·Original Article·

Expression of a novel dipeptidyl peptidase 8 (*DPP8*) transcript variant, *DPP8-v3*, in human testis

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Abstract

Aim: To investigate the role of a novel dipeptidyl peptidase 8 transcript variant (*DPP8-v3*) gene in testis development and/or spermatogenesis. **Methods:** A human testis cDNA microarray was hybridized with mRNA of human adult and fetal testes. Differentially expressed clones were sequenced and characterized and their expression was analyzed by real-time reverse transcription polymerase chain reaction (RT-PCR) and Southern-blot analysis. **Results:** A new transcript variant of the human dipeptidyl peptidase (*DPP8*), exhibiting a 5-fold higher expression level in human adult than that in fetal testes, was cloned and was named *DPP8* variant 3 (*DPP8-v3*). The full-length sequence of *DPP8-v3* was 3,030 bp, encoding a protein of 898 amino acids. **Conclusion:** *DPP8-v3* is a novel human *DPP8* transcript variant highly expressed in the adult testis. Similar to *DPPIV*, *DPP8-v3* may play a key role in the immunoregulation of testes and accordingly may influence spermatogenesis and male fertility. *(Asian J Androl 2005 Sep; 7: 245–255)*

1   Introduction

Testis development and/or spermatogenesis is a highly ordered process that is strictly regulated by various factors therein the orderly expression of genes is the intrinsic factor which may directly influence the process of spermatogenesis. In addition, androgen is the major player in the extrinsic regulation of germ cell development [1, 2]. Over the past decade it has been accepted that, directly or indirectly, immunology intrudes into nearly every aspect of mammalian reproduction [3]. It has been confirmed that testis development/spermatoge-

nesis requires the immune balance in the local environment of testis. On one hand, there is a perfect immune resistant system to protect the environment of spermatogenesis by diversiform immunocytes, such as macrophages and T-cells, in the testis. There is also a complicated network of cytokine in the testis. On the other hand, similar to other important organs, such as the brain and eye, the testis is an immune privilege organ – the immunoreaction against antigen is on the low level and inflammation is weak, which can avoid severe phlogistic harm on tissue structure. Especially, the mild immunoreaction prevents the formation of antibodies to spermatozoa in adult testes [4]. Therefore, there must be a complicated immune network in the testis to maintain its structure and function.

Dipeptidyl peptidase 8 (*DPP8*), which has been proven to be a member of the prolyl oligopeptidase S9B family, is probably a new candidate participating in the

regulation of immune balance in testis. *DPP8* was first cloned by Abbott *et al.* [5], who described it as a human postproline dipeptidyl aminopeptidase homologous to the dipeptidyl peptidase IV (*DPPIV*). A large number of studies have been done on the structure, biologic characteristics and potential functions of *DPPIV*, which have shown that it can participate in different biologic processes, such as chemokine biology, type-II diabetes and tumor biology [6, 7]. The critical role of *DPPIV* in immunoregulation has attracted researchers' attention especially. Previous studies all revealed that *DPPIV* was an important participant in immune regulation, especially in T-lymphocyte activation [5, 8]. Abbott *et al.* [5] have described the similarities between *DPP8* and *DPPIV* in tissue expression pattern and substrates, suggesting that *DPP8* may also have a potential role in T-cell activation and immune function. Three transcripts of the *DPP8* gene, which can be spliced by different mechanisms, have been identified. Northern-blot analysis has shown that although differentially expressed in different human tissues, it is only in the testis that the three transcripts are all detected, which implicates that *DPP8* plays a key role in the immune regulation of testes [5].

In a past study, we constructed cDNA microarrays from the human testis large insert cDNA library, containing 9216 genes, together with several housekeeping genes. On the basis of the cDNA microarray, we compared the expression of genes in the fetal and adult human testis at a high throughput [9]. A highly expressed novel human *DPP8* transcript variant, *DPP8-v3*, was found in adult testes. In this study, the characteristics and tissue distribution of this novel *DPP8* transcript variant, its expression in different developmental stages of testes and its possible correlation with testis development/spermatogenesis are discussed.

2　Materials and methods

2.1 Samples

Informed consent was received from either the participants or their kin and the approval to conduct this research was granted by the ethics committee of Nanjing Medical University before sample collection. Human testes from adult males (37–43 years old) were obtained from the Body Donor Center (Nanjing Medical University, Nanjing, China) and fetal testes from accidentally aborted 6-month-old fetuses (Clinical Reproductive Center, Nanjing Medical University, Nanjing, China).

2.2 Preparation of human testis cDNA microarray

The testis cDNA microarray was constructed as previously described [9]. Briefly, this microarray contained 9216 cDNA clones derived from a human testis 5'-STRETCH PLUS cDNA library (Clontech, Palo Alto, CA, USA; source of insert cDNA came from 25 Caucasians, aged from 20 to 65 years). The inserts were amplified by PCR using 5'-CCATTGTGTTGGTACCCGGG AATTCG-3' as a forward primer and 5'-ATAAGCTTGC TCGAGTCTAGAGTCGAC-3' as a reverse primer. In each 100 μL PCR reaction buffer (50 mmol/L KCl, 10 mmol/L Tris-HCl, pH8.4), 2 mmol/L $MgCl_2$, 0.15 mmol/L dNTPs, 4U Taq polymerase, 25 pmol of each primer and 20 ng of plasmid DNA were added. The reaction started with an initial denaturation at 95 °C for 4 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 3 min and additional extension of 10 min. PCR products were spotted on the membrane to make human testis cDNA microarray.

2.3 Screening of genes differentially expressed in fetal and adult testis

Total RNA of adult and fetal testes were extracted separately according to Trizol RNA isolation protocol (Gibco BRL, Grand Island, NY, USA) and quantified with a UV spectrometer. The Poly(A)+ mRNA was purified using an affinity column filled with poly (dT) resin (Qiagen, Hilden, Germany). The probes were prepared by incorporation of ^{33}P-labeled dATP in a reverse transcription reaction using 2 μL purified mRNA as the template, an oligo (dT) as the primer and moloney murine leukemin (M-MLV) reverse transcriptase. Each labeling reaction was carried out with 200 μCi [d-^{33}P]ATP following the manufacturer's instruction (NEN Life Science, Boston, MA, USA). Then the human testis cDNA microarray was hybridized with the ^{33}P-labeled human fetal and adult testis cDNA probes, respectively. The hybridization intensity of corresponding dots in adult and fetus were compared. If the difference of spot intensity in adult and fetus was more than 3-fold, higher or lower, this clone was considered as differentially expressed.

All differentially expressed cDNA plasmids were amplified, extracted and purified in mini-preps (QIAprep Spin Miniprep kit, Qiagen, Hilden, Germany). The full insert lengths were sequenced with an ABI auto-sequencer (model no. 377) at Huada Gene Center (Beijing,

China). The sequences were then blasted in GenBank (http://www.ncbi.nlm.nih.gov) by using the software Blast (http://www.ncbi.nlm.nih.gov/blast) to determine the homology among various species and locations in chromosomes. The nucleic and deduced amino acid sequences were also analyzed by using Gene Runner and SMART software (http://smart.embl-heidelberg.de/) [10].

2.4 Tissue distribution of DPP8-v3 gene

After sequence identification and analysis, a gene highly expressed in adult testis, named *DPP8-v3*, was identified. The expression profile of this gene was determined by PCR followed by Southern-blot analysis. Multiple tissue cDNA panels were from commercial human multiple tissue cDNA (MTC) panel I and II kit (Cat#K1420-1 and K1421-1, Clontech), including 16 kinds of human tissues (heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon and leukocytes). Primers were synthesized at BioAsia (Shanghai, China): P_1 5'-GAGTCAACCACCGTTCAC -3', P_2 5'- CTGTTTCC ATTGCTGCTG -3'. P_1, the upstream primer, was located at a specific 5' region of *DPP8-v3* and P_2, the downstream primer, was in the common region of *DPP8-v3* and its homologous genes. β-actin was used as the positive control of the cDNA templates, its upstream primer was 5'- CGGTTGGCCTTGGGGTTCAGGGGG -3' and downstream primer was 5'-ATCGTGGGGG CGCCCCAGGCACCA -3'. The PCR mixture (20 μL) contained 10 × PCR buffer 2 μL, 25 mmol/L Mg^{2+} 1.5 μL, 20 mmol/L dNTPs 0.15 μL, 5 U/μL Tag DNA polymerase 0.15 μL, 12.2 μL distilled water, 1 μL of each primer (5 pmol/μL), cDNA sample 2 μL. PCR was performed with an initial denaturation temperature at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 1 min and an additional extension at 72 °C for 7 min. PCR products were separated by electrophoresis on 1 % agarose gels and then transferred to Hybond TM-N⁺ nylon membranes (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK).

The *DPP8-v3* cDNA probe was labeled with the same primers used for the PCR. The template was plasmid pTrip1E2 × with *DPP8-v3* cDNA insert and Digoxigenin (Dig)-labeled dNTPs were used. Hybridization was performed according to the instruction manual of DIG High Prime DNA Labeling and Detection Starter Kit II (Cat. No.1585614, Roche Molecular Biochemicals, Indianapolis,

IN, USA). The hybridization filter was pre-hybridized for 30 min at 48 °C in DIG Easy Hyb working solution, which was followed by 4 h hybridization with a denatured Dig-labeled *DPP8-v3* probe. Then the filter was washed twice at room temperature in 2 × SSC–0.1 % SDS (5 min each), twice at 68 °C in 0.5 × SSC–0.1 % SDS (15 min each). Hybridization result was visualized with immunological detection through chemiluminescent substrate for alkaline phosphatase (CSPD).

2.5 Quantitative analysis of DPP8-v3 mRNA in different developmental stages of male testes

To compare the differential expression of the *DPP8-v3* gene in different development stages of the testis, fluorescent RT-PCR was performed. cDNA of male testes included adult (deceased people aged 37–43 years) ($n = 3$) and fetal (accidentally aborted 6-month-old) testes' cDNA ($n = 3$). Primers were the same as we used in the experiment of tissue distribution of *DPP8-v3* gene. Each reaction contained the cDNA template, 1 × SYBR Green PCR Master Mix (Applied Biosystems, Foster city, CA, USA) and 250 nmol/L of forward and reverse primers. The RT-PCR was performed using ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, USA) following thermal cycling conditions: 5 min at 94 °C, 30 s at 94 °C, 30 s at 55 °C and 1 min at 72 °C with 40 cycles. β-actin was run in parallel with the same template as positive control under the same procedure. Statistically significant differences among the groups were determined by Stata 7.0 software (UCLA Academic Technology, Los Angeles, CA, USA). $P < 0.05$ was considered statistically significant.

3 Results

3.1 Hybridization of human testis cDNA microarray identified DPP8-v3 gene

After hybridization and data analysis, genes differentially expressed in human adult and fetal testes were considered as testis development and/or spermatogenesis-related. A clone, named *DPP8-v3*, was identified. This gene was highly expressed in adult testes compared with fetal testes. The hybridization signal intensities were approximately 5.52-fold higher in adult (40.15) than those in fetal testes (6.16) (Figure 1).

3.2 Sequence identification and analysis of DPP8-v3 gene

Adult testis   **Fetal testis**
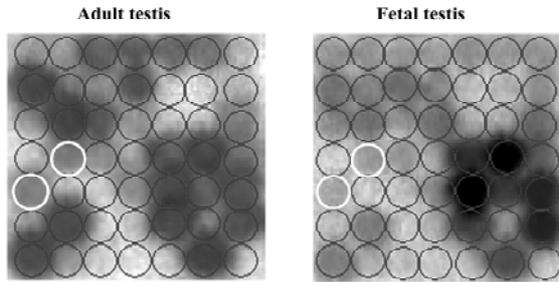
Figure 1. Partial cDNA hybridization images showing differential expression of the *DPP8-v3* gene in adult and fetal testes. White rings are duplicates of *DPP8-v3* cDNA. The hybridization intensity was 5.52-fold higher in adult (40.15) than that in fetal testes (6.16).

The full-length cDNA of *DPP8-v3* gene is 3,030 bp and it contains an open reading frame (161–2857 nt) that encodes a protein with 898 amino acids. The methionine at 161–163 nt was the initiation site because there was an upstream stop code TGA at 158–160 nt (Figure 2). The cDNA sequence of this clone was deposited with GenBank (accession number AY354202).

Blast search revealed that *DPP8-v3* was highly homologous to three other genes, all related to the homo sapiens *DPP8* gene family identified in humans: *DPP8* variant 1 (NM_130434), variant 2 (NM_017743) and variant 4 (NM_197961). Similar to *DPP8-v3*, these three transcripts are also localized in human chromosome 15

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GAGTCAACCACCGTTCACTGGGCGCCCCTTGAGCTCTGGGCGCTGGCCTCCTGGCTTCCA    60

CGCTTTGATGGTGAGGAAAGGAAAGATTCCTGTGAGAAGAGCAGGATGAGCAGAGGGATT   120

CTATGCTTGAAGTCGAGTCACTTGAAAAAGATCTCTTTGAATGTGGAAGAGATCTGAGCA   180
                                         M  W  K  R  S  E  Q

GATGAAAATAAAATCAGGAAAATGCAACATGGCAGCAGCAATGGAAACAGAACAGCTGGG   240
 M  K  I  K  S  G  K  C  N  M  A  A  A  M  E  T  E  Q  L  G

TGTTGAGATATTTGAAACTGCGGACTGTGAGGAGAATATTGAATCACAGGATCGGCCTAA   300
 V  E  I  F  E  T  A  D  C  E  E  N  I  E  S  Q  D  R  P  K

ATTGGAGCCTTTTTATGTTGAGCGGTATTCCTGGAGTCAGCTTAAAAAGCTGCTTGCCGA   360
 L  E  P  F  Y  V  E  R  Y  S  W  S  Q  L  K  K  L  L  A  D

TACCAGAAAATATCATGGCTACATGATGGCTAAGGCACCACATGATTTCATGTTTGTGAA   420
 T  R  K  Y  H  G  Y  M  M  A  K  A  P  H  D  F  M  F  V  K

GAGGAATGATCCAGATGGACCTCATTCAGACAGAATCTATTACCTTGCCATGTCTGGTGA   480
 R  N  D  P  D  G  P  H  S  D  R  I  Y  Y  L  A  M  S  G  E

GAACAGAGAAAATACACTGTTTTATTCTGAAATTCCCAAAACTATCAATAGAGCAGCAGT   540
 N  R  E  N  T  L  F  Y  S  E  I  P  K  T  I  N  R  A  A  V

CTTAATGCTCTCTTGGAAGCCTCTTTTGGATCTTTTTCAGGCAACACTGGACTATGGAAT   600
 L  M  L  S  W  K  P  L  L  D  L  F  Q  A  T  L  D  Y  G  M

GTATTCTCGAGAAGAAGAACTATTAAGAGAAAGAAAACGCATTGGAACAGTCGGAATTGC   660
 Y  S  R  E  E  L  L  R  E  R  K  R  I  G  T  V  G  I  A

TTCTTACGATTATCACCAAGGAAGTGGAACATTTCTGTTTCAAGCCGGTAGTGGAATTTA   720
 S  Y  D  Y  H  Q  G  S  G  T  F  L  F  Q  A  G  S  G  I  Y

TCACGTAAAAGATGGAGGGGCCACAAGGATTTACGGCAACAACCTTTAAGGCCCAATCTAGT   780
 H  V  K  D  G  G  P  Q  G  F  T  Q  Q  P  L  R  P  N  L  V

GGAAACTAGTTGTCCCAACATACGGATGGATCCAAAATTATGCCCTGCTGATCCAGACTG   840
 E  T  S  C  P  N  I  R  M  D  P  K  L  C  P  A  D  P  D  W

GATTGCTTTTATACATAGCAACGATATTTGGATATCTAACATCGTAACCAGAGAAGAAAG   900
 I  A  F  I  H  S  N  D  I  W  I  S  N  I  V  T  R  E  E  R
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Figure 2 (to be continued).

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GAGACTCACTTATGTGCACAATGAGCTAGCCAACATGGAAGAAGATGCCAGATCAGCTGG      960
        R   L   T   Y   V   H   N   E   L   A   N   M   E   E   D   A   R   S   A   G

AGTCGCTACCTTTGTTCTCCAAGAAGAATTTGATAGATATTCTGGCTATTGGTGGTGTCC     1020
        V   A   T   F   V   L   Q   E   E   F   D   R   Y   S   G   Y   W   W   C   P

AAAAGCTGAAACAACTCCCAGTGGTGGTAAAATTCTTAGAATTCTATATGAAGAAAATGA     1080
        K   A   E   T   T   P   S   G   G   K   I   L   R   I   L   Y   E   E   N   D

TGAATCTGAGGTGGAAATTATTCATGTTACATCCCCTATGTTGGAAACAAGGAGGGCAGA     1140
        E   S   E   V   E   I   I   H   V   T   S   P   M   L   E   T   R   R   A   D

TTCATTCCGTTATCCTAAAACAGGTACAGCAAATCCTAAAGTCACTTTTAAGATGTCAGA     1200
        S   F   R   Y   P   K   T   G   T   A   N   P   K   V   T   F   K   M   S   E

AATAATGATTGATGCTGAAGGAAGGATCATAGATGTCATAGATAAGGAACTAATTCAACC     1260
        I   M   I   D   A   E   G   R   I   I   D   V   I   D   K   E   L   I   Q   P

TTTTGAGATTCTATTTGAAGGAGTTGAATATATTGCCAGAGCTGGATGGACTCCTGAGGG     1320
        F   E   I   L   F   E   G   V   E   Y   I   A   R   A   G   W   I   P   E   G

AAAATATGCTTGGTCCATCCTACTAGATCGCTCCCAGACTCGCCTACAGATAGTGTTGAT     1380
        K   Y   A   W   S   I   L   L   D   R   S   Q   T   R   L   Q   I   V   L   I

CTCACCTGAATTATTTATCCCAGTAGAAGATGATGTTATGGAAAGGCAGAGACTCATTGA     1440
        S   P   E   L   F   I   P   V   E   D   D   V   M   E   R   Q   R   L   I   E

GTCAGTGCCTGATTCTGTGACGCCACTAATTATCTATGAAGAAACAACAGACATCTGGAT     1500
        S   V   P   D   S   V   T   P   L   I   I   Y   E   E   T   T   D   I   W   I

AAATATCCATGACATCTTTCATGTTTTTCCCCAAAGTCACGAAGAGGAAATTGAGTTTAT     1560
        N   I   H   D   I   F   H   V   F   P   Q   S   H   E   E   E   I   E   F   I

TTTTGCCTCTGAATGCAAAACAGGTTTCCGTCATTTATACAAAATTACATCTATTTTAAA     1620
        F   A   S   E   C   K   T   G   F   R   H   L   Y   K   I   T   S   I   L   K

GGAAAGCAAATATAAACGATCCAGTGGTGGGCTGCCTGCTCCAAGTGATTTCAAGTGTCC     1680
        E   S   K   Y   K   R   S   S   G   G   L   P   A   P   S   D   F   K   C   P

TATCAAAGAGGAGATAGCAATTACCAGTGGTGAATGGGAAGTTCTTGGCCGGCATGGATC     1740
        I   K   E   E   I   A   I   T   S   G   E   W   E   V   L   G   R   H   G   S

TAATATCCAAGTTGATGAAGTCAGAAGGCTGGTATATTTTGAAGGCACCAAAGACTCCCC     1800
        N   I   Q   V   D   E   V   R   R   L   V   Y   F   E   G   T   K   D   S   P

TTTAGAGCATCACCTGTACGTAGTCAGTTACGTAAATCCTGGAGAGGTGACAAGGCTGAC     1860
        L   E   H   H   L   Y   V   V   S   Y   V   N   P   G   E   V   T   R   L   T

TGACCGTGGCTACTCACATTCTTGCTGCATCAGTCAGCACTGTGACTTCTTTATAAGTAA     1920
        D   R   G   Y   S   H   S   C   C   I   S   Q   H   C   D   F   F   I   S   K

GTATAGTAACCAGAAGAATCCACACTGTGTGTCCCTTTACAAGCTATCAAGTCCTGAAGA     1980
        Y   S   N   Q   K   N   P   H   C   V   S   L   Y   K   L   S   S   P   E   D

TGACCCAACTTGCAAAACAAAGGAATTTTGGGCCACCATTTTGGATTCAGCAGGTCCTCT     2040
        D   P   T   C   K   T   K   E   F   W   A   T   I   L   D   S   A   G   P   L

TCCTGACTATACTCCTCCAGAAATTTTCTCTTTTGAAAGTACTACTGGATTTACATTGTA     2100
        P   D   Y   T   P   P   E   I   F   S   F   E   S   T   T   G   F   T   L   Y
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Figure 2 (to be continued).

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TGGGATGCTCTACAAGCCTCATGATCTACAGCCTGGAAAGAAATATCCTACTGTGCTGTT        2160
  G   M   L   Y   K   P   H   D   L   Q   P   G   K   K   Y   P   T   V   L   F
CATATATGGTGGTCCTCAGGTGCAGTTGGTGAATAATCGGTTTAAAGGAGTCAAGTATTT        2220
  I   Y   G   G   P   Q   V   Q   L   V   N   N   R   F  │K   G   V   K   Y   F
CCGCTTGAATACCCTAGCCTCTCTAGGTTATGTGGTTGTAGTGATAGACAACAGGGGATC        2280
 │R   L   N   T   L   A   S   L   G   Y   V   V   V   V   I   D   N   R   G   S │
CTGTCACCGAGGGCTTAAATTTGAAGGCGCCTTTAAATATAAAATGGGTCAAATAGAAAT        2340
 │C   H   R   G   L   K   F   E   G   A   F   K   Y   K   M   G   Q   I   E   I │
TGACGATCAGGTGGAAGGACTCCAATATCTAGCTTCTCGATATGATTTCATTGACTTAGA        2400
 │D   D   Q   V   E   G   L   Q   Y   L   A   S   R   Y   D   F   I   D   L   D │
TCGTGTGGGCATCCACGGCTGGTCCTATGGAGGATACCTCTCCCTGATGGCATTAATGCA        2460
 │R   V   G   I   H   G   W   S   Y   G   G   Y   L   S   L   M   A   L   M   Q │
GAGGTCAGATATCTTCAGGGGTTGCTATTGCTGGGGCCCCAGTCACTCTGTGGATCTTCTA        2520
 │R   S   D   I   F   R   V   A   I   A   G   A   P   V   T   L   W   I   F   Y │
TGATACAGGATACACGGAACGTTATATGGGTCACCCTGACCAGAATGAACAGGGCTATTA        2580
 │D   T   G   Y   T   E   R   Y   M   G   H   P   D   Q   N   E   Q   G   Y   Y │
CTTAGGATCTGTGGCCATGCAAGCAGAAAAGTTCCCCTCTGAACCAAATCGTTTACTGCT        2640
 │L   G   S   V   A   M   Q   A   E   K   F   P   S   E   P   N   R   L   L   L │
CTTACATGGTTTCCTGGATGAGAATGTCCATTTTGCACATACCAGTATATTACTGAGTTT        2700
 │L   H   G   F   L   D   E   N   V   H   F   A   H   T   S   I   L   L   S   F │
TTTAGTGAGGGCTGGAAAGCCATATGATTTACAGATCTATCCTCAGGAGAGACACAGCAT        2760
 │L   V   R   A   G   K   P   Y   D   L   Q   I   Y   P   Q   E   R   H   S   I │
AAGAGTTCCTGAATCGGGAGAACATTATGAACTGCATCTTTTGCACTACCTTCAAGAAAA        2820
 │R   V   P   E   S   G   E   H   Y   E   L   H   L   L   H   Y   L   Q   E   N │
CCTTGGATCACGTATTGCTGCTCTAAAAGTGATA *TAA* TTTTGACCTGTGTAGAACTCTCT      2880
 │L   G   S   R│  I   A   A   L   K   V   I
GGTATACACTGGCTATTTAACCAAATGAGGAGGTTTAATCAACAGAAAACACAGAATTGA        2940
TCATCACATTTTGATACCTGCCATGTAACATCTACTCCTGAAAATAAATGTGGTGCCATG        3000

CAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
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Figure 2. Nucleotide sequence and deduced amino acid sequence of the cDNA for *DPP8-v3*. Numbering of the nucleotide is shown on the right. Initiation and stop codons are in italic. N-terminal β-propeller domain is underlined and C-terminal peptidase S9 domain is boxed. PCR primers for the determination of expression profile are in shadow. Upstream primer is located in the specific region of *DPP8-v3*. Downstream primer is in the common region of *DPP8-v3* and its homologous genes.

(NT_086827.1|Hs15_86498) and transcribed from an identical gene. Transcript and splicing comparison of *DPP8-v3* with homologous genes indicated that the gene consists of 22 exons (Figure 3). Exon 2 was present only in *DPP8-v3*. Exon 3 was present in *DPP8-v3* and *DPP8* variant 4, exon 18 in *DPP8-v3* and *DPP8* vari-

ants 1 and 4, and exon 19 in *DPP8-v3* and *DPP8* variant 1. *DPP8-v3* lacked exon 1 and presented exon 22 shorter than other homologous genes. Due to changes in specific nucleotides, the sequences of proteins differed.

3.3 Homologous comparison and predicted feature of
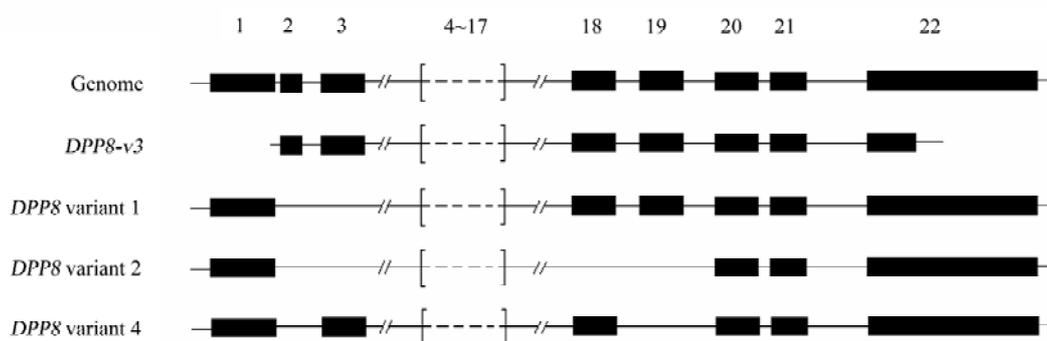
Figure 3. Transcript and splicing comparison of *DPP8-v3* with homologous genes. Homologues originate from one gene which consists of 22 exons. Exons 4–17 are the same in all transcripts. Exon 2 was present only in *DPP8-v3*; exon 3 was present in *DPP8-v3* and *DPP8* variant 4 (NM_197961); exon 18 was present in *DPP8-v3* and *DPP8* variants 4 and 1 (NM_130434); exon 19 was present in *DPP8-v3* and *DPP8* variant 1. *DPP8-v3* lacked exon 1. Exon 22 in *DPP8-v3* was shorter than that of homologous genes.

DPP8-v3 peptide sequence

DPP8-v3 gene encoded an 898 amino-acid protein with predicted molecular weight 103 kDa and isoelectric point 5.62. Analysis of the amino acid sequence by using SMART software revealed that the *DPP8-v3* protein has an N-terminal β-propeller and a C-terminal peptidase S9 domain located at amino acids 104-676 and 682-891, respectively (Figure 2).

Previous studies have shown that *DPP8* was a member of the dipeptidyl peptidase IV (*DPPIV*)-like gene family [5]. Therefore, the amino acid sequence and conserved domain of *DPP8-v3*, *DPP8* variants 1, 2 and 4 and human *DPPIV* were compared, which showed that various *DPP8* peptidase shared 17.59 % identity and 67.26 % similarity with *DPPIV*. They all presented at least one of the N-terminal β-propeller domain and the C-terminal peptidase S9 domain, characteristics of the *DPPIV* peptidase (Figures 4 and 5).

3.4 Expression profile of DPP8-v3 gene

PCR amplification of cDNAs from different human tissues followed by Southern-blot analysis showed that *DPP8-v3* gene was predominantly expressed in human testis and pancreas, weakly in the placenta and lung, almost imperceptibly in other organs (Figure 6).

3.5 Differential expression of DPP8-v3 in different development stages of male testis

Quantitative fluorescent RT-PCR indicated that the *DPP8-v3* gene was differentially expressed in human adult and fetal testes. The expression level was quantified according to the number of cycles (Ct value) before
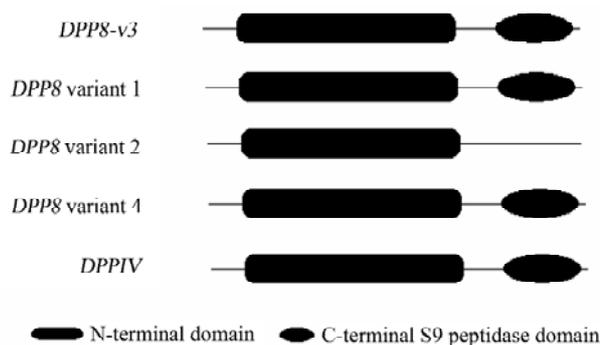
Figure 4. Comparison of *DPP8-v3* protein domains to *DPP8*-related homologues. N-terminal β-propeller and C-terminal peptidase S9 domain of the different proteins share similarity to the tertiary structure of the human dipepitidyl peptidase IV (*DPPIV*).

entering the exponential phase in the PCR reaction. This value was conversely related to the input copies of transcript. The Ct value of the *DPP8-v3* gene for human adult and fetal testes was 24.91 ± 0.16 and 34.24 ± 0.63, respectively; while the Ct value of β-actin, 18.34 ± 0.11 and 18.87 ± 0.80, respectively, was not significantly different between adult and fetal testes (Figure 7). So the expression level of the *DPP8-v3* gene in human adult testes was significantly higher than that in fetal testis ($P < 0.05$). This result accurately verified the result of hybridization of cDNA microarray and revealed a correlation between *DPP8-v3* expression and testicular development.

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DPP8 variant 2   ---------------MAAAMETEQLGVEIFETADCEENIESQDRPKLEPFYVERYSWSQLKKLLADTRKYHGYMMAKAP
DPP8 variant 4   MWKRSEQMKIKSGKCNMAAAMETEQLGVEIFETADCEENIESQDRPKLEPFYVERYSWSQLKKLLADTRKYHGYMMAKAP
DPP8-v3          MWKRSEQMKIKSGKCNMAAAMETEQLGVEIFETADCEENIESQDRPKLEPFYVERYSWSQLKKLLADTRKYHGYMMAKAP
DPP8 variant 1   ---------------MAAAMETEQLGVEIFETADCEENIESQDRPKLEPFYVERYSWSQLKKLLADTRKYHGYMMAKAP
DPPIV            ---MKTPWKVLLGLLGAAALVTIITVPVVLLNKGTDDATADSRKTYTLTDYLKNTYRLKLYS-----------LRWISD
                                **:       : * ::::..   : .  :*:.  .*    :  : *   .             :    :

DPP8 variant 2   HDFMFVKRNDPDGPHSDRIYYLAMSGENRENTLFYSEIPKTINRAAVLMLSWKPLLDLFQATLDYGMYSREEELLRERKR
DPP8 variant 4   HDFMFVKRNDPDGPHSDRIYYLAMSGENRENTLFYSEIPKTINRAAVLMLSWKPLLDLFQATLDYGMYSREEELLRERKR
DPP8-v3          HDFMFVKRNDPDGPHSDRIYYLAMSGENRENTLFYSEIPKTINRAAVLMLSWKPLLDLFQATLDYGMYSREEELLRERKR
DPP8 variant 1   HDFMFVKRNDPDGPHSDRIYYLAMSGENRENTLFYSEIPKTINRAAVLMLSWKPLLDLFQATLDYGMYSREEELLRERKR
DPPIV            HEYLYKQENN----------ILVFNAEYGNSSVFLENS--------------TFDEFGHSINDYSISPDGQFILLEYN-
               .     *::::: :.*:          *.:.:* .:.::* . :  .              : :: :: **.: .   : :** :

DPP8 variant 2   IGTVGIASYDYHQGSGTFLFQAGSGIYHVKDGGPQGFTQQPLRPNLVETSCPNIRMDPKLCPADPDWIAFIHSNDIWISN
DPP8 variant 4   IGTVGIASYDYHQGSGTFLFQAGSGIYHVKDGGPQGFTQQPLRPNLVETSCPNIRMDPKLCPADPDWIAFIHSNDIWISN
DPP8-v3          IGTVGIASYDYHQGSGTFLFQAGSGIYHVKDGGPQGFTQQPLRPNLVETSCPNIRMDPKLCPADPDWIAFIHSNDIWISN
DPP8 variant 1   IGTVGIASYDYHQGSGTFLFQAGSGIYHVKDGGPQGFTQQPLRPNLVETSCPNIRMDPKLCPADPDWIAFIHSNDIWISN
DPPIV            -----------YVKQWRHSYTASYDIYDLNK----------RQLITEERIPNNTQWVTWSPVG-HKLAYVWNNDIYVKI
                            :    .   . : *. .**.::.            *   :.*   **      .  .*.. . :*:: .***::.

DPP8 variant 2   IVTREERRLTYVHNELANMEEDARSAGVATFVLQEE-FDRYSGYWWCPKAETTPSGGKILRILYEENDESEVEIIHVTSP
DPP8 variant 4   IVTREERRLTYVHNELANMEEDARSAGVATFVLQEE-FDRYSGYWWCPKAETTPSGGKILRILYEENDESEVEIIHVTSP
DPP8-v3          IVTREERRLTYVHNELANMEEDARSAGVATFVLQEE-FDRYSGYWWCPKAETTPSGGKILRILYEENDESEVEIIHVTSP
DPP8 variant 1   IVTREERRLTYVHNELANMEEDARSAGVATFVLQEE-FDRYSGYWWCPKAETTPSGGKILRILYEENDESEVEIIHVTSP
DPPIV            EPNLPSYRITWTG------KEDIIYNGITDWVYEEEVFSAYSALWWS------PNGTFLAYAQFNDTEVPLIEYSFYSDE
                  . . *:*:.        :**      *:: :* :** *. **. **.        *.*   :      :::.: . .:*   . :.

DPP8 variant 2   MLETRRADSFRYPKTGTANPKVTFKMSEIMIDAEGRIIDVIDKELIQPFEILFEGVEYIARAGWTPEGKYAWSILLDRSQ
DPP8 variant 4   MLETRRADSFRYPKTGTANPKVTFKMSEIMIDAEGRIIDVIDKELIQPFEILFEGVEYIARAGWTPEGKYAWSILLDRSQ
DPP8-v3          MLETRRADSFRYPKTGTANPKVTFKMSEIMIDAEGRIIDVIDKELIQPFEILFEGVEYIARAGWTPEGKYAWSILLDRSQ
DPP8 variant 1   MLETRRADSFRYPKTGTANPKVTFKMSEIMIDAEGRIIDVIDKELIQPFEILFEGVEYIARAGWTPEGKYAWSILLDRSQ
DPPIV            SLQYPKTVRVPYPKAGAVNPTVKFFV--VNTDSLSSVTNATSIQITAPASMLIG-DHYLCDVTWATQER-----------
                  *:   ::  . ***:*:.**.*.*  :   : *: . : :.    :: * .:*: .  .*:. . *:.: :

DPP8 variant 2   TRLQIVLISPELFIPVEDDVMERQRLIESVPDSVTPLIIYEETTDIWINIHDIFHVFPQSHEEEIEFIFASECKTGFRHL
DPP8 variant 4   TRLQIVLISPELFIPVEDDVMERQRLIESVPDSVTPLIIYEETTDIWINIHDIFHVFPQSHEEEIEFIFASECKTGFRHL
DPP8-v3          TRLQIVLISPELFIPVEDDVMERQRLIESVPDSVTPLIIYEETTDIWINIHDIFHVFPQSHEEEIEFIFASECKTGFRHL
DPP8 variant 1   TRLQIVLISPELFIPVEDDVMERQRLIESVPDSVTPLIIYEETTDIWINIHDIFHVFPQSHEEEIEFIFASECKTGFRHL
DPPIV            -------------------ISLQWLRRIQNYSVMDICDYDESSGRWN------CLVARQHIEMSTTGWVGRFRPSEPHF
                   :. * * .    ** : *:*::. *     :..:. * *    :... :..    *:

DPP8 variant 2   YKITSILKESKYKRSSGCLPAPSDFKCPIKEEIAITSGEWEVLGRHGSNIQVDEVRRLVYFEGTKD-SPLEHHLYVVSYV
DPP8 variant 4   YKITSILKESKYKRSSGCLPAPSDFKCPIKEEIAITSGEWEVLGRHGSNIQVDEVRRLVYFEGTKD-SPLEHHLYVVSYV
DPP8-v3          YKITSILKESKYKRSSGGLPAPSDFKCPIKEEIAITSGEWEVLGRHGSNIQVDEVRRLVYFEGTKD-SPLEHHLYVVSYV
DPP8 variant 1   YKITSILKESKYKRSSGCLPAPSDFKCPIKEEIAITSGEWEVLGRHGSNIQVDEVRRLVYFEGTKD-SPLEHHLYVVSYV
DPPIV            TLDGNSFY--KIISNEEGYRHICYFQIDKKDCTFITKGTWEVIG-----IEALTSDYLYYISNEYKGMPGGRNLYKIQLS
                  . :  *   .. *    . *:     *:    **.* ***:*       *:.     * * :..  .  *  ::** :.

DPP8 variant 2   NPGEVTRLTDRGYSHSCCISQHCDFFISKYSNQKNP-HCVSLYKLSSPEDDPTCKTKEFWATILDSAGPLPDYTPPEIFS
DPP8 variant 4   NPGEVTRLTDRGYSHSCCISQHCDFFISKYSNQKNP-HCVSLYKLSSPEDDPTCKTKEFWATILDSAGPLPDYTPPEIFS
DPP8-v3          NPGEVTRLTDRGYSHSCCISQHCDFFISKYSNQKNP-HCVSLYKLSSPEDDPTCKTKEFWATILDSAGPLPDYTPPEIFS
DPP8 variant 1   NPGEVTRLTDRGYSHSCCISQHCDFFISKYSNQKNP-HCVSLYKLSSPEDDPTCKTKEFWATILDSAGPLPDYTPPEIFS
DPPIV            DYTKVTCLSCELNPERCQYYSVSFSKEAKYYQLRCSGPGLPLYTLHSSVNDKGLRVLEDNSALDKMLQNVQ--MPSKKLD
                  : :** *:. .  .. *  . .     :** : :. . :.**.* *. :*    :. * ::: .     :    *.: :.
```

Figure 5 (to be continued).

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(continued)

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DPP8 variant 2   FESTTGFTLYGMLYKPHDLQPGKKYPTVL-IYGGPQ-------------------------------------------
DPP8 variant 4   FESTTGFTLYGMLYKPHDLQPGKKYPTVLFIYGGPQVQLVNNRFKGVKYFRLNTLASLGYVVVVIDNRGSCHRGLKFEGA
DPP8-v3          FESTTGFTLYGMLYKPHDLQPGKKYPTVLFIYGGPQVQLVNNRFKGVKYFRLNTLASLGYVVVVIDNRGSCHRGLKFEGA
DPP8 variant 1   FESTTGFTLYGMLYKPHDLQPGKKYPTVLFIYGGPQVQLVNNRFKGVKYFRLNTLASLGYVVVVIDNRGSCHRGLKFEGA
DPPIV            FIILNETKFWYQMILPPHFDKSKKYPLLLDVYAGPCSQKADTVFR--LNWATYLASTENIIVASFDGRGSGYQGDKIMHA
                 *    . .::   :  * .:: .**** :* :*.**

DPP8 variant 2   ----------------------------------------------------------VAIAGAPVTLWIFYDTGYTERYMG
DPP8 variant 4   FKYKM----------------------------------------------------VAIAGAPVTLWIFYDTGYTERYMG
DPP8-v3          FKYKMGQIEIDDQVEGLQYLASRYDFIDLDRVGIHGWSYGGYLSLMALMQRSDIFRVAIAGAPVTLWIFYDTGYTERYMG
DPP8 variant 1   FKYKMGQIEIDDQVEGLQYLASRYDFIDLDRVGIHGWSYGGYLSLMALMQRSDIFRVAIAGAPVTLWIFYDTGYTERYMG
DPPIV            INRRLGTFEVEDQIEAAR-QFSKMGFVDNKRIAIWGWSYGGYVTSMVLGSGSGVFKCGIAVAPVSRWEYYDSVYTERYMG
                                                                          .** ***: * :**: *******

DPP8 variant 2   HPDQNEQGYYLGSVAMQAEKFPSEPNRLLLLHGFLDENVHFAHTSILLSFLVRAGKPYDLQIYPQERHSIRVPESGEHYE
DPP8 variant 4   HPDQNEQGYYLGSVAMQAEKFPSEPNRLLLLHGFLDENVHFAHTSILLSFLVRAGKPYDLQIYPQERHSIRVPESGEHYE
DPP8-v3          HPDQNEQGYYLGSVAMQAEKFPSEPNRLLLLHGFLDENVHFAHTSILLSFLVRAGKPYDLQIYPQERHSIRVPESGEHYE
DPP8 variant 1   HPDQNEQGYYLGSVAMQAEKFPSEPNRLLLLHGFLDENVHFAHTSILLSFLVRAGKPYDLQIYPQERHSIRVPESGEHYE
DPPIV            LPTPEDNLDHYRNSTVMSRAENFKQVEYLLIHGTADDNVHFQQSAQISKALVDVGVDFQAMWYTDEDHGIASSTAHQHIY
                 *   ::: :   . :: :.   :  .  **:** *:**** ::: : . ** .*  ::   *.:* *.*  . ::*

DPP8 variant 2   LHLLHYLQENLGSRIAALKVI
DPP8 variant 4   LHLLHYLQENLGSRIAALKVI
DPP8-v3          LHLLHYLQENLGSRIAALKVI
DPP8 variant 1   LHLLHYLQENLGSRIAALKVI
DPPIV            THMSHFIKQCFSLP-------
                 *: *:::: :.
```

Figure 5. Amino acid alignments of *DPP8-v3* and *DPP8*-related homologues to human dipepitidyl peptidase IV (*DPPIV*). *, high consensus amino acids; :, low consensus amino acids.
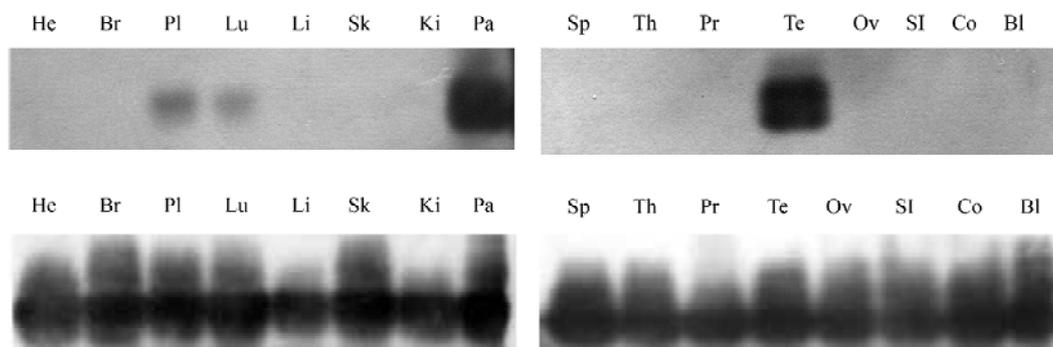
Figure 6. Tissue distribution of *DPP8-v3* and β-actin, used as positive control, after multi-tissue PCR followed by Southern blot analysis. Top: *DPP8-v3* was predominantly expressed in the testis and pancreas, weakly in the placenta and lung, and not readily detectable in samples from other tissues. Bottom: β-actin was expressed in all tissues. He, heart; Br, brain; Pl, placenta; Lu, lung; Li, liver; Sk, skeletal muscle; Ki, kidney; Pa, pancreas; Sp, spleen; Th, thymus; Pr, prostate; Te, testis; Ov, ovary; SI, small intestine; Co, colon; Bl, blood leukocyte.
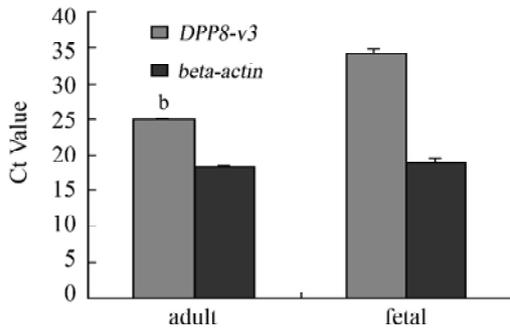
Figure 7. RT-PCR shows differential expression of *DPP8-v3* in human adult and fetal testes. *DPP8-v3* was highly expressed in adult testes compared with fetal testes ($^bP < 0.05$). The expression level of *DPP8-v3* was quantified according to the number of cycles (Ct value) before entering the exponential phase in the PCR reaction.

4   Discussion

In the present study, the testis cDNA microarray was used to identify the genes related to testis development and spermatogenesis [9]. A new gene, named *DPP8-v3*, was identified. The results not only showed that cDNA microarray was an efficient method to identify genes expression profiles, but also indicated that *DPP8-v3* was a novel *DPP8* transcript variant and may influence testis development/spermatogenesis by regulating immune state in testis.

The full length cDNA of *DPP8* gene was first identified by Abbott *et al.* [5]. By analyzing its sequence, tissue distribution and biological activity, which showed the similarities between *DPP8* and *DPPIV* in tissue expression pattern and substrates, *DPP8* was considered a new member of the *DPPIV*-like gene family. Thus it was speculated that *DPP8* has a potential role similar to *DPPIV* [5, 11]. *DPPIV* is a serine aminopeptidase with an ubiquitous tissue expression and significant upregulation on activated T-cell. In addition, human *DPPIV* is also known as the T-cell activation antigen CD26 [8, 12, 13]. A number of studies have indicated that *DPPIV*/CD26 is a key player in immunological processes and this role may involve both its enzyme activity and its non-catalytic activity, which is the ability to bind adenosine deaminase (ADA) [8, 14].

The tertiary structure of *DPPIV* contains an N-terminal β-propeller domain and a C-terminal peptidase S9 domain which perform ADA-binding and serine enzyme activity respectively (Figure 4). It has been proven that the non-catalytic activity of *DPPIV*, which binds to the soluble extracellular ADA, is able to reduce a local concentration of adenosine around T-cell and protect the T-cell from adenosine-mediated inhibition of proliferation. So *DPPIV* is considered as a costimulator of T-cell to participate in the immunoreaction [15, 16]. On the other hand, the role of enzyme activity of *DPPIV* within the immune system is gradually laid out. It was reported that *DPPIV* probably influenced chemotactic activity of some chemokines on Th2 lymphocytes and dendritic cells, but not on Th1 lymphocytes. These chemokines, including RANTES, eotaxin, monocyte drived chemokine (MDC) and stromal derived factor (SDF), share a conserved NH$_2$-X-Pro sequence (where X is any amino acid) at the NH$_2$ terminus, which conforms to the substrate specificity of *DPPIV* [17, 18]. Thus, *DPPIV* can mediate the N terminal truncation of these chemokines, resulting in alterations in their biologic activities, such as receptor specificity and chemotactic, potentially influence migration of Th2 lymphocyte and dendritic cells [11, 19, 20]. Thus it can be speculated that, during immunological process, *DPPIV* promotes T-cell proliferation and activation. Furthermore, it simultaneously regulates the chemotaxis of some lymphocyte to balance the intensity of immunoreaction.

Similarly, *DPP8* has the same N-terminal β-propeller domain and a C-terminal peptidase S9 domain as *DPPIV* (Figure 4), which suggests that *DPP8* is also the key player in immune regulation by its enzyme activity as well as no-catalytic activity. Furthermore, the immunoregulated role of *DPP8* may be especially important in testes. Three different transcripts of the *DPP8* gene have been identified: a major transcript of 5.0 kb and a minor transcript of 8.0 kb were present at either moderate or high levels in most examined tissues, including brain, liver, spleen, heart, testis, while a transcript of approximately 3.0 kb was detected only in the testis [5]. Thus, the testis was the only tissue to express these three *DPP8* transcripts, suggesting that immunoregulation is particularly complicated in this tissue. This is in accordance with the accepted idea that immunoreaction must attain a balance between immune resistance and immune protection in testes, which contributes to the maintenance of normal spermatogenesis.

One more *DPP8*-related transcript variant has been identified in the present study (Figure 3). It is possible that the expression of the different transcript variants may be used to regulate the levels of active DPP8 protein

[5]. The ability to immunoregulate diverse *DPP8* transcript variants may be different in testes. For instance, *DPP8* variant 2 only contains the N-terminal β-propeller domain and lacks the influence on chemotaxis of some lymphocyte. Thus, we speculate that the expression of so many *DPP8* transcript variants, with different immunoregulation activity in the testis, may regulate the immunological level and stabilize the immune balance in the mass. More transcript variants may be found in the future and their biologic mechanisms need to be further explored.

Spermatogenesis begins at puberty, so genes highly expressed in adult testes should be related to spermatogenesis. Maintenance of immune balance is very important in the process of spermatogenesis, which implies DPP8 protein must be abundant in adult testes. The *DPP8-v3* gene we have identified is just a novel *DPP8* transcript variant highly expressed in adult testes, so it would play a key role in spermatogenesis and male fertility as a member of the *DPP8* regulated network. Further study is required to provide more information and to obtain better understanding of the exact role and mechanism of action of *DPP8-v3* in spermatogenesis.

Acknowledgment

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