

Age-dependent Expression of Catspers in Human and Mice Sperm

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Abstract

Catspers are crucial for sperm functioning and its motility. This study brings forth the differential expression of Catsper 1, 2 and 4, irrespective of the species. The expression is studied in ejaculated human and mice testicular sperm with progression of age. Decreased expression of Catsper 1 was found in both the study subjects. In contrast, Catsper 4 expression decreased in human while Catsper 4 did not show any trend in mice. It can be concluded that the Catsper 1 can be used as a marker for aging and male infertility.

Keywords: Aging; Catsper; Sperm motility; Human; Mice; Fertility

Introduction

Calcium ions play an important role in almost every aspect of cell communication and sperm function such as, maturation, motility, and acrosome reaction (AR) in many species [1]. The voltage gated ion channels are the key part in maintaining the intracellular level of calcium in sperm cell. Catspers are novel voltage gated Ca^{2+} ion channel located specifically in the principal piece of the sperm tail, controls the influx of calcium ions into the spermatozoa, important for sperm motility. The gene coding for Ca^{2+} channel are exclusively expressed in the testis [2]. As the age progresses the sperm motility deteriorates and as Catsper is the gene responsible for maintaining the sperm motility it becomes important to further elucidate Catsper expression with the progression of age for which there has been not much studies reported. Previously, we suggested that the calcium level drops down with increase in age [3], we hypothesize that owing to the drop in calcium levels, Catsper expression may also show age dependent variations. The altered expression with age may be an important aspect to study in ageing and male infertility.

Materials and Methods

Study subject

(A) Semen samples of healthy fertile men of the reproductive age groups 21 to 40 years were obtained from Indian Sperm Tech Semen Bank and Research Institute Ahmedabad, India. The protocol was approved by Institutional Ethical Committee (IEC/NU/I/IS/04).

The inclusion criteria for collecting the samples were healthy, fertile men as identified by the pathology laboratory following assessment of sperm motility and viability. Samples of individuals with any kind of reproductive disorders, urinary tract infection (UTI), infertility and genetic disorder were excluded at the time of sample collection.

(B) Total of 24 Healthy male Swiss albino mice of age 12 weeks were procured from Animal Vaccine Institute, Gandhinagar, compelling to CPSCEA guidelines. Animals were grown up to 54 weeks. The average lifespan of mice is 1.5-2 years. This project (IS/PHD/13-1/032) was approved by Institutional Animal Ethics Committee (IAEC). The

Guidance for Care and Use of Animals for Scientific Research (Indian National Science Academy, 2000) was strictly followed. The animals were acclimatized for two months prior to the experiments. The food and water intake of the animals was monitored on daily basis.

Sample collection

(A) In humans the semen samples were collected as per the guidelines mentioned in WHO manual [4]. (B) In case of mice six animals of each age group were sacrificed at regular interval i.e., after they attained their age of 20 weeks, 32 weeks, 48 weeks and 54 weeks for the study using high concentration of anesthesia. The testicular sperm was isolated from testis by gently shearing the tissues and rupturing the tubules, allowing the sperm to float into the medium (0.9% N-Saline). The seminal fluid with the sperm was suspended in N-saline and further used for semen analysis [5].

RNA isolation from sperm

As, the sperm has less concentration of RNA, two semen samples were pooled so that the total sperm count is 10^{12} instead of 10^6 . Total RNA was isolated by using trizol (Sigma-Aldrich) from ejaculated human sperm and mice testicular sperm and then purity and integrity of the extracted RNA was measured by optical density (260/280-nm ratio) using nanodrop (Jenway, Genova nano; no. 53301, UK) and qualitatively analyzed by using gel electrophoresis on 1% agarose gel. The methods indicated well preserved integrity of the extracted RNA with little or no protein contamination.

Primer designing, cDNA synthesis and polymerase chain reaction (PCR)

Primers for Catsper 1 and 4 for human and 1 and 2 for mice were designed using standard softwares and used for expression studies (Table 1). Polymerase chain reaction (PCR) was performed with 25 μ L reaction mixture of 3 μ L of RT product, 12.5 μ L of PCR master mix (Emerald master mix, Clontech, Korea) which is the composition of 1.5 mmol/L $MgCl_2$, 2.5 U Taq DNA polymerase, 100 μ mol/L dNTP, 0.1 μ mol/L primer, and 1 \times Taq DNA polymerase magnesium-free buffer and as per the protocol mentioned in the manufacturers instruction manual.

HUMAN			
Gene name	Genebank accession no.	Sequence	Final amplification (base pairs)
Catsper1	NM_053054.3	F: TCATTCTCTGTGGGGTCCT	221
		R: AAGTGCTCTGGACGTAGTCG	
Catsper4	NM_198137.1	F: CTACACTCTCAGGGCGCTTC	365
		R: TGACGAACAGGTTGATGCCA	
MICE			
Catsper1	NM_139301.2	F: TGCAGCATTTGCGTGAGTTC	369
		R: RAGCGATAGAACCGCGTACTG	
Catsper2	NM_153075.3	F: AATGGGGCACATCACACT	239
		R: GGAGGTGGCAGATTGGTTCA	

Table 1: Detail of primers for human and mice Catsper.

Expression analysis

To check the PCR end product, electrophoresis was performed. Product were electrophoresed on a 1.5% ethidium bromide-stained agarose gel and saved as digital images taken in a gel doc system. The densitometry of the amplification was performed by using software – TotalLab 1.0 software.

Statistical analysis

Data were expressed as mean ± SD and the graphical representation of the gel images was done by using Graph pad Prism, version 6. To determine the significance of the values obtained; t-test and one way Anova was done in human and mice values respectively considering the number of groups each study subject has.

Results

A total of 102 human semen samples collected which were segregated into 21-30 and 31-40. Mice sperm samples were further divided into four age groups i.e., 20 weeks, 32 weeks, 48 weeks and 54 weeks. For the semen collection, the samples were collected from May Flower Hospital and Indian Spermtech Bank, Ahmedabad. The inclusion and exclusion criteria were followed as mentioned previously.

Both the mice and human sperms were ascertained for their fertility state and were found to be in the fertile range as per the WHO guidelines [4] (Data not shown).

Expression analysis of Catper1 and 4 for humans and Catsper 1 and 2 for mice were performed. Differential expression patterns were observed and further densitometric analysis was performed to quantify the band intensity. Catsper 1 in human and mice showed a decreasing trend with the progression of age indicating reduced sperm motility in aging human males and in aging male mice.

While Catsper 4 showed an increasing trend in humans but with a faint expression of bands. In contrast, Catsper 2 showed a non-significant trend in mice with progression of age (Figure 1). The Catsper 3 gene expression studies were performed in both the study

subjects. However, Catsper 3 gene expression was not obtained in both the study subjects.

The significant interference of Catsper 1, 2, 4 and other genes of the reproductive system in controlling the expression of Catsper3 should be investigated in future.

Discussion

The age slab of 21-40 was considered for this study as it's the highly reproductive age group. But as reported from our lab earlier [3], the mitochondrial stress levels were significantly high and the reduced semen parameters (mainly motility) were observed in the middle age of 31-35. So, the Catsper gene expression pattern would give the clear picture of the possible cause of the reduced sperm motility with the progression of age. The limitation with the human semen samples was the availability of semen samples beyond the age of 40 and the number of samples per age group. The gene expression studies in age slab of 12 weeks to 54 weeks (representing infant stage, puberty stage, adult stage and old age) would show significant role of Catsper in the aging mice.

Our results have revealed that Catsper 1 expression in both human and mice was found to be decreased with the progression. Ren et al. [2] described cloning and characterization of Catsper1, which has a key role in controlling sperm motility. Nikpoor et al. [6] reported a significant reduction in the level of Catsper gene expression (up to 3.5-fold difference) among patients which lack sperm motility as compared with patients whose infertility cannot be described.

In the present study, Catsper 2 did not show any trend in the mouse sperm. While Nikpoor et al. [6] reported lowered Catsper gene expression levels and deficient sperm motility in a proportion of subfertile patients.

Identification of Catsper 2, with several shared features suggested the possibility of the involvement of other factors in sperm motility [7].

In our study, the reduced Catsper 4 gene expression in human might be an age dependent event or could be attributed to the increased stress levels in men.

Catsper 1 could be explored as a target for contraceptive medicine and infertility treatment control sperm motility. The exact mode of action and the crosstalk involved needs to be further investigated. It could be concluded that.

The quantitative analysis of the gene expression should be studied further with Real Time-PCR for better understanding.

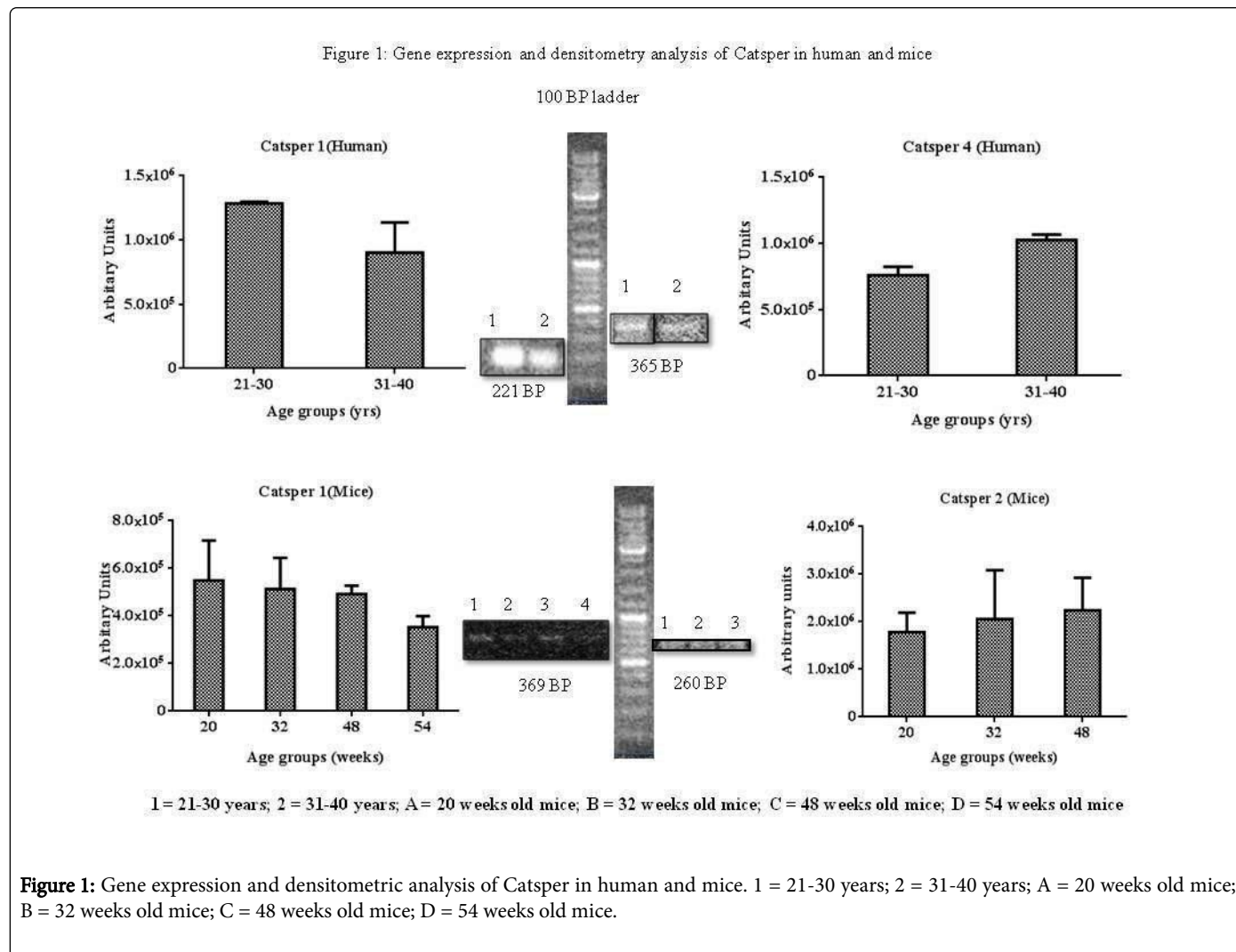


Figure 1: Gene expression and densitometric analysis of Catsper in human and mice. 1 = 21-30 years; 2 = 31-40 years; A = 20 weeks old mice; B = 32 weeks old mice; C = 48 weeks old mice; D = 54 weeks old mice.

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