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REVIEW

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Function of manchette and intra-manchette transport in spermatogenesis and male fertility

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Abstract

The manchette is a transient skirt-like structure consisting of microtubules (MTs) and filamentous actin (F-actin) surrounding the elongating sperm head during spermiogenesis. It is pivotal in sperm head shaping controlled by the acrosome-acroplaxome-manchette complex, acrosome formation, and flagellar assembly by microtubular-based protein delivery. Defects in the manchette frequently lead to teratozoospermia concomitant with oligozoospermia and asthenozoospermia, but the pathogenic mechanism underlying manchette function and its role in male infertility remain poorly understood. In this review, we systematically described the assembly and disassembly of the manchette, intra-manchette transport (IMT) and its regulatory model, the function and mechanism of manchette and IMT in regulating sperm head shaping and flagellar assembly during spermatogenesis; summarized the research progress of manchette-related genes related to male infertility; and listed the manchette-related proteins in knockout mouse models and clinical cases, which provide the theoretical basis for an in-depth understanding of the molecular mechanism of manchette involved in spermatogenesis and male fertility for understanding the potentially developing treatments for infertility and reproductive disorders.

Plain English Summary

The manchette regulates sperm head formation by mediating LINC complex-dependent transport during nuclear condensation, facilitating assembly of the AAM structure to stabilize the sperm head skeleton, and driving zipper-like movement to compress and maintain the nucleus after chromatin condensation. The manchette primarily regulates the assembly of flagellar accessory structures by exerting its storage and transportation functions. Intra-manchette transport (IMT) facilitates protein and vesicle transport, essential for nuclear condensation and sperm tail assembly, with defects leading to male infertility. Key proteins like KIF3A, HOOK1, and SPAG6 are involved in manchette function, with mutations causing abnormal sperm morphology and infertility. Mutations in manchette-related genes (e.g., CFAP43, CFAP65) are linked to severe asthenozoospermia and multiple morphological abnormalities of the sperm flagellum (MMAF).

Keywords Manchette, IMT, Spermatogenesis, Infertility, AAM axis, LINC complex

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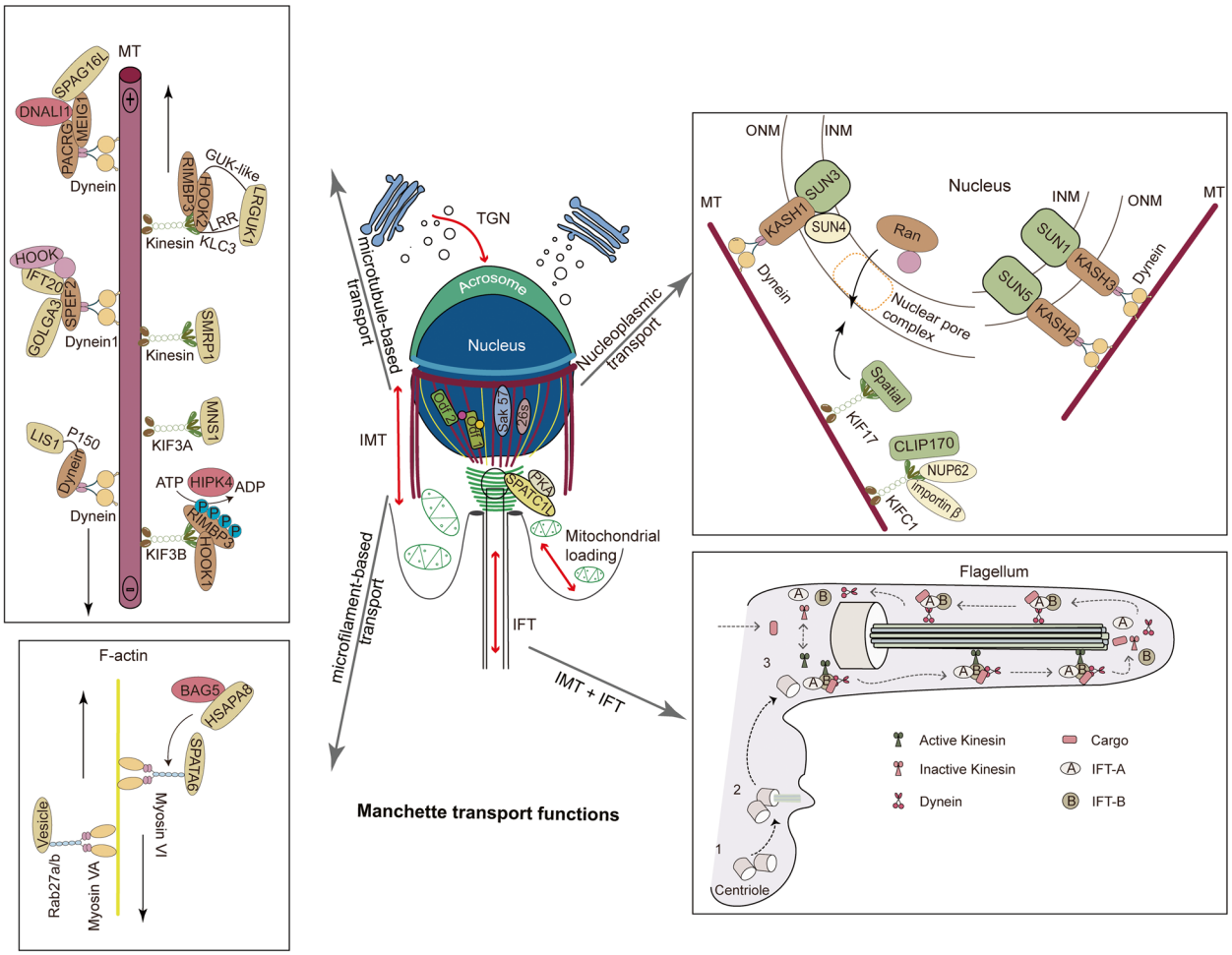
Graphical Abstract



Background

Spermatogenesis, a complex cellular differentiation process, comprises three principal stages: mitosis of the spermatogonia, two meiotic divisions of the spermatocytes, and sperm metamorphosis, culminating in the transformation of diploid spermatogonia into haploid elongated spermatozoa [1]. Among these stages, sperm metamorphosis is the latest and most evident morphological transformation, transforming round spermatids into elongated spermatids. This process encompasses four principal phases: acrosome ontogeny, tail development, sperm head emergence, and cytoplasmic condensation [2, 3]. Intriguingly, a temporary microtubule structure called manchette plays an essential role in the nuclear condensation and flagellar assembly which are the most significant transformations during spermiogenesis.

The manchette is a crucial transport platform of vesicles and proteins via intra-manchette transport (IMT), ensuring the correct formation of the sperm head, acrosome, and sperm tail during spermiogenesis. As a specialized component of the cytoskeleton in spermatids, the manchette not only connects to the nucleus through the linker of the nucleoskeleton and cytoskeleton (LINC) complex for nucleoplasmic transport, but also interacts with the acroframosome (AFS) and the acroplaxome to form the acroframosome-acroplaxome-manchette (AAM) axis for sperm head shaping. The IMT system orchestrates bidirectional cargo trafficking during spermiogenesis: (i) Anterograde transport mediates Golgi-derived vesicle sorting and acrosomal cap formation during the early phase; (ii) Retrograde coordination with IFT becomes critical during late spermiogenesis,

ensuring sperm tail elongation [4]. Three main types of IMT pathways are known: short-distance microfilament-based transport; long-distance microtubule-based bidirectional transport using motor complexes of kinesin-2 for anterograde (forward) movement and cytoplasmic dynein-2 for retrograde (backward) movement; and nucleoplasmic transport via LINC complexes facilitating the movement of proteins and other molecules between the nucleus and the cytoplasm.

Emerging studies utilizing gene-edited mouse models (knockout/mutation) have established a causal link between functional defects in manchette- or IMT-associated genes and phenotypic manifestations of teratozoospermia and male infertility. Concurrently, the integration of next-generation sequencing (NGS) technologies into clinical diagnostics has uncovered a growing repertoire of novel genes essential for manchette biogenesis and regulation dynamics, thereby identifying these loci as pathogenic contributors to idiopathic male infertility in humans.

Given the important roles of the manchette and IMT in spermatogenesis and male fertility, this review systematically introduces the formation and functions of the manchette and IMT in spermatogenesis and summaries mouse models and clinical case studies of manchette-related proteins and genes. This work aims to refine the molecular framework underlying manchette/IMT biology and serves as a foundation for exploring therapeutic strategies in human male infertility.

Methods

A comprehensive literature search was conducted using electronic databases, including PubMed and Google Scholar, to identify relevant English literature published up to June 2024. Search terms included combinations of keywords such as 'manchette', 'intra-manchette transport', 'spermatogenesis', 'sperm head shaping', 'flagellar assembly', and 'male infertility'. Additional articles were identified through a manual screening of the reference lists from the retrieved papers. A total of 2900 records were generated, of which 341 were duplicates. In total, 2035 studies were excluded because titles/abstracts were not relevant to the review. The remaining 524 records were assessed for full-text review, and 283 records were further excluded. Overall, 254 articles were included in this final review. While we endeavored to conduct a comprehensive literature search, certain limitations in the retrieval process should be acknowledged, including potential omissions due to database bias, keyword variations across fields, and incomplete inclusion of non-English studies.

Assembly and disassembly of manchette and the intra-manchette transport

Assembly and disassembly of manchette

In mammals, spermatogenesis occurs in the seminiferous tubules of the testes. The epithelium of the seminiferous tubules undergoes a repetitive series of changes known as the cycle of the seminiferous epithelium. In the mouse, the cycle of the seminiferous epithelium can be subdivided into 12 stages, and spermiogenesis can be subdivided into 16 steps [5]. During spermiogenesis, the sperm manchette first appears in step 8 spermatids in mouse whereas in step 2 in humans. The disassembly of the manchette occurs around steps 13–14 when the nucleus completes morphogenesis [6]. The period of manchette formation and depolymerization suggests that the manchette is a temporary structure during sperm morphogenesis, playing a key role in the important stages of sperm head shaping, acrosome formation, and flagellar assembly; and also suggests that its dynamic regulatory mechanism is closely related to manchette-related proteins. Abnormalities in the manchette are closely linked to a failure in shaping the sperm head, which consequently affects male fertility.

The manchette is primarily composed of three components: a perinuclear ring, an MT mantle with one end attached to the ring, and dense plaques located at the far end of the mantle [7] (Fig. 1C). The MT mantle is the main part of the manchette and is mainly composed of microtubules (MTs) and microfilaments. During manchette assembly, short MTs accumulate around the nucleus and connect to the perinuclear ring consisting of keratin 9 and δ-tubulin through their plus-ends; then they rapidly form a skirt-like structure containing up to 1,000 MTs parallel to the nucleus and surround only parts of the lower region of the sperm head, from the end of the acrosome to the flagellum (Table 1). Moreover, the skirt-like structure contains two types of MTs: those that lie in close apposition to the nuclear membrane and are physically attached, at multiple points, to the nuclear membrane via the LINC complexes, involving in

(See figure on next page.)

Fig. 1 Pattern diagram of the manchette structure with its associated transport function and connectivity function structures. **A** An F-actin cytoskeletal pathway involving the myosin Va/Rab27a/b complex for short-distance transport. **B** An MT cytoskeletal pathway involving the kinesin/dynein for long-distance transport and MT ultrastructural pattern diagram. **C** An enlarged pattern diagram of the dense plaques at the end of the manchette. **D** A diagram of two special linkers of the nucleoskeleton and cytoskeleton (LINC) complexes. **E** A model diagram of nucleoplasmic transport and its LINC complex structure

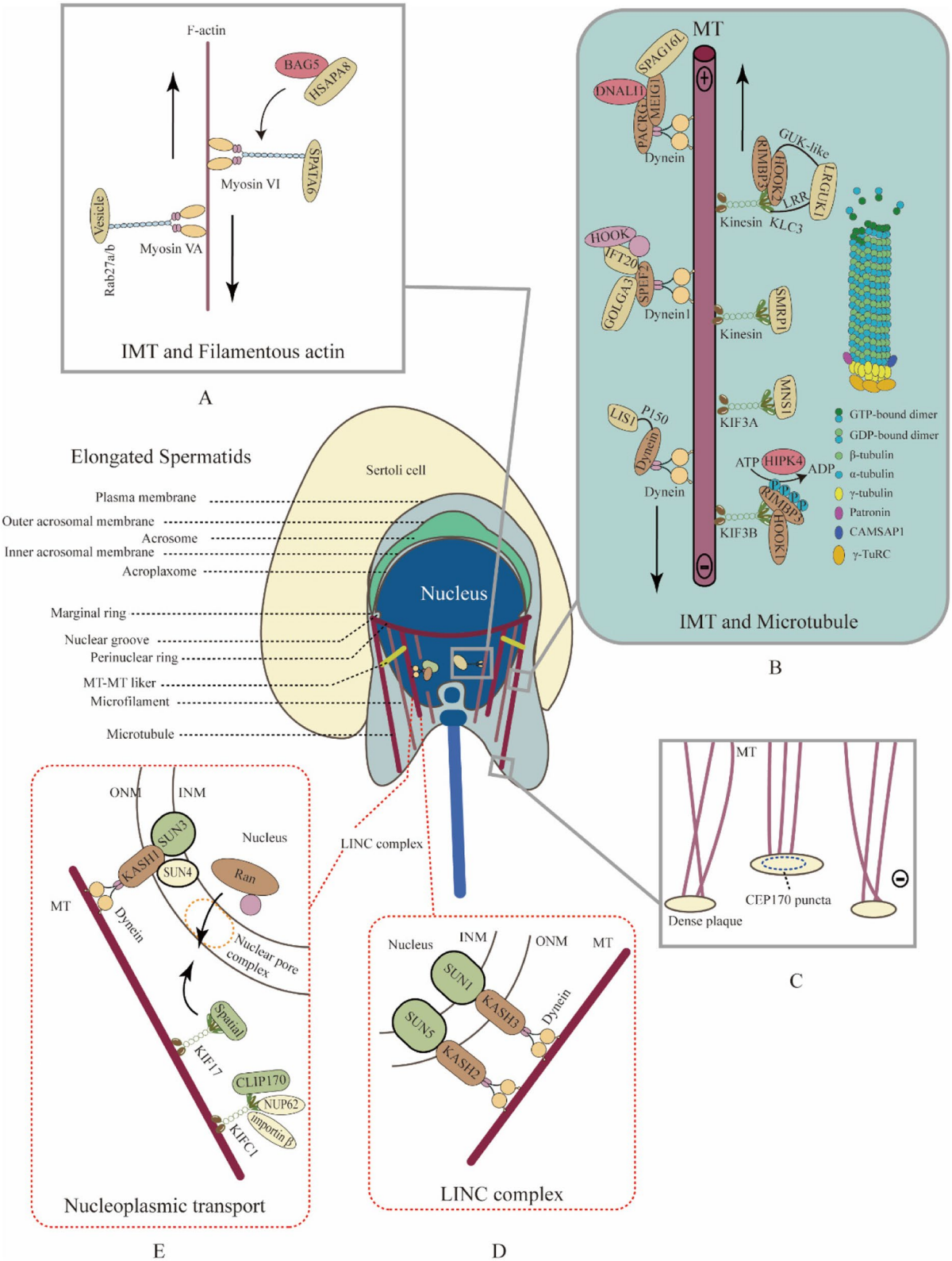


Fig. 1 (See legend on previous page.)

Table 1 The characteristics and major functions of manchettes

Structure	Cytoskeleton	Constituent proteins	Major functions	Reference
Perinuclear ring	Microtubule	keratin 9; δ-tubulin; perunuclear ring protein (62 KDa), etc.	As a noncentrosomal microtubule organizing center (MTOC) of manchette assembly; to form the manchette-perinuclear ring tight junction for sperm head shaping; to form perinuclear ring-nuclear linker complexes for zipper-like movement of the manchette	[6]
MT mantle	Microtubule; Microfilament	α-, β-, δ- and ε- tubulins; β-actin; vimentin; acetylated, tyrosinated, glutamylated alpha-tubulin, and an alpha-3/7 tubulin isoform, etc.	To transport protein and vesicle via the intra-manchette transport (IMT) pathway; with the acroframosome and acroplaxome to form the AAM axis; with the perinuclear ring to form the manchette-perinuclear ring tight junction; with LINC complexes to form manchette-nucleus linkage for nucleocytoplasmic transport	[6–9]
Dense plaques	No data	CEP170; KIF2A, etc.	Involved in manchette MT depolymerization	[7]

nucleus shaping, and those that are more radial and will ultimately project into the cytoplasmic lobes, playing an important role in flagellar assembly protein transport. Similarly, MTs are interconnected via linkers to form a net-like structure.

It is worth noting that a nucleator is required to initiate manchette MT formation. The origin of manchette MTs remains under debate. Two specific nucleation sites have been postulated: the perinuclear ring and the centrosome. However, their roles and mechanisms as noncentrosomal microtubule organizing centers (MTOCs) have not been elucidated. Three predominant hypotheses have been proposed to explain this process [6]. Firstly, the perinuclear ring might function as MTOC. This is supported by the initial appearance of MTs in the post-acrosomal region of mouse spermatids, which aligns with periodic densities in the perinuclear ring. However, but its MTOC identity is marked by δ-tubulin rather than the well-known γ-tubulin, indicating a specialized nucleation mechanism. Secondly, centrosomes could function as MTOC. Evidences include their universal MTOC marker γ-tubulin and an in vitro model where exogenous taxol and GTP nucleate manchette MTs with spermatid centrosomes. Thirdly, the perinuclear ring and the centrosome jointly fulfill MTOC. The manchette MTs are nucleated from the centrosomes with the plus-end extending toward the developing perinuclear ring and the perinuclear ring can capture the MTs near the centrosomes to rapidly form parallel MT arrays. Additionally, the perinuclear ring may capture MTs formed at other noncentrosomal nucleation sites and stabilize them near the perinuclear ring, such as the Golgi apparatus and existing MTs. Although the exact mechanism of manchette formation is not clear, the perinuclear ring's ability to connect and stabilize forming MTs and its correct formation are crucial for the organization of the manchette.

Therefore, it can be deduced that the manchette is a specialized microtubular structure whose formation and stabilization depend on the properties of microtubules and microtubule-associated modulation. The structure and polarity of MTs and microfilaments contribute to the unique cytoskeletal structure and function of the manchette. The MT of manchette is a hollow tube (22–25 nm in diameter) composed of 13 microtubulin protofilaments formed by head-to-tail heterodimers of α- and β-tubulin. Interestingly, γ-tubulin, altough not a part of the MT polymer, forms the γ-tubulin ring complex (γ-TuRC) which serves as a template for MT nucleation [10]. Unlike axonemal MTs, manchette MTs are oriented with their plus-ends toward the acrosome and minus-ends away. The plus-ends display faster growth rates, slower dissociation and kinesin-dependent transport, while minus-ends grow more slowly, dissociate rapidly, anchor to MTOCs, and require dynein [7, 11]. Moreover, most MT plus-ends are highly dynamic, regulated by microtubule plus-end-tracking proteins (+ TIPs), whereas minus-ends are mostly stable, only controlled by γ-TuRC [12, 13], Patronin [14], and CAMSAPs. It should be mentioned that CAMSAPs are a family of proteins that bind and stabilize noncentrosomal MTs post-nucleation that are released from γ-TuRC. Specifically, CAMSAP1 localizes at manchette MT minus-ends. Its loss disrupts dense plaque protein (CEP170, KIF2A) localization, impairing structural integrity and MT depolymerization [7] (Fig. 1C).

Manchette disassembly is primarily mediated by microtubule-severing proteins (MTSPs), a group of ATPases associated with diverse cellular activities (AAA + ATPase) superfamily members belonging to the

meiotic clade. These enzymes, which include katanins (KATNB1, KATNA1, KATNAL2), fidgetins, and spastin (SPAST), regulate microtubule dynamics through conserved microtubule-severing activity [15, 16]. Microtubule severing occurs at the minus-end of the manchette (near the sperm tail), mediated by the katanin complex specifically localized at this site. Katanin comprises a catalytic subunit (p60) and a regulatory subunit (p80), where p80 stabilizes p60 and regulates microtubule dynamics. Mutations in p80 cause delayed manchette elimination, sperm head/tail malformations, and mitochondrial sheath defects, leading to complete sperm immobility, short/absent tails, and structural abnormalities. Additionally, the WD40-repeat protein 62 (WDR62) facilitates katanin p80 recruitment to the manchette. Mutations in *Wdr62* impair manchette clearance, resulting in oligoasthenoteratozoospermia (OAT) phenotype [17]. The katanin isoforms KATNA1 and KATNAL1 orchestrate multiple processes in spermatogenesis, including meiotic spindle dynamics, cytokinesis, mid-body abscission, and spermatid remodeling events such as Golgi organization, acrosome formation, and manchette assembly [18]. KATNAL2, functioning either in partnership with KATNB1 or independently, coordinates these processes akin to other katanin isoforms. Mutations in *Katnal2* disrupt manchette architecture, leading to oligozoospermia and infertility in mice [19]. Parallel to katanins, SPAST mediates microtubule pruning and manchette dissolution essential for manchette development and sperm head shaping. SPAST specifically targets polyglutamylated microtubules within the manchette, controlling microtubule density and ensuring timely manchette degradation at spermiogenesis termination. In $Spast^{KO/KO}$ mice, the manchette was detached from most elongating spermatid nuclei with abnormal manchettes, including ectopic, abnormally dense, and excessively elongated manchettes [20]. In addition, *Axdnd1* [21], *Rsph6a* [22], etc. delayed manchette clearance in knockout mice, suggesting that these genes or proteins may be involved in manchette disassembly.

The intra-manchette transport and the regulatory model

The IMT serves as a central delivery system for spermiogenesis, orchestrating the targeted trafficking of cargoes (proteins/vesicles) to execute nuclear condensation and sperm tail assembly. IMT is functionally classified into three subtypes according to its structure and transport mechanisms: 1) an F-actin cytoskeletal pathway mediated by myosin motors (e.g., myosin Va/Rab27a/b) for short-distance transport, fascinating transverse acrosome extension and concentration; 2) an MT cytoskeletal pathway driven by kinesin-2 (anterograde) and cytoplasmic dynein-2 (retrograde) for long-distance bidirectional transport, essential for sperm head shaping and flagellar assembly and as the mainstay of nuclear deformation through the circular arrangement of MTs [8, 23]; 3) Nucleocytoplasmic transport via LINC complexes, bridging nuclear-cytoskeletal interactions to regulate protein shuttling and nuclear remodeling [9].

The kinesin-2 family, comprising heterotrimeric (e.g., KIF3) and homodimeric (e.g., KIF17) motors, is a dominant microtubule transport system in the manchette. It orchestrates nucleus and acrosome formation by delivering mitochondria, Golgi apparatus, and structural proteins via IMT. Concurrently, kinesin-2 also cooperates with intra-flagellar transport (IFT) machinery to assemble sperm flagella during spermiogenesis [24].

Cytoplasmic dynein, a minus-end-directed microtubule motor, with its cofactor dynactin and cargo adaptor forms a complex to aid in bidirectional intracellular transport. In spermiogenesis, cytoplasmic dynein bridges the cytoskeleton and nucleoskeleton via LINC proteins (e.g., SUN3-Nesprin1), transmitting mechanical forces to deform the spermatid nucleus [25–27]. Notably, dynein's role in IMT focuses more on structural remodeling than bulk cargo transport, contrasting with kinesin's predominant vesicle trafficking functions [28].

The LINC complex is a nuclear envelope protein bridge assembly that connects the nuclear contents to the cytoskeleton, consisting of conserved KASH–SUN protein bridges. The testis-specific Sad1/Unc84 homology (SUN) proteins localize to the inner nuclear membrane, including SUN1, SUN3, SUN4, and SPAG4L. Klarsicht/Anc1/Syne1 homology (KASH) proteins localize to the outer nuclear membrane, including Nesprin-1, Nesprin-2, Nesprin-3, and Nesprin-4. SUN domain proteins link to the C-terminal of KASH domain proteins to form the bridge. KASH proteins then link to the cytoskeleton by binding to MT motor proteins [9]. Three specific LINC complexes identified during spermatid elongation are: 1)SUN3-Nesprin-1, interacting with SUN4 and localized at the manchette-nucleus interface [29]; 2) SUN1-Nesprin-3, atypically non-nuclear localized at the anterior pole and potentially involved in basal body-nucleus attachment [26]; 3) SPAG4L/SPAG4Lβ-Nesprin-2, where SPAG4Lβ is a short transcript variant of SPAG4L [30] (Fig. 1D and E). In summary, the LINC complex is essential for coupling the manchette to the nucleus. This linkage is related to the specific polarized MTs arrangement of the manchette (upper positive and lower negative). Through this structural integration, the LINC complex achieves three critical functions: supporting the manchette movement to shape the nucleus; participating in the transport function of the IMT, and facilitating nucleoplasmic transport, which transports proteins in and out of the nucleus.

Myosin VI, a retrograde (minus-end-directed) motor, is uniquely associated with actin-rich structures critical for nuclear shaping—including the acroplaxome, manchette, and Sertoli cell actin hoops [31]. In contrast, all other myosins move towards the plus-end of actin (anterograde). For example, the actin-based motor protein myosin Va and its receptor, Rab27a/b transport Golgi-derived proacrosomal vesicles to the acrosome and along the manchette [32]. Spermatogenesis Associated 6 (SPATA6) is the component of the sperm head–tail coupling apparatus (HTCA) required for normal assembly of the sperm head–tail conjunction, involving in myosin-based microfilament transport through interaction with myosin subunits [33] (Fig. 1A).

Cargo proteins rely on adaptors rather than directly binding to motor proteins. These cargo adaptors, recruited primarily through the regulation of small GTPases of the Ras-related protein (RAB) family [34], form the structure of the motor-bridge-cargo proteins or manchette-related complexes for IMT and spermiogenesis. For example, kinesin-2 motor subunit KIF3A can bind to either KIF3B or the KIF1-binding protein (KBP) and is responsible for the meiosis specific nuclear structural 1 (MNS1) [35], and SMRP1 [36] transport within the manchette. Homeodomain-interacting protein kinase HIPK4 phosphorylates RIMBP3 forming KIF3B/HOOK1/RIMBP3 manchette-related complex [37] and regulates manchette during spermatogenesis [38]. The GUK-like domain of LRGUK1 binds to adaptor proteins HOOK2-RIMBP3 complex, and the LRR domain of LRGUK1 binds to motor protein KLC3 to form a multiprotein complex for transporting LRGUK1 via the AAM-tail axis, which is required for manchette function and male fertility [39]. LRGUK-1 is required for multiple aspects of sperm assembly, including acrosome attachment, sperm head shaping, and the initiation of axoneme growth to form the core of the sperm tail [40]. KLC3 subsequently plays a role in the plus-end-directed transport of mitochondria [26]. In contrast to well-investigated members of the kinesin family, the molecular mechanism of dynein-dynactin complex assembly and the role of dyneins in MT-based transport mechanisms remain unclear. It has been shown that the dynein-binding protein lissencephaly-1 (LIS1) binds dynactin's p150 subunit to constrain dynein-dynactin to ensure efficient complex formation [41]. Besides, DNALI1 recruits and stabilizes PACRG and co-localizes with PACRG in the manchette [42]. PACRG recruits MEIG1 to the manchette to form the MEIG1/PACRG complex for cargo transport, such as SPAG16L, to build sperm flagella [43]. Similarly, SPEF2 protein, which is required for cilia motility and formation of sperm tail structures, is present in the manchette before the localization to the sperm tail mid-piece. It is a link protein that interacts with Cytoplasmic dynein 1 to transport and attach IFT20 [44] and GOLGA3 [45]. The IFT20 protein, in turn, binds to HOOK and other IMT functional proteins (Fig. 1B).

The related cargo-bridging proteins in the manchette can also act as intermediaries during nucleocytoplasmic transport. For example, in mice, Kinesin-Like Protein KIF17 is phosphorylated by protein kinase A, which shuttles between the nucleus and cytoplasm for transporting cargo (e.g. Spatial [46]). Besides, Ran, a Ras-related GTPase, localized in the nucleus of round spermatids in rat and mouse testis and along the microtubules of the manchette in elongating spermatids is required for nucleoplasmic transport and for regulating the assembly of microtubules [47]. Similarly, the motor protein kinesin-like protein KIFC1 participates in nucleoplasmic transport with CLIP170, the nuclear pore protein NUP62, and the nuclear import factor importin β, involved in normal disintegration of the manchette, sperm head shaping, and acrosome biogenesis [6] (Fig. 1E).

Function and mechanism of manchette and IMT in spermiogenesis

The manchette is an important transient junctional platform during spermiogenesis, playing an integral role in sperm head formation and flagellar assembly. The transport function of IMT is manifested in two main ways: 1) upward kinesin-directed manchette-nucleus transport, and 2) downward kinesin-directed manchette-basal body transport, for the tail formation with both prograde and retrograde transportation. The manchette, a special cytoskeleton, can also interact with other structures to regulate sperm head metamorphosis. For example, it cooperates with the acroframosome and acroplaxome to form the AAM axis, with the perinuclear ring to form the manchette-perinuclear ring tight junction, and with LINC complexes to form manchette-nucleus linkage for transverse nuclear-cytoplasmic trafficking. However, the pathways underpinning manchette transport are still poorly understood.

Manchette regulates sperm head formation

The mouse sperm head transforms from a rounded nucleus to an elongated, sickle-shaped nucleus during spermatogenesis, requiring coordinated assembly of specific cytoskeletal structures, nuclear translocation, and chromatin condensation [48]. Proper nuclear reshaping is a critical step during sperm head formation. The manchette plays a central role in this process by: 1) mediating LINC complex-dependent transport during nuclear condensation [9], 2) facilitating assembly of the AAM structure [8], and 3) driving zipper-like microtubule sliding to compress the nucleus [25].

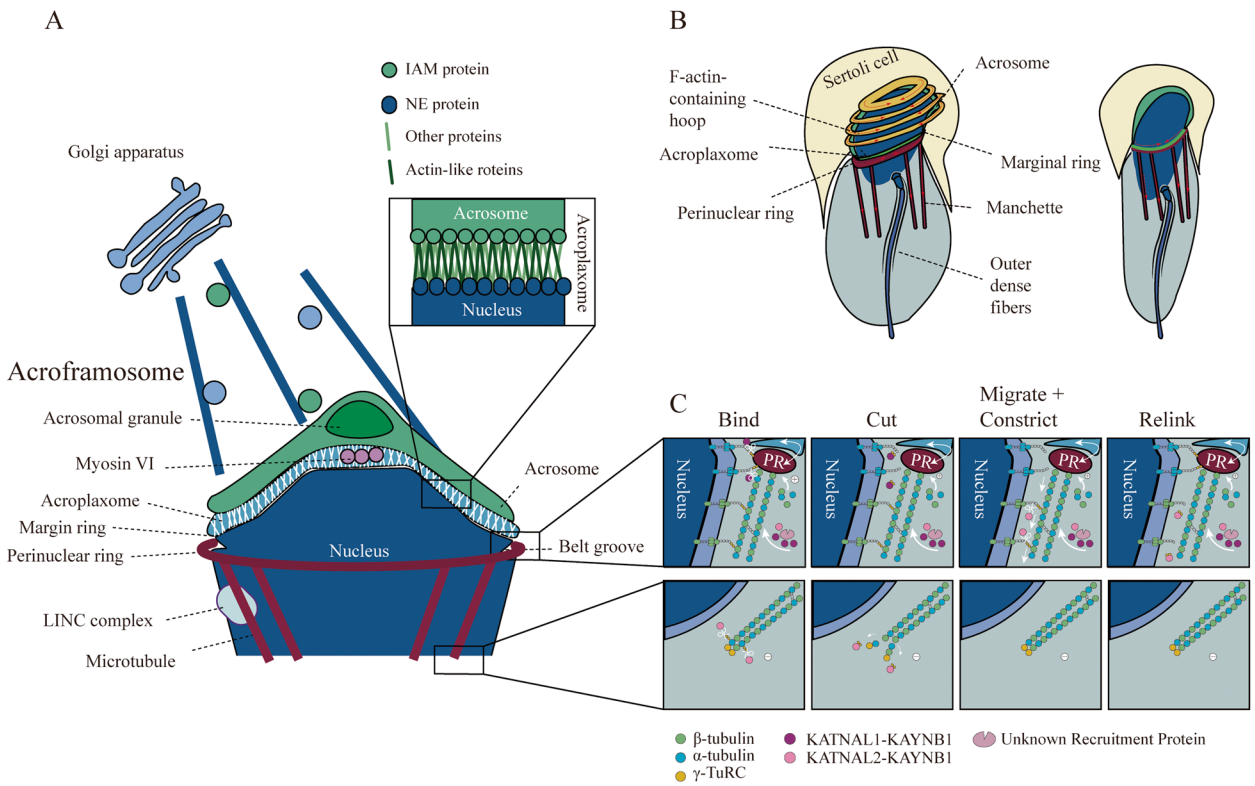


Fig. 2 Model diagram of the mechanism of acroframosome-acroplaxome-manchette (AAM) axis regulating sperm head shaping. **A** The structure of the AAM axis. **B** The constriction proceeds of the AAM axis. **C** The hypothesis of the mechanism of manchette migration. IAM: Inner acrosomal membrane. NE: Nuclear endometrium

Acroframosome (AFS), a novel cytoskeleton, serves as the microtubule-based framework of the acrosome. It directs vesicular and protein trafficking toward the nucleus. The ambient MTs of the acrosome emanate from the Golgi apparatus, which acts as MTOC in spermatids (Fig. 2A). The AFS was speculated to position the Golgi apparatus, help cargo transport from the Trans-Golgi Network (TGN) to the acrosome saccule, interact with the acroplaxome or subacrosomal chamber, and stabilize the linkages between them. Even though the AFS has an important role in sperm deformation, whether such a veritable AFS skeleton exists in mammalian spermatids remains obscure [8].

The acroplaxome is a plate-like structure composed of numerous microfilaments and associated motile molecules (e.g. myosin Va, VIIa, and VI [49]) anchored to the spermatid nucleus. The acroplaxome plate is rich in F-actin and keratin 5 (KRT5) and attended by two plaques and harbors actin-like proteins as well as several other proteins, including Ran GTPase, Hook1, dynactin p150Glued, cenexin-derived ODF2, testis-expressed profilin-3 and profilin-4, testis-expressed Fer tyrosine kinase (FerT), members of the ubiquitin–proteasome system and cortactin [50] (Fig. 2A). The acroplaxome not only anchors the acrosome to the nucleus during the spermatid head shaping but also anchors the proacrosomal sac to limit its size during acrosome formation [8]. Furthermore, it is also one of the essential cytoskeletons in acrosome formation.

Manchette with the AFS and acroplaxome forms an AAM cytoskeletal system. The AAM axis anchors the acrosome to the nucleus forming the axis of head-to-tail spermiogenesis to stabilize the sperm head skeleton. It also generates internal supportive forces to resist exogenous contractile forces exerted by the Sertoli cell-derived F-actin-containing ectoplasmic specialization structures on the sperm nucleus. The exact mechanism is unknown, but it is hypothesized that the two F-actin-rich rings, the marginal ring of the acroplaxome and the perinuclear ring which is the insertion point of the MTs of the manchette, may be regulated by Tyrosine phosphorylation and the Cortactin kinase pathway to remodel the F-actin network into progressive short diameters that act as endogenous contractions via a sleeve-like pattern. Moreover, these two rings also descend along the nucleus from the two-thirds position, mechanically shaping the distal half of the sperm head by constricting and maintaining after chromatin condensation. Simultaneously,

the manchette progressively ratchets down the nucleus towards the forming sperm tail via the dynamic 'unzippering' and 're-zippering' of microtubule-nuclear and perinuclear ring-nuclear linker complexes also known as the zipper-like movement of the manchette [51] (Fig. 2B). In the mouse, the movement moves toward the spermatid caudal site around steps 13–14 with some possible molecular motor, such as KIF3A, a subunit of heterotrimeric kinesin 2, and KIFC1, a minus-end directed microtubule-dependent motor [6]. Moreover, δ- and ε-tubulin exhibit manchette localization, with δ-tubulin observed at the perinuclear ring and both δ- and ε-tubulin at manchette MTs. δ- and ε-tubulin are candidates for linkers to the LINC complex and δ-tubulin may function in stabilizing MTs to the manchette perinuclear ring [52].

ε-tubulin TUBE1 [53] can contact the plus-end of β-tubulin, which may be adjacent to microtubules and make lateral interactions. TUBE1 is required to form and maintain axoneme structural integrity in sperm tails and manchette microtubule migration, length, and timely disassembly during spermiogenesis. The absence of TUBE1 results in nuclear membrane linkers remaining intact and microtubule pruning is diminished, ultimately leading to failure in manchette migration and hyper-extension of the manchette microtubules. TUBE1, an accessory for complex microtubule structures that facilitate regulatory processes, is a target of katanin severing. KATNAL1 targets TUBE1 by possibly interacting with an N-terminal microtubule interacting and trafficking domain (MIT) of TUBE1 which does not possess an acidic C-terminal tail, the target of traditional KATNA1-KATNB1 microtubule severing. In addition, unidentified protein(s) recruit KATNAL1 and KATNAL2. KATNAL1-KATNB1 complexes principally remodel microtubules near, or at the perinuclear ring, whereas KATNAL2-KATNB1 complexes target microtubules interfacing the nuclear membrane within the manchette bulk, during manchette disassembly.

Besides, STATHATOS GG, et al. [53] provided a hypothesis of the mechanism of manchette migration in the mouse, which occurs in four cycling phases: Bind, Cut, Migrate + Constrict, and Relink. This model proposes that the microtubule-nuclear and perinuclear ring-nuclear linkages are progressively remodeled by KATNAL2-KATNB1 and KATNAL1-KATNB1 complexes, respectively, in a TUBE1-dependent manner, following the AAM axis constriction process, to enable the manchette to rachet down the nucleus. In addition, tubulin subunits continue to be incorporated at the plus-ends of the skirt of the manchette microtubule (Fig. 2C).

Manchette regulates flagellar assembly

The structural integrity of the flagellum plays an important role in sperm motility and viability. Sperm flagellar assembly can be divided into three aspects: pre-assembly and transit of sperm tail components; axoneme assembly; and accessory structure assembly [54]. Among them, the manchette primarily regulates the assembly of flagellar accessory structures by exerting its storage and transportation functions [6]. Furthermore, the assembly of the sperm flagellum requires the replacement of axoneme components that transform at the flagellar end, the removal of transformation products from the flagellum, and the transport of membrane components such as signaling proteins, receptor proteins, and other proteins between the flagellum and the cytosol [48]. It is still unclear how these substances are delivered to specific locations but they depend upon IMT and IFT to transport cargo proteins and exchange materials between the extended sperm flagellum and the cytoplasm via the ciliary gate or annulus [55]. The manchette and sperm tail axoneme, both microtubular-based protein delivery platforms, share structural and functional homology. Notably, studies of flagellum and IFT have directly informed mechanistic understanding of manchette transport dynamics. This functional parallelism, combined with the IMT-dependent assembly of the head–tail coupling apparatus (HTCA), suggests coordinated involvement in the flagellar assembly by transporting proteins from head to tail [56]. The process consists of three main parts: 1) manchette storage; 2) IMT-based transportation; 3) IFT-based precision sorting.

During spermiogenesis, axoneme development precedes manchette formation. This temporal regulation is critical because the histone-to-protamine transition (HTP) and nuclear condensation block access of the transcriptional machinery to gene sequences, while flagellar assembly occurs in a compartment that lacks protein translational machinery. Therefore, mRNAs required for the terminal steps of germ cell remodeling are transcribed precociously, stored, and then, at the appropriate time, selectively translated in the cytoplasm and transported along cytoskeletal tracks to their ultimate destination [26] (Fig. 3A). In summary, this continuous and spatiotemporally restrained nature of spermiogenesis provides an outstanding model system to identify and decode cytoskeletal elements and the key protein and organelle transport mechanisms required to assemble the sperm. For example, during the flagellar assembly, Keratins (e.g., SAK57, ODF1, ODF2, and ODF3) [57], other keratin-associated proteins (KAPs) (e.g., the 26S proteasome and the Odf1-binding protein SPAG4) [58], and flagellar accessory structure-associated proteins (e.g., the FS protein AKAP4 and the ODF proteins) [26] are first translated in the cytoplasm and temporarily stored in the manchette before being sorted to the developing sperm tail via IMT. Furthermore, signaling proteins are

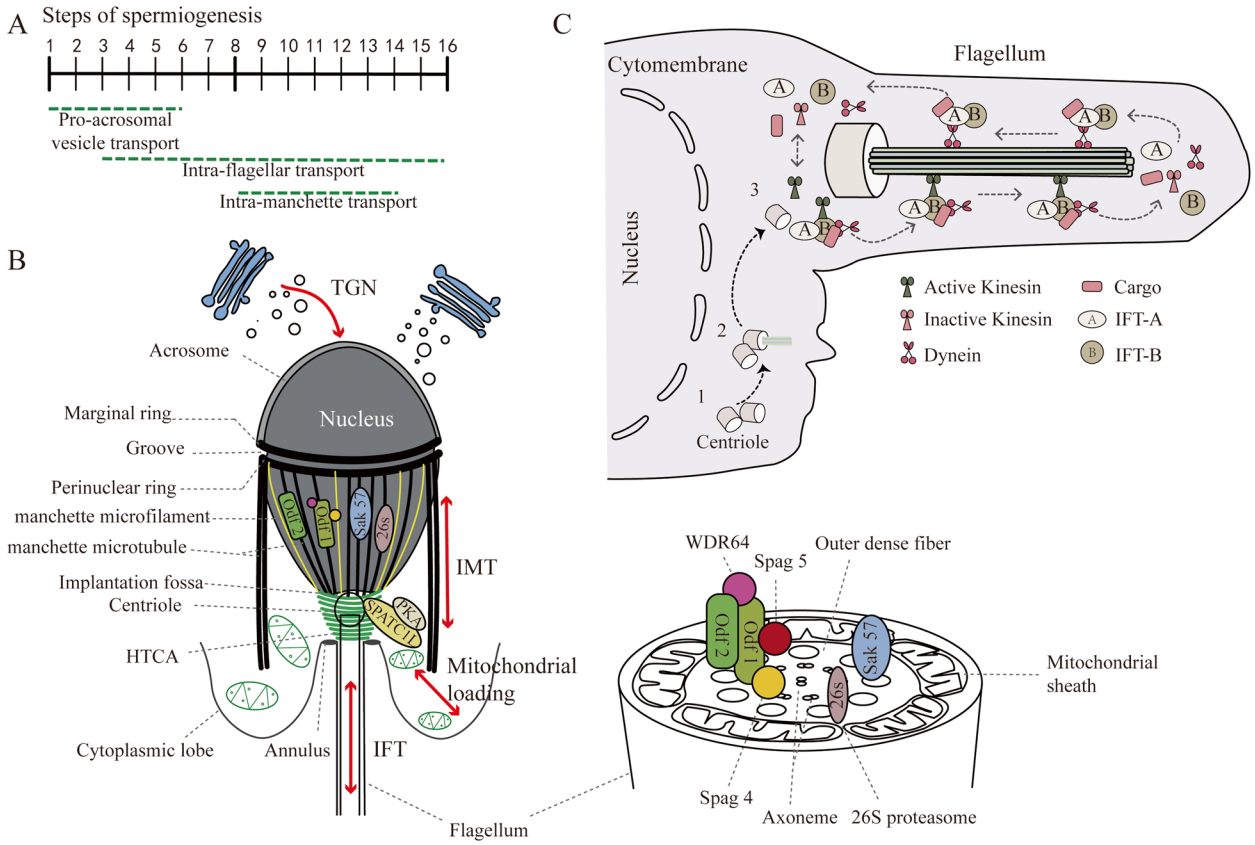


Fig. 3 Schematic diagram of protein transport systems during spermiogenesis. **A** A timeline to indicate the sequence of the three protein/vesicle transport pathways. **B** The multiple parallel cargo transport pathways and the precise location of some proteins in the flagella by IMT and IFT. **C** The formation of flagella and the main steps of IFT

also likely to be snapped to the manchette in an intimate connection with the elongating and condensing spermatid nucleus [58] (Fig. 3B).

The IFT, driven by kinesin-2 (anterograde) and dynein-2 (retrograde) motors, is a bidirectional transport machinery critical for flagellar assembly and maintenance. IFT complexes (IFT-A and IFT-B) serve as adaptors to recruit IMT cargo (e.g., tubulin, radial spoke proteins) and signaling molecules via IFT-B subunits (e.g., IFT88, IFT172) to the elongating flagellum for axoneme development [59, 60]. Cargo proteins are unloaded and the IFT complex is remodeled to activate retrograde motor proteins dynein-2 while inactivating prograde motor proteins [61] (Fig. 3C).

In addition, the HTCA is a centrosome-based structure consisting of two cylindrical microtubule-based centrioles and associated components. The proximal centriole inserts into the nuclear indentation, and the distal centriole gives rise to the sperm flagellum [62]. The failure of HTCA assembly and sperm tail formation causes male infertility. The manchette-associated protein BAG5, expressed in steps 9–16 of spermatids, is essential for sperm HTCA assembly. BAG5 forms a complex with HSPA8 to modulate the proteostasis of myosin proteins (myosin Va and myosin VI) and dynein proteins (DYNLT1, DCTN1, and DNAL1) by protein folding (Fig. 1A) and ablation of BAG5 in mice disrupts the functional motor proteins (myosin and dynein proteins) in sperm manchette structure, which results in some core proteins (e.g., SPATA6 [33]) involved in the assembly of HTCA fail to transport into HTCA, thereby leading to the separation of the sperm head and tail resulting in acephalic spermatozoa syndrome (ASS), one of the most severe teratozoospermia, which refers to sperm in the semen with intact flagella but without sperm head [62]. Additionally, spermatogenesis and centriole associated 1-like (SPATC1L) [63] are also associated with the regulatory subunit of protein kinase A (PKA) to stabilize the sperm head–tail junction. Disruption of *Spatc1 l* in mice causes male sterility through head–tail detachment, a structural defect that directly impairs sperm motility. (Fig. 3B).

Defects in manchette-related proteins and male infertility

Factors that perturb spermiogenesis lead to male infertility, which is generally manifested by a decreased sperm count (azoospermia or oligozoospermia), impaired sperm motility (asthenozoospermia), or a high proportion of morphologically abnormal sperm (teratozoospermia). A genetic defect is the most likely underlying cause of the pathology in ~15% of infertile men [64]. Despite the identification of numerous male sterility genes, the majority of genetic causes of male sterility remain uncharacterized. Mutations in genes associated with the manchette or IMT can lead to abnormal spermatogenesis, producing morphologically abnormal spermatozoa, generally characterized by the phenotype of asthenoteratozoospermia (ATZ). Among these, multiple morphological abnormalities of the sperm flagellum (MMAF) is the most severe. Proteins associated with MMAF include the AKAP, CCDC, CFAP, RSPH, TTC, DANI, LRRC, and DNAH families, among others [65]. Moreover, HOOK and its associated complexes [66], SPAG [67–70], and CEP [71–80] families play roles in the assembly and maintenance of the manchette. In addition to cytoskeletal and motor-related proteins, the SUN [29, 81–83], IFT [84, 85], and SEPT [68, 86–88] families, as well as STK36 [89], PACRG-MEIG1 protein complex [42], SPEF2 [45, 90–92], TENT5C [93], and other proteins also play a role in IMT. Experimental data have demonstrated that abnormal expression of all these proteins may contribute to spermatogenic disorders and members of each protein family often bind to each other to form specific protein complexes. Besides, our group collected the published literature to summarize a table about KO mouse models affecting the manchette formation in elongating spermatids and spermiogenic defects of human spermatids, which is detailed in an additional file [see Additional file 1/Table 2].

Defects in manchette formation and depolymerization-related proteins and male infertility

Hook family proteins

Hook family proteins serve as junctions, facilitating the transport of organelles and other cargo along MTs via kinesin-mediated processes. Additionally, they are implicated in centrosome-related cellular functions. There are three HOOK family members expressed in human, HOOK1, HOOK2, and HOOK3. HOOK1 is mainly involved in the assembly and stabilization of MT arrays during spermatogenesis [239]. HOOK2 mediates assembly of the dynein–dynactin complex and regulates mitotic progression and cytokinesis [240]. HOOK3 is mainly localized in the Golgi membrane and determines the normal paranuclear localization of the Golgi [174].

Abnormalities in Hook family members and their associated complexes can lead to spermatogenic disorders and infertility. For example, *Hook1* and *Rimbp3* KO mice present an ectopic manchette, abnormal sperm heads, and detached sperm tails characterized by a deformed nucleus and a detached acrosome [37]. Notably, its regulator homeodomain-interacting protein kinase 4 (HIPK4) is essential for spermiogenesis and male fertility in mice. HIPK4-null mouse exhibit sterility with OAT, and their male germ cells display disrupted F-actin-scaffolded acroplaxome during spermatid elongation and abnormal head morphologies in mature spermatozoa [171].

The HOOK family members may represent one cargo-specific link between the motors and cargo within the manchette in addition to acrosome and acroplaxome vesicle transport. However, the elucidation of the exact transport mechanisms and cargo/motor complexes during IMT of sperm tail proteins requires extensive further studies.

SPAG family

Sperm associated antigen 6 (SPAG6) was initially identified in a human testis cDNA expression library, localized to cytoplasmic vesicles in spermatocytes, the acrosome of round and elongating spermatids, and manchette. SPAG6 has multiple functions, as a structural component of the ciliary/flagellar axonemal centrioles and as a regulator of germ cell development during spermatogenesis. These functions depend on binding proteins such as Snapin, COPS5, SPAG16L, TCTE3, TAC1, etc. [58]. In *Spag6* knockout mice, disrupted central microtubule (MT) assembly in the axoneme during late spermatogenesis results in aberrant sperm morphology, including ATZ and MMAF phenotype [70]. Notably, SPAG16L protein, normally localized to the cytoplasm of round spermatids and manchette, fails to target the manchette in *Spag6*-deficient mice [218]. Hence, further studies are required to elucidate the binding interfaces between SPAG6 and partners (e.g., Snapin, COPS5) and their mechanistic roles in manchette regulation.

CEP family proteins

CEP family proteins constitute the pericentrosomal material. Abnormalities in these proteins can disrupt centrosomes, impairing microtubule formation in the manchette, ultimately leading to oligoasthenozoospermia (OA). Key members of this protein family include CEP41, CEP70, CEP131, TSGA10, CEP128, CEP135, CEP112, CEP170B, CEP19, CEP164, CEP78, CEP250.

Table 2 Proteins involved in manchette and the associated defective phenotype in mouse models and human spermatids

Protein	Localization	Interactions	Gene-defective Mouse phenotype	Human phenotype	Reference
ACTL7A	Golgi apparatus; subacrosomal layer; sperm head and tail	ACTR8; ACTR10; DCTN2; TES; ENAH; ACTL7B	Defective acrosome-acroplaxome-manchette complex; abnormal shaping of sperm heads; hindered development of manchette	Small head sperm	[94]
ACTN4	Perinuclear; manchette	SEPT14	Teratozoospermia; sperm head defects	No data	[95]
ADGB	Sperm flagellum	CFAP69; SPEF2	Reduced sperm concentration and motility; malformation of both elongating and elongated spermatids	AZS	[96]
AKAP3	Sperm fibrous sheath	CABYR; OVGP1; DNALI1	Lacked a complete fibrous sheath; male sterility	MMAF	[65, 97–100]
AKAP4	Sperm fibrous sheath	QRICH2; DNALI1	Abnormal sperm morphology, motility, and infertility	MMAF	[65, 98, 101–103]
ARL3	Manchette	ODA16	Abnormal head shape; lasso-like coiled tail; decapitation	No data	[104, 105]
ARP1	Manchette; centrosome; Golgi complex	No data	No data	No data	[106]
AXDND1	From the mid-pachytene spermatocytes to the early spermatids	SRP68	Abnormal nuclear shaping and manchette	No data	[107]
CAMSAP1	Caudal end of manchette	CEP170; KIF2A	OAT; deformed sperm nuclei and tails; infertile	Infertile	[7]
CCDC103	Midpiece of sperm tail	No data	MMAF	Absence of DA on their axonemes	[108, 109]
CCDC146	Centrosome; multiple microtubule-related organelles	IFT88; IFT20; KRT38; GMNN; RAB3IP; FANCM; TXLNA	Impacting the manchette, HTCA, and axoneme	MMAF	[110]
CCDC178	Testis	CFAP53; IFT88; KIF3 A	Severe oligospermia	No data	[111]
CCDC181	Manchette; sperm flagella	HOOK1; LRRC46; PP1	Male infertility; defective sperm head shaping and flagellum formation; MMAF phenotypes	No data	[66, 112, 113]
CCDC183	Axoneme	No data	MMAF	Male infertility	[114]
CCDC189	Testis; sperm axoneme	CABCOCO1; PABPC1; PABPC2	MMAF	No data	[115, 116]
CCDC34	Midpiece of sperm flagella	RABL2 A	MMAF	OAT	[117]
CCDC38	Manchette; sperm tail	CCDC42; CCDC146; IFT88; ODF2	Distorted manchette; multiple morphological abnormalities of the flagella of spermatozoa; infertility; MMAF	No data	[118]
CCDC39	Axonemes	No data	MMAF	MMAF	[92, 119]
CCDC40	Axoneme	No data	MMAF	No data	[120]
CCDC41	Mother centriole	CEP164; IFT20; Arl13b	No data	No data	[121]
CCDC42	Manchette; sperm tail; HTCA	ODF1; ODF2	Affecting the HTCA	No data	[122]

Table 2 (continued)

Protein	Localization	Interactions	Gene-defective Mouse phenotype	Human phenotype	Reference
CCDC65	Axoneme	No data	MMAF	AZS	[123]
CCDC87	Pachytene spermatocytes; Spermatids	No data	Morphological deformations in sperm; defective acrosome and nucleus formation; subfertility	No data	[124]
CEP112	Sperm neck; centrioles	hnRNPA2B1; EEF1 A1; EIF4 A1	OAT	Acephalic spermatozoa; OAT	[80, 125]
CEP128	Centrioles	ODF2	Dissociation of subdistal appendage components from the centriole; decreased the stability of centriolar microtubules	Infertility	[77, 78]
CEP131	Centrosome	BBS4	Short tail, disorganized sperm tail structures, ectopic, and elongated manchette	No data	[72]
CEP135	Centrosome	CCDC14	Centriole duplication defects	MMAF	[71, 75, 126]
CEP164	Centrioles; nucleus	CCDC41; TTBK2	Completely infertile; a substantial reduction in the number of late-stage germ cells	No data	[76, 121, 127]
CEP19	Centrioles	FOP; CEP350; RabL2	Abnormal crooked flagella; diminished sperm motility	No data	[73, 74, 85]
CEP170B	Microtubule	Liprin-α1/PP2 A; KIF2 A	No data	No data	[128]
CEP250	Centrioles	No data	Infertile; reduction in the spermatogonial pool and the meiotic blockade	No data	[129]
CEP41	Microtubule	No data	No data	No data	[130]
CEP70	Centrosomes	No data	Abnormal formation of flagella and acrosomes	No data	[131]
CEP78	Centrioles	USP16; CETN1	Complete infertility; aberrant sperm morphology; diminished sperm count; disorganization of sperm ultrastructure	OAT; MMAF	[79]
CFAP251	Sperm tail	No data	MMAF	MMAF	[132]
CFAP43	Axoneme	SEC13; CUL7	MMAF	MMAF	[133]
CFAP44	Axoneme	IFT140; WDR19	MMAF	MMAF	[133]
CFAP46	Axoneme	No data	MMAF	No data	[134]
CFAP47	Axoneme	No data	MMAF	No data	[135]
CFAP52	Axoneme	No data	Oligozoospermia	Infertility	[136]
CFAP53	Manchette; sperm tail	KIF3A; IFT88; CCDC42; CCDC178	MMAF; severe oligospermia	No data	[111, 137]

Table 2 (continued)

Protein	Localization	Interactions	Gene-defective Mouse phenotype	Human phenotype	Reference
CFAP58	Sperm tail	CFAP47; CCDC42; IFT88	MMAF; severe defects in the sperm tail, affects the manchette structure, abnormal sperm head shaping; increased in spermatozoa apoptosis;	No data	[134]
CFAP61	Axoneme	No data	MMAF	No data	[138]
CFAP65	Axoneme	No data	MMAF	MMAF	[139–141]
CFAP69	The midpiece of the sperm flagellum	SNTA1	Disruption of the flagellum structure	MMAF	[142]
CFAP70	Cytoskeleton; flagellum	WDR90; SPATA4; ITSN2; ITSN1; OBSL1	Disappearance of the flagella in the lumen and long spermatid nuclei	OAT; MMAF	[143]
CLIP170	Microtubule plus ends; perinuclear ring	LIS1	Irregular arrangement of manchette; subfertile; abnormal sperm heads	No data	[144, 145]
CNTROB	Cytoskeleton; microtubules; centrosome; centrioles	KRT5, tubulin	Ectopic and asymmetric perinuclear ring and manchette, detached centrosome, decapitated and disorganized tails	No data	[6]
DLEC1	Cytoplasm	TRiC; BBS; α- and β-tubulin	Head deformation, shortened tail, and abnormal manchette organization	No data	[146]
DNAH1	Flagellum axoneme	ZMYND12	MMAF	MMAF	[147, 148]
DNAH10	Sperm tail axoneme	No data	MMAF	MMAF	[149]
DNAH11	Sperm tail axoneme	DNAI1; DNAH5; DNAI2; CCDC114	MMAF	MMAF	[150–152]
DNAH12	Sperm tail axoneme	DNAH6	MMAF	MMAF	[153]
DNAH17	Sperm tail axoneme	No data	MMAF	MMAF	[154]
DNAH2	Flagellum axoneme	DNAH1; DNAH17	MMAF	MMAF	[155, 156]
DNAH3	Inner dynein arms	DNAH1; DNAH17	Infertile; the severe reduction in sperm movement with abnormal IDA and mitochondrial structure	ATZ; MMAF	[156, 157]
DNAH5	Axoneme	DNAH1; DNAH17	MMAF	MMAF	[156, 158]
DNAH6	Axoneme; sperm neck	DYNLT1	MMAF	MMAF; Globozoospermia; acephalic spermatozoa	[159]
DNAH7	Sperm tail axoneme	DNAH1; DNAH17	MMAF	MMAF	[156, 160]
DNAH8	Sperm tail axoneme	DNAH1; DNAH17	MMAF	MMAF	[156, 161]
DNAH9	Sperm tail axoneme	No data	MMAF	MMAF	[162, 163]
DNALI1	Manchette	PACRG	Impaired sperm spermiation; misshapen heads bent tails, enlarged midpiece, discontinuous accessory structure; AZS	AZS; MMAF	[42, 98]

Table 2 (continued)

Protein	Localization	Interactions	Gene-defective Mouse phenotype	Human phenotype	Reference
DRC1	Axoneme	No data	MMAF	MMAF	[164]
E-MAP-115	Microtubules	Kinesin 1	Ectopic manchette along regions of the nucleus that normally do not display manchette and tail appears normal	No data	[6]
FAM46C	Manchette	No data	Headless spermatozoa; male sterility	No data	[165]
FU	Manchette; the acrosome-acroplaxome complex	KIF27, ODF1	Periaxonemal abnormalities, manchette elongated and malformed, acroplaxome affected	No data	[89]
GGNBP2	Nucleus; cytoplasmic vesicle	GGN1; FANCL;	Smaller testes; azoospermic phenotype; irregularly shaped acrosomes, acrosome detachment, cytoplasmic remnant, ectopic manchette, and ill-formed head shape	No data	[166–168]
GOPC	trans-Golgi region	Frizzled5/8; USP8; Golgi160; RAB6 A; GRID2; BECN1; RHOQ; ACCN3; CFTR; CSPG5	Lack of acrosome, postacrosomal sheath, and posterior ring; misplaced perinuclear ring, ectopic, and manchette; Impaired mitochondrial sheath assembly	Globozoospermia; Teratozoospermia	[169, 170]
HIPK4	No data	RIMBP3	Cytoskeletal defects; abnormal sperm heads	No data	[38, 171]
HOOK1	Manchette	RIMBP3; CCDC181	Manchette elongated, knobbed-like shape of the head, weak head–tail connection, and bending of the tail	Severe teratozoospermia patients with decapitated and decaudated spermatozoa (DDS)	[20]
HOOK2	Centrosome	CEP110; Par6α	No data	No data	[172, 173]
HOOK3	Golgi membrane	No data	No data	No data	[174]
IFT140	Manchette	No data	Infertile; significantly reduced sperm number and motility; amorphous heads; short/bent flagella; swollen tail tips; vesicles along the flagella	Infertility; severe oligozoospermia, AZS, and OAT	[175, 176]
IFT144	Manchette	IFT140; IFT88	No data	Asthenoteratospermia	[177]
IFT172	Manchette	ODF2; AKAP4; IFT25; IFT57	Reduced spermatozoa number and viability; abnormal sperm head morphology and elongated manchette	No data	[178]
IFT20	Manchette; Golgi complex	SPATA1; GMAP210; SPEF2; BLOC-1; SPAG17	Infertile; significantly reduced sperm counts and motility; abnormally shaped elongating spermatid heads and bulbous round spermatids	No data	[45, 69, 179–182]

Table 2 (continued)

Protein	Localization	Interactions	Gene-defective Mouse phenotype	Human phenotype	Reference
IFT88	Manchette; flagellum; HTCA	GMAP210; HOOK1; CCDC38	No axoneme, disorganized tail components, malformed HTCA, ectopic perinuclear ring, and manchette elongated	No data	[84]
IQCG	Manchette	Calmodulin	Short tail and disorganized sperm tail structures, irregular nucleus	Asthenospermia	[183, 184]
IQCN	Mitochondrion	CDC42; ACTB; ACTG1	Abnormal manchette assembly; head deformity	TFF; autosomal-recessive fertilization failure	[185–187]
KATNAL2	Cytoplasm	KATNB1; STRIP1	Manchette defection; infertile; OAT phenotype	OAT	[188]
KATNB1	Cytoskeleton; microtubules; centrosome	Katanin p60	Affecting sperm tail motility; manchette elongated and knobbed-like sperm head	OAT; oligozoospermia	[18]
KDM3A/ JMJD1A/ JHDM2A	Nucleus	Tnp1; Prm1; Hsp90	Abnormal acrosome and manchette; the absence of implantation fossa	Oligozoospermia; Azoospermia; Globozoospermia	[189–191]
KIF17b	Manchette	Spatial/TBATA; PRAMEL1	No data	No data	[46, 192]
KIF27	Manchette	No data	No data	No data	[89]
KIF3A	Manchette; cytoskeleton; microtubules; centrosome; centriole	MGCRABGAP; KIF3B; KAP; MNS1; KBP; SMRP1	No axoneme, disorganized tail components, manchette elongated and knobbed-like sperm head	No data	[193]
KIF3B	Manchette; cytoskeleton	KIF3A; KIFAP3; KIFBP	No data	OAT	[194]
KIFC5A	Manchette	No data	No data	No data	[195]
LC8	No data	No data	No data	No data	[196]
LIS1	No date	Dynein	Infertile	No data	[144, 197, 198]
LRGUK1	Manchette	HOOK1-3; RIMBP3; KLC3	Short tail, acrosome, and acroplaxome detached, manchette mts unevenly distributed and elongated manchette	No data	[39]
LRRC23	Axonemal microtubules	RSPH9	Infertility; defective sperm motility	Infertile; defective sperm motility	[199]
LRRC46	Flagellum	No data	MMAF	MMAF	[65]
LRRC48	Flagellum	No data	AZS; infertility	AZS	[200]
LRRC4C	Flagellum	No data	No data	No data	[201]
LRRC50	Flagellum	No data	MMAF	MMAF	[65]
LRRC56	Flagellum	IFT88	No data	No data	[202, 203]
LRRC6	Flagellum	No data	MMAF	MMAF	[204]
LRRC69	Flagellum	No data	No data	No data	[205]
LRRC8A	Flagellum	No data	Germ cells degeneration; MMAF	Sertoli cell-only syndrome (SCOS)	[206]

Table 2 (continued)

Protein	Localization	Interactions	Gene-defective Mouse phenotype	Human phenotype	Reference
MEIG1	Manchette	PACRG; SPAG16	Disorganized sperm tail structures, disrupted manchette structure reported, and round or detached heads	No data	[207]
NRD convertase	cytoplasm of spermatids	No data	No data	No data	[208]
NUP210L	Nuclear envelope	BAF-L; NUP98; KIF20 A	Most spermatids arrest during nuclear elongation (step 10–11) with mislocalized NPCs and disorganized manchette microtubules that frequently invaginate the nucleus from the caudal pole	Globozoospermia and Androgen Insensitivity Syndrome	[209]
PACRG	Manchette	MEIG1	Disorganized sperm tail structures, disrupted manchette structure reported, and round or detached heads	No data	[210]
PFN3	Acrosome-acroplaxome-manchette complex	No data	Globozoospermia; infertility	No data	[211]
PFN4	Acrosome-acroplaxome-manchette complex	No data	Amorphous head shape; flagellar defects; reduced sperm motility	No data	[212]
RSPH1	Flagellum	No data	Primary ciliary dyskinesia	No data	[213]
RSPH3	Flagellum	No data	Primary ciliary dyskinesia	No data	[214]
RSPH4A	Flagellum	No data	Primary ciliary dyskinesia	No data	[215]
RSPH6A	Flagellum	AP5M1; ALS2; RANBP2; RSPH1; RSPH9	Short immotile spermatozoa; impairing manchette removal; disappearance of RSPH9	No data	[22]
RSPH9	Flagellum	No data	Primary ciliary dyskinesia	No data	[215]
SEPT10	Manchette	ADGB	No data	No data	[216]
SEPT12	Perinuclear regions; manchette	CDC42; SPAG4	Defective sperm heads	ATZ; oligoasthenozoospermia	[86, 87]
SEPT14	Perinuclear ring; manchette	SPAG4; LaminB1; SEPT9	Male infertility	Azoospermia	[88]
SPAG17	Axonemes	PCDP1; FT20	Abnormally long manchette structures; abnormal tail and head morphology	AZS	[69, 217]
SPAG4L	Nuclear membrane; endoplasmic reticulum	Nesprin-3; Nesprin-2; PMFBP1	Damage of head-to-tail linkage; ASS	acephalic spermatozoa	[67, 82, 83]
SPAG6	Sperm tail	STK36; Snapin; SPINK2; COPS5; SPAG16L	Abnormal spermatogenesis	AZS; MMAF	[70, 218]

Table 2 (continued)

Protein	Localization	Interactions	Gene-defective Mouse phenotype	Human phenotype	Reference
Spastin	Cytoplasm; cytoskeleton; microtubules; centrosome	NUP133; NUP107; NUP43; CHMP1B; IST1	Complete loss of functional germ cells; extreme abnormalities in manchette structure, acrosome biogenesis, and catastrophic loss of nuclear integrity	No data	[20]
SPAST	Manchette	No data	Ectopic, abnormally dense, and excessively elongated manchettes; detachment of the manchette from most elongating spermatid nuclei	No data	[20]
SPATC1L	Sperm neck	PKA	Male sterility; separation of sperm heads from tails	acephalic spermatozoa	[63, 219]
SPEF2	Sperm flagellum	Cytoplasmic dynein 1; GOLGA3; IFT20	Short tail, elongated manchette, and disorganized sperm tail structures	MMAF; PCD	[45, 90, 91]
SPEM1	Manchette	RANBP17, UBQLN1	Head bend back, mid-piece wrapped around the head and retained cytoplasm	Teratozoospermia	[220–222]
SPATA20	Extracellular; mitochondrion; nucleus	PSMC3; NDUFA10	Smaller testes, reduced sperm counts, decreased sperm motility, and deformed spermatozoa; aberrant manchette	No data	[223]
SSP411	Round and elongated spermatid	No data	smaller testes, reduced sperm counts, decreased sperm motility, deformed spermatozoa, and abnormal sperm heads	No data	[223, 224]
STK33	Perinuclear region; spermatogenic epithelia	CALML3; CALM3; CDC37	Severely malformed and immotile spermatozoa; disordered structural tail elements; tight, straight, and elongated manchette; sterile; defects in the mitochondrial sheath, fibrous sheath, outer dense fiber, and axoneme; subfertile; oligoasthenozoospermia	AZS; MMAF; Oligoasthenozoospermia	[225–227]
STK36	Manchette; acrosome acroplaxome complex	ODF1; KIF27	Infertility; reduced sperm count; abnormal sperm head shaping; deception; motility defects; structural abnormalities in the acrosome, manchette, and sperm tail accessory structures	No data	[89]
SUN1	No data	No data	No data	No data	No data
SUN2	No data	No data	No data	No data	No data

Table 2 (continued)

Protein	Localization	Interactions	Gene-defective Mouse phenotype	Human phenotype	Reference
SUN3	Inner nuclear membrane	SUN4	Globozoospermia; the loss of manchette, and coiled tails	No data	[81]
SUN4	Inner nuclear membrane	SUN3; ODF1; SON4; SEPT12; Nesprin-1	Globozoospermia; severely disorganized manchette, and coiled tails	No data	[29, 68, 228]
TENT5C	Manchette	No data	increased numbers of anucleate; morphologically abnormal manchette; reduced fertilization capacity; infertility	No data	[93]
TSGA10	Centrioles; sperm tails	GRP78; NSUN2,	Reduced sperm motility; disordered mitochondrial sheath formation	Infertility; acephalic spermatozoa	[229, 230]
TTC21A	Nucleus	No data	Asthenoteratospermia	Asthenoteratospermia	[231]
TTC29	Flagellum axoneme	No data	Asthenoteratospermia	Asthenoteratospermia	[232]
TUBB4B	Cytoplasmic; microtubules; mitotic spindles; manchette; axonemes	No data	Male infertility; failure to produce sperm cells; delayed perinuclear ring and manchette migration towards the basal body	No data	[233, 234]
TUBE1	Cytoplasm; cytoskeleton; microtubules; centrosome	KATNAL1; KATNAL2; KATNB1	Sterile; spermiation (sperm release) failure; mispositioning of the basal body on the nuclear membrane	No data	[53]
UBE2B	Meiotic chromatin regions,	RAD18; HR6B; H2 A	Mislocation of the longitudinal columns of the FS, head shape and MS abnormalities, acrosomal defects, and ectopic manchette	Azoospermia; Oligozoospermia	[235–237]
WDR64	Manchette, midpiece of the flagellum	ODF1	Decreased sperm motility	No data	[238]

AZS Asthenozoospermia, *MMAF* Multiple morphological abnormalities of the flagellum, *OAT* Oligoasthenoteratozoospermia, *HTCA* sperm head–tail coupling apparatus, *IDA* Inner dynein arm, *ATZ* Asthenoteratozoospermia, *NPCs* Nuclear pore complexes, *ASS* Azoospermia with sertoli cell syndrome

CEP41, a centrosomal protein predominantly expressed in the testis, interacts with the testicular spermatocyte centrosome replication-associated protein CEP63 to regulate the stability of the number of spermatocyte centrosomes by controlling CEP63 recruitment. Its deficiency triggers excessive centrosome duplication in spermatocytes, disrupting its MTOC functions and impairing sperm tail formation [130]. Similarly, CEP70 [131] and CEP131 [72] are critical for cilia formation, centriole replication, and genome stability. CEP70 deficiency causes acrosomal and flagellar defects, leading to infertility despite residual sperm production. CEP131 deficiency disrupts manchette assembly and IMT, arresting spermatogenesis at stage 9 and resulting in infertility.

The absence of CEP135 [75] and CEP112 [125] leads to MMAF and ASS, respectively, in human. CEP135 is a highly helical centrosomal protein that plays a scaffold-like role in centriole formation as an essential component of centrioles and participates in the formation of MT organization.

TSGA10, CEP128, and CEP78 are critically linked to male infertility in humans and mice. TSGA10 ensures proper mitochondrial sheath organization in spermatozoa. Its dysfunction correlates with reduced sperm motility in male patients [229, 230]. CEP128 regulates gene expression and TGF-β/BMP signaling pathway phosphorylation during spermatogenesis [78]. CEP78 is essential for centriole development of germ cells. Its deficiency disrupts sperm ultrastructure, causing morphological abnormalities, low sperm counts, and infertility [79].

The male KO mice of *Cep19* [73], *Cep164* [76], *Cep250* [129], *Cep55* [241], and *Cetn1* [242] were infertile. *Cep19* KO mice exhibited spermatozoa with persistent flagellar curvature and severely impaired motility. *Cep164* KO mice showed a marked depletion of late-stage germ cells.

CEP170B, a novel cortical microtubule- and microtubule-negative end-binding protein depending on Liprin-α1/PP2 A, antagonizes the protective effect of CAMSAPs on the minus ends of MTs with KIF2A and causes the disassembly of MT minus-ends [128]. Therefore, we hypothesize that CEP170B is likely to be involved in the regulation of manchette and play a role in sperm shaping.

CFAP family proteins

The CFAP family is a group of proteins associated with the development and functions of cilia and flagella and plays important roles in cilia and flagellar biogenesis, structural assembly, and maintenance of motility. Defects in the proteins of the CFAP family members lead to abnormal flagellar assembly and abnormal spermatozoa viability or morphology, resulting in severe asthenozoospermia (AZS) and male infertility. Most of them manifest MMAF, such as CFAP43, CFAP44 [133], CFAP70 [143, 243], CFAP69 [142], CFAP251 [132], CFAP47 [244], CFAP65 [139], CFAP58 [134], CFAP46, CFAP61 [138]. It can also manifest as severe oligoasthenoteratozoospermia (sOAT), such as CFAP46 and CFAP61 [138] and oligozoospermia, such as CFAP70 [143, 243], CFAP43 [23], CFAP53 [137], CFAP70 [143, 243], CFAP65 [140], CFAP52 [245], and CFAP58 [134], which are closely related to manchette to maintain normal manchette structure in addition to regulating the transport and assembly of flagellar components.

Defects in IMT related proteins and infertility

Kinesin family proteins

Members of the kinesin family localized in the manchette are KIF3A, KIF3B, KIF17b [46], KIF27 [89], and KIFC5A [195]. They can function in manchette or IMT with each other or with other proteins to form protein complexes. KIF3A is a subunit of heterotrimeric kinesin 2, an N-kinesin, MT positive end-directed motor protein required for sperm tail shaping as well as nucleus shaping in spermatogenesis. The depletion of KIF3A causes severe impairments in sperm tail formation, manchette organization, and the shaping of sperm heads [193]. KIF3B gene silent variant leading to sperm morphology and motility defects and male infertility [194]. KIF17b colocalizes with Spatial, also known as TBATA, in the manchette and the principal piece of the sperm tail [46] and participates in nucleoplasmic transport and sperm tail formation with PRAMEL1 [192], involved in nuclear head shaping.

Dynein and dynamin-related proteins

The mechanisms of dynamin in spermatogenesis have been poorly studied. Loss of Dynein Regulatory Complex Subunit 1 (DRC1), leads to MMAF and male infertility in humans and mice [164]. The dynein axonemal heavy chain (DNAH) family genes encode the dynein axonemal heavy chain, which is involved in cell motility. Genomic variations of DNAH family members have been frequently reported in male infertility with multiple morphological abnormalities of the sperm flagella in humans and mice, including DNAH1 [147], DNAH2 [155], DNAH3 [157], NADH5 [158, 246], NADH6 [159], DNAH7 [160], DNAH8 [161], DNAH9 [162, 163], DNAH10 [149], DNAH11 [150, 151], DNAH12 [153], and DNAH17 [154].

Besides, it is known that some abnormalities of dynamin-related proteins can lead to spermatogenesis disorders, such as CLIP170, ARP1, ARL3, LC8, and LIS1. CLIP170 is a plus-end tracking protein that mediates the association of dynein/dynactin to microtubule plus ends, and it also binds to kinetochores in a dynein/

dynactin-dependent fashion, both via its C-terminal domain implicated in the control of microtubule dynamics, dynactin localization, and the linking of endosomes to microtubules [144]. *Clip170* KO mice show a highly irregular arrangement of manchette resulting in subfertility and abnormal sperm heads [145]. ARP1, a protein of the dynactin complex, localizes the manchette in addition to the centrosome and Golgi complex to bind dynein and cargos as a regulated adapter [106]. ADP ribosylation factor-like 3 (ARL3), a Ras-related small GTP-binding protein, located in the manchette, binds to ODA16 and dissociates ODA16 from the IFT complex [104]. ARL3 and LC8 facilitated the detachment of dynactin from dynein [196]. In vivo RNAi-mediated ARL3 knockdown mice, the sperms had an abnormal head shape, a lasso-like coiled tail, or decapitation [105]. LIS1, a protein implicated in brain development, acts in several processes mediated by the dynein/dynactin pathway by interacting with dynein and other proteins [144]. $Lis1^{GT/GT}$ mice by gene trap integration are infertile [197, 198].

SEPTs

Septin genes encode well-preserved polymerizing GTP-binding cytoskeletal proteins. The cellular functions of SEPTs consist of mitosis, cytoskeletal remodeling, cell polarity, and vesicle trafficking through interactions with various types of cytoskeletons. Among them, closely related to IMT are SEPT14-ACTN4 protein complexes, SEPT10, and SEPT12. ACTN4, an actin-binding protein, forms a complex with SEPT14 and co-localizes in the perinuclear region and manchette of early elongating spermatids. SEPT14 mutations disrupt ACTN4-actin dynamics by mislocalizing and fragmenting ACTN4 signals, ultimately causing sperm head defects and teratozoospermia [95]. Androglobin (ADGB), a chimeric mammalian globin, mediates SEPT10 proteolysis in a calmodulin-dependent manner for regulating IMT and flagellar formation processes. In *Adgb*-null mice, SEPT10 mislocalization disrupts manchette and sperm annulus assembly, leading to structural and functional defects in spermiogenesis [216]. CDC42 negatively regulates the polymerization of testis-specific SEPT12. Mutations in *Sept12* disrupt this regulation, leading to teratozoospermia or oligozoospermia [87]. SEPTIN12 interacts with NDC1 to form a complex essential for spermatogenesis, likely coordinating nuclear-cytoplasmic transport or acrosome-manchette dynamics [87, 247].

SUN family proteins

The SUN (Sad1/UNC-84) family is defined by a conserved SUN domain amino acid sequence at the C-terminal end of the peptide chain, a transmembrane region near the N-terminal end, and a coiled-coil region between the transmembrane region and the SUN domain that mediates interactions with KASH-domain proteins to form LINC complexes. In humans, five SUN members have been identified: SUN1, SUN2, SUN3, SUN4 (also known as SPAG4), and SPAG4L (also known as SUN5 or TSARG4). SUN4 and SUN3 are critical for manchette-nuclear membrane tethering during spermiogenesis. *Sun4* KO mice exhibit round-headed spermatozoa due to manchette-nuclear detachment and failed nuclear elongation [248]. *Sun3* KO mice display more severe defects, manchette loss, defective chromatin condensation, disorders of the acrosomal body and flagellar structure, and a highly disorganized arrangement of the manchette [81], highlighting SUN3's role in cytoskeletal-nuclear coordination. SPAG4L is a new nuclear membrane and endoplasmic reticulum protein that plays an important role in the meiotic process. Mutations in *SPAG4L* (reported in 33–47% of ASS patients) disrupt sperm head–tail coupling, underscoring its conserved role in spermiogenesis [83].

IFT family

Mutations in the IFT subunit commonly disrupt flagellar assembly and function, but emerging evidence highlights their critical roles in manchette-mediated processes during spermatid elongation. For example, IFT172 [178], IFT140 [175], IFT144 [177], IFT20, and IFT88 [84] are localized in the manchette and may be involved in spermatogenesis by regulating the IMT process. While further research is needed to fully elucidate these processes, existing data corroborate the conclusions above, as outlined below. Knockdown of *Ift172* significantly reduced the number of spermatozoa in the epididymal tail, with morphological defects, reduced viability, abnormal sperm head morphology, and elongated manchette [178]. IFT20 deletion manifested infertile with significantly reduced sperm counts and motility, abnormally shaped elongating spermatid heads, and bulbous round spermatids [179]. IFT20 also interacted with other proteins such as GMAP210 [180], CCDC41 [121], SPATA1 [181], SPEF2 [45], BLOC-1 [182], and SPAG17 [69]. IFT140 interacts with IFT88 and IFT144. IFT140 deficiency is manifested by decreased sperm motility. *Mutations in WDR19* (encoding IFT144) cause asthenoteratozoospermia, characterized by reduced sperm motility and abnormal morphology [177]. IFT140 interacts with IFT88/IFT144 to maintain flagellar structural integrity. IFT140 is abnormally clustered in the head and neck of spermatozoa, and IFT88 is abnormally localized in the neck of the spermatozoa, which may lead to abnormal expression of other sperm components, especially IFT components, e.g., SPAG6, resulting in disruption and disorganization of MT structure. IFT140 deficiency causes

infertility with severely reduced sperm count and motility, abnormal sperm morphology: amorphous heads, short/bent flagella, swollen tail tips, and flagellar vesicles in mouse [175]; and causes infertility, characterized by severe oligozoospermia, AZS, and OAT in humans [176]. IFT88 deletion is manifested as flagellar abnormalities [84]. Additionally, IFT-associated proteins TTC family induce asthenoteratospermia in humans and mice, such as TTC29 [232] and TTC21A [231].

STK family

Serine/threonine kinases domain-containing proteins play important functions in sperm flagella and male fertility. However, the roles of these proteins in human reproduction remain poorly understood, and it has not been reported whether their variants are associated with human AZS [225].

STK33 is an MMAF-associated gene. Patients homozygous for STK33 variants exhibit reduced sperm motility, high-frequency flagellar morphological defects, and complete disorganization of flagellar ultrastructures [225]. Mechanistically, STK33 regulates the phosphorylation of fibrous sheath (FS) components AKAP3 and AKAP4 (A-kinase anchoring protein 3/4), which are essential for FS assembly during spermiogenesis [226]. In *Akap3* [97] or *Akap4* [101] null mice, sperm lack a fully assembled FS, causing male sterility.

The Fused (Fu) protein, encoded by the *Stk36* gene, is a novel manchette-related protein to regulate spermatid head shaping, tail morphogenesis, and peri-axonemal structure formation. It interacts with ODF1 and KIF27, and its loss in male germ cells results in infertility, characterized by reduced sperm count, abnormal sperm head shaping, decapitation, motility defects, and structural abnormalities in the acrosome, manchette, and sperm tail accessory structures [89].

PACRG-MEIG1

MEIG1/PACRG/DNALI1 complex is located in the manchette and involved in cargo transport, such as SPAG16L, to build sperm flagella [43]. DNALI1, an axonemal component, is a newly identified causative gene for AZS in both humans and mice. DNALI1 loss led to the asymmetrical development of sperm fibrous sheath and severely disrupted the transport and assembly of the FS proteins, especially AKAP3 and AKAP4, during flagellogenesis [98]. MEIG1 mutation diminishes the ability of MEIG1 to stabilize PACRG, such as W50 A, K57E, F66 A, and Y68 A [249]. Among them, homozygous mutated Y68 examined were completely infertile, and sperm count was dramatically reduced [207]. MEIG1 also determines the manchette localization of IFT20 and IFT88 [84].

LRRC family

The Leucine-rich repeat protein (LRRC) family includes four members reported to cause MMAF phenotypes: *Lrrc6* [204], *Lrrc46* [65], *Lrrc50* [65], *Lrrc8a* [206]. Genetic defects of LRRC6 have been associated with primary ciliary dyskinesia (PCD) and asthenozoospermia due to abnormal ultrastructure of ciliated axonemes in humans and mice. LRRC46 is specifically expressed in the testes of adult mice and is essential for sperm flagellum biogenesis. The knockout of *Lrrc46* in mice resulted in typical MMAF phenotypes, including sperm with short, coiled, and irregular flagella, reduced total sperm counts, impaired sperm motility, and led to completely infertile. The deletion of *Lrrc50* causes dynein arm defects, immotile cilia, and male infertility. LRRC8A-dependent VRAC activity is essential for male germ cell development and fertility. Human patients with a rare LRRC8A hypomorphic mutation are possibly linked to Sertoli cell-only syndrome (SCOS), a male sterility disorder characterized by the loss of germ cells. Whether it plays a role in MT formation and MT transport remains to be investigated.

Besides, there are other members reported to cause infertility: *Lrrc23* [199], *Lrrc48* [200], and *Lrrc56* [202, 203]. LRRC23, a radial spoke RS3 head component, is essential for RS head assembly and flagellar motility in mammalian spermatozoa. It interacts with RSPH9, another radial spoke protein. *Lrrc23* variants disrupt its localization to the sperm tail, resulting in asthenozoospermia and infertility in mice. The nexin-dynein regulatory complex (N-DRC) is a large protein complex in the sperm flagellum that connects adjacent doublets of microtubules. LRRC48 is a component of the N-DRC, and its variant causes asthenozoospermia and male infertility. LRRC56, a protein associated with intraflagellar transport, interacts with IFT88. Its mutations cause mucociliary clearance and laterality defects in *Trypanosoma brucei*. Its role in mammals remains speculative, but it may play a role in dynein transport during cilia assembly and involve the manchette-mediated flagellar assembly system.

Moreover, the testis-specific genes *LRRC4C* [201] and *LRRC69* [205] have been identified as novel candidate risk factors for male infertility. However, their functional roles in spermatogenesis and mechanistic contributions to germ cell development remain uncharacterized. Given that other LRRC family members (e.g., LRRC6, LRRC46) are known to maintain axonemal ultrastructure and dynein complex function to ensure fertility, it is plausible that LRRC4C and LRRC69 may function through analogous mechanisms in late-stage spermiogenesis; however, this hypothesis awaits validation via gain- or

loss-of-function experiments. Therefore, further studies are needed to define, such as the molecular interplay between LRRC4C/LRRC69 and key spermatogenic pathways; their potential roles in microtubule dynamics or manchette-flagellum coordination.

CCDC family proteins

Members of the coiled-coil domain-containing (CCDC) protein family possess the conserved coiled-coil motif that is responsible for molecular recognition and protein refolding. Recently, several *Ccdc* genes have been found to play important roles in sperm functions and male fertility. Among them, the *Ccdc* genes associated with MMAF include the *Ccdc34* [117], *Ccdc38* [250], *Ccdc39* [92, 119], *Ccdc40* [120], *Ccdc65* [123], *Ccdc103* [108], *Ccdc146* [110], *Ccdc183* [114], and *Ccdc189* [115].

the testis-specific protein CCDC38 interacts with CCDC42 [118] and CCDC146 [250], localizing on the manchette and sperm tail during spermiogenesis. Inactivation of CCDC38 in male mice results in a distorted manchette, multiple morphological abnormalities of the flagella of spermatozoa, and eventually male infertility. It also interacts with IFT88 and ODF2 to transport ODF2. CCDC42 localizes to the manchette, HTCA, and tail and interacts with ODF1 and ODF2 in the formation of the male germ cell cytoskeleton [122]. CCDC146 is predominantly expressed in the testes, and the knockout of this gene resulted in flagellum and manchette organization defects, finally leading to MMAF-like phenotype and complete infertility in male mice. It also interacts with IFT88 and IFT20. Besides, CCDC181 seems to interact with HOOK1 and directly with MTs localizing to the microtubular manchette of elongating spermatids and playing a role in mediating ciliary motility [66].

Ccdc87, a testis-specific gene, is predominantly expressed in pachytene spermatocytes and spermatids and is involved in the maintenance of sperm morphology and the regulation of initial sperm motility. CCDC87 protein is important for the formation of a normal sperm head. Deletion of this protein led to morphological deformations in sperm, displayed defective acrosome and nucleus formation, and resulted in subfertility in *Ccdc87* knockout mice [124].

CCDC178 is predominantly expressed in the testis, interacts with IFT88 and KIF3A, participates in the assembly of manchette, and plays a critical role in regulating sperm head shaping and flagellum biogenesis during spermiogenesis. CCDC178 knockout led to defects in IMT and the disruption of the apical ectoplasmic specialization resulting in complete male infertility with an oligoasthenospermia-like phenotype [111]. Besides, CFAP53 also interacts with KIF3A and IFT88 to participate in both IMT and IFT during sperm flagellum biogenesis [137]. This suggests that there may be some connection between CCDC178 and CFPA53.

CCDC189 is a radial-spoke-associated protein involved in sperm flagellum formation through its interactions with CABCOCO1 and intra-flagellar transport proteins. *Ccdc189*-deficient mice carried coiled, curved, or short flagella, which are typical MMAF phenotypes, resulting in OAT and male infertility [115, 116].

Other proteins

Spef2 gene KO mouse resulted in IMT disorders, leading to sperm head and tail malformations, decreased sperm counts, and MMAF [90, 91]

Terminal Nucleotidyl Transferase 5C (TENT5C), a non-classical RNA polyadenyl polymerase family, is localized in the manchette. TENT5C knockout mice show a normal manchette structure but increased numbers of anucleate and morphologically abnormal manchette and reduced fertilization capacity, leading to infertility [93].

Profilins (PFNs) are key regulatory proteins for actin polymerization in cells and are encoded in humans and mice by four *Pfn* genes. The testes-specific PFN3 and PFN4 are localized in the acroplaxome-manchette complex of developing spermatozoa. The deficient mice of PFN3 and PFN4 are subfertile, displaying type II globozoospermia with sperm displaying severe impairment in manchette formation leading to an amorphous sperm head shape, reduced sperm motility resulting from flagellum deformities, the dysregulation of the autophagic flux impairing acrosome biogenesis, and cytoplasm removal defect [211, 212].

SPEM1 is one of the spermiogenesis-essential proteins encoded by a testis-specific gene exclusively expressed in the developing spermatids and localized in the manchette. Inactivation of *Spem1* in mice results in deformed spermatozoa characterized by"head-bent-back"abnormalities. SPEM1 interacts with UBQLN1 and RANBP17 and transports them in the manchette which then respectively plays a role in the regulation of protein ubiquitination during spermiogenesis [220] and has a role in sex chromosome inactivation during the meiotic phase of spermatogenesis and IMT during spermiogenesis [221].

IQCN regulates MT nucleation during manchette assembly via calmodulin and related calmodulin-binding proteins, which resulted in head deformity with aberrant oocyte activation factor PLCζ [185]. Deficiency in IQCN led to the loss of its interaction with CDC42, resulting in impaired manchette-related functions and sperm flagellar assembly, causing male infertility in humans and mice [186].

N-arginine dibasic (NRD) convertase is a novel metalloendopeptidase that selectively cleaves at the N terminus of arginine residues in paired basic amino acids [251]. The enzyme localizes within the cytoplasm of spermatids and associates with MTs of the manchette and axoneme to establish MT structure and maintain its function [208].

The lysine demethylase Kdm3a (KDM3A) is required for male fertility, sex determination, and metabolic homeostasis through its nuclear role in chromatin remodeling. Mutations in KDM3A can lead to azoospermia or oligospermia [189].

Family with sequence similarity 46, member C (FAM46C) is a highly conserved non-canonical RNA polyadenylation polymerase to fasten the sperm head and flagellum in spermatids. It is abundantly expressed in human and mouse testes and localized to the manchette. Gene knockout of FAM46C in mice resulted in male sterility, characterized by the production of headless spermatozoa in the testes [165].

Golgi-associated PDZ and coiled-coil motif-containing protein (GOPC) is a Golgi protein that plays a role in vesicular transport and intracellular protein trafficking and degradation. Mice deficient in GOPC protein have globozoospermia and are infertile due to abnormalities in the acrosome, disordered structure of the manchette and acroplaxome, and abnormal localization of the manchette and perinuclear ring [169].

Radial spokes, which are regularly spaced along cilia, sperm, and flagella axonemes, consist of a thin'stalk'and a bulbous'head'that form a signal transduction scaffold between the central pair of microtubules and dynein. Mutations in radial spoke head (RSPH) component proteins cause primary ciliary dyskinesia, a disease arising from dysmotility of motile cilia and sperm, including RSPH1 [213], RSPH3 [214], RSPH6A [22], RSPH4A, and RSPH9 [215].

SSP411 might belong to a testis-specific thioredoxin family and is predominantly localized to round and elongated spermatids, with maximal expression at stages VII-XII [224]. The manchette is aberrant in $Ssp411^{PB/PB}$ spermatids. $Ssp411^{PB/PB}$ males are sterile. These males have smaller testes, reduced sperm counts, decreased sperm motility, deformed spermatozoa, and abnormal sperm heads [223].

AXDND1 is exclusively expressed in the round and elongating spermatids in humans and mice as a novel testis-enrich gene essential for spermiogenesis and male fertility probably by regulating the manchette dynamics, spermatid head shaping, sperm flagellum assembly. Axdnd1 knockout males are sterile with reduced testis size caused by increased germ cell apoptosis and sloughing, exhibiting phenotypes consistent with OAT [252].

Actin like 7 A (ACTL7A), a member of a highly conserved actin-related proteins (ARPs) family, interacts with various cytoskeletal proteins and is an indispensable protein for subacrosomal-associated F-actin formation, acrosomal anchoring, and male fertility. In *Actl7a*-KO mice, the deletion of ACTL7A damaged the formation of the AAM complex by inhibition of autophagy via PI3 K/AKT/mTOR signaling pathway activation which resulted in the accumulation of PDZ and LIM domain 1 (PDLIM1), leading to abnormalities in the shaping of sperm heads, resulting in small head sperm [94]. *Actl7b* deficiency leads to mislocalization of LC8 type dynein light chains and severe spermatid defects, such as detached acrosomes, disrupted membranes, and flagella malformations finally resulting in spermatogenic arrest and infertility in *Actl7b* KO male mice [253].

Many others may cause defects in spermatogenesis that have not been published, such as CEP83, and those under investigation by our group, such as OFD1.

Conclusions

Sperm malformation is one of the main reasons for male infertility. The major process of spermiogenesis is sperm head shaping and flagellar assembly in which the manchette is a crucial structure. Firstly, the manchette provides a platform for protein and vesicle transport of sperm head shaping and flagellar assembly, and for the assembly of mitochondria into the mid-piece of sperm. Secondly, the manchette interacts with the cytoskeleton and centrosome forming manchette-perinuclear ring linkage, manchette-nuclear linkage, and AAM axis to balance morphology of the sperm head. Finally, the manchette orchestrates IMT and IFT, coupling head-to-tail cargo delivery into an integrated system essential for sperm morphogenesis. Real-time tracking of IMT-mediated cargo movement in this system offers a powerful approach to decode the potential regulation mechanisms of spermiogenesis.

Interfering with the assembly and disassembly of the manchette and the normal regulation of IMT may result in a range of spermatogenic disorders, but the precise mechanisms remain to be elucidated. The specificity of the short-term presence of manchette during sperm formation precludes the possibility of accurately identifying the manchette-associated proteins through cryo-electron microscopy or sequencing, among other techniques. As biological techniques continue to evolve, an increasing number of proteins associated with manchette function will be validated. Currently, over 100 manchette-associated proteins have been identified through the knockout animal models obtained by CRISPR-Cas9 technology, genotypic phenotyping, and interactions with proteins by coimmunoprecipitation (Co-IP) experiments and mass

spectrometry or proteome profile. However, the specific mechanisms of interaction for these proteins remain unclear.

Furthermore, the functions of manchette MTs are heterogeneous and are primarily distinguished by post-translational modifications (PTM) of MT proteins. These modifications include the acetylation and detyrosination of α-tubulin C-terminus and the Glutamylation or polyglutamylation of α- and β-tubulin C-terminus. Nevertheless, further evaluation is required to map the heterogeneity of sperm-collared MTs [254].

Further research is required to gain a deeper understanding of the mechanisms underlying manchette assembly and disassembly, especially the regulatory mechanisms of the MTOC in greater detail. It is also essential to study the dynamic changes of the MT or other cytoskeleton, examine the interactions between MTs and motor proteins or other related proteins, and elucidate the specific molecular and transport mechanisms of motor proteins that transport cargoes via bridge proteins. To gain a deeper understanding of how mutations impact the structure and function of the manchette, leading to male infertility. This will also provide a crucial theoretical foundation for the clinical diagnosis and treatment of male infertility resulting from spermatogenic disorders, as well as the development of novel contraceptive targets for men in the future.

Abbreviations

+ TIPs	Microtubule plus-end-tracking proteins
AAA	ATPases associated with diverse cellular activities
AAM	Acroframosome-acroplaxome-manchette
AFS	Acroframosome
ASS	Acephalic spermatozoa syndrome
ATZ	Asthenoteratozoospermia
AZS	Asthenozoospermia
F-actin	Filamentous actin
HTCA	Head-tail coupling apparatus
HTP	Histone-to-protamine transition
IFT	Intra-flagellar transport
IMT	Intra-manchette transport
KBP	KIF1-binding protein
KHC	Kinesin heavy chain
KIFs	Kinesin superfamily proteins
KLC	Kinesin light chain
LINC	Linker of the nucleoskeleton and cytoskeleton
MAPs	Microtubule-associated proteins
MMAF	Multiple morphological abnormalities of the sperm flagellum
MNS1	Meiosis specific nuclear structural 1
MT	Microtubule
MTOC	Microtubule organizing center
MTSPs	Microtubule severing proteins
OA	Oligoasthenozoospermia
OAT	Oligoasthenoteratozoospermia
PTM	Post-translational modifications
RAB	Ras-related protein
SPAST	Spastin
WDR62	WD40 repeat protein 62

Supplementary Information

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Supplementary Material 1.

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Authors' contributions
T.G. collected the data and wrote the manuscript; B.W. and T.G. developed the project. T.G., Y.L., J.L. and Y. Z. designed the graphs and tables. B.W. and T.G. reviewed and finalized the manuscript; All authors read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

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Not applicable.

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Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Hess RA, Renato de Franca L. Spermatogenesis and cycle of the seminiferous epithelium. Adv Exp Med Biol. 2008;636:1–15.
2. O'Donnell L. Mechanisms of spermiogenesis and spermiation and how they are disturbed. Spermatogenesis. 2014;2:e979623.
3. Oehninger S, Kruger TF. Sperm morphology and its disorders in the context of infertility. F&S Reviews. 2021;1:75–92.
4. Kierszenbaum AL. Intramanchette transport (IMT): managing the making of the spermatid head, centrosome, and tail. Mol Reprod Dev. 2002;1:1–4.
5. Ahmed EA, de Rooij DG. Staging of mouse seminiferous tubule cross-sections [M]. Meiosis. 2009:263–277.
6. Lehti MS, Sironen A. Formation and function of the manchette and flagellum during spermatogenesis. Reproduction. 2016;4:R43-54.
7. Hu W, Zhang R, Xu H, Li Y, Yang X, Zhou Z, Huang X, Wang Y, Ji W, Gao F, et al. CAMSAP1 role in orchestrating structure and dynamics of manchette microtubule minus-ends impacts male fertility during spermiogenesis. Proc Natl Acad Sci U S A. 2023;45:e2313787120.

8. Wei YL, Yang WX. The acroframosome-acroplaxome-manchette axis may function in sperm head shaping and male fertility. Gene. 2018;660:28–40.
9. Kmonickova V, Frolikova M, Steger K, Komrskova K. The role of the LINC complex in sperm development and function. Int J Mol Sci. 2020;21(23):9058.
10. Zheng Y, Wong ML, Alberts B, Mitchison T. Nucleation of microtubule assembly by a gamma-tubulin-containing ring complex. Nature. 1995;6557:578–83.
11. Jakobs MAH, Zemel A, Franze K. Unrestrained growth of correctly oriented microtubules instructs axonal microtubule orientation. Elife. 2022;11:e77608.
12. Brito C, Serna M, Guerra P, Llorca O, Surrey T. Transition of human γ-tubulin ring complex into a closed conformation during microtubule nucleation. Science. 2024;6685:870–6.
13. Rai D, Song Y, Hua S, Stecker K, Monster JL, Yin V, Stucchi R, Xu Y, Zhang Y, Chen F, et al. CAMSAPs and nucleation-promoting factors control microtubule release from γ-TuRC. Nat Cell Biol. 2024;3:404–20.
14. Goodwin SS, Vale RD. Patronin regulates the microtubule network by protecting microtubule minus ends. Cell. 2010;2:263–74.
15. Wacławek E, Włoga D. Microtubule severing proteins - structure and activity regulation. Postepy Biochem. 2016;1:46–51.
16. McNally FJ, Roll-Mecak A. Microtubule-severing enzymes: From cellular functions to molecular mechanism. J Cell Biol. 2018;12:4057–69.
17. Ho UY, Feng CA, Yeap YY, Bain AL, Wei Z, Shohayeb B, Reichelt ME, Homer H, Khanna KK, Bowles J, et al. WDR62 is required for centriole duplication in spermatogenesis and manchette removal in spermiogenesis. Commun Biol. 2021;1:645.
18. Dunleavy JEM, Graffeo M, Wozniak K, O'Connor AE, Merriner DJ, Nguyen J, Schittenhelm RB, Houston BJ, O'Bryan MK. The katanin A-subunits KATNA1 and KATNAL1 act co-operatively in mammalian meiosis and spermiogenesis to achieve male fertility. Development. 2023;150(22):dev201956.
19. Dunleavy JEM, Okuda H, O'Connor AE, Merriner DJ, O'Donnell L, Jamsai D, Bergmann M, O'Bryan MK. Katanin-like 2 (KATNAL2) functions in multiple aspects of haploid male germ cell development in the mouse. PLoS Genet. 2017;11:e1007078.
20. Cheers SR, O'Connor AE, Johnson TK, Merriner DJ, O'Bryan MK, Dunleavy JEM. Spastin is an essential regulator of male meiosis, acrosome formation, manchette structure and nuclear integrity. Development. 2023;150(6):dev201183.
21. Houston BJ, Nguyen J, Merriner DJ, O'Connor AE, Lopes AM, Nagirnaja L, Friedrich C, Kliesch S, Tüttelmann F, Aston KI, et al. AXDND1 is required to balance spermatogonial commitment and for sperm tail formation in mice and humans. Cell Death Dis. 2024;7:499.
22. Abbasi F, Miyata H, Shimada K, Morohoshi A, Nozawa K, Matsumura T, Xu Z, Pratiwi P, Ikawa M. RSPH6A is required for sperm flagellum formation and male fertility in mice. J Cell Sci. 2018;131(19):jcs221648.
23. Yu Y, Wang J, Zhou L, Li H, Zheng B, Yang S. CFAP43-mediated intramanchette transport is required for sperm head shaping and flagella formation. Zygote. 2021;1:75–81.
24. Scholey JM. Kinesin-2: a family of heterotrimeric and homodimeric motors with diverse intracellular transport functions. Annu Rev Cell Dev Biol. 2013;29:443–69.
25. Dunleavy JEM, O'Bryan MK, Stanton PG, O'Donnell L. The cytoskeleton in spermatogenesis. Reproduction. 2019;2:R53-r72.
26. Pleuger C, Lehti MS, Dunleavy JE, Fietz D, O'Bryan MK. Haploid male germ cells-the grand central station of protein transport. Hum Reprod Update. 2020;4:474–500.
27. Wang L, Bu T, Wu X, Li L, Sun F, Cheng CY. Motor proteins, spermatogenesis and testis function. Adv Protein Chem Struct Biol. 2024;141:381–445.
28. Cianfrocco MA, DeSantis ME, Leschziner AE, Reck-Peterson SL. Mechanism and regulation of cytoplasmic dynein. Annu Rev Cell Dev Biol. 2015;31:83–108.
29. Calvi A, Wong AS, Wright G, Wong ES, Loo TH, Stewart CL, Burke B. SUN4 is essential for nuclear remodeling during mammalian spermiogenesis. Dev Biol. 2015;2:321–30.
30. Li X, Wu Y, Huang L, Yang L, Xing X. SPAG4L/SPAG4Lβ interacts with Nesprin2 to participate in the meiosis of spermatogenesis. Acta Biochim Biophys Sin (Shanghai). 2019;7:669–76.
31. Zakrzewski P, Lenartowski R, Rędowicz MJ, Miller KG, Lenartowska M. Expression and localization of myosin VI in developing mouse spermatids. Histochem Cell Biol. 2017;4:445–62.
32. Kierszenbaum AL, Tres LL, Rivkin E, Kang-Decker N, van Deursen JM. The acroplaxome is the docking site of Golgi-derived myosin Va/Rab27a/b-containing proacrosomal vesicles in wild-type and Hrb mutant mouse spermatids. Biol Reprod. 2004;5:1400–10.
33. Yuan S, Stratton CJ, Bao J, Zheng H, Bhetwal BP, Yanagimachi R, Yan W. Spata6is required for normal assembly of the sperm connecting piece and tight head–tail conjunction. Proc Natl Acad Sci U S A. 2015;112(5):E430.
34. Cross JA, Dodding MP. Motor-cargo adaptors at the organelle-cytoskeleton interface. Curr Opin Cell Biol. 2019;59:16–23.
35. Leslie JS, Rawlins LE, Chioza BA, Olubodun OR, Salter CG, Fasham J, Jones HF, Cross HE, Lam S, Harlalka GV, et al. MNS1 variant associated with situs inversus and male infertility. Eur J Hum Genet. 2020;1:50–5.
36. Matsuoka Y, Miyagawa Y, Tokuhiro K, Kitamura K, Iguchi N, Maekawa M, Takahashi T, Tsujimura A, Matsumiya K, Okuyama A, et al. Isolation and characterization of the spermatid-specific Smrp1 gene encoding a novel manchette protein. Mol Reprod Dev. 2008;6:967–75.
37. Zhou J, Du YR, Qin WH, Hu YG, Huang YN, Bao L, Han D, Mansouri A, Xu GL. RIM-BP3 is a manchette-associated protein essential for spermiogenesis. Development. 2009;3:373–82.
38. Liu X, Zang C, Wu Y, Meng R, Chen Y, Jiang T, Wang C, Yang X, Guo Y, Situ C, et al. Homeodomain-interacting protein kinase HIPK4 regulates phosphorylation of manchette protein RIMBP3 during spermiogenesis. J Biol Chem. 2022;9:102327.
39. Okuda H, DeBoer K, O'Connor AE, Merriner DJ, Jamsai D, O'Bryan MK. LRGUK1 is part of a multiprotein complex required for manchette function and male fertility. Faseb j. 2017;3:1141–52.
40. Yan W, Liu Y, DeBoer K, de Kretser DM, O'Donnell L, O'Connor AE, Merriner DJ, Okuda H, Whittle B, Jans DA, et al. LRGUK-1 is required for basal body and manchette function during spermatogenesis and male fertility. PLoS Genet. 2015;11(3):e1005090.
41. Singh K, Lau CK, Manigrasso G, Gama JB, Gassmann R, Carter AP. Molecular mechanism of dynein-dynactin complex assembly by LIS1. Science. 2024;6690:eadk8544.
42. Yap YT, Li W, Huang Q, Zhou Q, Zhang D, Sheng Y, Mladenovic-Lucas L, Yee SP, Orwig KE, Granneman JG, et al. DNALI1 interacts with the MEIG1/PACRG complex within the manchette and is required for proper sperm flagellum assembly in mice. Elife. 2023;12:e79620.
43. Li W, Tang W, Teves ME, Zhang Z, Zhang L, Li H, Archer KJ, Peterson DL, Williams DC Jr, Strauss JF 3rd, et al. A MEIG1/PACRG complex in the manchette is essential for building the sperm flagella. Development. 2015;5:921–30.
44. Sironen A, Hansen J, Thomsen B, Andersson M, Vilkki J, Toppari J, Kotaja N. Expression of SPEF2 during mouse spermatogenesis and identification of IFT20 as an interacting protein. Biol Reprod. 2010;3:580–90.
45. Lehti MS, Zhang FP, Kotaja N, Sironen A. SPEF2 functions in microtubule-mediated transport in elongating spermatids to ensure proper male germ cell differentiation. Development. 2017;14:2683–93.
46. Saade M, Irla M, Govin J, Victorero G, Samson M, Nguyen C. Dynamic distribution of Spatial during mouse spermatogenesis and its interaction with the kinesin KIF17b. Exp Cell Res. 2007;3:614–26.
47. Kierszenbaum AL, Gil M, Rivkin E, Tres LL. Ran, a GTP-binding protein involved in nucleocytoplasmic transport and microtubule nucleation, relocates from the manchette to the centrosome region during rat spermiogenesis. Mol Reprod Dev. 2002;1:131–40.
48. Yogo K. Molecular basis of the morphogenesis of sperm head and tail in mice. Reprod Med Biol. 2022;1:e12466.
49. Zakrzewski P, Rędowicz MJ, Buss F, Lenartowska M. Loss of myosin VI expression affects acrosome/acroplaxome complex morphology during mouse spermiogenesis†. Biol Reprod. 2020;3:521–33.
50. Kierszenbaum AL, Rivkin E, Tres LL. Cytoskeletal track selection during cargo transport in spermatids is relevant to male fertility. Spermatogenesis. 2011;3:221–30.
51. Russell LD, Russell JA, MacGregor GR, Meistrich ML. Linkage of manchette microtubules to the nuclear envelope and observations of the role of the manchette in nuclear shaping during spermiogenesis in rodents. Am J Anat. 1991;2:97–120.

52. Stathatos GG, Dunleavy JEM, Zenker J, O'Bryan MK. Delta and epsilon tubulin in mammalian development. Trends Cell Biol. 2021;9:774–87.
53. Stathatos GG, Merriner DJ, O'Connor AE, Zenker J, Dunleavy JE, O'Bryan MK. Epsilon tubulin is an essential determinant of microtubule-based structures in male germ cells. EMBO Rep. 2024;6:2722–42.
54. Lehti MS, Sironen A. Formation and function of sperm tail structures in association with sperm motility defects. Biol Reprod. 2017;4:522–36.
55. Gadadhar S, Hirschmugl T, Janke C. The tubulin code in mammalian sperm development and function. Semin Cell Dev Biol. 2023;137:26–37.
56. Wu B, Gao H, Liu C, Li W. The coupling apparatus of the sperm head and tail†. Biol Reprod. 2020;5:988–98.
57. Tres LL, Kierszenbaum AL. Sak57, an acidic keratin initially present in the spermatid manchette before becoming a component of paraaxonemal structures of the developing tail. Mol Reprod Dev. 1996;3:395–407.
58. Kierszenbaum AL. Spermatid manchette: Plugging proteins to zero into the sperm tail. Mol Reprod Dev. 2001;4:347–9.
59. Taschner M, Weber K, Mourão A, Vetter M, Awasthi M, Stiegler M, Bhogaraju S, Lorentzen E. Intraflagellar transport proteins 172, 80, 57, 54, 38, and 20 form a stable tubulin-binding IFT-B2 complex. EMBO J. 2016;7:773–90.
60. Lacey SE, Foster HE, Pigino G. The molecular structure of IFT-A and IFT-B in anterograde intraflagellar transport trains. Nat Struct Mol Biol. 2023;5:584–93.
61. Lacey SE, Pigino G. The intraflagellar transport cycle. Nat Rev Mol Cell Biol. 2025;3:175–92.
62. Gan S, Zhou S, Ma J, Xiong M, Xiong W, Fan X, Liu K, Gui Y, Chen B, Zhang B, et al. BAG5 regulates HSPA8-mediated protein folding required for sperm head-tail coupling apparatus assembly. EMBO Rep. 2024;4:2045–70.
63. Kim J, Kwon JT, Jeong J, Kim J, Hong SH, Kim J, Park ZY, Chung KH, Eddy EM, Cho C. SPATC1L maintains the integrity of the sperm head-tail junction. EMBO Rep. 2018;19(9):e45991.
64. Xavier MJ, Salas-Huetos A, Oud MS, Aston KI, Veltman JA. Disease gene discovery in male infertility: past, present and future. Hum Genet. 2020;1:7–19.
65. Yin Y, Mu W, Yu X, Wang Z, Xu K, Wu X, Cai Y, Zhang M, Lu G, Chan WY, et al. LRRC46 Accumulates at the Midpiece of Sperm Flagella and Is Essential for Spermiogenesis and Male Fertility in Mouse. Int J Mol Sci. 2022;23(15):8525.
66. Schwarz T, Prieler B, Schmid JA, Grzmil P, Neesen J. Ccdc181 is a microtubule-binding protein that interacts with Hook1 in haploid male germ cells and localizes to the sperm tail and motile cilia. Eur J Cell Biol. 2017;3:276–88.
67. Jiang XZ, Yang MG, Huang LH, Li CQ, Xing XW. SPAG4L, a novel nuclear envelope protein involved in the meiotic stage of spermatogenesis. DNA Cell Biol. 2011;11:875–82.
68. Yeh CH, Kuo PL, Wang YY, Wu YY, Chen MF, Lin DY, Lai TH, Chiang HS, Lin YH. SEPT12/SPAG4/LAMINB1 complexes are required for maintaining the integrity of the nuclear envelope in postmeiotic male germ cells. PLoS One. 2015;3:e0120722.
69. Kazarian E, Son H, Sapao P, Li W, Zhang Z, Strauss JF, Teves ME. SPAG17 is required for male germ cell differentiation and fertility. Int J Mol Sci. 2018;19(4):1252.
70. Xu C, Tang D, Shao Z, Geng H, Gao Y, Li K, Tan Q, Wang G, Wang C, Wu H, et al. Homozygous SPAG6 variants can induce nonsyndromic asthenoteratozoospermia with severe MMAF. Reprod Biol Endocrinol. 2022;1:41.
71. Ohta T, Essner R, Ryu JH, Palazzo RE, Uetake Y, Kuriyama R. Characterization of Cep135, a novel coiled-coil centrosomal protein involved in microtubule organization in mammalian cells. J Cell Biol. 2002;1:87–99.
72. Hall EA, Keighren M, Ford MJ, Davey T, Jarman AP, Smith LB, Jackson IJ, Mill P. Acute versus chronic loss of mammalian Azi1/Cep131 results in distinct ciliary phenotypes. PLoS Genet. 2013;12:e1003928.
73. Shalata A, Ramirez MC, Desnick RJ, Priedigkeit N, Buettner C, Lindtner C, Mahroum M, Abdul-Ghani M, Dong F, Arar N, et al. Morbid obesity resulting from inactivation of the ciliary protein CEP19 in humans and mice. Am J Hum Genet. 2013;6:1061–71.
74. Mojarad BA, Gupta GD, Hasegan M, Goudiam O, Basto R, Gingras AC, Pelletier L. CEP19 cooperates with FOP and CEP350 to drive early steps in the ciliogenesis programme. Open Biol. 2017;7(6):170114.
75. Sha YW, Xu X, Mei LB, Li P, Su ZY, He XQ, Li L. A homozygous CEP135 mutation is associated with multiple morphological abnormalities of the sperm flagella (MMAF). Gene. 2017;633:48–53.
76. Siller SS, Sharma H, Li S, Yang J, Zhang Y, Holtzman MJ, Winuthayanon W, Colognato H, Holdener BC, Li FQ, et al. Conditional knockout mice for the distal appendage protein CEP164 reveal its essential roles in airway multiciliated cell differentiation. PLoS Genet. 2017;12:e1007128.
77. Kashihara H, Chiba S, Kanno SI, Suzuki K, Yano T, Tsukita S. Cep128 associates with Odf2 to form the subdistal appendage of the centriole. Genes Cells. 2019;3:231–43.
78. Zhang X, Wang L, Ma Y, Wang Y, Liu H, Liu M, Qin L, Li J, Jiang C, Zhang X, et al. CEP128 is involved in spermatogenesis in humans and mice. Nat Commun. 2022;1:1395.
79. Zhang X, Zheng R, Liang C, Liu H, Zhang X, Ma Y, Liu M, Zhang W, Yang Y, Liu M, et al. Loss-of-function mutations in CEP78 cause male infertility in humans and mice. Sci Adv. 2022;40:eabn0968.
80. Zhang X, Huang G, Jiang T, Meng L, Li T, Zhang G, Wu N, Chen X, Zhao B, Li N, et al. CEP112 coordinates translational regulation of essential fertility genes during spermiogenesis through phase separation in humans and mice. Nat Commun. 2024;1:8465.
81. Gao Q, Khan R, Yu C, Alsheimer M, Jiang X, Ma H, Shi Q. The testis-specific LINC component SUN3 is essential for sperm head shaping during mouse spermiogenesis. J Biol Chem. 2020;19:6289–98.
82. Zhang Y, Liu C, Wu B, Li L, Li W, Yuan L. The missing linker between SUN5 and PMFBP1 in sperm head-tail coupling apparatus. Nat Commun. 2021;1:4926.
83. Zhang Y, Yang L, Huang L, Liu G, Nie X, Zhang X, Xing X. SUN5 Interacting With Nesprin3 Plays an Essential Role in Sperm Head-to-Tail Linkage: Research on Sun5 Gene Knockout Mice. Front Cell Dev Biol. 2021;9:684826.
84. Yap YT, Shi L, Zhang D, Huang Q, Siddika F, Wang Z, Li W, Zhang Z. MEIG1 determines the manchette localization of IFT20 and IFT88, two intraflagellar transport components in male germ cells. Dev Biol. 2022;485:50–60.
85. Boegholm N, Petriman NA, Loureiro-López M, Wang J, Vela MIS, Liu B, Kanie T, Ng R, Jackson PK, Andersen JS, et al. The IFT81-IFT74 complex acts as an unconventional RabL2 GTPase-activating protein during intraflagellar transport. EMBO J. 2023;18:e111807.
86. Kuo YC, Lin YH, Chen HI, Wang YY, Chiou YW, Lin HH, Pan HA, Wu CM, Su SM, Hsu CC, et al. SEPT12 mutations cause male infertility with defective sperm annulus. Hum Mutat. 2012;4:710–9.
87. Huang CY, Wang YY, Chen YL, Chen MF, Chiang HS, Kuo PL, Lin YH. CDC42 negatively regulates testis-specific SEPT12 polymerization. Int J Mol Sci. 2018;19(9):2627.
88. Vahabi Barzi N, Kakavand K, Sodeifi N, Ghezelayagh Z, Sabbaghian M. Expression and localization of Septin 14 gene and protein in infertile men testis. Reprod Biol. 2020;2:164–8.
89. Nozawa YI, Yao E, Gacayan R, Xu SM, Chuang PT. Mammalian Fused is essential for sperm head shaping and periaxonemal structure formation during spermatogenesis. Dev Biol. 2014;2:170–80.
90. Liu C, Lv M, He X, Zhu Y, Amiri-Yekta A, Li W, Wu H, Kherraf ZE, Liu W, Zhang J, et al. Homozygous mutations in SPEF2 induce multiple morphological abnormalities of the sperm flagella and male infertility. J Med Genet. 2020;1:31–7.
91. Tu C, Nie H, Meng L, Wang W, Li H, Yuan S, Cheng D, He W, Liu G, Du J, et al. Novel mutations in SPEF2 causing different defects between flagella and cilia bridge: the phenotypic link between MMAF and PCD. Hum Genet. 2020;2:257–71.
92. Aprea I, Wilken A, Krallmann C, Nöthe-Menchen T, Olbrich H, Loges NT, Dougherty GW, Bracht D, Brenker C, Kliesch S, et al. Pathogenic gene variants in CCDC39, CCDC40, RSPH1, RSPH9, HYDIN, and SPEF2 cause defects of sperm flagella composition and male infertility. Front Genet. 2023;14:1117821.
93. Brouze M, Czarnocka-Cieciura A, Gewartowska O, Kusio-Kobiałka M, Jachacy K, Szpila M, Tarkowski B, Gruchota J, Krawczyk P, Mroczek S, et al. TENT5-mediated polyadenylation of mRNAs encoding secreted proteins is essential for gametogenesis in mice. Nat Commun. 2024;15(1):5331.
94. Zhang Y, Tang J, Wang X, Sun Y, Yang T, Shen X, Yang X, Shi H, Sun X, Xin A. Loss of ACTL7A causes small head sperm by defective

acrosome-acroplaxome-manchette complex. Reprod Biol Endocrinol. 2023;1:82.
95. Lin YH, Huang CY, Ke CC, Wang YY, Lai TH, Liu HC, Ku WC, Chan CC, Lin YH. ACTN4 mediates SEPT14 mutation-induced sperm head defects. Biomedicines. 2020;8(11):518.
96. Qu R, Zhang Z, Wu L, Li Q, Mu J, Zhao L, Yan Z, Wang W, Zeng Y, Liu R, et al. ADGB variants cause asthenozoospermia and male infertility. Hum Genet. 2023;6:735–48.
97. Xu K, Yang L, Zhang L, Qi H. Lack of AKAP3 disrupts integrity of the subcellular structure and proteome of mouse sperm and causes male sterility. Development. 2020;147(2):dev181057.
98. Wu H, Liu Y, Li Y, Li K, Xu C, Gao Y, Lv M, Guo R, Xu Y, Zhou P, et al. DNALI1 deficiency causes male infertility with severe asthenozoospermia in humans and mice by disrupting the assembly of the flagellar inner dynein arms and fibrous sheath. Cell Death Dis. 2023;2:127.
99. Li YF, He W, Mandal A, Kim YH, Digilio L, Klotz K, Flickinger CJ, Herr JC, Herr JC. CABYR binds to AKAP3 and Ropporin in the human sperm fibrous sheath. Asian J Androl. 2011;2:266–74.
100. Zhao Y, Kan FWK. Human OVGP1 enhances tyrosine phosphorylation of proteins in the fibrous sheath involving AKAP3 and increases sperm-zona binding. J Assist Reprod Genet. 2019;7:1363–77.
101. Fang X, Huang LL, Xu J, Ma CQ, Chen ZH, Zhang Z, Liao CH, Zheng SX, Huang P, Xu WM, et al. Proteomics and single-cell RNA analysis of Akap4-knockout mice model confirm indispensable role of Akap4 in spermatogenesis. Dev Biol. 2019;2:118–27.
102. Turner RM, Musse MP, Mandal A, Klotz K, Jayes FC, Herr JC, Gerton GL, Moss SB, Chemes HE. Molecular genetic analysis of two human sperm fibrous sheath proteins, AKAP4 and AKAP3, in men with dysplasia of the fibrous sheath. J Androl. 2001;2:302–15.
103. Zhang G, Li D, Tu C, Meng L, Tan Y, Ji Z, Cheng J, Lu G, Lin G, Zhang H, et al. Loss-of-function missense variant of AKAP4 induced male infertility through reduced interaction with QRICH2 during sperm flagella development. Hum Mol Genet. 2021;2:219–31.
104. Huang Y, Dong X, Sun SY, Lim TK, Lin Q, He CY. ARL3 GTPases facilitate ODA16 unloading from IFT in motile cilia. Sci Adv. 2024;36:eadq2950.
105. Qi Y, Jiang M, Yuan Y, Bi Y, Zheng B, Guo X, Huang X, Zhou Z, Sha J. ADP-ribosylation factor-like 3, a manchette-associated protein, is essential for mouse spermiogenesis. Mol Hum Reprod. 2013;5:327–35.
106. Fouquet J, Kann M, Souès S, Melki R. ARP1 in Golgi organisation and attachment of manchette microtubules to the nucleus during mammalian spermatogenesis. J Cell Sci. 2000;113:877–86.
107. Hiradate Y, Harima R, Yanai R, Hara K, Nagasawa K, Osada M, Kobayashi T, Matsuyama M, Kanno SI, Yasui A, et al. Loss of Axdnd1 causes sterility due to impaired spermatid differentiation in mice. Reprod Med Biol. 2022;1:e12452.
108. Zubair M, Khan R, Ma A, Hameed U, Khan M, Abbas T, Ahmad R, Zhou JT, Shah W, Hussain A, et al. A recurrent homozygous missense mutation in CCDC103 causes asthenoteratozoospermia due to disorganized dynein arms. Asian J Androl. 2022;3:255–9.
109. Pereira R, Oliveira ME, Santos R, Oliveira E, Barbosa T, Santos T, Gonçalves P, Ferraz L, Pinto S, Barros A, et al. Characterization of CCDC103 expression profiles: further insights in primary ciliary dyskinesia and in human reproduction. J Assist Reprod Genet. 2019;8:1683–700.
110. Muroňová J, Kherraf ZE, Giordani E, Lambert E, Eckert S, Cazin C, Amiri-Yekta A, Court M, Chevalier G, Martinez G, et al. Lack of CCDC146, a ubiquitous centriole and microtubule-associated protein, leads to non-syndromic male infertility in human and mouse. Elife. 2024;12:RP86845.
111. Dong F, Wang X, Huang T, Yin Y, Xiao S, Ma Y, Wei H, Wu B, Zhang R, Wang L, Wang G, et al. CCDC178-mediated cytoskeleton assembly is required for spermiogenesis in mice. Genes Dis. 2024;6:101132.
112. Zi X, Li Q, Yue Lu, Lyu Q, Guo H, Meng X, Zhou J, Zhao H. CCDC181 is required for proper spermiogenesis in mice. J Genet Genomics. 2024;11:1327–30.
113. Zhang XJ, Hou XN, Zhou JT, Shi BL, Ye JW, Yang ML, Jiang XH, Xu B, Wu LM, Shi QH. CCDC181 is required for sperm flagellum biogenesis and male fertility in mice. Zool Res. 2024;5:1061–72.
114. Wang Y, Chen J, Huang X, Wu B, Dai P, Zhang F, Li J, Wang L. Gene-knockout by iSTOP enables rapid reproductive disease modeling and phenotyping in germ cells of the founder generation. Sci China Life Sci. 2024;5:1035–50.
115. Zhou H, Yang F, Li G, Yuan L, Ge T, Niu C, Zheng Y. CCDC189 depletion leads to oligo-astheno-teratozoospermia and male infertility in mice†. Biol Reprod. 2024;4:800–14.
116. Wang M, Kang J, Shen Z, Hu Y, Chen M, Cui X, Liu H, Gao F. CCDC189 affects sperm flagellum formation by interacting with CABCOCO1. Natl Sci Rev. 2023;9:nwad181.
117. Cong J, Wang X, Amiri-Yekta A, Wang L, Kherraf ZE, Liu C, Cazin C, Tang S, Hosseini SH, Tian S, et al. Homozygous mutations in CCDC34 cause male infertility with oligoasthenoteratozoospermia in humans and mice. J Med Genet. 2022;7:710–8.
118. Zhang R, Wu B, Liu C, Zhang Z, Wang X, Wang L, Xiao S, Chen Y, Wei H, Jiang H, et al. CCDC38 is required for sperm flagellum biogenesis and male fertility in mice. Development. 2022;149(11):dev200516.
119. Chen D, Liang Y, Li J, Zhang X, Zheng R, Wang X, Zhang H, Shen Y. A novel CCDC39 mutation causes multiple morphological abnormalities of the flagella in a primary ciliary dyskinesia patient. Reprod Biomed Online. 2021;5:920–30.
120. Xu Y, Yang B, Lei C, Yang D, Ding S, Lu C, Wang L, Guo T, Wang R, Luo H. Novel compound heterozygous variants in CCDC40 associated with primary ciliary dyskinesia and multiple morphological abnormalities of the sperm flagella. Pharmgenomics Pers Med. 2022;15:341–50.
121. Joo K, Kim CG, Lee MS, Moon HY, Lee SH, Kim MJ, Kweon HS, Park WY, Kim CH, Gleeson JG, et al. CCDC41 is required for ciliary vesicle docking to the mother centriole. Proc Natl Acad Sci U S A. 2013;15:5987–92.
122. Tapia Contreras C, Hoyer-Fender S. CCDC42 Localizes to Manchette, HTCA and tail and interacts with ODF1 and ODF2 in the formation of the male germ cell cytoskeleton. Front Cell Dev Biol. 2019;7:151.
123. Jreijiri F, Cavarocchi E, Amiri-Yekta A, Cazin C, Hosseini SH, El Khouri E, Patrat C, Thierry-Mieg N, Ray PF, Dulioust E, et al. CCDC65, encoding a component of the axonemal Nexin-Dynein regulatory complex, is required for sperm flagellum structure in humans. Clin Genet. 2024;3:317–22.
124. Wang Tongtong, Yin Qianqian, Ma Xuehao, Tong Ming-Han, Zhou Yuchuan. Ccdc87 is critical for sperm function and male fertility†. Biol Reprod. 2018;99(4):817.
125. Sha Y, Wang X, Yuan J, Zhu X, Su Z, Zhang X, Xu X, Wei X. Loss-of-function mutations in centrosomal protein 112 is associated with human acephalic spermatozoa phenotype. Clin Genet. 2020;2:321–8.
126. González-Martínez J, Cwetsch AW, Martínez-Alonso D, López-Sainz LR, Almagro J, Melati A, Gómez J, Pérez-Martínez M, Megías D, Boskovic J, et al. Deficient adaptation to centrosome duplication defects in neural progenitors causes microcephaly and subcortical heterotopias. JCI Insight. 2021;6(16):e146364.
127. Čajánek L, Nigg EA. Cep164 triggers ciliogenesis by recruiting Tau tubulin kinase 2 to the mother centriole. Proc Natl Acad Sci U S A. 2014;28:E2841-2850.
128. Guan C, Hua S, Jiang K. The CEP170B-KIF2A complex destabilizes microtubule minus ends to generate polarized microtubule network. EMBO J. 2023;11:e112953.
129. Floriot S, Bellutti L, Castille J, Moison P, Messiaen S, Passet B, Boulanger L, Boukadiri A, Tourpin S, Beauvallet C, et al. CEP250 is required for maintaining centrosome cohesion in the germline and fertility in male mice. Front Cell Dev Biol. 2021;9:754054.
130. Prassanawar SS, Sarkar T, Panda D. CEP41, a ciliopathy-linked centrosomal protein, regulates microtubule assembly and cell proliferation. J Cell Sci. 2024;137(13):jcs261927.
131. Liu Q, Guo Q, Guo W, Song S, Wang N, Chen X, Sun A, Yan L, Qiao J. Loss of CEP70 function affects acrosome biogenesis and flagella formation during spermiogenesis. Cell Death Dis. 2021;12(5):478.
132. Auguste Y, Delague V, Desvignes JP, Longepied G, Gnisci A, Besnier P, Levy N, Beroud C, Megarbane A, Metzler-Guillemain C, et al. Loss of Calmodulin- and Radial-Spoke-Associated Complex Protein CFAP251 Leads to Immotile Spermatozoa Lacking Mitochondria and Infertility in Men. Am J Hum Genet. 2018;3:413–20.
133. Tang S, Wang X, Li W, Yang X, Li Z, Liu W, Li C, Zhu Z, Wang L, Wang J, et al. Biallelic Mutations in CFAP43 and CFAP44 Cause Male Infertility with Multiple Morphological Abnormalities of the Sperm Flagella. Am J Hum Genet. 2017;6:854–64.
134. Wei X, Wang X, Yang C, Gao Y, Zhang Y, Xiao Y, Ju Z, Jiang Q, Wang J, Liu W, et al. CFAP58 is involved in the sperm head shaping and flagellogenesis of cattle and mice. Development. 2024;151(7):dev202608.

135. Liu C, Tu C, Wang L, Wu H, Houston BJ, Mastrorosa FK, Zhang W, Shen Y, Wang J, Tian S, et al. Deleterious variants in X-linked CFAP47 induce asthenoteratozoospermia and primary male infertility. Am J Hum Genet. 2021;2:309–23.
136. Jin HJ, Ruan T, Dai S, Geng XY, Yang Y, Shen Y, Chen SR. Identification of CFAP52 as a novel diagnostic target of male infertility with defects of sperm head-tail connection and flagella development. Elife. 2023;12:RP92769.
137. Wu B, Yu X, Liu C, Wang L, Huang T, Lu G, Chen ZJ, Li W, Liu H. Essential role of CFAP53 in sperm flagellum biogenesis. Front Cell Dev Biol. 2021;9:676910.
138. Huang T, Yin Y, Liu C, Li M, Yu X, Wang X, Zhang H, Muhammad T, Gao F, Li W, et al. Absence of murine CFAP61 causes male infertility due to multiple morphological abnormalities of the flagella. Sci Bull (Beijing). 2020;10:854–64.
139. Zhang X, Shen Y, Wang X, Yuan G, Zhang C, Yang Y. A novel homozygous CFAP65 mutation in humans causes male infertility with multiple morphological abnormalities of the sperm flagella. Clin Genet. 2019;6:541–8.
140. Wang W, Tian S, Nie H, Tu C, Liu C, Li Y, Li D, Yang X, Meng L, Hu T, et al. CFAP65 is required in the acrosome biogenesis and mitochondrial sheath assembly during spermiogenesis. Hum Mol Genet. 2021;23:2240–54.
141. Li W, Wu H, Li F, Tian S, Kherraf ZE, Zhang J, Ni X, Lv M, Liu C, Tan Q, et al. Biallelic mutations in CFAP65 cause male infertility with multiple morphological abnormalities of the sperm flagella in humans and mice. J Med Genet. 2020;2:89–95.
142. Dong FN, Amiri-Yekta A, Martinez G, Saut A, Tek J, Stouvenel L, Lorès P, Karaouzène T, Thierry-Mieg N, Satre V, et al. Absence of CFAP69 Causes Male Infertility due to Multiple Morphological Abnormalities of the Flagella in Human and Mouse. Am J Hum Genet. 2018;4:636–48.
143. Jin HJ, Wang JL, Geng XY, Wang CY, Wang BB, Chen SR. CFAP70 is a solid and valuable target for the genetic diagnosis of oligo-astheno-teratozoospermia in infertile men. EBioMedicine. 2023;93:104675.
144. Coquelle FM, Caspi M, Cordelières FP, Dompierre JP, Dujardin DL, Koifman C, Martin P, Hoogenraad CC, Akhmanova A, Galjart N, et al. LIS1, CLIP-170's key to the dynein/dynactin pathway. Mol Cell Biol. 2002;9:3089–102.
145. Akhmanova A, Mausset-Bonnefont AL, van Cappellen W, Keijzer N, Hoogenraad CC, Stepanova T, Drabek K, van der Wees J, Mommaas M, Onderwater J, et al. The microtubule plus-end-tracking protein CLIP-170 associates with the spermatid manchette and is essential for spermatogenesis. Genes Dev. 2005;20:2501–15.
146. Okitsu Y, Nagano M, Yamagata T, Ito C, Toshimori K, Dohra H, Fujii W, Yogo K. Dlec1 is required for spermatogenesis and male fertility in mice. Sci Rep. 2020;1:18883.
147. Yu W, An M, Xu Y, Gao Q, Lu M, Li Y, Zhang L, Wang H, Xu Z. Mutational landscape of DNAH1 in Chinese patients with multiple morphological abnormalities of the sperm flagella: cohort study and literature review. J Assist Reprod Genet. 2021;8:2031–8.
148. Dacheux D, Martinez G, Broster Reix CE, Beurois J, Lores P, Tounkara M, Dupuy JW, Robinson DR, Loeuillet C, Lambert E, et al. Novel axonemal protein ZMYND12 interacts with TTC29 and DNAH1, and is required for male fertility and flagellum function. Elife. 2023;12:RP87698.
149. Tu C, Cong J, Zhang Q, He X, Zheng R, Yang X, Gao Y, Wu H, Lv M, Gu Y, et al. Bi-allelic mutations of DNAH10 cause primary male infertility with asthenoteratozoospermia in humans and mice. Am J Hum Genet. 2021;8:1466–77.
150. Zhu D, Zhang H, Wang R, Liu X, Jiang Y, Feng T, Liu R, Zhang G. Association of DNAH11 gene polymorphisms with asthenozoospermia in Northeast Chinese patients. Biosci Rep. 2019;39(6):BSR20181450.
151. Guo S, Tang D, Chen Y, Yu H, Gu M, Geng H, Fang J, Wu B, Ruan L, Li K, et al. Association of novel DNAH11 variants with asthenoteratozoospermia lead to male infertility. Hum Genomics. 2024;1:97.
152. Loges NT, Antony D, Maver A, Deardorff MA, Güleç EY, Gezdirici A, Nöthe-Menchen T, Höben IM, Jelten L, Frank D, et al. Recessive DNAH9 loss-of-function mutations cause laterality defects and subtle respiratory ciliary-beating defects. Am J Hum Genet. 2018;6:995–1008.
153. Geng H, Wang K, Liang D, Ni X, Yu H, Tang D, Lv M, Wu H, Li K, Shen Q, et al. Further evidence from DNAH12 supports favorable fertility outcomes of infertile males with dynein axonemal heavy chain gene family variants. iScience. 2024;7:110366.
154. Whitfield M, Thomas L, Bequignon E, Schmitt A, Stouvenel L, Montantin G, Tissier S, Duquesnoy P, Copin B, Chantot S, et al. Mutations in DNAH17, Encoding a Sperm-Specific Axonemal Outer Dynein Arm Heavy Chain, Cause Isolated Male Infertility Due to Asthenozoospermia. Am J Hum Genet. 2019;1:198–212.
155. Hwang JY, Nawaz S, Choi J, Wang H, Hussain S, Nawaz M, Lopez-Giraldez F, Jeong K, Dong W, Oh JN, et al. Genetic Defects in DNAH2 Underlie Male Infertility With Multiple Morphological Abnormalities of the Sperm Flagella in Humans and Mice. Front Cell Dev Biol. 2021;9:662903.
156. Navapour L, Mogharrab N, Parvin A, Rezaei Arablouydareh S, Movahedpour A, Jebraeily M, Taheri-Anganeh M, Ghasemnejad-Berenji H. Identification of high-risk non-synonymous SNPs (nsSNPs) in DNAH1 and DNAH17 genes associated with male infertility: a bioinformatics analysis. J Appl Genet. 2025;2:333–346.
157. Wang X, Shen G, Yang Y, Jiang C, Ruan T, Yang X, Zhuo L, Zhang Y, Ou Y, Zhao X, et al. DNAH3 deficiency causes flagellar inner dynein arm loss and male infertility in humans and mice. Elife. 2024;13:RP96755.
158. Fliegauf M, Olbrich H, Horvath J, Wildhaber JH, Zariwala MA, Kennedy M, Knowles MR, Omran H. Mislocalization of DNAH5 and DNAH9 in respiratory cells from patients with primary ciliary dyskinesia. Am J Respir Crit Care Med. 2005;12:1343–9.
159. Tu C, Nie H, Meng L, Yuan S, He W, Luo A, Li H, Li W, Du J, Lu G, et al. Identification of DNAH6 mutations in infertile men with multiple morphological abnormalities of the sperm flagella. Sci Rep. 2019;1:15864.
160. Gao Y, Liu L, Shen Q, Fu F, Xu C, Geng H, Lv M, Li K, Tang D, Song B, et al. Loss of function mutation in DNAH7 induces male infertility associated with abnormalities of the sperm flagella and mitochondria in human. Clin Genet. 2022;2:130–5.
161. Liu C, Miyata H, Gao Y, Sha Y, Tang S, Xu Z, Whitfield M, Patrat C, Wu H, Dulioust E, et al. Bi-allelic DNAH8 Variants Lead to Multiple Morphological Abnormalities of the Sperm Flagella and Primary Male Infertility. Am J Hum Genet. 2020;2:330–41.
162. Tang D, Sha Y, Gao Y, Zhang J, Cheng H, Zhang J, Ni X, Wang C, Xu C, Geng H, et al. Novel variants in DNAH9 lead to nonsyndromic severe asthenozoospermia. Reprod Biol Endocrinol. 2021;1:27.
163. Yan F, Zhi W, Wei Y, Dai L, Xu W, Zheng R. Novel variants in DNAH9 are present in two infertile patients with severe asthenospermia. J Hum Genet. 2025;2:105–11.
164. Zhang J, He X, Wu H, Zhang X, Yang S, Liu C, Liu S, Hua R, Zhou S, Zhao S, et al. Loss of DRC1 function leads to multiple morphological abnormalities of the sperm flagella and male infertility in human and mouse. Hum Mol Genet. 2021;21:1996–2011.
165. Zheng C, Ouyang YC, Jiang B, Lin X, Chen J, Dong MZ, Zhuang X, Yuan S, Sun QY, Han C. Non-canonical RNA polyadenylation polymerase FAM46C is essential for fastening sperm head and flagellum in mice†. Biol Reprod. 2019;6:1673–85.
166. Liu L, He Y, Guo K, Zhou L, Li X, Tseng M, Cai L, Lan ZJ, Zhou J, Wang H, et al. Ggnbp2-Null mutation in mice leads to male infertility due to a defect at the spermiogenesis stage. Am J Pathol. 2017;11:2508–19.
167. Zhao QG, Lu BS, Huang PT. Functions of FANCL in primordial germ cell formation and Fanconi anemia. Yi Chuan Xue Bao. 2005;9:993–1000.
168. Guo K, He Y, Liu L, Liang Z, Li X, Cai L, Lan ZJ, Zhou J, Wang H, Lei Z. Ablation of Ggnbp2 impairs meiotic DNA double-strand break repair during spermatogenesis in mice. J Cell Mol Med. 2018;10:4863–74.
169. Bizkarguenaga M, Gomez-Santos L, Madrid JF, Sáez FJ, Alonso E. Increase of germ cell nuclear factor expression in globozoospermic Gopc(-/-) knockout mice. Andrology. 2019;3:319–28.
170. Ito C, Akutsu H, Yao R, Kyono K, Suzuki-Toyota F, Toyama Y, Maekawa M, Noda T, Toshimori K. Oocyte activation ability correlates with head flatness and presence of perinuclear theca substance in human and mouse sperm. Hum Reprod. 2009;10:2588–95.
171. Crapster JA, Rack PG, Hellmann ZJ, Le AD, Adams CM, Leib RD, Elias JE, Perrino J, Behr B, Li Y, et al. HIPK4 is essential for murine spermiogenesis. Elife. 2020;9:e50209.
172. Szebenyi G, Hall B, Yu R, Hashim AI, Krämer H. Hook2 localizes to the centrosome, binds directly to centriolin/CEP110 and contributes to centrosomal function. Traffic. 2007;1:32–46.

173. Pallesi-Pocachard E, Bazellieres E, Viallat-Lieutaud A, Delgrossi MH, Barthelemy-Requin M, Le Bivic A, Massey-Harroche D. Hook2, a microtubule-binding protein, interacts with Par6α and controls centrosome orientation during polarized cell migration. Sci Rep. 2016;6:33259.
174. Walenta JH, Didier AJ, Liu X, Krämer H. The Golgi-associated hook3 protein is a member of a novel family of microtubule-binding proteins. J Cell Biol. 2001;5:923–34.
175. Zhang Y, Liu H, Li W, Zhang Z, Zhang S, Teves ME, Stevens C, Foster JA, Campbell GE, Windle JJ, et al. Intraflagellar transporter protein 140 (IFT140), a component of IFT-A complex, is essential for male fertility and spermiogenesis in mice. Cytoskeleton. 2018;2:70–84.
176. Wang X, Sha YW, Wang WT, Cui YQ, Chen J, Yan W, Hou XT, Mei LB, Cui-cui Y, Wang J. Novel IFT140 variants cause spermatogenic dysfunction in humans. Mol Genet Genomic Med. 2019;7(9):e920.
177. Ni X, Wang J, Lv M, Liu C, Zhong Y, Tian S, Wu H, Cheng H, Gao Y, Tan Q, et al. A novel homozygous mutation in WDR19 induces disorganization of microtubules in sperm flagella and nonsyndromic asthenoteratospermia. J Assist Reprod Genet. 2020;6:1431–9.
178. Zhang S, Liu Y, Huang Q, Yuan S, Liu H, Shi L, Yap YT, Li W, Zhen J, Zhang L, et al. Murine germ cell-specific disruption of Ift172 causes defects in spermiogenesis and male fertility. Reproduction. 2020;4:409–21.
179. Zhang Z, Li W, Zhang Y, Zhang L, Teves ME, Liu H, Strauss JF 3rd, Pazour GJ, Foster JA, Hess RA, et al. Intraflagellar transport protein IFT20 is essential for male fertility and spermiogenesis in mice. Mol Biol Cell. 2016;23:3705–16.
180. Susan K, Dutcher, John A, Follit, San Agustin JT, Xu F, Julie A, Jonassen, Rajeev Samtani, Lo CW, Pazour GJ. The Golgin GMAP210/TRIP11 Anchors IFT20 to the Golgi Complex. PLoS Genet. 2008;12:e1000315.
181. Zhang L, Zhen J, Huang Q, Liu H, Li W, Zhang S, Min J, Li Y, Shi L, Woods J, et al. Mouse spermatogenesis-associated protein 1 (SPATA1), an IFT20 binding partner, is an acrosomal protein. Dev Dyn. 2020;4:543–55.
182. Monis WJ, Faundez V, Pazour GJ. BLOC-1 is required for selective membrane protein trafficking from endosomes to primary cilia. J Cell Biol. 2017;7:2131–50.
183. Li RK, Tan JL, Chen LT, Feng JS, Liang WX, Guo XJ, Liu P, Chen Z, Sha JH, Wang YF, et al. Iqcg is essential for sperm flagellum formation in mice. PLoS One. 2014;5:e98053.
184. Zhu Y, Fan LQ, Zhou D, Zhang P, Xu F. Expression of IQCG in the human testis and its correlation with asthenospermia. Zhonghua Nan Ke Xue. 2018;4:304–10.
185. Dai J, Li Q, Zhou Q, Zhang S, Chen J, Wang Y, Guo J, Gu Y, Gong F, Tan Y, et al. IQCN disruption causes fertilization failure and male infertility due to manchette assembly defect. EMBO Mol Med. 2022;12:e16501.
186. Li Q, Wang Y, Zheng W, Guo J, Zhang S, Gong F, Lu GX, Lin G, Dai J. Biallelic variants in IQCN cause sperm flagellar assembly defects and male infertility. Hum Reprod. 2023;7:1390–8.
187. Wang Y, Chen G, Tang Z, Mei X, Lin C, Kang J, Lian J, Lu J, Liu Y, Lan F, et al. Loss-of-function mutations in IQCN cause male infertility in humans and mice owing to total fertilization failure. Mol Hum Reprod. 2023;29(7):018.
188. Wei X, Liu W, Zhu X, Li Y, Zhang X, Chen J, Isachenko V, Sha Y, Lu Z. Biallelic mutations in KATNAL2 cause male infertility due to oligo-asthenoteratozoospermia. Clin Genet. 2021;4:376–85.
189. Kasioulis I, Syred HM, Tate P, Finch A, Shaw J, Seawright A, Fuszard M, Botting CH, Shirran S, Adams IR, et al. Kdm3a lysine demethylase is an Hsp90 client required for cytoskeletal rearrangements during spermatogenesis. Mol Biol Cell. 2014;8:1216–33.
190. Hojati Z, Soleimanpour E, Javadirad SM, Nasr-Esfahani MH. Identification of Two Novel Mutations in KDM3A Regulatory Gene in Iranian Infertile Males. Iran Biomed J. 2019;3:220–7.
191. Okada Y, Scott G, Ray MK, Mishina Y, Zhang Y. Histone demethylase JHDM2A is critical for Tnp1 and Prm1 transcription and spermatogenesis. Nature. 2007;7166:119–23.
192. Liu WS, Lu C, Mistry BV. Subcellular localization of the mouse PRAMEL1 and PRAMEX1 reveals multifaceted roles in the nucleus and cytoplasm of germ cells during spermatogenesis. Cell Biosci. 2021;1:102.
193. Lehti MS, Kotaja N, Sironen A. KIF3A is essential for sperm tail formation and manchette function. Mol Cell Endocrinol. 2013;1–2:44–55.
194. Heydari R, Seresht-Ahmadi M, Mirshahvaladi S, Sabbaghian M, Mohseni-Meybodi A. KIF3B gene silent variant leading to sperm morphology and motility defects and male infertility†. Biol Reprod. 2022;4:766–74.
195. Navolanic PM, Sperry AO. Identification of isoforms of a mitotic motor in mammalian spermatogenesis. Biol Reprod. 2000;5:1360–9.
196. Jin M, Yamada M, Arai Y, Nagai T, Hirotsune S. Arl3 and LC8 regulate dissociation of dynactin from dynein. Nat Commun. 2014;5:5295.
197. Nayernia K, Vauti F, Meinhardt A, Cadenas C, Schweyer S, Meyer BI, Schwandt I, Chowdhury K, Engel W, Arnold HH. Inactivation of a testis-specific Lis1 transcript in mice prevents spermatid differentiation and causes male infertility. J Biol Chem. 2003;48:48377–85.
198. Drusenheimer N, Nayernia K, Meinhardt A, Jung B, Arnold HH, Engel W. Overexpression of Lis1 in different stages of spermatogenesis does not result in an aberrant phenotype. Cytogenet Genome Res. 2011;4:269–82.
199. Hwang JY, Chai P, Nawaz S, Choi J, Lopez-Giraldez F, Hussain S, Bilguvar K, Mane S, Lifton RP, Ahmad W, et al. LRRC23 truncation impairs radial spoke 3 head assembly and sperm motility underlying male infertility. Elife. 2023;12:RP90095.
200. Qin J, Wang J, Chen J, Xu J, Liu S, Deng D, Li F. Homozygous variant in DRC3 (LRRC48) gene causes asthenozoospermia and male infertility. J Hum Genet. 2024;8:401–9.
201. Eggers S, DeBoer KD, van den Bergen J, Gordon L, White SJ, Jamsai D, McLachlan RI, Sinclair AH, O'Bryan MK. Copy number variation associated with meiotic arrest in idiopathic male infertility. Fertil Steril. 2015;1:214–9.
202. Bonnefoy S, Watson CM, Kernohan KD, Lemos M, Hutchinson S, Poulter JA, Crinnion LA, Berry I, Simmonds J, Vasudevan P, et al. Biallelic mutations in LRRC56, encoding a protein associated with intraflagellar transport, cause mucociliary clearance and laterality defects. Am J Hum Genet. 2018;5:727–39.
203. Bonnefoy S, Alves AA, Bertiaux E, Bastin P. LRRC56 is an IFT cargo required for assembly of the distal dynein docking complex in Trypanosoma brucei. Mol Biol Cell. 2024;35(8):ar106.
204. Shi S, Tang X, Long S, Yang J, Wang T, Wang H, Hu T, Shi J, Huang G, Qiao S, et al. A novel homozygous LRRC6 mutation causes male infertility with asthenozoospermia and primary ciliary dyskinesia in humans. Androl. 2025;3:459–72.
205. Kikas T, Punab AM, Kasak L, Poolamets O, Vihljajev V, Pomm K, Reiman M, Tjagur S, Korrovits P, Punab M, et al. Microdeletions and microduplications linked to severe congenital disorders in infertile men. Sci Rep. 2023;1:574.
206. Bao J, Perez CJ, Kim J, Zhang H, Murphy CJ, Hamidi T, Jaubert J, Platt CD, Chou J, Deng M, et al. Deficient LRRC8A-dependent volume-regulated anion channel activity is associated with male infertility in mice. JCI Insight. 2018;3(16):e99767.
207. Li W, Huang Q, Zhang L, Liu H, Zhang D, Yuan S, Yap Y, Qu W, Shiang R, Song S, et al. A single amino acid mutation in the mouse MEIG1 protein disrupts a cargo transport system necessary for sperm formation. J Biol Chem. 2021;5:101312.
208. Hospital V, Chesneau V, Balogh A, Joulie C, Seidah NG, Cohen P, Prat A. N-arginine dibasic convertase (nardilysin) isoforms are soluble dibasic-specific metalloendopeptidases that localize in the cytoplasm and at the cell surface. Biochem J. 2000;349(Pt 2):587–97.
209. Ali MAD, Longepied G, Lévy N, Metzler-Guillemain C, Mitchell M. Nucleoporin NUP210L and BAF-paralogue BAF-L together ensure microtubule organization and nuclear integrity in spermatids. J Clin Anat. 2025;1. https://doi.org/10.31579/2834-5134/067.
210. Zhang Z, Shen X, Gude DR, Wilkinson BM, Justice MJ, Flickinger CJ, Herr JC, Eddy EM, Strauss JF 3rd. MEIG1 is essential for spermiogenesis in mice. Proc Natl Acad Sci U S A. 2009;40:17055–60.
211. Umer N, Arévalo L, Phadke S, Lohanadan K, Kirfel G, Sons D, Sofia D, Witke W, Schorle H. Loss of Profilin3 impairs spermiogenesis by affecting acrosome biogenesis, autophagy, manchette development and mitochondrial organization. Front Cell Dev Biol. 2021;9:749559.

212. Umer N, Phadke S, Shakeri F, Arévalo L, Lohanadan K, Kirfel G, Sylvester M, Buness A, Schorle H. PFN4 is required for manchette development and acrosome biogenesis during mouse spermiogenesis. Development. 2022;149(16):dev200499.
213. Onoufriadis A, Shoemark A, Schmidts M, Patel M, Jimenez G, Liu H, Thomas B, Dixon M, Hirst RA, Rutman A, et al. Targeted NGS gene panel identifies mutations in RSPH1 causing primary ciliary dyskinesia and a common mechanism for ciliary central pair agenesis due to radial spoke defects. Hum Mol Genet. 2014;13:3362–74.
214. Jeanson L, Copin B, Papon JF, Dastot-Le Moal F, Duquesnoy P, Montantin G, Cadranel J, Corvol H, Coste A, Désir J, et al. RSPH3 mutations cause primary ciliary dyskinesia with central-complex defects and a near absence of radial spokes. Am J Hum Genet. 2015;97(1):153–62.
215. Castleman VH, Romio L, Chodhari R, Hirst RA, de Castro SC, Parker KA, Ybot-Gonzalez P, Emes RD, Wilson SW, Wallis C, et al. Mutations in radial spoke head protein genes RSPH9 and RSPH4A cause primary ciliary dyskinesia with central-microtubular-pair abnormalities. Am J Hum Genet. 2009;2:197–209.
216. Keppner A, Correia M, Santambrogio S, Koay TW, Maric D, Osterhof C, Winter DV, Clerc A, Stumpe M, Chalmel F, et al. Androglobin, a chimeric mammalian globin, is required for male fertility. Elife. 2022;11:e72374.
217. Xu X, Sha YW, Mei LB, Ji ZY, Qiu PP, Ji H, Li P, Wang T, Li L. A familial study of twins with severe asthenozoospermia identified a homozygous SPAG17 mutation by whole-exome sequencing. Clin Genet. 2018;2:345–9.
218. Liu Y, Zhang L, Li W, Huang Q, Yuan S, Li Y, Liu J, Zhang S, Pin G, Song S, et al. The sperm-associated antigen 6 interactome and its role in spermatogenesis. Reproduction. 2019;2:181–97.
219. Li YZ, Li N, Liu WS, Sha YW, Wu RF, Tang YL, Zhu XS, Wei XL, Zhang XY, Wang YF, et al. Biallelic mutations in spermatogenesis and centriole-associated 1 like (SPATC1L) cause acephalic spermatozoa syndrome and male infertility. Asian J Androl. 2022;1:67–72.
220. Bao J, Zhang J, Zheng H, Xu C, Yan W. UBQLN1 interacts with SPEM1 and participates in spermiogenesis. Mol Cell Endocrinol. 2010;1–2:89–97.
221. Bao J, Wu Q, Song R, Jie Z, Zheng H, Xu C, Yan W. RANBP17 is localized to the XY body of spermatocytes and interacts with SPEM1 on the manchette of elongating spermatids. Mol Cell Endocrinol. 2011;2:134–42.
222. Sethi S, Mehta P, Andrabi W, Mitra K, Rajender S. SPEM1 gene mutation in a case with sperm morphological defects leading to male infertility. Reprod Sci. 2024;10:3102–11.
223. Liu M, Ru Y, Gu Y, Tang J, Zhang T, Wu J, Yu F, Yuan Y, Xu C, Wang J, et al. Disruption of Ssp411 causes impaired sperm head formation and male sterility in mice. Biochim Biophys Acta Gen Subj. 2018;3:660–8.
224. Shi HJ, Wu AZ, Santos M, Feng ZM, Huang L, Chen YM, Zhu K, Chen CL. Cloning and characterization of rat spermatid protein SSP411: a thioredoxin-like protein. J Androl. 2004;4:479–93.
225. Ma H, Zhang B, Khan A, Zhao D, Ma A, Zhou J, Khan I, Khan K, Zhang H, Zhang Y, et al. Novel frameshift mutation in STK33 is associated with asthenozoospermia and multiple morphological abnormalities of the flagella. Hum Mol Genet. 2021;21:1977–84.
226. Yu W, Li Y, Chen H, Cui Y, Situ C, Yao L, Zhang X, Lu S, Liu L, Li L, et al. STK33 phosphorylates fibrous sheath protein AKAP3/4 to regulate sperm flagella assembly in spermiogenesis. Mol Cell Proteomics. 2023;6:100564.
227. Martins LR, Bung RK, Koch S, Richter K, Schwarzmüller L, Terhardt D, Kurtulmus B, Niehrs C, Rouhi A, Lohmann I, et al. Stk33 is required for spermatid differentiation and male fertility in mice. Dev Biol. 2018;1:84–93.
228. Yang K, Adham IM, Meinhardt A, Hoyer-Fender S. Ultra-structure of the sperm head-to-tail linkage complex in the absence of the spermatid-specific LINC component SPAG4. Histochem Cell Biol. 2018;1:49–59.
229. Ye Y, Wei X, Sha Y, Li N, Yan X, Cheng L, Qiao D, Zhou W, Wu R, Liu Q, et al. Loss-of-function mutation in TSGA10 causes acephalic spermatozoa phenotype in human. Mol Genet Genomic Med. 2020;7:e1284.
230. Luo G, Hou M, Wang B, Liu Z, Liu W, Han T, Zhang D, Zhou X, Jia W, Tan Y, et al. Tsga10 is essential for arrangement of mitochondrial sheath and male fertility in mice. Andrology. 2021;1:368–75.
231. Liu W, He X, Yang S, Zouari R, Wang J, Wu H, Kherraf ZE, Liu C, Coutton C, Zhao R, et al. Bi-allelic Mutations in TTC21A Induce Asthenoteratospermia in Humans and Mice. Am J Hum Genet. 2019;4:738–48.
232. Liu C, He X, Liu W, Yang S, Wang L, Li W, Wu H, Tang S, Ni X, Wang J, et al. Bi-allelic Mutations in TTC29 Cause Male Subfertility with Asthenoteratospermia in Humans and Mice. Am J Hum Genet. 2019;6:1168–81.
233. Dodd DO, Mechaussier S, Yeyati PL, McPhie F, Anderson JR, Khoo CJ, Shoemark A, Gupta DK, Attard T, Zariwala MA, et al. Ciliopathy patient variants reveal organelle-specific functions for TUBB4B in axonemal microtubules. Science. 2024;6694:eadf5489.
234. Sanzhaeva U, Wonsettler NR, Rhodes SB, Ramamurthy V. TUBB4B is essential for the expansion of differentiating spermatogonia. Sci Rep. 2024;1:20889.
235. Huang I, Emery BR, Christensen GL, Griffin J, Peterson CM, Carrell DT. Novel UBE2B-associated polymorphisms in an azoospermic/oligozoospermic population. Asian J Androl. 2008;3:461–6.
236. An JY, Kim EA, Jiang Y, Zakrzewska A, Kim DE, Lee MJ, Mook-Jung I, Zhang Y, Kwon YT. UBR2 mediates transcriptional silencing during spermatogenesis via histone ubiquitination. Proc Natl Acad Sci U S A. 2010;5:1912–7.
237. Zhang Q, Mou LS, Gui YT, Cai ZM. UBE2B gene and male infertility: an update. Zhonghua Nan Ke Xue. 2014;4:367–71.
238. Zhang Y, Xing X, Huang L, Su Y, Liu G, Zhang X, Yang Y. WDR64, a testis-specific protein, is involved in the manchette and flagellum formation by interacting with ODF1. Heliyon. 2024:19:e38263.
239. Mendoza-Lujambio I, Burfeind P, Dixkens C, Meinhardt A, Hoyer-Fender S, Engel W, Neesen J. The Hook1 gene is non-functional in the abnormal spermatozoon head shape (azh) mutant mouse. Hum Mol Genet. 2002;14:1647–58.
240. Dwivedi D, Kumari A, Rathi S, Mylavarapu SVS, Sharma M. The dynein adaptor Hook2 plays essential roles in mitotic progression and cytokinesis. J Cell Biol. 2019;3:871–94.
241. Sinha D, Kalimutho M, Bowles J, Chan AL, Merriner DJ, Bain AL, Simmons JL, Freire R, Lopez JA, Hobbs RM, et al. Cep55 overexpression causes male-specific sterility in mice by suppressing Foxo1 nuclear retention through sustained activation of PI3K/Akt signaling. Faseb j. 2018;9:4984–99.
242. Avasthi P, Scheel JF, Ying G, Frederick JM, Baehr W, Wolfrum U. Germline deletion of Cetn1 causes infertility in male mice. J Cell Sci. 2013;126(Pt 14):3204.
243. Chen J, Wang Y, Wu B, Shi H, Wang L. Experimental and molecular support for Cfap70 as a causative gene of "multiple morphological abnormalities of the flagella" with male infertility†. Biol Reprod. 2023;4:450–60.
244. Liao HQ, Guo ZY, Huang LH, Liu G, Lu JF, Zhang YF, Xing XW. WDR87 interacts with CFAP47 protein in the middle piece of spermatozoa flagella to participate in sperm tail assembly. Mol Hum Reprod. 2022;29(1):gaac042.
245. Tapia Contreras C, Hoyer-Fender S. The WD40-protein CFAP52/WDR16 is a centrosome/basal body protein and localizes to the manchette and the flagellum in male germ cells. Sci Rep. 2020;1:14240.
246. Horváth J, Fliegauf M, Olbrich H, Kispert A, King SM, Mitchison H, Zariwala MA, Knowles MR, Sudbrak R, Fekete G, et al. Identification and analysis of axonemal dynein light chain 1 in primary ciliary dyskinesia patients. Am J Respir Cell Mol Biol. 2005;1:41–7.
247. Lai TH, Wu YY, Wang YY, Chen MF, Wang P, Chen TM, Wu YN, Chiang HS, Kuo PL, Lin YH. SEPT12-NDC1 Complexes Are Required for Mammalian Spermiogenesis. Int J Mol Sci. 2016;11:e72374.
248. Pasch E, Link J, Beck C, Scheuerle S, Alsheimer M. The LINC complex component Sun4 plays a crucial role in sperm head formation and fertility. Biol Open. 2015;12:1792–802.
249. Li W, Walavalkar NM, Buchwald WA, Teves ME, Zhang L, Liu H, Bilinovich S, Peterson DL, Strauss JF 3rd, Williams DC Jr, et al. Dissecting the structural basis of MEIG1 interaction with PACRG. Sci Rep. 2016;6:18278.
250. Ma Y, Wu B, Chen Y, Ma S, Wang L, Han T, Lin X, Yang F, Liu C, Zhao J, et al. CCDC146 is required for sperm flagellum biogenesis and male fertility in mice. Cell Mol Life Sci. 2023;1:1.
251. Chesneau V, Prat A, Segretain D, Hospital V, Dupaix A, Foulon T, Jégou B, Cohen P. NRD convertase: a putative processing endoprotease

associated with the axoneme and the manchette in late spermatids. J Cell Sci. 1996;109:2737–45.
252. Ma Q, Cao C, Zhuang C, Luo X, Li X, Wan H, Ye J, Chen F, Cui L, Zhang Y, et al. AXDND1, a novel testis-enriched gene, is required for spermiogenesis and male fertility. Cell Death Discov. 2021;1:348.
253. Merges GE, Arévalo L, Kovacevic A, Lohanadan K, de Rooij DG, Simon C, Jokwitz M, Witke W, Schorle H. Actl7b deficiency leads to mislocalization of LC8 type dynein light chains and disruption of murine spermatogenesis. Development. 2023;150(21):dev201593.
254. Janke C, Magiera MM. The tubulin code and its role in controlling microtubule properties and functions. Nat Rev Mol Cell Biol. 2020;6:307–26.

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