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# Expression of katanin p80 in human spermatogenesis

Christiane Pleuger, M.Sc.,[a] Daniela Fietz, D.V.M.,[a] Katja Hartmann, D.V.M.,[a] Wolfgang Weidner, M.D.,[b] Sabine Kliesch, M.D.,[c] Moira K. O'Bryan, Ph.D.,[d] Adriaan Dorresteijn, Ph.D.,[e] and Martin Bergmann, Ph.D.[a]

[a] Institute for Veterinary, Anatomy, Histology and Embryology, [b] Clinic for Urology, Pediatric Urology and Andrology, and [e] Institute for General Zoology and Developmental Biology, Justus Liebig University, Giessen, Germany; [c] Department of Clinical Andrology, Centre for Reproductive Medicine and Andrology, University Hospital Münster, Münster, Germany; and [d] The Development and Stems Cells program of Monash Biomedicine Discovery Institute and The Department of Anatomy and Developmental Biology, Monash University, Clayton, Victoria, Australia

**Objective:** To define the stage-by-stage expression of *KATNB1* during human spermatogenesis.
**Design:** Gene expression analysis, histologic and immunohistochemical evaluation.
**Setting:** University research laboratories and andrological clinic.
**Patient(s):** Eighty human testicular biopsy samples: 43 showing normal spermatogenesis, 9 with maturation arrest at level of spermatocytes, 8 with maturation arrest at level of spermatogonia, and 20 with a Sertoli cell only syndrome.
**Intervention(s):** None.
**Main Outcome Measure(s):** Evaluation of katanin p80 expression in normal as well as impaired spermatogenesis on mRNA (RT-PCR, RT-qPCR, and in situ hybridization) and protein level (immunohistochemistry/immunofluorescence).
**Result(s):** *KATNB1* messenger RNA is exclusively expressed in germ cells, and quantitatively reduced in maturation arrests at the level of spermatogonia. The KATNB1 protein was detected in type B spermatogonia entering meiosis and in the Golgi complex of pachytene spermatocytes. Immediately before the first meiotic division, it is colocalized with the cleaving centriole. It was also detected in early round spermatids in the dictyosome.
**Conclusion(s):** The expression and localization of KATNB1 support a role in spindle formation. The localization of KATNB1 in early round spermatids suggests an involvement in the formation of microtubule-based structures during spermiogenesis (manchette and flagellum). These data are consistent with the demonstrated role of KATNB1 in mouse meiosis, nuclear shaping, and flagellum formation of sperm and suggest the strong conservation of function even between distantly related species. (Fertil Steril® 2016;■: ■–■. ©2016 by American Society for Reproductive Medicine.)
**Key Words:** Katanin p80, microtubules, meiosis, spermatogenesis, spermiogenesis

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In male germ cell differentiation, rapid transformation of the microtubule scaffold is necessary to form the spindle apparatus during mitosis (spermatogonia) and meiosis (spermatocytes). In addition, the modification of microtubules is essential for the formation of the flagellum and the manchette, with the latter being a temporary microtubule-based structure that determines the sperm head shape and assists in the tail formation and elongation (1). Many defects of spermatogenesis are caused by a failure of microtubule dynamics (reviewed by O'Donnell and O'Bryan [2]). Our laboratory has recently identified a member of the microtubule severing proteins, the katanins, as a group of critical regulators of microtubule dynamics and male fertility in the mouse (1, 3).

The katanins are named after the Japanese samurai sword based on the demonstrated role in microtubule severing (4). The eponymous katanin complex consists of an enzymatic A-subunit (p60 subunit), encoded by *KATNA1*, and a regulatory B-subunit (p80 subunit), encoded by *KATNB1*. The severing function of katanin is carried out by the 60-kDa A subunit, a member of the AAA (ATPase Associated with diverse cellular Activities) protein family (5). After ATP binding at the carboxy-terminal domain, the p60 forms a 14–16 nm hexameric ring structure whereby the amino-terminal domains bind to the carboxy-terminal ends of tubulin subunits. The ATP hydrolysis initiates a conformational

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Reprint requests: Christiane Pleuger, M.Sc., Institute for Veterinary Anatomy, Histology and Embryology, Justus Liebig University, Frankfurter Strasse 98, 35392 Giessen, Germany (E-mail: christiane.pleuger@vetmed.uni-giessen.de).

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alteration that leads to destabilization and the subsequent severing of tubulin polymers (6). This process can be modulated by the regulatory p80 subunit. The KATNB1 protein consisting of an amino-terminal WD40 repeat domain, a central proline-rich domain, and a carboxy-terminal domain that interacts specifically with the amino-terminal domain of the enzymatic p60 subunit and targets it to the centrosome (5, 7). Depending on the cellular context, the p80 subunit enhances or inhibits the severing function of p60 (7, 8).

The whole katanin complex plays an important role for microtubule reorganization during cell division, in both the assembly and disassembly of the interphase network, before and during the spindle formation, indicated by specific interactions between the p80 subunit and the centrosomal proteins. A higher concentrated activity of katanin at spindle poles indicates an involvement in severing of microtubule minus ends from their attachment in the pericentriolar matrix (9–11). In addition, katanin is involved in the formation and differentiation of microtubule-based structures, like cilia, in various eukaryotic organisms (12–14). Orthologues of p60 and p80 were described in species of all kingdoms (reviewed by Roll-Mecak and McNally [15]).

Investigations of the katanin orthologues in *Chlamydomonas* (p80 orthologues PF15 [14]) and *Tetrahymena thermophila* (12) show a conserved role of the katanin complex during the assembly and disassembly of microtubule-based structures (cilia and flagella). Mutations in *Chlamydomonas* orthologues of katanin p80 reveal a defective formation of the central microtubule in the axoneme of the flagellum (14). In *Tetrahymena thermophila* the influence of katanin depends on the type and localization of microtubules. Whereas the severing complex negatively regulates nonciliary microtubules by reducing the polymer mass and destabilizing the internal network, the activity of katanin increases the mass of ciliary microtubules in the same cell (12). There was further evidence in trypanosomatids (*Leishmania major* and *Trypanosoma brucei*) for an influence of katanin in the regulation of flagellum length, which are consistent with the function in *Chlamydomonas* and *Tetrahymena* (13). These results indicate a conserved role of katanin in ciliogenesis (12–14).

Furthermore, investigations in *Caenorhabditis elegans* (16), *Drosophila melanogaster* (17), and *Xenopus laevis* (18) show an additional function of katanin during cell division and spindle formation. Katanin orthologous MEI-1 (p60) and MEI-2 (p80) in *C. elegans* regulates the spindle length during metaphase in oogenesis. The p80 orthologue MEI-2 ensures the targeting of MEI-1 to the spindle poles during meiosis (16). *X. laevis* katanin also regulates the microtubule modification to control the meiotic spindle length in oocytes (18). The katanin complex has a participating function in the process of "Pacman-mediated" shortening of the spindle microtubule in *D. melanogaster* during mitosis. Due to this shortening, chromosomes are being moved to the cell poles (17).

On the basis of the role of katanin during ciliogenesis and cell division in other organisms, we generated the *Taily* mouse line with a missense mutation in the highly conserved WD40 domain of the *Katnb1* gene, encoding the p80 subunit. Homozygous *Taily* males are infertile as a consequence of severely compromised meiosis, decreased sperm production, and the presence of sperm with abnormal head shape and missing progressive motility (1). This phenotype is comparable to the human oligoasthenoteratozoospermia (OAT, reviewed by Cooper [19]). The study showed an influence of katanin p80 to the formation of the meiotic spindle, especially during metaphase and anaphase. Furthermore, the formation of microtubule-based structures during spermiogenesis, notably the manchette and its role in nuclear shaping and the axoneme and its role in sperm motility (1). Subsequently, O'Donnell et al. (20) assessed patients with OAT for *KATNB1* variants applying direct sequencing analysis and immunofluorescence staining of the katanin p80 subunit. For this purpose, the whole protein encoding region, exon-intron-boundaries and 5′ and 3′ of the untranslated region in the *KATNB1* gene were sequenced in 100 men with OAT and 100 proven fertile men. The results revealed 37 *KATNB1* variants, whereof 10 variants were exclusively found in patients with OAT, but results did not show a significant correlation with the fertility status of the Australian men investigated (20). In the present study we aimed to analyze the *KATNB1* expression pattern in human testicular biopsies on messenger RNA (mRNA) and protein level.

## MATERIALS AND METHODS

### Testicular Tissue

Testis biopsies were collected, with consent, from patients attending the Centre of Reproductive Medicine and Andrology of the University Hospital in Münster and the Department of Urology and Andrology of the University Hospital in Giessen were indicated according to Bergmann and Kliesch (21) because of obstructive (re-fertilization procedure after vasectomy) and nonobstructive azoospermia. The current study has been approved by the ethical review committee of the Medical Faculty of the Justus-Liebig-University Giessen (decision 187b/09).

In total 80 human testicular biopsy specimens were used for this study. The testis tissue was immersed in Bouin fixative and embedded in paraffin. For histologic evaluation of spermatogenesis, 5-$\mu$m thick sections were stained with hematoxylin and eosin (H & E) and evaluated followed by score count analysis according to Bergmann and Kliesch (21). Stages of spermatogenesis were assigned as stated by Clermont (22). For comparative analysis of mRNA expression between different germ cell differentiation stages, we used 43 biopsy samples with normal spermatogenesis, 9 with a maturation arrest at the level of spermatocytes, 8 with a maturation arrest at the level of spermatogonia, and 20 showing a Sertoli cell only syndrome.

To assess the ultracellular morphology of germ cells, especially of spermatocytes immediately before the first meiotic division, testis biopsy specimens were fixed in glutaraldehyde and prepared for transmission electron microscopy (TEM) as described previously by Bergmann et al. (23). Testis biopsy specimens were immediately fixed by immersion in 5.5% glutaraldehyde in 0.05 mol/L cacodylate buffer adjusted to pH 7.35 (at least 3 hours), postfixed in osmium tetroxide ($OsO_4$, 1 hour), and embedded in Epon 812. Ultrathin sections