Mutation in Exon 6 of the Human VDAC3 (Porin Type 3) Gene in the Sperm with Low Motility

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Summary

Porins (Voltage dependent anion channels = VDACs), pore-forming protein abundant in the outer mitochondrial membrane, has been suggested play a role in the regulation of the ATP transport for sperm motility. In this study we analyzed exon 6 of the human VDAC3 gene in sperm with low motility from 30 asthenozoospermic patient compared to normal sperm obtained from 5 fertile man. Sperm samples with normal motility and low motility were collected after sperm preparation by swim-up methods. Iso-lated sperm DNA was amplified with specific primer for exon 6 of hVDAC3 gene, and subsequently sequenced. We found that there was an A to C mutation in 8 patients and A to G mutation in one patient, that leads to amino acid substitution from isoleucine to leucine and from lysine to glutamic acid at position 131 and 173 of the VDAC3 polypeptide.

Introduction

Asthenozoospermic condition in which sperm motility less than 50 % of total sperm, might caused by infection in male reproductive tract, defect in sperm maturation at epididimis, morphological and biochemical damages in aksonem of the sperm tail or failure in mitochondria function. Porin also known as Voltage Dependent Anion Channel (VDAC) is pore-form 30-35 kDa protein located in outer mitochondrial membrane of eukaryota. There are three subtypes of VDAC protein i.e VDAC1, VDAC2 and VDAC3 which now have been identified, each of them is highly con-
served in human, rat and mouse\(^3\). From “knock-out mouse” study that was conducted by Sampson et al.\(^4\) which deleted the last four exon of mouse VDAC3 gene, it has been reported that mutant male mice are healthier but infertile. The mutant mice have low sperm motility compared with the wild type mice, but they have a normal amount of sperm. It is suggested that porin play an important role in sperm movement. From preliminary functional study using anti-VDAC2 polyclonal antibody it is demonstrated that these antibodies can reduce the sperm motility until 10% compared with control group, but it is statistically not significant\(^5\). In this study, exon 6 of human VDAC3 gene was analyzed on the sperm with low motility from asthenozoospermia patient and normally motile sperm from fertile man.

**Material and Methods**

1. **Samples collection**

   The sperms with low motility were collected from bottom-middle layer of swim-up method in Kramer’s medium from asthenozoospermic semen. Sperms with quick progressive movement are collected from upper layer of swim-up methods from normozoospermic semen which were obtained from fertile men.

2. **Analysis of exon 6 human VDAC3 gene**

   After the collection of sample, total DNA from sperm was isolated with Wizard Purification Kit (Promega, USA). The fragment of human VDAC3 gene was amplified using PCR core kit (Promega) with primer 5' ACATGTGTGTGCAGCTGGAA '3 and 5' GAAGAGTGGAAGTTGAG '3. Amplification was carried out for 35 cycles and annealing temperature at 55 °C for 45 seconds. Amplified fragment of the human VDAC3 gene was then sequenced subsequently using big dye terminator mix through the ABI sequencer.

**Results**

Amplified DNA fragment of exon 6 human VDAC3 gene in the size of 230 bp from sperm with low motility that was obtained from 8 asthenozoospermia patients who have mutation in VDAC3 gene and from normal motility sperm that obtained from fertile man as well as amplified DNA fragment with â-actin as positive control are shown (fig. 1)

In this study we found that among 30 asthenozoospermia patients, 8 of them showed nucleotide substitution from A to C in exon 6 of VDAC3 gene and one other showed substitution from A to G. Table 1 showed the type of nucleotide, codon as well as amino acid substitution and position of VDAC3 polypeptide of those 8 asthenozoospermia patients. Figure 2 showed
Figure 1. PCR product of 230 bp from exon 6 of human VDAC3 gene were detected from sperm with normal motility (N3) as well as from sperm with low motility obtained from 8 asthenozoospermia patients (A4, A5, A6, A7, A10, A13, A21, and A34). PCR product of 661 bp from β-actin primer from normozoospermic sperm was also found. M = Marker 100 bp.

Figure 2. Sequences of amplified DNA fragment of exon 6 human VDAC3 gene from sperm with normal motility that was obtained from fertile man (A) and from sperm with low motility that was obtained from asthenozoospermia patient (B). The substitution of amino acid at isoleucine (ATA) to leucine (CTA) is shown in square.

Figure 3. Sequences of amplified DNA fragment of exon 6 human VDAC3 gene from sperm with normal motility that was obtained from fertile man (A) and from sperm with low motility that was obtained from asthenozoospermia patient (B). The substitution of amino acid at lysine (AAG) to glutamic acid (GAG) is shown in square.
that there is an A to C substitution which makes a substitution of ATA (Ile) to CTA (Leu). We compared also data sequence asthenozoospermic sperm with gene bank for exon 6 of *Homo sapiens* VDAC3 gene. Nucleotide substitution from A to G makes a substitution of AAG (Lys) to GAG (Glu) (Fig.3).

The substitution of amino acid in the position 131 and 173 of human VDAC3 polypeptide is suggested will make alteration of the primary structure or the charge of channel in VDAC3 protein. This alteration might influence the ATP flux from mitochondria to axoneme, subsequently energy consumption for sperm movement. It has been known that mammalian VDACs regulate of ATP transport from mitochondria to cytoplasm⁶,⁷.

### Conclusion

In this study we found two kind of nucleotide substitution in exon 6 of human VDAC3 gene in sperm with low motility from asthenozoospermia patients, which make consequence a substitution of amino acids in VDAC3 polypeptide.

### References

4. Sampson M J, Decker WK, Beaudet AL, Ruitenbeek W, Armstrong D,

<table>
<thead>
<tr>
<th>Sample</th>
<th>Normal motility</th>
<th>Low motility</th>
<th>Nucleotide substitution</th>
<th>Codon substitution</th>
<th>Amino acid substitution</th>
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<tr>
<td>A4</td>
<td>0</td>
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<td>ATA → CTA</td>
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<tr>
<td>A5</td>
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<td>A → C</td>
<td>ATA → CTA</td>
<td>Ile 131 Leu</td>
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<tr>
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<td>45</td>
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<tr>
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<td>37</td>
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<td>ATA → CTA</td>
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<td>27</td>
<td>A → C</td>
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<td>Ile 131 Leu</td>
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Table 1. The percentage of sperm motility as well as the type of nucleotide, codon substitution and position of VDAC3 polypeptide of 8 asthenozoosperma patient.

