still an issue of discussion and the lively debate on the use of artificial oocyte activation should encourage physicians to remain cautious. Successful applications of ICSI in patients with acephalic spermatozoa are overall in favor of a good prognosis for this therapeutic strategy. However, the ICSI outcome of some *TSGA10* mutated ASS individuals was compromised, suggesting that some differences may exist between the individual genotypes as it was observed in the *MMAF* phenotype [10]. Indeed, the impact on the centriole function could vary, depending on the gene defects, and recent advances in the role of the distal centriole in human zygote development may explain such discrepancies between genes. Additional correlation studies should be performed to consider the individual genotypes in the counseling of ASS patients and the identification of further ASS genes may help in better defining the prognosis of ASS patients for ICSI.

## **Conclusion**

In conclusion, we are certain that the efforts of the international community to regularly increase the list of genes associated with infertility should expand our basic knowledge of mammalian spermatogenesis. A clearer understanding of the physiopathology should provide better advice and new hope for treatments that should soon be available to many more patients, especially those with the most severe defects, such as sperm head defects.

## **Practice points**

- Teratozoospermia is defined as morphological defects of the spermatozoa including sperm head defects and accounts among the most severe form of male infertility.
- Genetic defects are the main causes of severe head sperm defect such as globozoospermia, macrozoospermia and acephalic spermatozoa.
- Homozygous deletions of the *DPY19L2* gene are the main genetic cause of globozoospermia and should be investigated first in patients with this sperm phenotype.

- Punctual mutations in *AURKC* are the only genetic causes known of macrozoospermia and molecular analysis of this gene in clinical practice should begin with screening of the recurrent c.144delC in exon 3 and c.744C>A in exon 6.
- Six genes are currently associated with acephalic spermatozoa but mutations in *SUN5* remain the most frequent genetic defects in this sperm phenotype. Whole-exome sequencing appears to be an interesting approach to investigate patients with acephalic spermatozoa.
- ICSI is formally contraindicated for patients with *AURKC* mutations due to the high and invariable risk of aneuploidy.
- ICSI in globozoospermia is still an issue of discussion and the use of artificial oocyte activation should be performed with caution.
- ICSI in patients with acephalic spermatozoa are overall in favor of a good prognosis for this therapeutic strategy.

### **Research agenda**

- Numerous other genes are likely to be involved in these three phenotypes and it is critical to pursue studies to improve our genetic understanding of the molecular pathogenesis of sperm head defects.
- Beyond the simple identification of candidate genes, functional work and/or phenotypic characterization using animal models should be performed to decipher the physiopathological mechanisms leading to the phenotype.
- A better understanding of the physiopathology of sperm head defects should provide a basis for the development of therapeutic solutions tailored to the individual defects.
- Correlation studies should be performed to take into account the individual genotypes of patients to better define the ICSI prognosis of patients with sperm head defects.

## Acknowledgments

None

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## TABLES

**Table 1** List of mutations previously identified in the 5 genes confidently associated in globozoospermia.

Gene name	Chromosome position (hg19)	Coding sequence variant	Protein variant	Overall number of studied patients	Overall number of mutated patients	Reference
<b>SPATA 16</b>	3:172,889,357-173,141,235	c.848G>A	p.Arg283Gln	29 globozoospermic men including 15 unrelated men	3 brothers from a consanguineous family	[23]
		Ex2del	n.d	16 globozoospermic men including 6 patients from 3 families	2	[25]
<b>DPY19L2</b>	12:63,558,913-63,669,201	c.153_189del	p.Trp52SerfsTer7	69 globozoospermic men	1	[45]
		c.368A>G	p.His123Arg	9 globozoospermic men	1	[43]
		c.575A>G	p.His192Arg	69 globozoospermic men	1	[45]
		c.586G>C	p.Glu196Gln		1	

		c.715G>C	p.Gly239Arg	15 globozoospermic men	1	[16]
		Ex5_6del	n.d	54 globozoospermic men	2	[38]
		Ex5_7del	n.d		1	
		Ex7del	n.d	72 globozoospermic men	4	[40,44]
		c.869G>A	p.Arg290His	169 globozoospermic men	5	[37-39,45]
		c.892C>T	p.Arg298Cys	145 globozoospermic men	6	[38,42,45]
		c.893G>A	p.Arg298His	69 globozoospermic men	1	[45]
		c.925C>A	p.Gln309Lys		1	
		c.989T>C	p.Leu330Pro	15 globozoospermic men	1	[39]
		c.1024C.T	p.Met358Lys	34 globozoospermic men including 31 unrelated men	1	[37]



		c.1033C>T	p.Gln345Ter	54 globozoospermic men	1	[38]
		c.1073T.A	p.Gln342Ter	34 globozoospermic men including 31 unrelated men	2	[37]
		c.1182-1184delATC	p.Leu394_Ser395delinsPhe	9 globozoospermic men	1	[43]
		c.1182-1186delATCTT	p.Leu394PhefsTer19		1	
		c.1183delT	p.Ser395LeufsTer9	123 globozoospermic men	3	[38,45]
		c.1218 + 1G>A	p.del372-384	54 globozoospermic men	1	[38]
		c.1438G>A	p.Glu480Lys	69 globozoospermic men	1	[45]
		c.1478C>G	p.Thr493Arg	54 globozoospermic men	1	[38]
		c.1532delA	p.Lys511RfsTer8	15 unrelated globozoospermic men	2	[39]
		c.1553-1554delAT	p.Tyr518CysfsTer14	9 globozoospermic men	1	[43]

		c.1579_1580 +4 delAGGTAAinsTCAT	n.d	21 globozoospermic men including 18 unrelated men	1	[42]
		c.1580+1G>A	p.512_527delfsTer5	69 globozoospermic men	1	[45]
		c.[1679delT; 1681_1682delAC]	p.Leu560Ter	15 unrelated globozoospermic men	1	[39]
		c.1720C>T	p.Arg574Ter	9 globozoospermic men	1	[43]
		c.1840G>T	p.Glu614Ter	69 globozoospermic men	1	[45]
		c.2038A>T	p.Lys680Ter	54 globozoospermic men	1	[38]
		c.2126+5G>A	n.d	9 globozoospermic men	1	[43]
<b>PICK1</b>	22:38,056,311- 38,075,704	c.198G>A	p.Gly393Arg	1	3	[49]
<b>ZPBP1</b>	7:49,846,355- 50,121,329	c.50G>C	p.Arg17Pro	381 unrelated teratozoospermic men	2	[50]
		c.550A>G	p.Ser184Gly		1	

		c.650A>T	p.His217Leu		1	
		c.673G>A	p.Gly225Ser		1	
		c.860T>C	p.Ile287Thr		1	
		c.755A>G	p.Asn252Ser		1	
		c.1056+1G>A	n.d		1	
		c.1056+8T>A	n.d		1	
		Ex5_7del	n.d		1	
		Ex6_7del	n.d		1	
		Ex7del	n.d		3	
		Ex7del, ins144	n.d		2	
		c.931C>T	p.Gln311Ter	15 globozoospermic men	1	[16]
<b>CCDC62</b>	12:122,773,621-122,827,528	c.442C > T/ c.847C > T	p.Gln148Ter/p.His283Tyr	15 globozoospermic men	1	[16]

**Table 2** List of mutations previously identified in the 6 genes implicated in acephalic spermatozoa syndrome.

Gene name	Chromosome position (hg19)	Coding sequence variant	Protein variant	Cohort description	Mutated patients	Reference
SUN5	20:31571580-31592240	c.824C>T	p.Thr275Met	17 unrelated men	1	[64]
		c.851C>G	p.Ser284Ter		1	
		c.340G>A	p.Gly114Arg		1	
		c.1066C>T/ c.485T>A	p.Arg356Cys / p.Met162Lys		1	
		c.381delA/ c.824C>T	p.Val128SerfsTer7 / p.Thr275Met		1	
		c.381delA/ c.781G>A	p.Val128SerfsTer7 / p.Val261Met		1	
		c.216G>A/ c.1043A>T	p.Trp72Ter / p.Asn348Ile		1	

		c.425+1G>A/c.1043A>T	p.Asn348Ile / n.d		1	
		GRCh38 chr20:32995761_32990672 delinsTGGT	p.Leu143SerfsTer30	3 related men	3	[65]
		c.829C>T	p.Gln277Ter	3 unrelated men	1	[66]
		c.1067G>A	p.Arg356His		1	
		c.211+1_211+2 dupGT	p.Ser71Cysfs11Ter		1	
		c.381delA	p.Val128SerfsTer7	15 unrelated men	5	[67]
		c.781G>A/ c.1043A>T	p.Val261Met / p.Asn348Ile	3 unrelated men	1	[68]
		c.425+1G>A/c.475C>T	p.Arg159Ter / n.d		1	
		c.772C>T	p.Arg258Cys	11 unrelated men	1	[69]

<b>PMFBP1</b>	16:72,112,157-72,176,878	c.1462C>T	p.Gln488Ter	4 unrelated men + 2 brothers from a consanguineous family	2	[75]
		c.2404C>T	p.Gln802Ter		1	
		c.2725C>T	p.Arg909Ter		1	
		c.2725C>T/ c.2092delG	p.Arg909Ter / p.Ala698ProfsTer7		1	
		c.2561_2562del	p.Lys854ArgfsTer5	2 unrelated men	1	[76]
		c.2561_2562del/ c.327T>A	p.Lys854ArgfsTer5 / p.Trp109Ter		1	
		c.361C>T/ c.2089-1G>T	p.Gln121Ter / p.Ile697LeufsTer257	11 unrelated men	1	[69]
<b>TSGA10</b>	2:98,997,261-99,154,964	c.211delG	p.A71HfsTer12	1 man from a consanguineous family	1	[77]
		c.1739A>C	p.Gln580Pro	11 unrelated men	1	[69]
		c.545dupT	p.Ala183SerfsTer10	1 man from a consanguineous	1	[78]

				family		
<b>DNAH6</b>	2:84,516,455-84,819,589	c.7706G>A/ c.2454A>T	p.Arg2569His / p.Glu818Asp	1 man	1	[82]
<b>BRDT</b>	1:91,949,371-92,014,426	c.G2783A	p.Gly928Asp	1 man	1	[86]
<b>CEP112</b>	17:65,635,537-66,192,133	c.496C>T	p.Arg166Ter	2 unrelated men	1	[89]
		c.2074C>T/ c.2104C>T	p.Arg692Trp / p.Arg702Cys		1	

n.d: not determined

## FIGURE LEGENDS

**Fig. 1** Electron microscopy analysis of spermatozoa from different types of sperm head defects. (A) Control spermatozoa presents with a normal-shaped head and a flagellum joined together by the connecting piece. (B) Magnification of a normal-shaped sperm head showing the nucleus (N) surrounded by the acrosome (Ac). OAM: outer acrosomal membrane, IAM: inner acrosomal membrane. (C) A typical globozoospermic spermatozoa from a *DPY19L2* deleted patient presents with a round-shaped head, absent or atrophied acrosome and an absence of mitochondria in the midpiece (D) Typical acephalic spermatozoa from a *SUN5* mutated patient. Tailless spermatozoa contain a slightly enlarged nucleus with a typical acrosome structure but lack the implantation fossa and basal plate. (E) A typical macrospermic spermatozoon from a patient with a homozygous *AURKC* c.144del mutation presents with a large head, irregular head outline and multiple flagella.

**Fig. 2** Known proteins involved in acrosome biogenesis whose functional absence leads to globozoospermia in human. Acrosome biogenesis includes several steps including protein processing within the endoplasmic reticulum, vesicle trafficking from the Golgi apparatus, vesicle fusion, acrosome integrity, interaction of the acrosomal and nuclear membranes with the acroplaxome, and maintaining integrity of the acrosomal matrix.

**Fig. 3** Location of mutations in the intron-exon structure and in the protein representation of the five genes (*DPY19L2*, *PICK1*, *SPATA16*, *ZBP1*, and *CCDC62*) associated with globozoospermia in humans. Exons are indicated as a black box, untranslated region as a clear box, introns as a line and the localization of the identified point mutations is shown by a line and the span of the identified genomic deletions is indicated by a line with two arrows at the end. Colored boxes indicate the known identified domains of the corresponding proteins.

**Fig. 4** Mutational frequency of *DPY19L2*, *PICK1*, *SPATA16*, *ZBP1*, and *CCDC62* mutations among 388 globozoospermic patients reported in the literature. The distribution of *DPY19L2* mutations are also reported (right side).

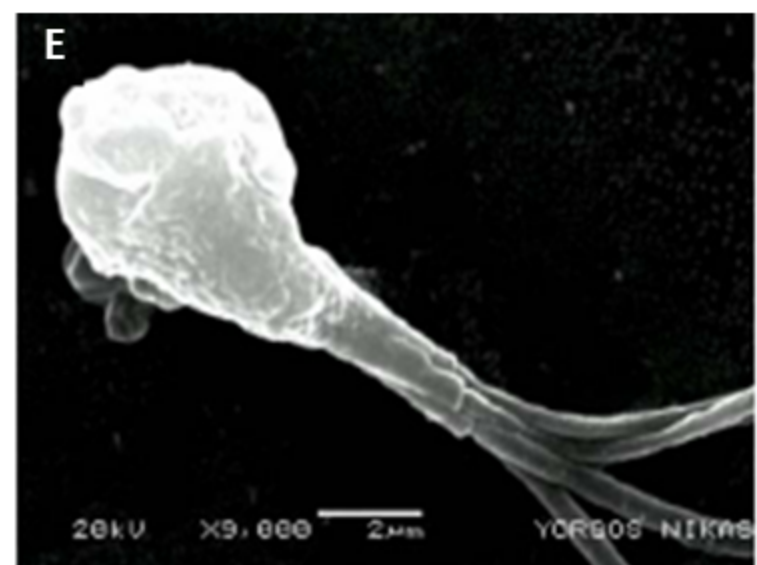
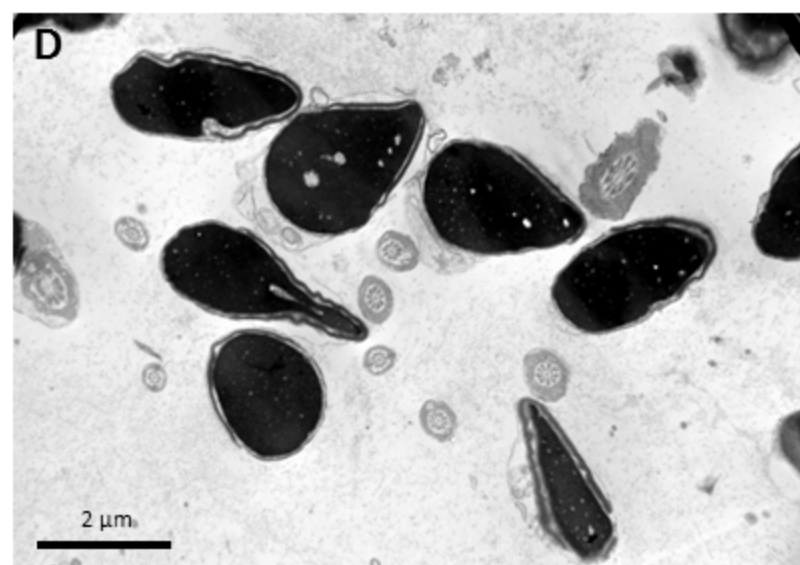
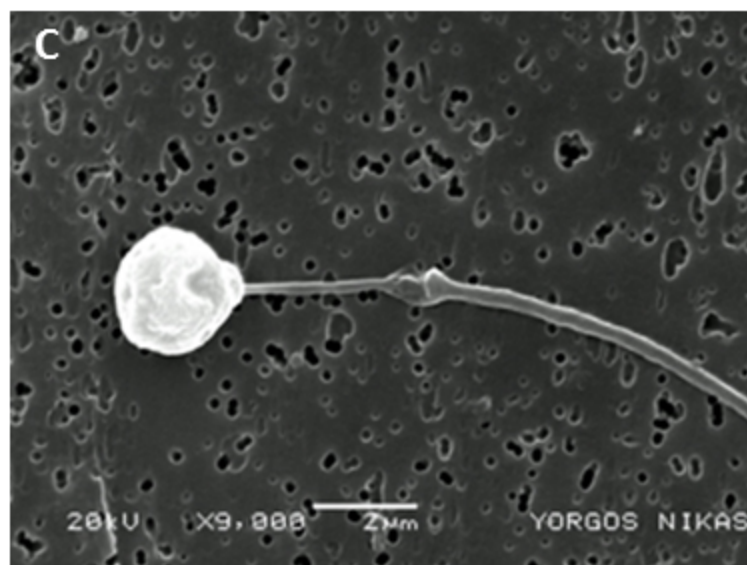
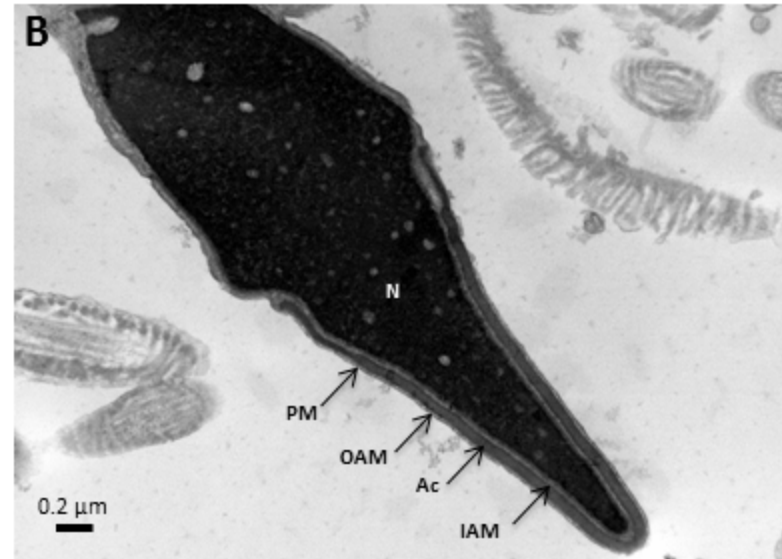
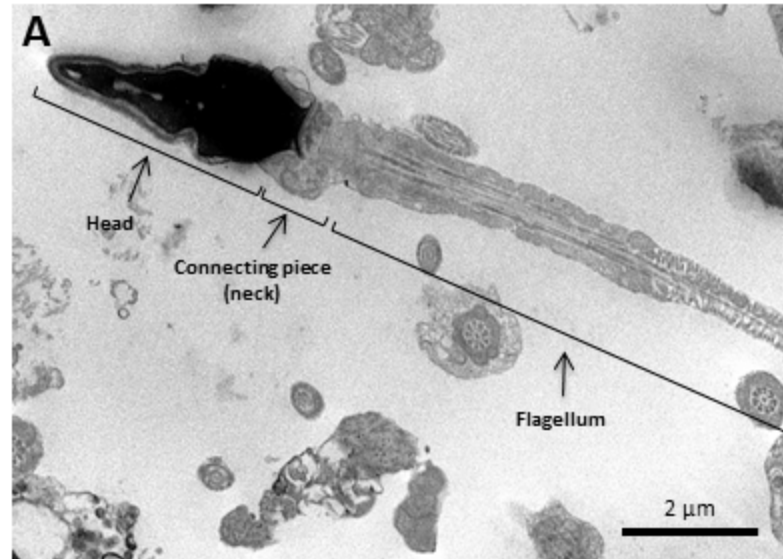
**Fig. 5** Location of mutations in the intron-exon structure and in the protein representation of the seven genes (*SUN5*, *PMFBP1*, *TSAG10*, *DNAH6*, *BRDT*, and *CEP112*) associated with acephalic spermatozoa syndrome in human. Exons are indicated as a black box, untranslated region as a clear box, introns as a line and the localization of the identified point mutations is shown by a line. Colored boxes indicate the known identified domains of the corresponding proteins.

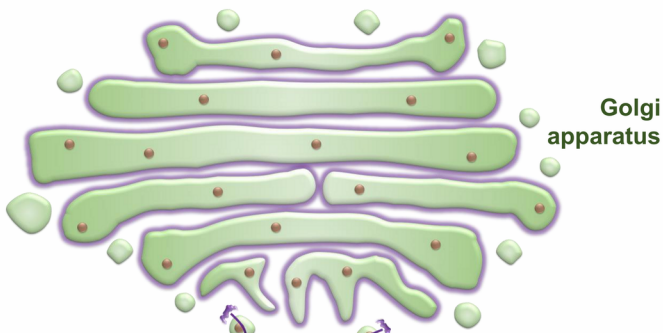


**Fig. 6** Mutational frequency of *SUN5*, *PMFBP1*, *TSAG10*, *DNAH6*, *BRDT*, and *CEP112* mutations among 66 ASS patients reported in the literature. The detailed distribution of *SUN5* mutations are also reported (right side).

**Fig. 7** Location of *AURKC* mutations in the intron-exon structure and the protein representation associated with macrozoospermia in human. Exons are indicated as a black box, untranslated region as a clear box, introns as a line, and the localization of the identified point mutations is shown by a line and the span of the identified genomic deletions is indicated by a line with two arrows at the end. Colored boxes indicate the known identified domains of the *AURKC* protein.

E





**Golgi apparatus**

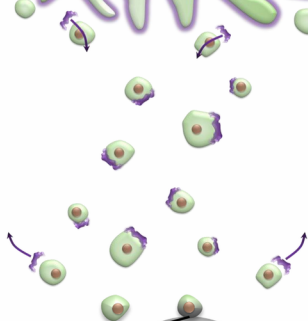
### **SPATA16**

Acrosome integrity

### **PICK1**

Vesicles trafficking and fusion

**Pro-acrosomal vesicles**



### **ZBPB1**

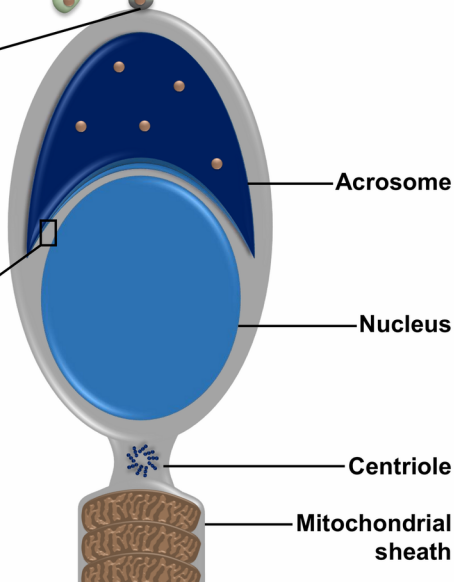
Vesicles fusion

### **CCDC62**

Co-expressed with ZBPB1

### **DPY19L2**

Interaction with nuclear envelope



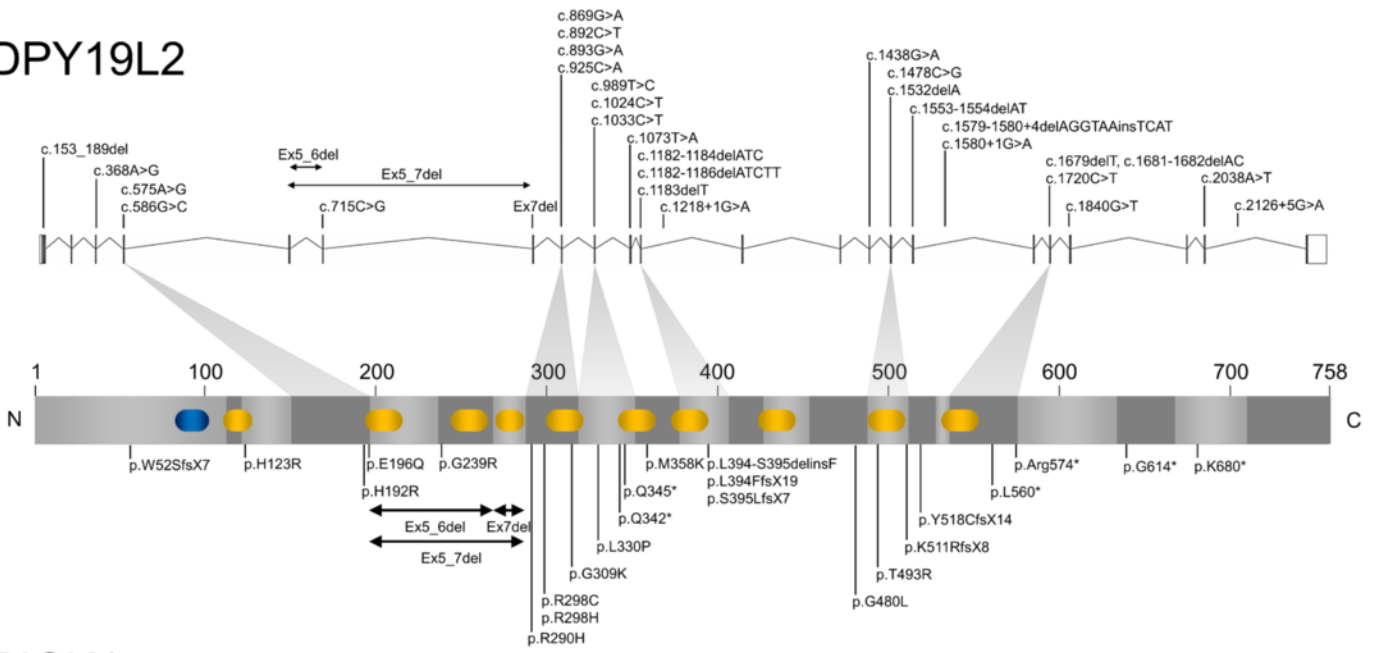
**Acrosome**

**Nucleus**

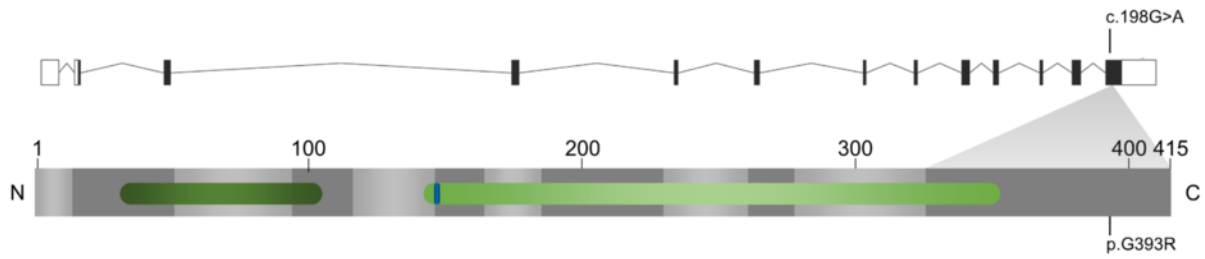
**Centriole**

**Mitochondrial sheath**

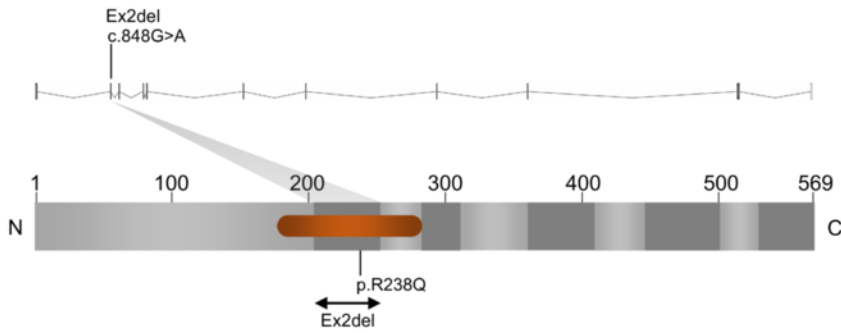
## DPY19L2



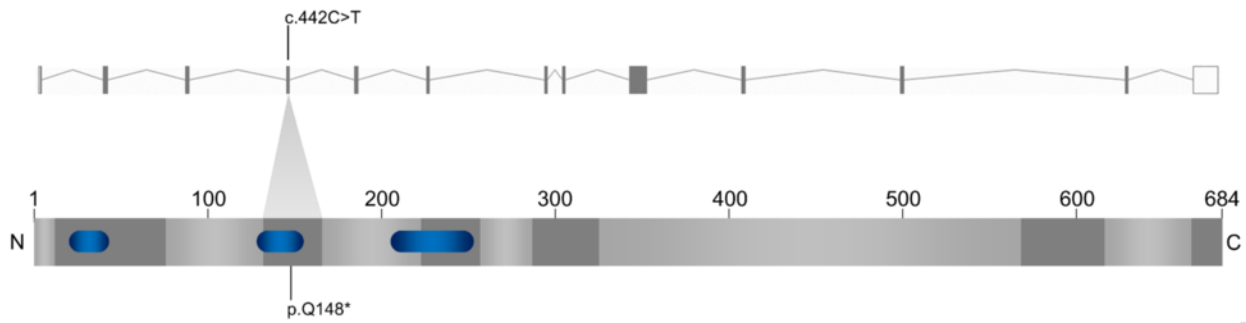
## PICK1



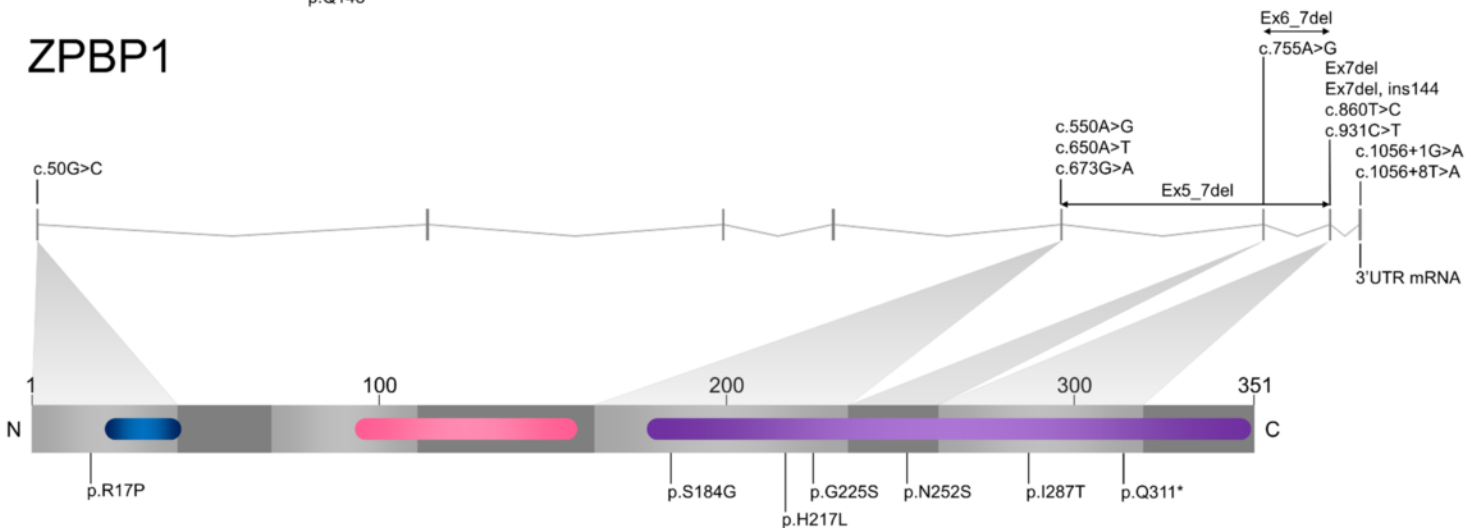
## SPATA16



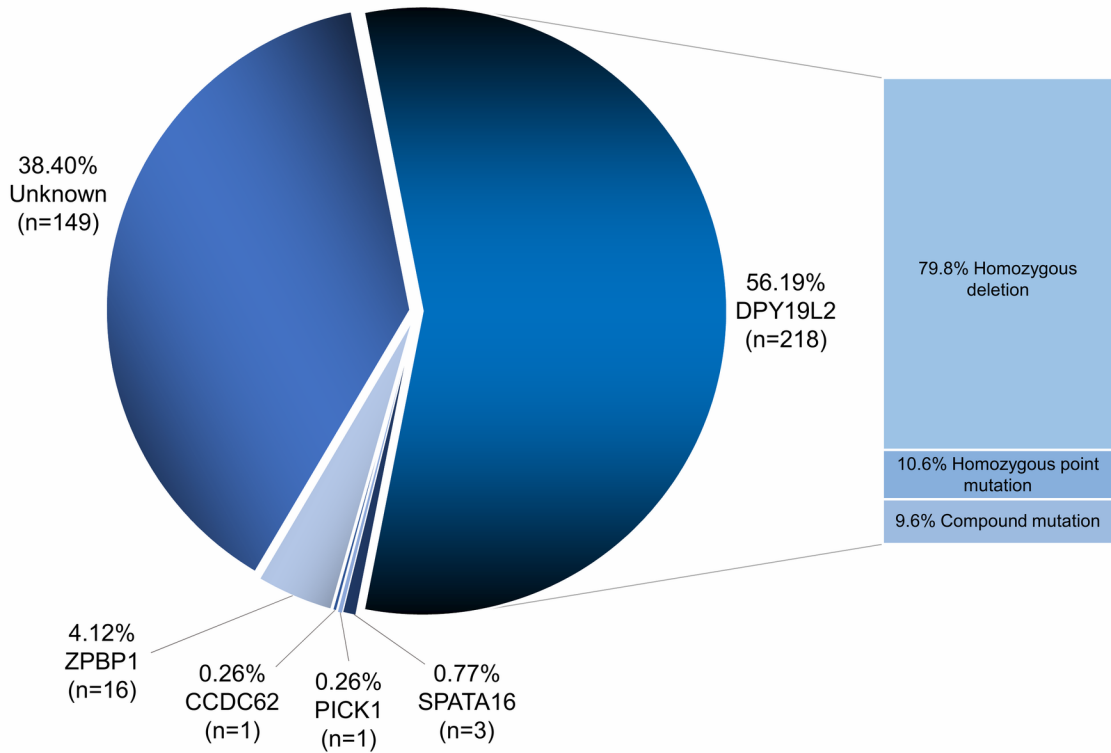
## CCDC62



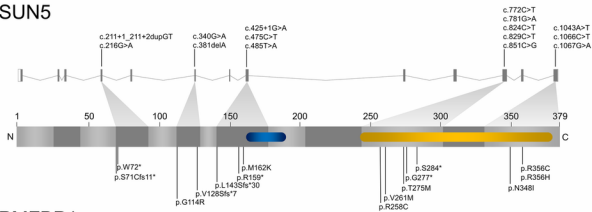
## ZPBP1



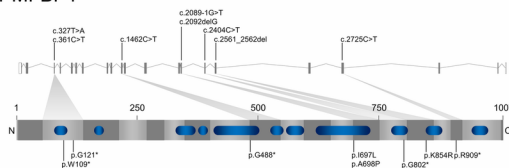
- Coiled-coiled domain
- Transmembrane helices
- PDZ domain
- AH/BAR domain
- Tetratricopeptide-like helical domain
- Immunoglobulin-like domain
- Zona-pellucida-binding domain



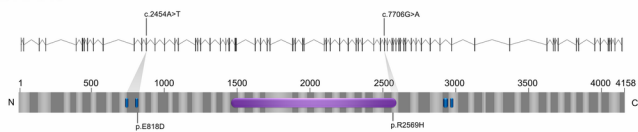
## SUN5



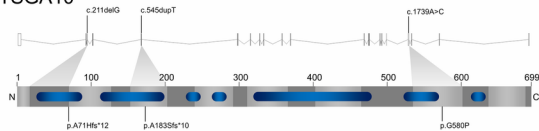
## PMFBP1



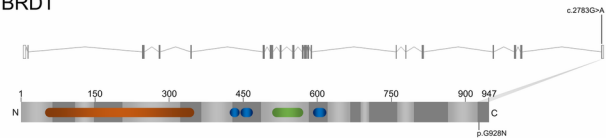
## DNAH6



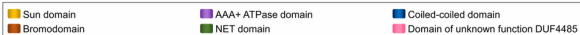
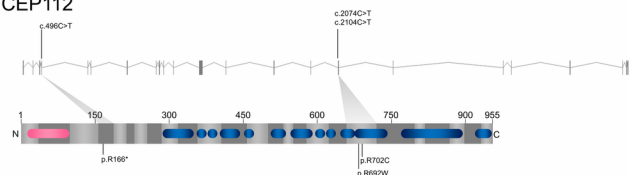
## TSGA10

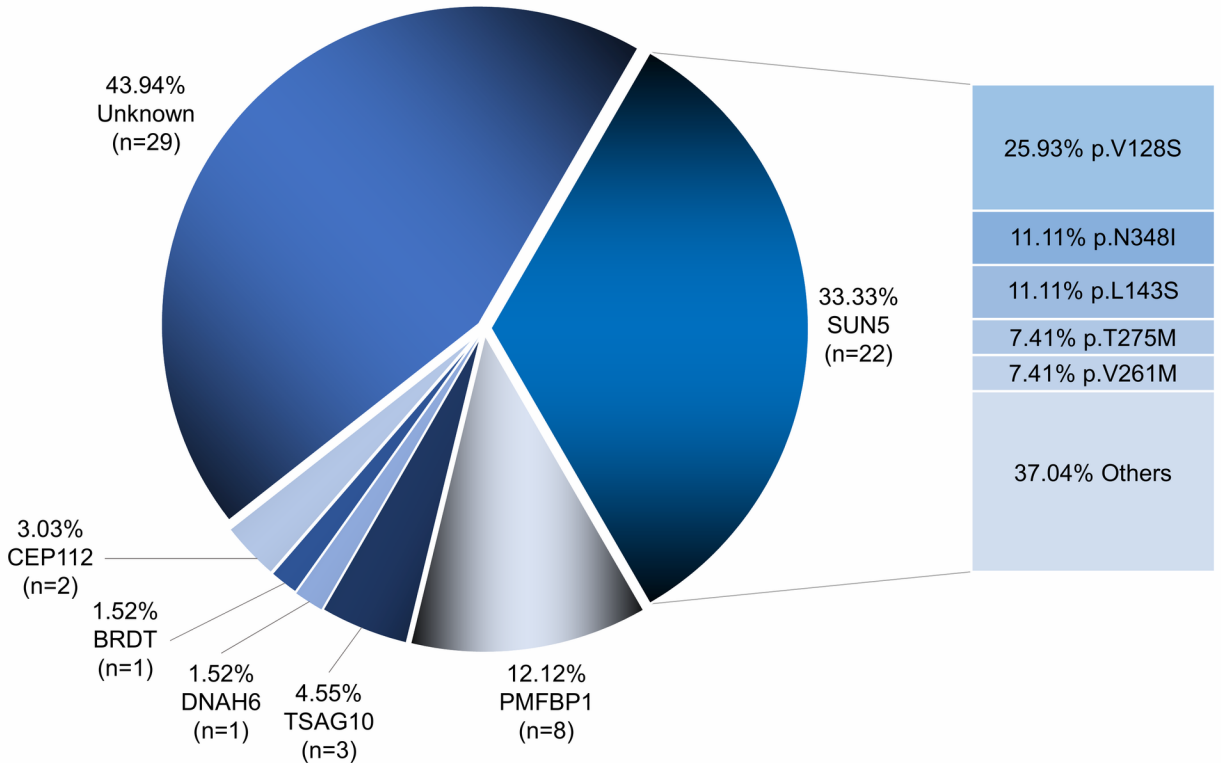


## BRDT



## CEP112





# AURKC

