Chapter 34
CatSper in Male Infertility

Takuya Nishigaki, Ana Laura González-Cota and Gerardo José Orta Salazar

Abstract In 2001, two groups independently reported different components of a novel Ca^{2+} channel named CatSper, which is expressed only in the testis and localized in the sperm flagellum. Now, we know that CatSper is a sperm-specific Ca^{2+} channel composed of four distinct pore-forming subunits accompanied with, at least, three auxiliary subunits. Although there is no heterologous expression system to study this CatSper channel, the elimination of any single subunit ever tried in transgenic mice results in male infertility, which indicates that each individual subunit is essential for the correct channel assembly. Whole-cell patch clamp recordings directly taken from spermatozoa revealed that CatSper is a moderately voltage-dependent Ca^{2+} channel and is activated by intracellular alkalinization and several extracellular ligands, i.e., progesterone and prostaglandin E in human spermatozoa. The spermatozoa of CatSper null mice exhibit a defect in hyperactivated flagellar motility, a vigorous flagellar movement required for fertilization under physiological conditions. In agreement with this, there are some families suffering from male infertility correlated with mutations in CatSper-related genes.

34.1 Introduction

The motility propelled by the flagellum is a special feature of spermatozoa and any defects in this function can lead to male infertility (Darszon et al. 2011). The flagellar beat is generated by the axoneme, which is also found in the cilium (or cilia) of epithelial cells in the whole body (Lindemann and Lesich 2010). Therefore, a defective mutation of a certain component in the axoneme can cause a

T. Nishigaki (✉) · A. L. González-Cota · G. J. Orta Salazar
Departamento de Genética del Desarrollo y Fisiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México (UNAM), Avenida Universidad #2001, Colonia Chamilpa 62210 Cuernavaca, Morelos, México
e-mail: takuya@ibt.unam.mx

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malfunction in sperm flagellar beating leading to male infertility accompanied with diminution of ciliary motion such as primary ciliary dyskinesia (Munro et al. 1994). On the other hand, there are several external factors that modulate the axoneme function such as ATP, pH, Ca^{2+}, and cAMP (protein phosphorylation depending on cAMP). Spermatozoa possess multiple sperm-specific proteins that control these factors: glycolytic enzymes (Miki et al. 2004), sodium-proton exchanger (Wang et al. 2003), Ca^{2+} channel (Ren et al. 2001), Ca^{2+}-ATPase (Okunade et al. 2004), adenylyl cyclase (Buck et al. 1999), and protein kinase (Nolan et al. 2004), etc. Therefore, a mutation in those proteins can cause male infertility as a result of the abnormal regulation of sperm motility without any other defects in the body. In this chapter, we review the structure and the function of CatSper, a sperm-specific Ca^{2+} channel essential to male fertility, and some reports of male infertility related to mutations that affect this channel.

34.2 Structure of CatSper

As seen in Fig. 34.1, CatSper is composed of four pore-forming α subunits (Ren et al. 2001; Quill et al. 2001; Arias et al. 2003; Lobley et al. 2003), which have six transmembrane segments, and at least three auxiliary subunits, β (Liu et al. 2007), γ (Wang et al. 2009), and δ (Chung et al. 2011). In the fourth segment (S4) of α1 and α2 subunit, four or five positively charged residues are found every three amino acids, which is a typical feature of the S4 segment of voltage-gated channels. In contrast, α3 and α4 have only two (Fig. 34.1c), suggesting a correlation with the moderate voltage dependence of this channel described later. One of the striking features of CatSper is that the pore of the channel is composed of four separated polypeptides instead of a single polypeptide as known in other voltage-gated Ca^{2+} channels. Another interesting feature is that α1 subunit has many histidine residues in its N-terminal cytoplasmic domain (Fig. 34.1b), which is proposed to function as a pH sensor (Kirichok et al. 2006). All α subunits have a pore-forming loop with the typical Ca^{2+} selective channel motif ([T/S] × [D/E] × W) (Fig. 34.1d). Interestingly, CatSper uses only aspartic acids in this motif (DDDD) instead of a mixture of aspartic acids and glutamic acids (EEDD) found in T-type Ca^{2+} channel (Ca_v3) or only glutamic acids (EEEE) found in other voltage-gated Ca^{2+} channels (Ca_v1 and Ca_v2) in vertebrates (Senatore et al. 2013), which suggests a distinct cation selectivity of CatSper compared with typical voltage-gated Ca^{2+} channels.

At present, three membrane proteins (β, γ, and δ) have been reported as auxiliary subunits of CatSper. The β subunit has two transmembrane segments and γ and δ have a single transmembrane segment (Liu et al. 2007; Wang et al. 2009; Chung et al. 2011) (Fig. 34.1a). All three auxiliary subunits have large extracellular domains, which may serve as receptors for extracellular ligands, in contrast to small cytoplasmic tails.

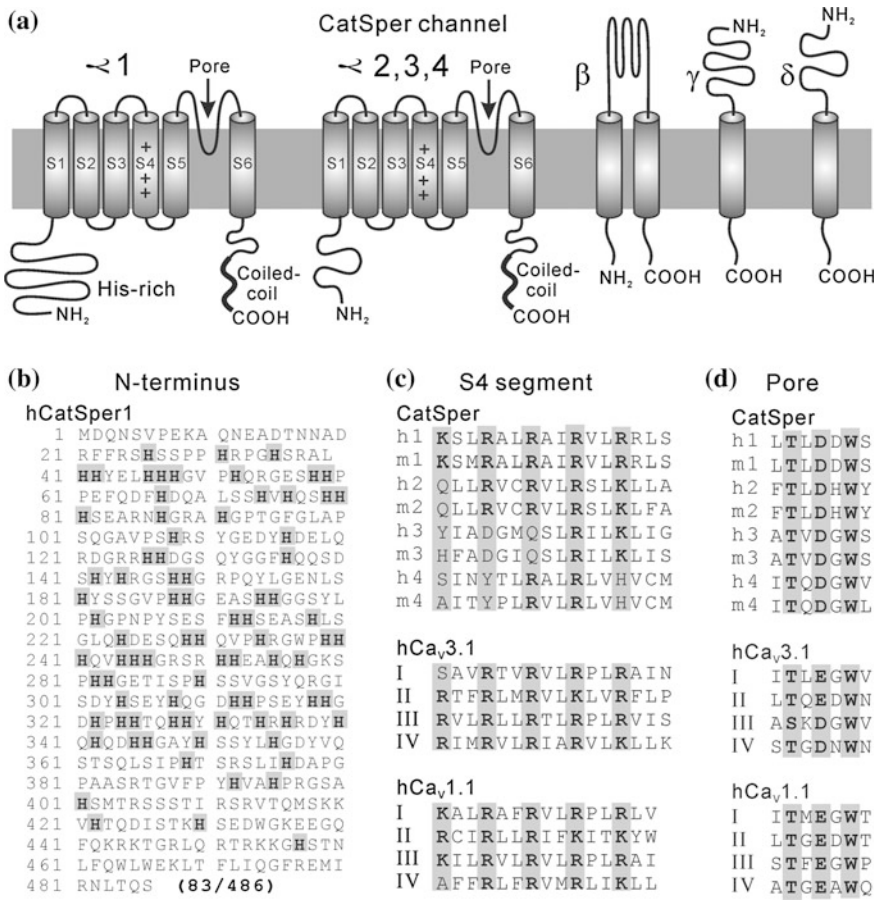

Fig. 34.1 Structure of CatSper. **a** Topology of pore-forming α subunits (1 − 4) and auxiliary subunits (*β*, *γ*, and *δ*). **b** Amino acid sequence of cytoplasmic N-terminal region of mouse CatSper is shown with histidine residue as *bold* and *gray* shadow. **c** Amino acid sequences of S4 segments of human CatSper (h1 − h4), mouse CatSper (m1 − m4), human $Ca_v3.1$ ($hCa_v3.1$, I − IV) (Perez-Reyes et al. 1998) and human $Ca_v1.1$ ($hCa_v1.1$, I − IV) (Tanabe et al. 1987) are aligned with *gray* shadows in every three residues. Arginine and Lysine are expressed as *bold*. **d** Amino acid sequences of the pore region are aligned as in (**c**). Three important residues for Ca^{2+} selective channels (T/S, E/D and W) are shown as *bold* and *gray* shadow

34.3 Aspects of Evolution

Amino acid sequences of all subunits of CatSper show a high level of diversity among different species (Table 34.1), which is a common feature of proteins specifically found in gametes (Swanson and Vacquier 2002). In general, transmembrane segments conserve their amino acid sequences, but relatively large cytoplasmic domains found in CatSper 1, 2, and 3 (α1, α2, and α3) and even large extracellular

Table 34.1 Amino acid identity of CatSper subunits between mouse and human

CatSper subunit	% of identity
α1	55
α2	65
α3	66
α4	72
β	56
γ	55
δ	51

domains found in all auxiliary subunits (β, γ, and δ) show increased diversity in their amino acid sequences. As a consequence, CatSper4 (α4) subunit, which have relatively small cytoplasmic domains, conserve their amino acid sequences better than any other subunits (Jin et al. 2005) (Table 34.1). The diversity found in α1 subunit is mainly due to a variety of amino acid sequences found in the histidine-rich N-terminal domain. This domain also shows many indels (insertion and deletion) even among species of primates although its biological significance is unknown (Podlaha and Zhang 2003). The diversity found in the auxiliary subunits could reflect a difference of ligand specificity between species.

Another very peculiar point of CatSper in terms of evolution is its mosaic distribution in metazoa as shown in Fig. 34.2 (Cai and Clapham 2008). For example, among vertebrates, neither aves (birds) and amphibians nor teleosts (fish) have CatSper (all α and β subunits) although cartilaginous fishes (ray) possess this channel. On the other hand, arthropoda (insects) and nematoda (*C. elegans*) do not have CatSper, but echinoderms (sea urchin) and cnidaria (sea anemone) conserve this channel. It is worth noting that the δ subunit is found only in mammals and reptiles but not in other species (Chung et al. 2011), indicating that this subunit has a unique function in mammals and reptiles and is not an essential subunit for CatSper channel in many other species.

In the case of the fruit fly, *Drosophila melanogaster*, TRPP2 channel (PKD2 channel) is localized on the distal tip of the sperm flagellum and a targeted mutation of this channel results in male infertility without apparent alterations in sperm morphology (Watnick et al. 2003). A detailed analysis of sperm motility in this mutant revealed that those spermatozoa swim backwards in the female reproductive tract (Kottgen et al. 2011), which clearly indicates a crucial role of TRPP2 channel for sperm motility regulation in this species. It would be interesting to identify what type of Ca^{2+} channels are involved in sperm motility regulation in species that lack CatSper in their genome.

34.4 Biophysical Properties

Elimination of any single subunit of CatSper that has been attempted (all α and δ subunits), in transgenic mice, results in male infertility accompanied with the lack of CatSper current in the spermatozoa (Qi et al. 2007; Chung et al. 2011; Jin et al.

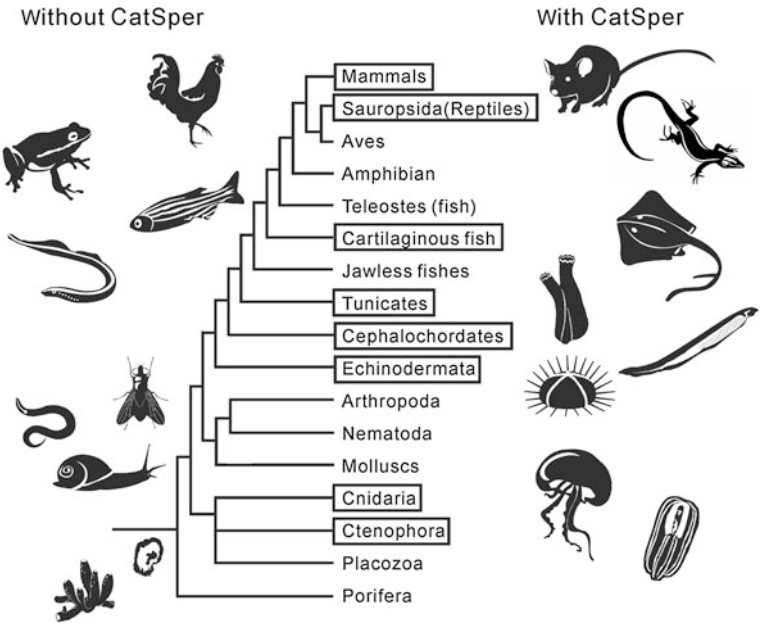

Fig. 34.2 Distribution of CatSper $\alpha(1-4)$ and β subunits in metazoan. This figure indicates the distribution of CatSper orthologs in whole metazoan species, which was revealed by the bioinformatic analysis of genome DNA sequences (Cai and Clapham 2008). The species which conserve CatSper genes are in box. In order to recognize each species easily, animal images are illustrated separating species possessing CatSper on the *right side* and those lacking this channel on the *left*. Some animal images do not correspond to the exact species whose genome sequences are determined, but they represent popular species from the same taxonomic phylum or class

2007; Kirichok et al. 2006), indicating that each subunit is indispensable for the proper channel assembly and function. In CatSper1 (α1 subunit) null mice, all other subunits including β, γ, and δ are missing in the mature spermatozoa (Liu et al. 2007; Wang et al. 2009; Chung et al. 2011), suggesting that only the correctly assembled CatSper channel can be transferred to the appropriate place, the plasma membrane of the principal piece in the flagellum. When they fail to assemble a functional channel, they are likely to be degraded during spermatogenesis. On the other hand, all attempts ever made to express a functional CatSper channel in heterologous systems have been in vain, which indicates that there might still be an unidentified auxiliary subunit, including germ cell-specific chaperons. Otherwise, a certain particular environment created by a particular lipid compositions or special scaffolding proteins might be necessary for a proper CatSper assembly and function.

While there is no heterologous expression system for CatSper yet, the biophysical properties of this channel had remained unknown until the development of the whole-cell patch clamp recording directly from mouse spermatozoa was reported (Kirichok et al. 2006). The trick of this technique is to use the

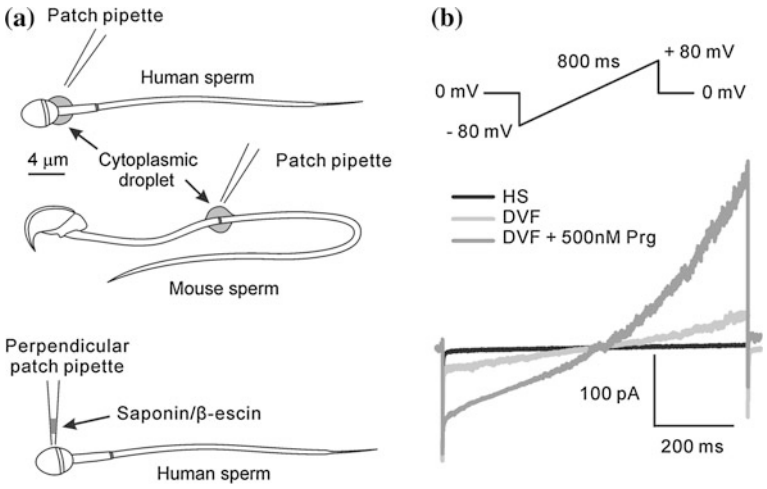

Fig. 34.3 Techniques of whole-cell patch clamp to spermatozoa and the typical CatSper current ($I_{CatSper}$). **a** *Upper* part illustrates the sites of cytoplasmic droplet (*gray shadow*) where patch pipettes should be attached to, in human and mouse spermatozoa. *Lower* part illustrates a perforated whole-cell patch clamp after on-cell patch clamp mode (Orta et al. 2012). **b** A representative $I_{CatSper}$ of human spermatozoon in response to a voltage-ramp, indicated above, under different conditions: standard solution for human sperm (HS, *black*), divalent cation-free solution (DVF, *gray*), and DVF in presence of 500 nM of progesterone (DVF + 500 nM Prg, *dark gray*)

cytoplasmic droplet, a residual cytoplasm occasionally found in mature spermatozoa (Fig. 34.3a), as a target for the patch clamp pipette. Even so, it is still difficult to do due to the small size and the motility of the cell (Lishko et al. 2013). Alternatively, it is possible to record a whole-cell current from mature spermatozoa using a perforated patch clamp on the sperm head (Orta et al. 2012). Although this method may be even more difficult to perform, it has the advantage of retaining the intracellular components of the cell. In addition, this method can be applied independently of the cytoplasmic droplet, which is not always present in mature spermatozoa.

Because of its small Ca^{2+} conductance and Ca^{2+}-dependent inactivation, CatSper current has been studied using monovalent cation current (Na^+ or Cs^+) in divalent cation-free (DVF) media (Fig. 34.3b). In this condition, the voltage dependence of the channel is not so prominent (Kirichok et al. 2006), which is at least partially explained by the surface-potential hypothesis; a shift of voltage-dependent curve toward the negative side in the absence of divalent cations (Hille 1991). Monovalent cation currents through CatSper ($I_{CatSper}$) can be inhibited by addition of low concentration of Ca^{2+} to the medium with IC_{50} of 65 nM in mouse (Kirichok et al. 2006) and 1.2 μM in human (Smith et al. 2013), which is a typical feature of a Ca^{2+} selective channel. Voltage dependence of CatSper was determined in the presence of Ba^{2+}, which does not induce the rapid inactivation of the

channel as Ca^{2+}. Using the tail current of Ba^{2+}, it was determined that the CatSper channel is a moderate voltage-dependent channel with a slope factor $k \sim 30$ for mouse (Kirichok et al. 2006) and ~ 20 for human (Lishko et al. 2011). It is a striking feature, in both murine and human CatSper, that the voltage-dependent curve markedly shifts to a more negative values by intracellular alkalinization, which is considered a physiological signal to activate this channel (Kirichok et al. 2006; Lishko et al. 2010).

Recently, it was reported that progesterone and prostaglandin E activate CatSper in human sperm but not in murine sperm (Fig. 34.3b) (Lishko et al. 2011; Strunker et al. 2011; Smith et al. 2013). Both hormones are physiological ligands for human sperm and have been known to immediately increase the intracellular concentration of Ca^{2+} ($[Ca^{2+}]i$) (Thomas and Meizel 1989; Shimizu et al. 1998; Schaefer et al. 1998). The action of these ligands on spermatozoa had been a great mystery for a long time in the field of reproduction. On the other hand, several artificial compounds, such as odorants (bourgeonal and undecanal) and cyclic nucleotide analogs (8-Br-cGMP and 8-Br-cAMP), also activate human CatSper, indicating that human CatSper functions as a polymodal chemosensor (Brenker et al. 2012). In the case of murine sperm, it is demonstrated that albumin (BSA) and zona pellucida increase the $[Ca^{2+}]i$ in a CatSper-dependent manner (Xia and Ren 2009a, b). The difference of ligand specificity between these species may be attributed to the diversity of the amino acid sequences of auxiliary subunits of CatSper (β, γ, and δ), which have large extracellular domains and are supposed to function as receptors for extracellular ligands.

At present, there are no specific blockers for CatSper. HC-056456 was reported as the first CatSper blocker (IC_{50}: ~ 3 μM) although its specificity is unknown (Carlson et al. 2009). Mibefradil and NNC 55-0396, both T-type voltage-gated Ca^{2+} channel blockers, in the 10–20 μM range inhibit efficiently the CatSper current (Strunker et al. 2011; Lishko et al. 2011) and Na^{+} influx induced by removal of external Ca^{2+} in human spermatozoa (Torres-Flores et al. 2011). However, Mibefradil and NNC 55-0396 elevate the intracellular pH of spermatozoa by an unknown mechanism (Brenker et al. 2012). MDL12330A, known as an adenylyl cyclase inhibitor, was recently found to block CatSper (Brenker et al. 2012). Therefore, a more specific blocker for CatSper without any secondary effects, which potentially functions as a male contraceptive, is eagerly desired in the field of reproduction.

34.5 Physiological Function

Spermatozoa recovered from the oviduct show a vigorous flagellar movement called hyperactivated motility, which is indispensable for the fertilization and characterized by asymmetric, large amplitude, and low frequent flagellar beating (Suarez 2008) as illustrated in Fig. 34.4a. Several experimental evidences suggest that hyperactivated motility has four major roles in the process of the fertilization

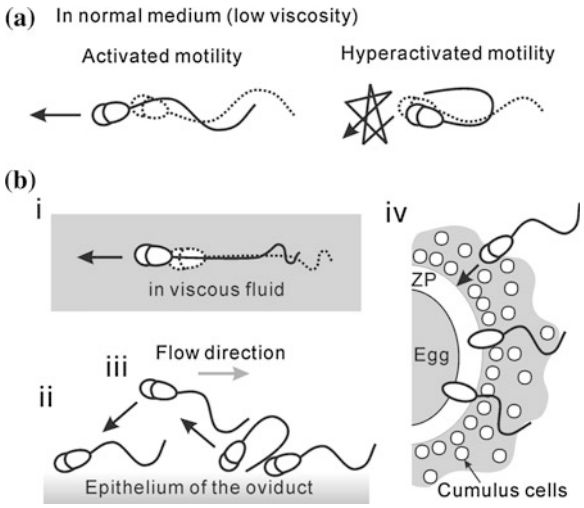

Fig. 34.4 Roles of hyperactivated motility in fertilization. **a** In normal experimental medium, immediately after spermatozoa are ejaculated they manifest a symmetric flagellar bend with high beat frequency called activated motility. In contrast, the hyperactivated motility is characterized by an asymmetric flagellar bend with low beat frequency. **b** Hyperactivated motility is required for four processes in mammalian fertilization: **i** Generate a propulsive force in viscous (or viscoelastic) environments, **ii** escape from the initial part of the isthmus of the oviduct, named sperm reservoir, **iii** swim against fluid flow, called rheotaxis, and **iv** penetrate through extracellular matrix of cumulus cells and the egg, named zona pellucida (ZP)

(Fig. 34.4b); (i) keep progressive motility in viscous environment (Suarez et al. 1991), (ii) detach from the initial part of the oviduct, lower isthmus, also called sperm reservoir (Suarez 1987), (iii) swim against fluid flow direction (rheotaxis) (Miki and Clapham 2013), and (iv) penetrate through extracellular matrix of the oocyte (Stauss et al. 1995). Murine spermatozoa lacking CatSper lose all these capacities together with fertility (Quill et al. 2003; Ren et al. 2001; Ho et al. 2009; Miki and Clapham 2013; Carlson et al. 2003), which confirms the physiological significance of hyperactivated sperm motility in this species. Therefore, the most obvious physiological role of CatSper is to induce and maintain the hyperactivated motility of the spermatozoa (Carlson et al. 2003). However, it is not completely understood what the physiological trigger(s) of sperm hyperactivation may be. There are several possibilities: (1) an increase in bicarbonate (HCO_3^-) concentration (Maas et al. 1977), which activates soluble adenylyl cyclase (Buck et al. 1999), (2) an increase in the fluid pH (Maas et al. 1977), (3) a specific ligand such as progesterone and prostaglandin E in human spermatozoa (Lishko et al. 2011; Strunker et al. 2011), (4) a decrease in Zn^{2+} concentration, which releases inhibition of voltage-gated proton channel (Lishko et al. 2010), and (5) a decrease in temperature (Bahat et al. 2005), which activates TRPM8 channel (De Blas et al. 2009). It is most likely that hyperactivated sperm motility is induced by several factors simultaneously and/or intermittently, which are generated concomitantly

upon ovulation. Some of these factors are common among different species, but some are species-specific as the case of progesterone and prostaglandin E, which activate human CatSper but not murine. Further studies are required to understand the mechanism of induction of hyperactivation under physiological conditions.

Although the physiological roles of CatSper in mouse sperm are well established, those in human sperm are still not fully understood. In humans, not only Ca^{2+} influx through CatSper but also its release from intracellular Ca^{2+} stores is indispensable to maintain hyperactivation (Harper et al. 2004; Kirkman-Brown et al. 2004; Alasmari et al. 2013b). So far, it is known that CatSper is critical for sperm hyperactivation induced by progesterone (Lishko et al. 2011; Strunker et al. 2011; Smith et al. 2013; Alasmari et al. 2013a; Senatore et al. 2013; Servin-Vences et al. 2012) and this channel also seems essential for sperm to penetrate viscous media (Alasmari et al. 2013a). The positive correlation found in the success rate of in vitro fertilization (IVF) and progesterone-induced intercellular Ca^{2+} increase (Alasmari et al. 2013a) would be a helpful information to understand the role of CatSper and to establish a diagnostic test to evaluate sperm capacity for fertilization.

On the other hand, progesterone and prostaglandin E were initially identified as physiological ligands to induce the acrosome reaction (AR) in human sperm (Osman et al. 1989; Schaefer et al. 1998; Shimizu et al. 1998). In murine model, it was reported that the egg coat (zona pellucida) induces an increase in intracellular Ca^{2+} in wild type but not in CatSper1 null mice spermatozoa (Xia and Ren 2009b) although the AR still can be induced by zona pellucida in the same transgenic mice. Even though there are some contradictory results, CatSper could play a role in the AR, which may provide another reason for the positive correlation between CatSper activity and the success rate of IVF (Alasmari et al. 2013a).

34.6 Channelopathy

In mouse, all α subunits and δ subunit were demonstrated to be essential for CatSper channel assembly and the absence of this channel results in male infertility due to a defect in sperm motility regulation, namely, lack of hyperactivated sperm motility. Therefore, a similar function of human orthologs might be expected in human sperm. As anticipated, mutations of two loci that encode CATSPER1 and CATSPER2 have been reported from some families associated with male infertility (Avidan et al. 2003; Avenarius et al. 2009; Zhang et al. 2007). Coincidentally, all of the patients were found to have a homozygous mutation, an autosomal recessive mutation, through a consanguineous marriage. This is probably owing to the fact that the pore-forming α subunit of CatSper is composed of hetero-tetramers but not homo-tetramers like a voltage-gated K^+ channel. Thus, a dominant negative mutation of α subunit of CatSper should be difficult to encounter.

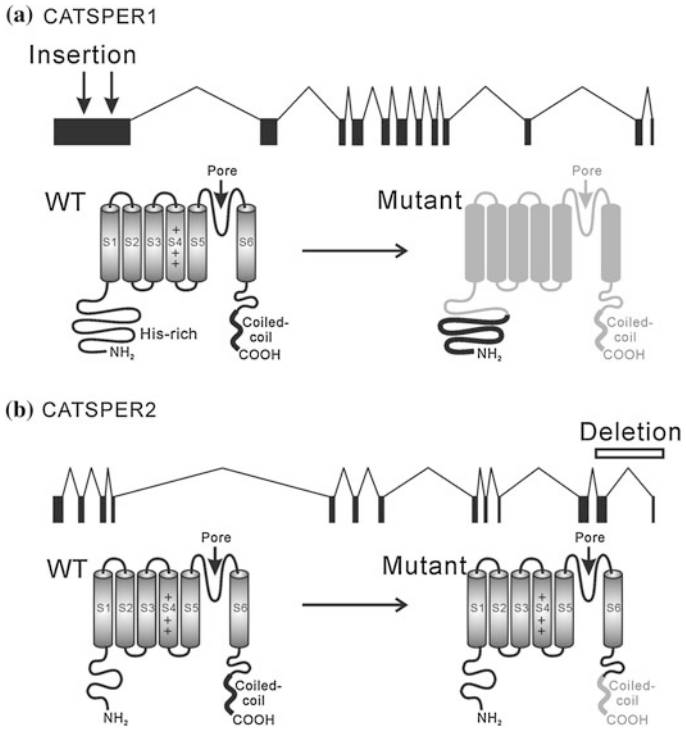

Fig. 34.5 Sites of CATSPER mutations correlated to human infertility and their predicted proteins. **a** The genome structure of CATSPER1 is illustrated as 12 exons (*black bars*) and introns (*polygonal lines*). The *arrows* indicate the two separated insertion mutations found in male infertile patients. Both mutants have some extra bases in the first exon, which encodes the His-rich N-terminal cytoplasmic domain, and are supposed to produce truncated proteins by a frame shift of mRNA as illustrated (*light gray*). **b** Genome structure of CATSPER2 (13 exons). The *white* box over the last two exons represents the deleted region of this gene found in infertile patients (French family). This region encodes a coiled-coil motif of the cytoplasmic C-terminal domain (*light gray*)

In 2009, two consanguineous Iranian families were identified as carriers of a similar, but distinct, insertion mutation in exon 1 of CATSPER1 gene (Fig. 34.5a) localized at chromosome 11q13.1 (c.539-540insT or c.948-949insATGGC) (Avenarius et al. 2009), which encodes the histidine-rich N-terminal cytoplasmic domain. Both mutations cause frame shifts and generate premature stop codons. As a consequence, they are predicted to produce proteins lacking all the transmembrane segments and the pore-forming loop, as illustrated in Fig. 34.5a, almost equivalent to a deletion of the whole α1 subunit. As is expected, the male homozygous carriers of these mutations are infertile although there is no detailed information about the sperm function of these patients.

Another locus of mutation of male infertility related to CatSper, as the most studied case, is found on the chromosome 15p15, which is also characterized with

non-syndromic deafness. This syndrome (male infertility and deafness) was first identified in a French family carrying a homozygous deletion of ~70 kb in the chromosome 15p15 which affects three contiguous genes: the first 24 exons of IP6 K, a kinase expressed ubiquitously; the entire coding sequence of SRTC, a protein mainly expressed on inner ear stereocilia which is related to the deafness; and the last two exons of CATSPER2 (Avidan et al. 2003) (Fig. 34.5b). This mutation removes the last 225 bases of the mRNA which encode the cytoplasmic C-terminal region of CatSper2 including a coiled-coil region (Fig. 34.5b) predicted to be necessary for protein–protein interactions to form the channel pore complex (Quill et al. 2001; Lobley et al. 2003). As mentioned above, all pore-forming subunits ($\alpha 1 - 4$) of CatSper are needed to assemble the functional murine CatSper channel (Qi et al. 2007). Therefore, CatSper2 and, in consequence, the entire CatSper channel complex had been expected to be absent in the CatSper2-deficient patient. Recently, this prediction was finally confirmed by detailed analysis of spermatozoa of one of these patients (II-2 in Avidan et al. 2003) using immunostaining and electrophysiological techniques (Smith et al. 2013). Namely, CatSperβ was unable to be detected by anti-CatSperβ antibody and CatSper current was absent from the spermatozoa of this patient. Furthermore, progesterone did not amplify any ionic current although the activities of other channels, K^+ channel (Slo3) and voltage-gated H^+ channel (Hv), were retained (Smith et al. 2013). Therefore, this study supports the idea that the principal Ca^{2+} channel in human sperm is CatSper and it is activated by progesterone. This locus is likely to be a hot spot to provoke a deletion mutation because a duplicated copy of these genes is located adjacently as pseudogenes (Avidan et al. 2003). In agreement with this, it was reported that there are three more Iranian families with similar delete mutations in this locus (Zhang et al. 2007) although the entire CATSPER2 gene was deleted in all three cases of Iranian families (Table 34.2). Combined, these reports provide strong evidences that link CatSper deficiencies to male infertility.

However, the phenotype of spermatozoa from the patients possessing the deletion mutation of the chromosome 15p15 is not exactly coincident with the phenotype of spermatozoa of transgenic mice lacking CatSper-related genes. In murine models, CatSper mutations did not cause any defects in spermatogenesis (number of matured spermatozoa) or sperm shape (Ren et al. 2001; Qi et al. 2007; Chung et al. 2011). Moreover, those mouse spermatozoa manifest normal-activated flagellar motility characterized by symmetric, low amplitude, and high frequent flagellar beating although they do not undergo hyperactivation (Carlson et al. 2003). In contrast, those patients suffer from asthenoteratospermia; disorders in sperm morphology and motility. Taking the phenotype of the transgenic mice into account, defects in sperm morphology and basal motility found in the patients with CATSPER2 mutation (Avidan et al. 2003; Zhang et al. 2007) could be attributed to a defect of another gene, including IP6 K, rather than different functions of CatSper between mouse and human. To date, there are only a few reported clinical cases that link CatSper to male infertility; two having defects on CATSPER1 (Avenarius et al. 2009) and four on CATSPER2 (Avidan et al. 2003; Zhang et al. 2007). Since all reported patients come from consanguineous families,

Table 34.2 Clinical cases of infertility correlated to CatSper mutations

Mutated gene	Affected families	Number of patient	Mutation (Locus)	Associated defects	Reference
CatSper1 (α1)	Iranian L-1025	2	c.539-540insT (11q13.1)	Asthenoteratospermia/ low sperm count	Avenarius et al. (2009)
CatSper1 (α1)	Iranian L-968	1	c.948-949insATGGC (11q13.1)	Asthenoteratospermia/ low sperm count	Avenarius et al. (2009)
CatSper2 (α2)	French	3	Del(15) ~70 kb Deletion of the last two exons (12 and 13) (15q15.1-15q15.3)	Deafness/ asthenoteratospermia/ no CatSper current	Avidan et al. (2003); Smith et al. (2013)
CatSper2 (α2)	Iranian D_SM	4	Del(15) ~100 kb Deletion of the entire gene (15q15.1-15q15.3)	Deafness/ asthenoteratospermia	Zhang et al. (2007)
CatSper2 (α2)	Iranian L-705	1	Del(15) ~100 kb Deletion of the entire gene (15q15.1-15q15.3)	Deafness/ asthenoteratospermia	Zhang et al. (2007)
CatSper2 (α2)	Iranian L-1014	2	Del(15) ~90 kb Deletion of the entire gene (15q15.1-15q15.3)	Deafness/ Asthenoteratospermia	Zhang et al. (2007)

they could also carry defects on other genes. Considering some distinct phenotypes between the transgenic mice and human patients, it is difficult to conclude the deficiencies on CatSper as the direct cause of male infertility in these patients. Further information about patients of male infertility and CatSper-related gene mutations is required to answer this question.

On the other hand, although genetic analysis has not been carried out, a systematic analysis of sperm Ca^{2+} responses and hyperactivation from healthy donors and sub-fertile patients were recently reported (Alasmari et al. 2013a). In this work, sub-fertile patients were classified into two groups; patients who could fertilize by in vitro fertilization (IVF) (Steptoe and Edwards 1978) and those who required intra-cytoplasmic sperm injection (ICSI) (Palermo et al. 1992). ICSI is an advanced technique of assisted reproduction and usually applied for male patients who failed in IVF. While spermatozoa from almost all donors and IVF patients manifest progesterone-induced intracellular Ca^{2+} increase, spermatozoa of 27 % ICSI patients failed to respond to progesterone. Considering that CatSper is activated by progesterone in human spermatozoa (Lishko et al. 2011; Strunker et al. 2011), roughly 27 % of ICSI patients have some defects in CatSper. Genetic analysis of those patients would contribute to the understanding of the correlation between mutations in CatSper-related genes and male infertility.

34.7 Conclusion

Although it is possible to record CatSper channel current by whole-cell patch clamping directly from spermatozoa, it is still a difficult technique. On the other hand, in vitro spermatogenesis is not so efficient and is a time-consuming process (~40 days) (Sato et al. 2011). Currently, mutagenesis of a CatSper-related gene requires an approach using transgenic animals (murine models), which is a very expensive project. Therefore, to establish a heterologous expression system for CatSper channel is of primary interest and the most important obstacle to overcome in order to promote the study of CatSper channel research.

CatSper is an essential channel for male fertility, but there are only a few reports about male infertility correlated to alteration of CatSper genes. There are several reasons to explain this situation. One of them would be owing to the fact that genetic defects in male fertility had not been inherited to the next generation, which could have prevented genetic research about male infertility in general. This situation has been altered by application of intra-cytoplasmic sperm injection (ICSI), one of advanced assisted reproduction techniques (ARTs). Since ICSI is getting to be a popular ART these days (Wong and Ledger 2013), CatSper mutations will accumulate in the future (Devroey and Van Steirteghem 2004). Nevertheless, this defect is supposed to affect only spermatozoa not other organs in contrast to the case of patients of ciliopathies (Munro et al. 1994). Considering the advance of technology for whole-genome sequencing, the lists of gene alterations of CatSper channel are supposed to be amplified soon in the future, which may also contribute to the understanding of the structure and function relationship of CatSper channel.

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