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SHORT COMMUNICATION

Molecular cloning and characterization of a novel splice variant of human *ZNF300* gene, which expressed highly in testis⋆

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Abstract
Zinc finger protein, one of the most important transcription factors, plays an essential role in regulating gene expression. C_2H_2 type zinc protein with KRAB domain contains two parts, one is C_2H_2 zinc finger motif which is use to binding to the DNA, while the other is KRAB associated box which mostly performs as a transcription repressor. In this study, we report the cloning and characterization of a novel splice variant of the human *ZNF300* gene (*ZNF300-B*). The *ZNF300-B* gene cDNA is 2293 bp in length, encoding a putative protein with 619 amino acid residues. *ZNF300-B* gene is mapped to chromosome 5q32–5q33 with seven exons. Reverse transcription polymerase chain reaction (RT-PCR) analysis showed that *ZNF300-B* and *ZNF-300* were expressed highly in human testis.

Keywords: ZNF300 *gene, transcription factor, splice variant, RT-PCR*

Database accession number: *Dq908918*

Introduction

Zinc finger protein which belongs to a protein superfamily is one of the most important DNA sequence-specific transcription regulators and plays an essential role in transcriptional controlling genes expression (Collins et al. 2001; Wu et al. 2001). Generally, this protein contains two motifs, one is DNA-binding domain and the other is transcriptional activation or repression domain (Medugno et al. 2005).

According to the conserved DNA-binding domain, the Zinc finger protein family can be divide into several types including C_2H_2 type, C_3H type, C_4 type, glucocorticoid receptor, GATA-1 type, GAL4 type, and LIM family (Wu et al. 2001; Yin et al. 2006). Among them, C_2H_2 type zinc finger protein is regarded as one of the largest subfamily of nucleic acid binding proteins (Dai et al. 2003; Gou et al. 2004). The C_2H_2 zinc finger motif consists of two cycteine and two histidine residues, and is defined with the consensus sequence of Φ–X–C–X(2–4)–C–X3–Φ–X5–Φ–X2–H–X(3,4)–H, where Φ represents a hydrophobic residue and X represents any amino acid, each finger unit often repeats several times (Pecasse et al. 1986; Li et al. 1999; Urrutia 2003). The zinc ion binding C_2H_2 type zinc finger motif initially found in the *Krüppel* gene of *Drosophlia* (Preiss et al. 1985) and TF III A transcription factor of *Xenopus laevis* (Miller et al. 1985). A Zn^{2+} can connect with the two cycteine and two histidine residues and the domain folds as a finger which can interact with the DNA, thus C_2H_2 motifs function as the DNA-binding domains (Pavletich and Pabo 1991;

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Theunissen et al. 1992; Grondin et al. 1997; Urrutia 2003).

KRAB domain which acts as the transcriptional repressor can be found in about one-third of the human C_2H_2 zinc finger proteins, and locates at the amino-terminal end of this protein (Collins et al. 2001). A part of the KRAB motifs consist of A and B boxes, the A box plays a key role in repression by protein–protein interacting with the co-repressors, and the B box enhances repression effect although the mechanisms are unknown (Vissing et al. 1995; Urrutia 2003; Hering et al. 2004).

Here we report on the molecular cloning and characterization a novel splice variant of human *ZNF300* gene. The *ZNF300-B* gene, which contains a KRAB A + B box and 12 C_2H_2 zinc finger motifs, is highly expressed in the human testis while *ZNF300* is mainly expressed in heart, skeletal muscle, and brain (Gou et al. 2004).

Materials and methods

Cloning and sequencing of ZNF300-B cDNA

A cDNA library was constructed using human fetal brain mRNA purchased from Clontech. The modified pBluescript II SK (+) vector was constructed by introducing 2 *Sfi* I recognition sites. After *Sfi* I digestion, cDNAs greater than 500 bp were ligated into the *Sfi* I A and *Sfi* I B sites of pBluescript II SK (+) vector. Double-strand cDNAs were prepared with a SMART™ PCR cDNA Synthesis Kit following manufacturer's instructions.

Sequence analysis and chromosomal location

DNA sequence and putative protein sequence homology searches and comparisons were carried out using the BLAST tool at the National Center for Biotechnology Information (NCBI, http://www.ncbi.nln.nih.gov/BLAST). Multiple sequence

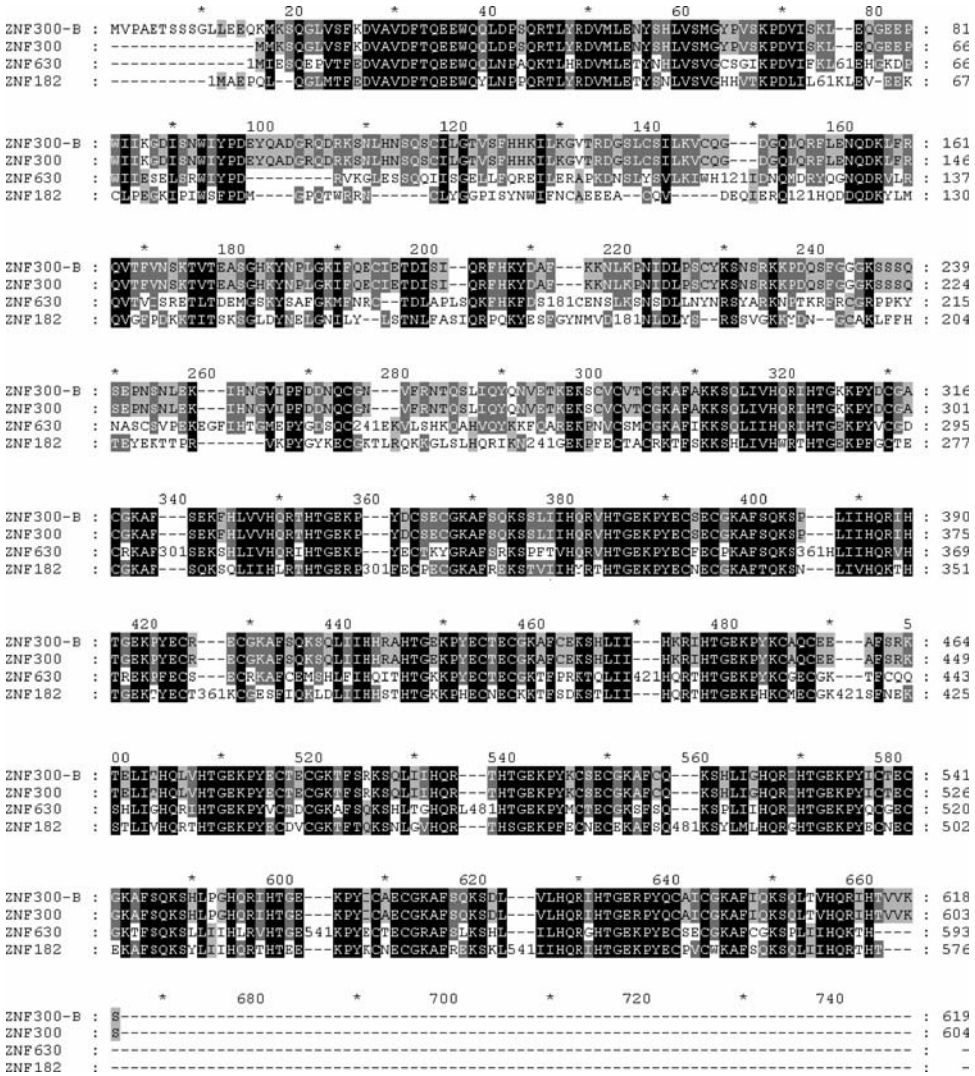

Figure 1. Protein sequence alignment of human ZNF300-B with ZNF300 (AAI17249, from *Homo sapiens*), ZNF630 (AAI12140, from *Homo sapiens*), and ZNF182 (AAI14101, from *Bos taurus*). Conserved amino acids are shaded.

alignment analysis was performed by GeneDoc program (http://www.psc.edu/biomed/genedoc/). A BLAST-N search against the human genome was performed to locate the chromosomal mapping of *ZNF300-B*. Relative analysis softwares include Gene Runner, Primer Premier5.0 et al.

Reverse transcription PCR

One human multiple tissue cDNA (MTC) panel (Clontech) were used as PCR template according to the manufacturer's protocol. The primers of *ZNF300-B* gene were shown as follows: sense primer (5′-TCTGCATGACTTGTCCTGGGA-3′, corresponding to nucleotides 165–185) and antisense primer (5′-CTGCAGCTGACCATCACCTTGAC-3′, corresponding to nucleotides 665–687). Twenty-four cycles (for GAPD) or 36 cycles (for *ZNF300-B*) of amplifications (30 s at 94°C, 30 s at 65°C, 1 min at 74°C) were performed using Taqplus polymerase (Sangon) in a volume of 50 μl. The PCR products were then resolved on 1.5% metaphor agarose.

Result and discussion

Cloning and sequence analysis of the ZNF300-B *gene*

A human fetal brain cDNA library was constructed in the lab. By large-scale sequencing, we cloned a novel splice variant of human *ZNF300* gene (*ZNF300-B*). The cDNA length is 2293 bp, containing an open reading frame from nucleotide 238 to 2097 bp, encoding a 619 amino acids predicted protein. The molecular mass of putative protein is 67.3 kDa. The sequence start codon (ATG) and stop codon (TAA) was found at the position 238–240 and 2095–2097 bp, respectively. In the N-terminal region, there is a KARB domian with A and B boxes. Meanwhile, the C-terminal is conserved with 12 C_2H_2 zinc finger motifs. Amino acids alignment was showed in Figure 1. The region spanning between amino acids residues 19 and 94 of ZNF300-B shows homology to the KRAB domain of other zinc finger proteins. The amino acids sequence reveals that ZNF300-B contains 12 C_2H_2 zinc finger motifs, and consistent with the consensus sequence Cys–X(2–4)–Cys–X12–His–X(3–5)–His.

Chromosome location of the ZNF300-B *gene*

By searching the human genome database, the *ZNF300-B* gene was located in contig NT_029289.10. The comparison result showed that the *ZNF300-B* gene located on the human chromsome 5q32–33 spanning about 9.6 kb of human genomic DNA (Figure 2). *ZNF300-B* gene has seven exons and six introns, while *ZNF300* gene has six exons. The 17 amino acids encoded by the third exon containing the start codon (ATG) and party of the fourth exon in *ZNF300-B*, were absent in *ZNF300* (Figure 3). All the sequences at the exon–intron junction were in consensus with the AG–GT rule, except one variant nucleotide, which is underlined in Table I.

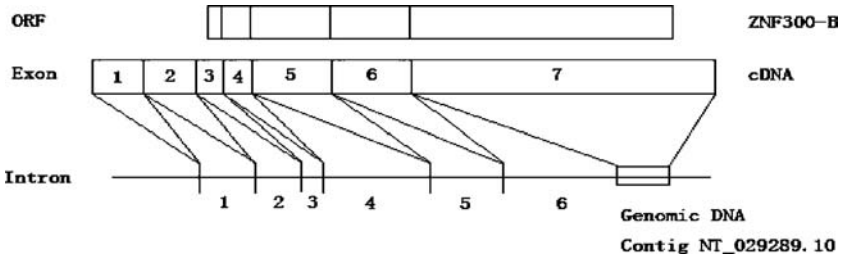

Figure 2. Genomic structure of human *ZNF300-B* gene.

ZNF300-B:

……GATGTTTTGATACCCCCATATAAAATAATCTGTCTGTCATGGGAGCCCCCACGTCCT
CTGAAATAGTTCAGCTCTGTCTGCATGACTTGTCCTGGGACTGAGGAAAATTTACCAG**g**
tatttaccttcctg……gtgatcatattat**ag**GTACAAGAGGAGTAAAGAATGATCCCTCCTCCACATGGT
GCCTGCTGAGACTTCT**gt**aaggctcaaacaacc……tttgctttttc**ag**TCATCTGGCCTTTTGGAAGAG
CAAAAAATGATGAAGTCCCAGG……

ZNF300:

……GATGTTTTGATACCCCCATATAAAATAATCTGTCTGTCATGGGAGCCCCCACGTCCT
CTGAAATAGTTCAGCTCTGTCTGCATGACTTGTCCTGGGACTGAGGAAAATTTACCAG**g**
tatttaccttcctg……tttgctttttc**ag**TCATCTGGCCTTTTGGAAGAGCAAAAAATGATGAAGTCCCA
GG……

Figure 3. Sequence analysis of the alternative splicing of *ZNF300*. Boxed nucleotides represent the 55 bp exon of *ZNF300-B*. The sequences at exon–intron junctions were consistent with the AG–GT consensus (showed in black).

Table I. Nucleotide sequence of the exon–intron junctions of the ZNF300-B gene. The intron sequence is shown in lowercase and the exon sequence in uppercase letters. Invariant nucleotide (ag/gt) are in black. Variant nucleotide (ga) is underlined.

3′ Splice acceptor	Exon	Size (bp)	5′ Splice donor	Intro	Size (bp)
	1	88	GTGCGGTGAAGG*gt*gagtctgtgg	1	726
gtttccttcc*ag*ATGTTTTGATAC	2	114	AAAATTTACCAG*gt*atttaccttc	2	480
atcatattat*ag*GTACAAGAGGAG	3	55	GCTGAGACTTCT*gt*aaggctcaaa	3	146
ttgctttttc*ag*TCATCTGGCCTT	4	42	ATGAAGTCCCAG*gt*gagttgcttt	4	4586
ttactattac*ag*GGGTTAGTATCA	5	127	TGGTCTCAATGG*gt*aaggatggct	5	243
tctgtttaat*ag*GGTATCCAGTTT	6	123	TGGGAGACAAGG*ga*agtgaaatat	6	1087
ttcttctttt*ag*ACAGGAAGAGTA	7	1746	ACATTTTTAATA—		

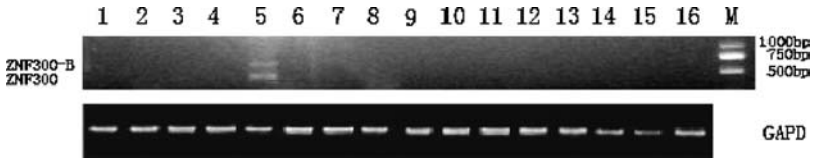

Figure 4. Reverse transcription PCR analysis of the *ZNF300-B* cDNA with human tissues. GAPD was used as a control. 1, Blood; 2, colon; 3, small intestine; 4: ovary; 5, testis; 6, prostate; 7, thymus; 8, spleen; 9, pancreas; 10, kidney; 11, skeletal muscle; 12, liver; 13, Lung; 14, placenta; 15, brain; 16, heart; M, marker.

Tissue distribution of the ZNF300-B *gene*

Reverse transcription RCR analysis was performed with the human multiple tissue cDNA (MTC) panel (Clontech), to investigate the expression pattern of *ZNF300-B* gene. The expected size of PCR product was 520 bp. While with the same primers, *ZNF300* was detected of 460 bp. The *ZNF300-B* expressed at a high levels in testis (Figure 4). The result indicates that *ZNF300-B* might play an essential role during meiosis and spermatogenesis. Further study will focus on the function and the structure of the *ZNF300-B* gene in human.

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References

Collins T, Stone JR, Williams AJ. 2001. All in the family: The BTB/POZ, KRAB, and SCAN domains. Mol Cell Biol 21: 3609–3615.

Dai J, Li Y, Ji C, Jin F, Zheng Z, Wang X, Sun X, Xu X, Gu S, Xie Y, Mao Y. 2003. Characterization of two novel KRAB-domain-containing zinc finger genes, ZNF460 and ZNF461, on human chromosome 19ql3.1 → ql3.4. Cytogenet Genome Res 103: 74–78.

Gou D, Wang J, Gao L, Sun Y, Peng X, Huang J, Li W. 2004. Identification and functional analysis of a novel human KRAB/C_2H_2 zinc finger gene ZNF300. Biochim Biophys Acta 1676:203–209.

Grondin B, Cote F, Bazinet M, Vincent M, Aubry M. 1997. Direct interaction of the KRAB/Cys2-His2 zinc finger protein ZNF74 with a hyperphosphorylated form of the RNA polymerase II largest subunit. J Biol Chem 272:27877–27885.

Hering TM, Kazmi NH, Huynh TD, Kollar J, Xu L, Hunyady AB, Johnstone B. 2004. Characterization and chondrocyte differentiation stage-specific expression of KRAB zinc-finger protein gene ZNF470. Exp Cell Res 299:137–147.

Li XA, Kokame K, Okubo K, Shimokado K, Tsukamoto Y, Miyata T, Kato H, Yutani C. 1999. Cloning and characterization of a novel human gene encoding a zinc finger protein with 25 fingers. Biochim Biophys Acta 1489:405–412.

Medugno L, Florio F, De Cegli R, Grosso M, Lupo A, Costanzo P, Izzo P. 2005. The Kruppel-like zinc-finger protein ZNF224 represses aldolase A gene transcription by interacting with the KAP-1 co-repressor protein. Gene 359:35–43.

Miller J, McLachlan AD, Klug A. 1985. Repetitive zinc-binding domains in the protein transcription factor IIIA from *Xenopus oocytes*. EMBO J 4:1609–1614.

Pavletich NP, Pabo CO. 1991. Zinc finger-DNA recognition: Crystal structure of a Zif268–DNA complex at 2.1 Å. Science 252:809–817.

Pecasse F, Beck Y, Ruiz C, Richards G. 1986. Kruppel-homolog, a stage-specific modulator of the prepupal ecdysone response, is essential for Drosophila metamorphosis. Cell 47:1025–1032.

Preiss A, Rosenberg UB, Kienlin A, Seifert E, Jäckie H. 1985. Molecular genetics of Krüppel, a gene required for segmentation of the *Drosophila* embryo. Nature 313:27–32.

Theunissen O, Rudt F, Guddat U, Mentzel H, Pieler T. 1992. RNA and DNA binding zinc fingers in Xenopus TFIIIA. Cell 71: 679–690.

Urrutia R. 2003. KRAB-containing zinc-finger repressor proteins. Genome Biol 4:231.

Vissing H, Meyer WK, Aagaard L, Tommerup N, Thiesen HJ. 1995. Repression of transcriptional activity by heterologous KRAB domains present in zinc finger proteins. FEES Lett 369:153–157.

Wu H, Zhang S, Qiu W, Zhang G, Xia Q, Xiao C, Huang X, Huang M, Agen P, Fan T, Yang J, Milunsky A. 2001. Isolation, characterization, and mapping of a novel human KRAB zinc finger protein encoding gene ZNF463. Biochim Biophys Acta 1518:190–193.

Yin G, Ji C, Zeng L, Wang Z, Wang J, Shen Z, Wu T, Gu S, Xie Y, Mao Y. 2006. Cloning and characterization of a novel KRAB-domain-containing zinc finger gene (ZNF284L). Mol Biol Rep 33:137–144.