Full Length Research Paper

CatSper ion channels: Bioinformatics analysis in *Homo sapiens*

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Due to the availability of huge amount of molecular biology data, our main focus was to determine the protein structures, functions and their role in different molecular pathways. The 3-D structure prediction of protein is important in medicine and biotechnology. Molecular docking not only finds the interaction between proteins but also the accurate models of energy of these interacting proteins and helps in further designing of the better drug for that particular protein. The drug targeting is either to inhibit, restore or for the modification of the protein structure. CatSper protein family is calcium ion permeable channels, located in the plasma membrane of sperm tail. It contains a conserved domain of six trans-membrane helices in their protein sequence. These four CatSper proteins (1 to 4) assemble and form tetramer, calcium selective channel. It has been found that all members of CatSper protein family (1-4) have a role in hyperactivation in sperm and fertilization processes. As a result of deletion of certain regions (bps) containing these genes along with some other genes, male infertility occurs. We have predicted and analyzed the 3D structures of all members of CatSper protein family in this article. Docking of predicted 3D structures of CatSper protein family, with calcium ion was also performed to verify their interactions.

Key words: CatSper, bioinformatics analysis, infertility, cation channel.

INTRODUCTION

Spermatozoa initially have the potential for motility in the epididymis. They gain the capacity in the female reproductive tract (Yanagimachi, 1994), where they get hyperactivated motility and other attributes facilitating fertilization (Eisenbach and Giojalas, 2006). Hyperactivated sperm tail motion appears different under different conditions but basically, it changes from symmetric and low amplitude to asymmetric and large amplitude (Cooke and Saunders, 2002; Yue et al. 2003; Suarez and Ho, 2003). In order for fertilization, hyperactivation is needed, providing the force to the sperm cell so that it can move from the oviductal reservoir and enter the umulus and zona pellucida which surrounds the egg (Yanagimachi, 1994; Suarez and Ho, 2003; Suarez and Pacey, 2006).

Spermatazoa contain calcium ion permeable protein channels called the CatSper family (CatSper1–4) localized in the plasma membrane of the tail of the sperm. CatSper channels are named after the first putative cation channels that are expressed specifically in the membrane of the sperm. Each CatSper’s protein contain six-transmembrane (6TM1) domain for calcium ions and seem specific for sperm cells and a coiled-coil protein-protein interaction domains in their intra cellular C-terminal tail, suggesting that they may interact directly or indirectly to form a functional tetramer (Lobley et al., 2003). After the studies of knock out mice models of this family, it has been seen that the sperms have the normal morphology with the ability of capacitation and acrosome reaction but lost the hyperactivation phenomena. That is why CatSper1 and CatSper2 are targeted in infertile males as they are essential for the hyperactivation of sperm cells during fertilization (Carlson et al., 2003; Quill et al., 2001; Ren et al., 2001; Quill et al., 2003). The ion transport domain has sequence identity ranging between 21.6 and 26.5% among CatSper members (Lobley et al., 2003). All CatSpers have shown close relatedness to the 6TM sodium channel (NaVBP) in bacteria. The S4 transmembrane segment is present in all the members of CatSper family. In this segment, positively charged amino...
acids are interspersed between every three amino acids. The only member, CatSper1 contains a remarkable abundance of histidine residues in the amino terminus.

All CatSper family has shown functionality in male sperm motility. CatSper1 was identified during the sequences homology searches to the voltage-gated Ca²⁺-selective channels (Ren et al., 2001). The mouse mutant model for CatSper1 protein produced an infertile mouse confirming that absence of CatSper1 affects the sperm motility. In the CatSper1 mutant (Lys180->LysfsX8 and Asp317->MetfsX18), all transmembrane domains and P-loop were lacking, forming a truncated protein that abolished the CatSper1 activity (Avenarius et al., 2009). The CatSper2 is being named as the human autosomal nonsyndromic male infertility gene due to its important role in the sperm cells (Ho et al., 2001). During the study of a French family suffering from asthenoteratozoospermia (poor motility) and nonsyndromic deafness along with congenital dyserythropoietic anemia type 1 (CDA1), there was CDA1 mutation and deletion of the proximal copy of 106 kb tandem repeat at chromosome 15q15. This location also encodes four other genes named KIAA0377, CKMT1, STRC and CATSPER2. The observed nonsyndromic deafness and male infertility phenotypes may be due to the lack of functional stereocilin and CatSper2 because STRC are expressed in the inner ear and both testis, while CATSPER2 is a cation channel of sperm. The involvement of CatSper2 in asthenoteratozoospermia is the first human autosomal gene defect to be associated with nonsyndromic male infertility (Verpy et al., 2001; Avidan et al., 2003; Zhang et al., 2007; Clapham and Garbers, 2005). Apart from the topology and sequence similarity, mutant mice model of CatSper3 and CatSper4 also confirmed their role in the sperm motility during fertilization, leading to the infertility of male.

As a result of their important role in male infertility, their structures were predicted using bioinformatics tools and software’s that will help to understand their interactomics and further drug targeting on them. Protein-protein interaction has functional consequences, that is, signal transduction, signal transfusion, etc. So, docking of CatSper proteins (1-4) with calcium ions is performed for the verification of their interactions and connectivity.

**MATERIALS AND METHODS**

**Sequences alignment**

Using ClustalW multiple sequence alignment program, sequence alignment of CatSper protein family was performed and dendogram was constructed.

**3D structure prediction**

For the 3D structure prediction, the CatSper1-4, the proteins sequences were submitted to the online CPHmodels-3.0, I-TESSER, 3Djigsaw, Modeller, SWISS MODEL (Arnold et al. 2006) and SAM T08 servers.

**3D structure analysis**

Bioinformatic tools were used to check the validity of each structure in a focused manner. Pdb files for each member of CatSper family were further analyzed for model quality using protparam, proscale, RAMPAGE, WHATIF server. Ramachadran plots were drawn for all of them, respectively.

**Protein-protein docking using Hex 6.0**

Pdb files of CatSper family were used as a receptor and calcium as a ligand in HEX 6.0 docking server along with docking parameters.

**RESULTS**

**Sequences alignment**

CatSper member’s ion transport domain has sequence identity ranging between 21.6 and 26.5% among CatSper members (Lobley et al., 2003). Dendogram showed that CatSper1 was more closely related to CatSper3 than CatSper2 and CatSper4 (Figure 1).

**3D structure prediction and analysis**

The best models were selected on the basis of minimum Z-score and RAMPAGE results (He et al., 2010; Benkert et al., 2008). ViewerLite has been used for structures
visualization (Figures 2A, B, C and D). All structures showed good Z-score but the number of their residue in favored regions varied. It is concluded that CatSper family are stable and have good half life (Table 1).

**Protein-protein docking using Hex 6.0:**
The docking results of CatSper family with calcium ion (Figure 3A, B, C and D) showed good E-value (Table 1).

**DISCUSSION**

*In silico* analysis of protein sequences using bioinformatics tools, has lots of advantages as compared to the wet lab analysis. *In silico* analysis is always less time consuming, easy to operate and obviously cost effective. There has been no specific identification of the missense mutations in the CatSper protein family. The infertility of male is because of the deletion in the region of chromosome 15q15 with the genes including CatSper2.
Table 1. Bioinformatics analysis of structure and sequence of CatSper protein family.

<table>
<thead>
<tr>
<th>Property</th>
<th>CatSper1</th>
<th>CatSper2</th>
<th>CatSper3</th>
<th>CatSper4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of amino acids</td>
<td>780</td>
<td>530</td>
<td>398</td>
<td>472</td>
</tr>
<tr>
<td>Z-score</td>
<td>0.411</td>
<td>0.748</td>
<td>0.699</td>
<td>0.465</td>
</tr>
<tr>
<td>Energy-value</td>
<td>-49.39</td>
<td>-51.55</td>
<td>-70.59</td>
<td>-52.48</td>
</tr>
<tr>
<td>Half life (hour)</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Instability index</td>
<td>45.60</td>
<td>53.60</td>
<td>42.25</td>
<td>52.84</td>
</tr>
<tr>
<td>Theoretical PI</td>
<td>7.22</td>
<td>6.82</td>
<td>5.88</td>
<td>5.15</td>
</tr>
<tr>
<td>Number of atoms</td>
<td>12434</td>
<td>8855</td>
<td>6563</td>
<td>7639</td>
</tr>
<tr>
<td>Extinction co-efficient</td>
<td>85845</td>
<td>89630</td>
<td>44600</td>
<td>70275</td>
</tr>
<tr>
<td>Aliphatic Index</td>
<td>70.91</td>
<td>112.51</td>
<td>101.61</td>
<td>106.57</td>
</tr>
<tr>
<td>Formula</td>
<td>C_{4032}H_{6046}N_{1196}O_{1144}S_{16}</td>
<td>C_{2834}H_{4479}N_{759}O_{785}S_{18}</td>
<td>C_{6128}H_{3296}N_{529}O_{588}S_{23}</td>
<td>C_{6445}H_{3830}N_{642}O_{702}S_{20}</td>
</tr>
<tr>
<td>Grand average of hydropathicity</td>
<td>-0.615</td>
<td>0.072</td>
<td>0.162</td>
<td>0.059</td>
</tr>
<tr>
<td>Disorder region prediction</td>
<td>1-416, 682 - 725, 752 - 759</td>
<td>1-100 and 340-530</td>
<td>1 - 19, 386 - 398</td>
<td>2 - 55, 318 - 343, 358 - 365, 412 - 470</td>
</tr>
<tr>
<td>Molecular weight (Da)</td>
<td>90090.6</td>
<td>62041.3</td>
<td>46422.1</td>
<td>54092.3</td>
</tr>
<tr>
<td>Residue in favored region (%)</td>
<td>93.5</td>
<td>95.4</td>
<td>84.1</td>
<td>92.1</td>
</tr>
<tr>
<td>Residue in allowed region (%)</td>
<td>5.2</td>
<td>3.0</td>
<td>12.7</td>
<td>4.6</td>
</tr>
</tbody>
</table>

and STRC involved in the deafness and infertility syndrome. Analyzed data of CatSper protein family revealed that CatSper2, along with STRC, has the most important role in the rare genetic diseases like male infertility and non-syndromic deafness. The 3D structure prediction had shown that the ion transport domain of CatSper family, if mutated can really affect the functionality of these proteins. The introduction of the helix breaker amino acids in the domain can definitely disturb the 3D structure of the CatSper family. The disorder region identification will help to know the intensity of mutation in protein, that is, if mutation lies in the predicted disordered region, there is a chance of malfunctioning of this protein family. Docking of CatSper protein family with calcium ion has been performed for structure validation.

In future, Pocket identification and biomolecular docking of these predicted structures will help to know interactomics of the CatSper protein family with other proteins in different pathways. Biomolecular drug targets can be designed on the basis of the 3D structures, binding pockets and binding sites of the CatSper family.

Further studies should be carried out for the better understanding of the CatSper protein family interaction with ligand (Ca ion or some other proteins) and their role in other pathways along with the fertilization pathway.

**Recommendation**

CatSper family can be targeted for non-hormonal contraceptives like for high blood pressure and migraine. In future, further research should be carried out in the sperm motility and fertilization pathways where it has a
key role to play. Computer based protein docking and wet lab-based techniques should be used in developing new bioactive drugs for targeting infertility.

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REFERENCES


Figure 3. Docking of CatSper1 (A), CatSper2 (B), CatSper3 (C) and CatSper4 (D) with calcium ion.


