ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 7, Issue, 10, pp. 13959-13963, October, 2016

International Journal of Recent Scientific Research

Research Article

CORRELATION BETWEEN CATSPER 1 GENE MUTATION AND HUMAN MALE INFERTILITY IN NORTH INDIA

Hena Naqvi[1,2,3] ., Shyam Pyari Jaiswar[1]., Mohammad Kaleem Ahmad[2]., Syed Rizwan Hussain[2]., Farzana Mahdi[3] and Abbas Ali Mahdi[2]

[1]Department of Obstetrics & Gynaecology, Department of Biochemistry, King George's Medical University(KGMU), Lucknow, Uttar Pradesh, India- 226003
[2]Molecular Cell Biology Lab, Department of Biochemistry, King George's Medical University(KGMU), Lucknow, Uttar Pradesh, India- 226003
[3]Central Research Lab, Department of Biochemistry, Era's Lucknow Medical College and Hospital (ELMC&H), Lucknow, Uttar Pradesh, India- 226003

ARTICLE INFO

Article History:

Received 17th July, 2016
Received in revised form 12th August, 2016
Accepted 04th September, 2016
Published online 28th October, 2016

Key Words:

CatSper, Asthenozoospermia, Sperm motility, Hyperactivation

ABSTRACT

Background: The cationic channel of sperm is a small family of ion channels, named after the first putative cation channel of sperm (CatSper). It is a sperm-specific, pH-sensitive, voltage-gated calcium channel, Ca^{2+}selective and located in the principal piece of the flagellum. CatSper 1 gene is located on chromosome 11q13.1 and is involved in sperm motility and hyperactivation (HA). We designed our study with an aim to investigate the CatSper 1 gene mutation in infertile men and correlated it with clinico-pathological findings.

Materials and methods: We performed mutational analysis of the CatSper 1 gene in 100 infertile men and 100 healthy controls. To screen mutations in CatSper 1 gene, we performed PCR followed by Single Stranded Conformational Polymorphism (SSCP). Samples showing shifts in bands were direct DNA sequenced.

Results: On analysis of mutational screening of CatSper 1 gene, we found one silent mutation in exon 2 in 10 asthenozoospermic males due to a single nucleotide change (**C**GG → **A**GG) at codon 457. This is a silent mutation as there is no alteration in amino acid sequence. In addition, at exon 5, change in nucleotide from CA**G** → CA**T** inferred change of amino acid from glutamine to histidine at codon 624 (Gln**624**His) in three asthenozoospermic males. This is a missense mutation.

Conclusions: On analysis of mutational screening of CatSper 1 gene, at exon 2 and 5 there was a silent and missense mutation respectively. In case of change of amino acid from glutamine to histidine (Gln**624**His) both the amino acids are polar and positively charged. However histidine is aromatic due to which there might be changes in structural conformation of protein product formed. All the mutations were found in different cases. It is important to note that these predictions have been made on the basis of the identified DNA alterations at the genomic level and that additional studies are required for confirmation at the transcriptional or translational level.

INTRODUCTION

The cationic channel of sperm (CatSper) belongs to a petite family of ion channels. CatSper channels (CatSper1, 2, 3 & 4) are named after the first putative cation channel of sperm (1; 2). It is a sperm-specific (1), Ca^{2+} selective (2), pH-sensitive (3), voltage-gated calcium channel (1), which is located in the principal piece of the flagellum (2; 4; 5). It is an evident fact that CatSper is analogous to other voltage-gated calcium channels as in the absence of divalent cations. CatSper can also conduct monovalent cations like sodium (Na^{+}) and caesium (Cs^{+}); yet, the affinity of CatSper to divalent cations especially Ca^{2+} is much higher (6). Additionally possible activation of CatSper channels occurs only in the presence of agonists or physiological stimuli whereas under normal physiological conditions CatSper channels remain active at a nominal level only when sperm membrane potential and intracellular pH stay poised (3). Female steroid hormone progesterone galvanizes the activity of CatSper in human (but not mouse) sperm cells (7; 8). This fact was further supported by the study in which men whose sperm-responsiveness towards progesterone was

**Corresponding author: Hena Naqvi*
Department of Obstetrics & Gynaecology, Department of Biochemistry, King George's Medical University(KGMU), Lucknow, Uttar Pradesh, India- 226003

low, exhibited reduced natural (9; 10) and assisted reproduction (11; 12) fertility

CatSper 1, gene is located on chromosome 11q13.1. The importance of CatSper1 channel in sperm motility was first ascertained in 2001[2] whereas its functional aspect as a calcium transporter was confirmed later in 2006 [3]. Furthermore its association with human male fertility was also revealed in two consanguineous Iranian families [13]. Despite the fact that ample of progress has been made in context to the physiological function of CatSper in mouse sperm functions, the scenario regarding human spermatozoa is still not clear. Basically in mice, disrupting genomic sequence of any one of the four CatSper channels (CatSper1/2/3/4) leads to infertility due to impaired sperm motility and failure of attaining HA (2; 14; 15). In addition to this, preliminary findings in CatSper-null mice condemned any participation of CatSper in the tyrosine phosphorylation escalation during sperm capacitation (Carlson *et al*., 2003; Quill *et al*., 2003); a recent report by Chung *et al*., 2014 (16) verified a strong contribution of CatSper channel in the tyrosine phosphorylation escalation during sperm capacitation. The function of CatSper in HA is debatable in human spermatozoa as in *in-vitro* study, no elevations in HA parameters were recorded in all samples when channel was stimulated either by elevating pH_i or with progesterone hormone (17,18; 5). Similarly in *in-vitro* studies performed by incubating channels with available CatSper inhibitors; inconsistent results were obtained (19, 20,5).

Another study reported degradation or mislocalization of CatSper channel in non-motile mouse spermatozoa (16). Furthermore, a study demonstrated that a higher percentage of spermatozoa express CatSper1 after swim-up selection and that a positive correlation exists between CatSper1 protein expression, evaluated by western blot analysis, and the percentage of progressive motility, whilst this inference was obtained in a small number of subjects (5). Overall these studies imply that low, absent or mislocalized CatSper expression in spermatozoa may be involved in a reduced ability to initiate the calcium signalling cascade.

In view of the vital role of CatSper in human sperm functions, we examined any correlation between male infertility and CatSper 1 gene in north Indian population. To accomplish this, we screened different exonic regions of the CatSper 1 gene.

MATERIALS AND METHODS

Semen collection and preparation

Semen samples were obtained according to World Health Organization (WHO) criteria (21) from a total of 100 consecutive patients undergoing routine semen analysis for couple infertility, in the Department of Obstetrics and Gynaecology, KGMU, Lucknow and Department of Gynaecology, ELMC&H, Lucknow, after obtaining patient's written informed consent to allow use of the remaining semen after completion of the analysis for research purposes.

Table 1 List of primers of CatSper 1 gene.

Forward Primer	Reverse Primer
CT-1, Ex-1A F: 5' CCT TTT CTT GAC CTC GGT TTC CTC 3'	**CT-1, Ex-1A R:** 5' ACG TTG GAG CTC ATC ATG GTA GTC 3'
CT-1, Ex-1B F: 5' TCC CAT GTC CAC CAA TCT CAC CAC 3'	**CT-1, Ex-1B R:** 5' GCC GGT GGG TCT GGT GGT AGT G 3'
CT-1, Ex-1C F: 5' CCA CCT TAG CGG GCT CCA ACA C 3'	**CT-1, Ex-1C R:** 5' TGC GGG GCC GAG ATC AGG T 3'
CT-1, Ex-2 F: 5' CCA GCA AGC AGG CAG GAG TGA C 3'	**CT-1, Ex-2 R:** 5' GCC ATC TAC TTC CCA GGG GTG AG 3'
CT-1, Ex-3&4 F: 5' GCC GGG GAG AGC CTG GGA ATC 3'	**CT-1, Ex-3&4 R:** 5' CCT GCG CCC CCA TCC CTT GAG 3'
CT-1, Ex-5&6 F: 5' TCA CCC AAG TCA CCA ACA AGT CAG 3'	**CT-1, Ex-5&6 R:** 5'CGA CCT GCT CCC TTC CCA TAC 3'
CT-1, Ex-7&8&9 F: 5' GCT CAG TGA CCC CAT CGG CAA AG 3'	**CT-1, Ex-7&8&9 R:** 5' TGT GGA AGG AGG TTA GGG GGA TGG 3'
CT-1, Ex-10 F: 5' TCG TGC TGC ATG CCC GGA GAA G 3'	**CT-1, Ex-10 R:** 5' TGC ACC CAC AGG CTC ATC CAC AAG 3'
CT-1, Ex-11&12 F: 5' TGG GGA GGA GGC AGG CAA GAA G 3'	**CT-1, Ex-11&12 R:** 5' CGC TGG TAG GGC TGG AGG GGA TC 3'

Table 2 PCR programs of different exons of CatSper 1 gene.

Exons	Length (bp)	Step 1 (Denaturation)	Step 2 (Annealing)			Cycles	Step3 (Extension)
Exon1 A	639	94^0C → 8 mins	94^0C → 1 min	68^0C → 1 min 10 secs	78^0C → 1 min 10 secs	40	78^0C → 10 mins
Exon1 B	303	94^0C → 8 mins	94^0C → 1 min	61^0C → 1 min	74^0C → 1 min	35	74^0C → 8 mins
Exon1 C	700	94^0C → 8 mins	94^0C → 1 min	67^0C → 1 min	76^0C → 1 min	40	76^0C → 8 mins
Exon 2	453	94^0C → 8 mins	94^0C → 1 min	70^0C → 1 min 10 secs	78^0C → 1 min 10 secs	35	78^0C → 8 mins
Exon3&4	598	94^0C → 8 mins	94^0C → 1 min	68^0C → 1 min 15 secs	79^0C → 1 min 10 secs	35	79^0C → 8 mins
Exon5&6	624	94^0C → 8 mins	94^0C → 1 min	68^0C → 1 min 10 secs	76^0C → 1 min 10 secs	38	76^0C → 8 mins
Exon 7&8&9	694	94^0C → 8 mins	94^0C → 1 min	67^0C → 1 min 20 secs	78^0C → 1 min 15 secs	40	78^0C → 10 mins
Exon 10	361	94^0C → 10 mins	94^0C → 1 min	66.5^0C → 1 min 10 secs	76^0C → 1 min 10 secs	35	76^0C → 10 mins
Exon 11&12	660	94^0C → 8 mins	94^0C → 1 min	69.5^0C → 1 min 15 secs	78^0C → 1 min 15 secs	38	78^0C → 12 mins

The Institutional Review Board and Ethics Committee of KGMU, Lucknow, approved this study. Semen parameters were assessed by optical microscopy and samples were divided into two groups: Asthenozoospermia (n = 50), Normozoospermia (n = 50), on the basis of progressive (a + b) sperm motility, 32% and viability 58%, as the fifth centile of reference values of WHO (21). Asthenozoospermic men showed a reduced motility, a lower sperm concentration and total number per ejaculate and worse sperm morphology than normozoospermic men. The control group comprised of age-matched healthy men who had previously initiated at least one pregnancy and exhibited a normal semen profile defined as in by WHO (21).

Sperm preparation and Isolation of DNA

Semen samples were collected in an autoclaved container in aseptic conditions. To rule out the possibility of contamination by residual cells in the samples used for the study, the spermatozoa was individually purified by two sequential centrifugations (20 min at 300g, 25^0C) through 45:90 discontinuous gradients of Percoll (Ostermeier *et. al.* 2002). The 90% fraction of the second centrifugation through the Percoll gradient was pooled for DNA extraction. The majority of sperms were isolated in the sediment. The sediment was collected and washed with 1X PBS. Genomic DNA extraction for molecular genetic studies was performed using a commercially available Semen extraction kit (Bangalore Genei, India) and was stored at -20°C. DNA concentration was measured with a Nanodrop ND-100 Spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE).

Genomic DNA PCR Amplification

Primers for CatSper 1 gene were designed using software. PCR was performed in a gradient thermocycler (ABI, USA) using thin walled 0.2ml PCR tubes. The final volume of PCR reaction mixture was 25µl containing 10-40ng genomic DNA, 1 picomole each of forward and reverse primers, 2X PCR master mix (ABI, USA) at concentration of 1X in final reaction volume. Amplification was carried out using different primer (Table 1) set for different exons and different PCR programs (Table 2) for different exonic regions of CatSper 1 gene. 1µl of amplified product was examined by electrophoresis on 2% agarose gel, stained with ethidium bromide.

Single- Stranded Conformational Polymorphism (SSCP)

Amplified PCR products of different exons were further analysed for shifts by SSCP method according to protocol developed by (22). For this purpose 10% polyacrylamide gel was prepared and poured into pre-prepared gel cassette (18 x 16 cm and 1 mm spacers), properly sealed with agarose. The gel was allowed to polymerize for about 15 minutes and then 20 minutes pre-run was given. Electrophoresis was carried out on a Bangalore Genei Mini gel electrophoresis system. Prior to loading, the samples were denatured at 94°C for 5 minutes and then snap-cooled. Generally 10µl of PCR products were mixed with 10µl of formamide dye and loaded in each well. Loading should be done in the ratio of 1:1 (Dye: PCR Product). The gel was run in 1X TBE (Tris Borate EDTA) buffer that was pre-cooled at 4°C. The gel tank was placed in a cold room. The voltage was set to 200 Volt and the gel was run for about 9 hours. After electrophoresis, the gel was stained with silver stain.

Samples showing abnormal SSCP profiles compared with the sample obtained from the normal individual were further purified and sent for sequencing (Figure 1).

Analysis of Sequencing

Samples showing altered position on SSCP gels were purified followed by automated sequencing for confirmation of mutation. The PCR product of each sample was first purified and then submitted in 25 µl reaction volume with 10 picomoles of appropriate primer. The sequencing was performed by automated direct DNA sequencing technique, which incorporates fluorescently labelled di-deoxy-nucleotides during cycle sequencing and separates the resulting products by capillary electrophoresis for detection on an ABI 3730XL DNA Analyser (Applied Biosystems, USA). Multiple alignment and sequence analysis were done using BLAST (Basic Local Alignment Search Tool), BioEdit and FinchTV. Mutations were reconfirmed by sequencing amplicons in both directions and in independent second samples.

RESULTS

Our study included 100 infertile male cases and 100 normal fertile male controls. The mean age of onset for infertile male cases and normal fertile male controls was 35.5 ± 1.30. The amplified products were detected by SSCP- PAGE for detection of shift variations/ mutations.

SSCP analysis

100 amplified products were detected by SSCP- PAGE for detection of shift variations/ mutations. Shifts were found in 35 samples of asthenozoospermia.

Genomic Analysis

We evaluated different exonic regions of CatSper1 gene by sequencing. The sequencing chromatogram showed a change in nucleotide in exon 2 and exon 5.

In exon 2 we found one silent mutation in 10 Asthenozoospermic males due to a single nucleotide change (**C**GG → **A**GG) but no change of amino acid at codon 457 (Figure 2).

In exon 5 we found one missense mutation in 3 Asthenozoospermic males due to a single nucleotide change (CA**G** → CA**T**) as well as change of amino acid from glutamine to histidine at codon 624 (Figure 3).

DISCUSSION

CatSper, a highly conserved region in humans and mice is a family of four sperm specific voltage-gated cation channels [13,14]. Its vital role in fertilization activities in mouse sperm is well defined (2; 23). In our study we found one silent mutation in exon 2 of CatSper1 gene in 10 asthenozoospermic males. There was change in nucleotide from **C**GG → **A**GG at codon 457 but there was no alteration in amino acid. However, mutation in exon 5 in three asthenozoospermic males, change in nucleotide from CA**G** → CA**T** results in change of amino acid from glutamine to histidine at codon 624 (Gln**624**His)

resulting in a missense mutation. Both the amino acids are polar and positively charged. However histidine is aromatic amino acid due to which there might be changes in structural conformation of protein product formed. All the mutations were found in different cases. It is essential to annotate that these predictions have been made on the basis of the identified DNA alterations at the genomic level and that further studies are entailed for confirmation at the transcriptional or translational level.

In human the importance of CatSper in sperm motility is still confounding. Studies reveal that knockout genes result in lack of expression of the channel in the mature sperm and also in acquiring of HA (24; 14). In a study, male patient showing sperm defects and a relegated fertility during routine semen analysis was categorised as asthenozoospermic. The defects included low sperm count, increase in abnormal spermatozoa morphology, reduced sperm motility and a reduction in semen volume. On further evaluation at genomic level, insertion mutations in CatSper 1 was detected. (13). Secondly consequences of mutations of CatSper1 gene is a truncated protein reported in two different families in the population (25; 13) but its association with sperm motility in these subjects is ill-defined (26, 27). In contrast to this, a recent study using the patch clamp technique has documented the absence of CatSper currents in sperm from a man carrying CatSper1 mutations (26). Thus relationship between CatSper mutations and asthenozoospermia is still mystifying. A recent study by Tamburino *et al* 2015(28) depicts that the sperm derived from asthenozoospermic men express average levels of CatSper 1 protein and the levels are highly correlated with the progressive motility of the semen samples. This indicates a link between expression and function of CatSper channels and motility of spermatozoa, further proposing an association of these channels in the pathogenesis of asthenozoospermia(5, 29, 30, 31), providing a possible elucidation for the reduced [Ca^{2+}] ion increase in response to Progesterone observed in oligoasthenospermic subjects (10). Overall, these results specify that during spermatogenensis, any variations in CatSper genes or its protein regulation will result in different levels of expression of the functional channel in mature sperm and subsequent modifications in their motility.

Likewise heterozygous mutations of CatSper 1-4 genes reported in (6 out of 30) asthenozoospermic males (which have not been found in normozoospermic men) (32) propose that variations in expression or function of channel may also be due to mutations.

Furthermore, it has recently been shown that CatSper domains were not kerbed to the principal piece of the sperm tail in the non-motile mouse spermatozoa. These domains were also disintegrated.

(16).Tamburrino *et al*., 2015(28) examined CatSper1 localization in sperms obtained from asthenozoospermic and normozoospermic men and found that although CatSper1 was mostly expressed in the principal piece, non-canonical localization of the protein was also observed and this was higher in asthenozoospermic men. This result indicates that, besides a lower percentage of sperm cells expressing CatSper1, patients with low sperm motility may also display a non-functional localization.

Overall all these studies entirely support our findings. Although CatSper genes are present autosomally, they act as a polymodal, chemosensory calcium channel and play a vital role in the regulation of sperm hyperactivation. It seems that Ca^{2+} influx via CatSper and release of intracellular Ca^{2+} stores interact in a complex manner to make fertilization possible. A specific mutation in CatSper gene could be used as a male infertility marker but till date only arbitrary mutation findings have restricted our conclusion though its role in sperm function has been clearly defined.

References

1. Quill TA, Ren D, Clapham DE, Garbers DL. A voltage-gated ion channelexpressed specifically in spermatozoa. Proc Natl Acad Sci USA 2001; 98:12527–12531.
2. Ren D, Navarro B, Perez G, JacksonAC, Hsu S, Shi Q, Tilly JL, Clapham DE. A sperm ion channel required for sperm motility and male fertility. Nature2001; 413:603–609.
3. Kirichok Y, Navarro B, Clapham DE. Whole-cell patch-clamp measurementsof spermatozoa reveal an alkaline-activated Ca2+ channel. Nature 2006; 439:737–740.
4. Smith, J.F., Syritsyna, O., Fellous, M., Serres, C., Mannowetz, N., Kirichok, Y., Lishko, P.V., 2013. Disruption of the principal, progesterone-activated sperm Ca2+ channel in a CatSper2-deficient infertile patient. PNAS 110, 6823–6828.
5. Tamburrino L, Marchiani S,Minetti F, FortiG, MuratoriM, Baldi E. TheCatSpercalcium channel in human sperm: relation with motility and involvement inprogesterone-induced acrosome reaction. Hum Reprod 2014; 29:418–428.
6. Singh AP, Singh R. CatSper channel, sperm function and male fertility. Reproductive BioMedicine Online (2015) **30**, 28–38.
7. Lishko PV, Botchkina IL, Kirichok Y. Progesterone activates the principalCa2+ channel of human sperm. Nature 2011; 471:387–391.
8. Stru¨nker T, Goodwin N, Brenker C, Kashikar ND, Weyand I, Seifert R,Kaupp UB. The CatSper channel mediates progesterone-induced Ca2+influx in human sperm. Nature 2011; 47:382–386.
9. Tesarik J, Mendoza C. Defective function of a nongenomic progesteronereceptor as a sole sperm anomaly in infertile patients. Fertil Steril 1992; 58:793–797.
10. Falsetti C, Baldi E, Krausz C, Casano R, Failli P, Forti G. Decreasedresponsiveness to progesterone of spermatozoa in oligozoospermicpatients. *J Androl* 1993; 14:17–22.
11. Krausz C, Bonaccorsi L, Maggio P, Luconi M, Criscuoli L, Fuzzi B, Pellegrini S,Forti G, Baldi E. Two functional assays of sperm responsiveness toprogesterone and their predictive values in in-vitro fertilization. Hum Reprod 1996; 11:1661–1667.
12. Jacob A, Hurley I, Mandel FS, Hershlag A, Cooper GW, Benoff S. Humansperm non-nuclear progesterone receptor expression is a novel markerfor fertilization outcome. Mol Hum Reprod 1998; 4:533–542.

13. Avenarius MR, Hildebrand MS, Zhang Y, Meyer NC, Smith LL, Kahrizi K,Najmabadi H, Smith RJ. Human male infertility caused by mutations inthe CATSPER1 channel protein. *Am J Hum Genet* 2009; 84:505–510.
14. Qi H, Moran MM, Navarro B, Chong JA, Krapivinsky G, Krapivinsky L, Kirichok Y, Ramsey IS, Quill TA, Clapham DE. All four CatSper ionchannel proteins are required for male fertility and sperm cellhyperactivated motility. Proc Natl Acad Sci USA 2007; 104:1219–1223.
15. Jin J, Jin N, Zheng H, Ro S, Tafolla D, Sanders KM, Yan W. Catsper3 andCatsper4 are essential for sperm hyperactivated motility and malefertility in the mouse. Biol Reprod 2007; 77:37–44.
16. Chung JJ, Shim SH, Everley RA, Gygi SP, Zhuang X, Clapham DE. Structurallydistinct Ca (2+) signaling domains of sperm flagella orchestrate tyrosinephosphorylation and motility. Cell 2014;1 57:808–822.
17. AlasmariW, Barratt CL, Publicover SJ, Whalley KM, Foster E, Kay V, Martinsda Silva S, Oxenham SK. The clinical significance of calcium-signallingpathways mediating human sperm hyperactivation. Hum Reprod 2013a; 28:866–876.
18. Alasmari W, Costello S, Correia J, Oxenham SK, Morris J, Fernandes L,Ramalho-Santos J, Kirkman-Brown J, Michelangeli F, Publicover S *et al*.Ca2+ signals generated by CatSper and Ca2+ stores regulate different behaviors in human sperm. *J Biol Chem* 2013b; 288:6248–6258.
19. Sagare-Patil V, Galvankar M, Satiya M, Bhandari B, Gupta SK, Modi D.Differential concentration and time dependent effects of progesteroneon kinase activity, hyperactivation and acrosome reaction in human spermatozoa. *Int J Androl* 2012; 35:633–644.
20. Sagare-Patil V, Vernekar M, Galvankar M, Modi D. Progesterone utilizes thePI3K-AKT pathway in human spermatozoa to regulate motility and hyperactivation but not acrosome reaction. Mol Cell Endocrinol 2013; 374:82–91.
21. World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen, 5th edn. Cambridge, UK: Cambridge University Press, 2010.
22. Hussain SR, Naqvi H, Mahdi F, Bansal C, Babu SG. KIT proto-oncogene exon 8 deletions at c\don 419 are highly frequent in acute myeloid leukaemia with inv(16) in Indian population. Mol Biotechnol. 2013; 54(2): 461-8.
23. Shukla, K.K., Mahdi, A.A., Rajender, S., 2012. Ion channels in sperm physiology, male fertility and infertility. *J. Androl*. 33, 777–788.
24. Carlson AE, Westenbroek RE, Quill T, Ren D, Clapham DE, Hille B,Garbers DL, Babcock DF. CatSper1 required for evoked Ca2+ entry and control of flagellar function in sperm. Proc Natl Acad Sci USA 2003; 100:14864–14868.
25. AvidanN,TamaryH,DganyO, CattanD, ParienteA, ThulliezM, BorotN, Moati L, Barthelme A, Shalmon L *et al*. CATSPER2, a human autosomalnonsyndromic male infertility gene. *Eur J Hum Genet* 2003; 11:497–502.
26. Hildebrand MS, Avenarius MR, Fellous M, Zhang Y, Meyer NC, Auer J, Serres C, Kahrizi K, Najmabadi H, Beckmann JS *et al*. Genetic male infertility and mutation of CATSPER ion channels. *Eur J Hum Genet* 2010; 18:1178–1184.
27. Jaiswal D, Singh V, Dwivedi US, Trivedi S, Singh K. Chromosome microarrayanalysis: a case report of infertile brothers with CATSPER gene deletion. Gene 2014; 542:263–265.
28. Tamburrino L, Marchiani S, Vicini E, Muciaccia B, Cambi M, Pellegrini S, Forti G, Muratori M, Baldi E. Quantification of CatSper1 expression in human spermatozoa and relation to functional parameters. Human Reproduction, Vol.30, No.7 pp. 1532–1544, 2015
29. Nikpoor P, Mowla SJ, Movahedin M, Ziaee SA, Tiraihi T. CatSper gene expression in postnatal development of mouse testis and in subfertile men with deficient sperm motility. Hum Reprod 2004; 19:124–128.
30. Li HG, Ding XF, Liao AH, Kong XB, Xiong CL. Expression of CatSper familytranscripts in the mouse testis during post-natal development and human ejaculated spermatozoa: relationship to sperm motility. Mol Hum Reprod2007; 13:299–306.
31. Bhilawadikar R, Zaveri K, Mukadam L, Naik S, Kamble K, Modi D, Hinduja I.Levels of Tektin 2 and CatSper 2 in normozoospermic and oligoasthenozoospermic men and its association with motility, fertilization rate, embryoquality and pregnancy rate. *J Assist Reprod* Genet 2013; 30:513–523.
32. Visser L,WesterveldGH, Xie F, van Daalen SK, van der Veen F, LombardiMP, Repping S. A comprehensive gene mutation screen in men with asthenozoospermia. Fertil Steril 2011; 95:1020–1024.e1–9.
33. Mohammadi S, Movahedin M, Mowla SJ. Up-regulation of CatSper genesfamily by selenium. Reprod Biol Endocrinol 2009; 7:126.
34. Mohammadi S, Jalali M, Nikravesh MR, Fazel A, Ebrahimzadeh A, Gholamin M, Sankian M. Effects of vitamin-E treatment on Cat Spergenes expression and sperm quality in the testis of the aging mouse. *Iran J Reprod Med* 2013; 11:989–998.
35. Wang Y, Shi J, Li L, Liu D, Li L, Tang C, Li J. Adverse effects of 2,2′,4,4′-tetrabromodiphenyl ether on semen quality and spermatogenesis inmale mice. Bull Environ Contam Toxicol 2013; 90:51–54.
36. Amini M, Shirinbayan P, Behnam B, Roghani M, Farhoudian A, Joghataei MT, Koruji M. Correlation between expression of CatSper family and spermprofiles in the adult mouse testis following Iranian Kerack abuse. Andrology 2014; 2:386–393.
37. Park EH, Kim do R, Kim HY, Park SK, Chang MS. Panax ginseng induces theexpression of CatSper genes and sperm hyperactivation. *Asian J Androl* 2014; 16:845–851.

****