



Short communication

The structure and functional analysis of canine T-cell receptor beta region

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ABSTRACT

VDJ recombination is a key process in T-cell receptor (TCR) and immunoglobulin (Ig) molecules development. Comparison of ENSEMBL and GenBank database information revealed major differences in dog T-cell receptor beta (TRB) region annotations. ENSEMBL based genomic alignment of dog TRB sequence with human sequence and annotation showed a very similar structure of TRB. However, there is only one cluster of DJC segments in dogs. In dog, 38 V segments are followed by 1 D segment, 6 J segments and 1 C segment. Like humans and mice, dogs have another V segment opposite in orientation downstream of the C segment. V segments anticipated were analyzed using the RT-PCR and capillary electrophoresis. Thirty-one of them were identified in samples of thymus and spleen RNA and thus believed to be subjected to chromosomal rearrangement and RNA splicing. We identified and analyzed probable structure of canine TCR beta region, which is different when compared to sequences published in GenBank or ENSEMBL databases.

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1. Introduction

As man's best friend, veterinary medicine can often have a focus on the canine species. Dogs suffer from various diseases that involve the immune system. A very important problem is a group of autoimmune disorders (Felsburg, 1994; Pedersen, 1999), which can involve both primary immunodeficiency (Giger et al., 1987; Felsburg et al., 1999) and autoimmunity (Lewis et al., 1965). Moreover, the dog has also been used as a biomedical model, namely in studies on hematopoietic stem cell transplantation (Ladiges et al., 1990; Wagner and Storb, 1996; Kirk, 2003).

T-cell receptors (TCRs) are heterodimer surface structures expressed on the surface of T lymphocytes. They are responsible for a specific recognition of antigen fragments presented by antigen-presenting cells via major histocompatibility complex (Kuby, 1997). TCRs can be sub-

divided into two different categories based on the protein chains used: $\alpha\beta$ TCR and $\gamma\delta$ TCR. Canines, humans and mice belong to animal species in which $\alpha\beta$ TCR T lymphocytes are the predominant lymphocyte population in both peripheral blood and lymphoid organs (Faldyna et al., 2001, 2005). Maturation of T lymphocytes in the thymus involves rearrangement of chromosomal locuses coding protein chains of TCR by a mechanism closely related to chains of immunoglobulins. This process assembles mature TCR genes from individual V (variable), D (diversity) and J (joining) segments. Production of functional VDJ β rearrangement allows assembly and expression of pre-TCR receptor, which drives T lymphocyte precursors proliferation and maturation. Subsequently, the rearrangement of TCR α locus is activated (Jackson and Krangel, 2006).

Public genome databases constitute a repository of biological research data research done throughout the world. Veterinary medicine embarked on the dog genome sequence shortly after the human genome sequence had been published. The poodle's genome sequence (Kirkness et al., 2003) represents 1.5 $\times$ coverage of the entire dog

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Table 1

RSSs identified, primers used and V segments expression analyzed as presence of RT-PCR products on capillary electrophoresis.

5' RSS					Segment					3' RSS					Primer		Primer sequence		RT-PCR ^a
From	Nonamer	N12	Heptamer	To		From	Heptamer	N23	Nonamer	To	Primer	5' position	5' sequence	3'					
					LOC482754	9993634	CACAGTG	N23	ATACAAACC	9993596	V1	9993677	ATGTGACCCAGAGCAGAACC					Yes	
					ENSCAFG 0.24754	9942224	CATCCTG	N23	CAACCAAAA	9942186	V2	9942453	GTACTGGTATCAGCAGAGGGC					Yes	
					TRBV2	9940885	ATCTGTG	N23	GAATAATGT	9940847	V3	9940905	GTAGAGCCAGCCAGAGATG					No	
											V3.2	9940939	AGCTGGAAGGTCTGATGGAT					No	
					TRBV3-1	9938361	CACAGCC	N23	GCACAAACC	9938323	V4	9938535	CCTCAAGCAACTGCTGAAGATC					Yes	
					TRBV4-1	9935521	CACTTTG	N23	CAAAAGGTA	9935483	V5	9935599	GAATGGAGACAGGGTCTCTGAATC					Yes	
					ENSCAFG 0.30817	9934877	CACAGCC	N23	GCACAAACA	9934839	V6	9935092	CAACATCTGGGACATGATGAG					Yes	
					TRBV17		nd		nd		V7	9933606	CAGAAAGTGGAGTTTCTAATTTTATCTACAG					No	
					TRBV7-1	9933437	CACAGCC	N23	ACACAGACC	9933399	V8	9933476	GGGAGACTCAGCCATGTACC					No	
											V8.2	9933542	GGATCGATTCTCAGCTGGAAA					No	
					ENSCAFG 0.30815	9927728	CACAGCC	N23	GCACAAACC	9927690	V9	9927829	TTTCTCACTGAGACATCTGACAAAT					Yes	
					TRBV4-2	9924205	CACAGCC	N23	GCACAAACA	9924167	V10	9924377	GTCAAGAGCCACCGAAGCTTAT					Yes	
					Similar to TRBV7-1	9922764	CACAGCC	N23	ACACAAACC	9922573	V11	9922810	CAGAGCTGGGAGACTCAACC					No	
											V11.2	9922873	CAAGGATCAATTCTCAGCTGGAT					Yes	
					Similar to TRBV3-1	9919382	CACAGCC	N23	GCACAAACC	9919344	V12	9919437	TCAACTCTCTGGAGACAGGAGAC					Yes	
					ENSCAFG 0.03814	9915980	CACAGCC	N23	GCACAAACC	9915942	V13	9916057	CAGCTCCAGGTTCTACCTTCA					Yes	
					TRBV7-2	9914607	CACAGCC	N23	GCACAAGCT	9914569	V14	9914696	CAGCTGAGTTGCCCTGACAGA					No	
											V14.2	9914737	TTCCCTCAGAGAAGGCTGAC					No	
					Similar to XM_851617	9912379	CACAATA	N23	TCGGAAAGA	9912341	V15	9912435	TGAAGTCAACCAAGCAAC					No	
											V15.2	9912555	GAGACCATACCTGAACTCTTGA					No	
					ENSCAFG 0.03812	9903489	CACAGCC	N23	ACAAAAACC	9903451	V16	9903695	CCCTGAGATGTTCCCTTATCTCT					Yes	
					TRBV6-8	9898425	CACAGCG	N23	ACAAAAAT	9898387	V17	9898497	GGAGCTTCCTTCTCAAGCTG					Yes	
					TRBV7-9	9894674	CACAGTG	N23	TTACAAACC	9894636	V18	9894694	CTCTGTGCCAGCAGTCTCAC					Yes	
					TRBV6-6	9891547	CACAGCT	N23	ACCGAAAGC	9891509	V19	9891640	ACGTCTCCCGAAATGTGAAG					Yes	
					TRBV5-8	9885867	CACAGCC	N23	GCACAAGCC	9885829	V20	9885966	GATTCTCAGGGCAGCAGTTC					Yes	
					TRBV13	9879267	CACAGCC	N23	ACTCAAACT	9879229	V21	9879461	ATCTCGTCTCTGGACACAACACT					Yes	
					TRBV10-3	9874468	CACAGCA	N23	ACATAAAGG	9874430	V22	9874535	TCCCTCTCTACTGGACTCT					Yes	
					TRBV11-3	9866975	CACCGCG	N23	ACATAAAAC	9866937	V23	9867066	CAGAGAGGCCCAAAGGAGTAG					No	
											V23.2	9867138	GGATCTCCCGATTCACTTTG					Yes	
					TRBV12-4	9860125	CACAACA	N23	GCAGAAACC	9860087	V24	9860147	ACCTATGTGGCAGCAGGGTA					No	
											V24.2	9860192	CATCTGAAGATCCAGCCAAC					Yes	
					TRBV14	9852768	CACAGTA	N23	GCAGAAACC	9852730	V25	9852864	CTCAGCAGAGATGCCTGACA					Yes	
					TRBV15	9847639	CACGGAG	N23	GCACAAACC	9847601	V26	9847704	TCTCTTAGCATCCGCTCACC					Yes	
					TRBV23-1	9844042	CACAGTG	N23	CACAAACAT	9844004	V27	9844172	ATTCTTTCCAGGATAAGCGTGT					Yes	
					TRBV18	9833441	CACATCA	N23	GCACAAACG	9833403	V28	9833523	CCGAAGACGGACTTAGCATC					Yes	
					TRBV19	9830161	CACAGTG	N23	GCAGAAATG	9830123	V29	9830374	GTGTGACTCTGGAATGTGAACAG					Yes	
					TRBV20-1	9825013	CACAGCC	N23	ACAAGAAC	9824975	V30	9825156	CCTTACCCTTATGGTGACCTCTA					Yes	
					TRBV21-1	9817815	CATAGTG	N23	CACAAACGC	9817777	V31	9817909	CAGCTACATGCCCAAGAAC					Yes	
					LOC482750	9813345	CACAGCG	N23	ACACAAACT	9813313	V32	9813434	AGAGGAGAAGGGGCTGTTC					No	
											V32.2	9813512	CTGCGGCTGATTACCTCTC					No	
					TRBV24-1	9804427	CACAGTG	N23	ACAGAAAGC	9804389	V33	9804567	CTACGGCTGATCTCCTACTCCT					Yes	
					TRBV25-1	9799153	CACAGTG	N23	ATAAAAGG	9799115	V34	9799186	GACATCCCTGTACTCTGTGC					Yes	
					XM_851617	9792290	CACAGCA	N23	ACAAGAAAG	9792252	V35	9792518	TTGGGACAGGAAAGAAATTAAC					Yes	
					TRBV27	9782989	CACAGCG	N23	ACAGAAAGG	9782951	V36	9783089	GGGTCTCTCGGAAAGAGAAG					No	
											V36.2	9783112	GGAGATGTCCTGATGGTA					No	
					TRBV28	9773836	CACAGTG	N23	ACAAGATGC	9773798	V37	9773924	TCGAGGAAGAAGAAGGATGC					Yes	
					TRBV29-1	9767293	CACAGCG	N23	ACAAGAAC	9767255	V38	9767484	TACCAAGTCACCTTGATTTCT					Yes	

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