Assignment¹ of the bovine tumor protein D52 gene (*TPD523X*) to the distal half of BTA14 with somatic and radiation cell hybrid panel mapping

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¹ To our knowledge, this is the first time this gene has been mapped in cattle.

Rationale and significance

TPD52 belongs to the D52 gene family. D52 proteins act as signaling molecules, which may play a role as regulators of cell proliferation (Nourse et al., 1998). It is also suggested that the gene product acts differently in normal and tumor affected tissues and between fetal and adult tissues (e.g. Byrne et al., 1995; Tiacci et al., 2005). Chen et al. (1996) identified overexpression of the gene in tumor derived cell lines from multiple cancers. *TPD52* in human and mouse is located on HSA8q21 and MMU3A1-A2, respectively. A bovine partial gene sequence of *TPD52* was identified with the strategy of comparative mapping by annotation and sequence similarity (COMPASS) and according to the gene assignment in human a locus was predicted on *Bos taurus* (BTA) chromosome 14 (Ma et al., 1998). Several independent investigations give experimental proof that BTA14 is associated with different quantitative traits influencing milk production (Khatkar et al., 2004). The physical assignment of the bovine *TPD52* gene by somatic cell and radiation cell hybrid mapping techniques presented in this report will enhance our knowledge about loci in trait associated BTA14 chromosome segments.

Materials and methods

Primer sequences *TPD52*-F 5'-CGTTGTATTTGTTATTTATCAAG-TTGT-3' and *TPD52*-R 5'-TCCCAAGTAAATCTAGTCATGC-3' were designed from bovine EST BP230009B10C3 (Soares normalized bovine placenta *Bos taurus* cDNA clone BP230009B10C3 5' mRNA sequence). The bovine sequence (Acc. No. AW462495) is similar to the bovine genome shotgun sequence AAFC01061142 (Identities 488/496 bp; 98%; Expect = 0.0) as well as to reference sequences in human (Acc. No. NM_005079; Identities 408/488 bp; 83%; Expect = e-130) and mouse (Acc. No. NM_009412; Identities = 113/148 bp; 76%; Expect = 4e-14). PCR was performed using HotStar-Taq-Polymerase (Qiagen). PCR conditions were 94°C for 30 s, 58°C for 30 s and 72°C for 45 s repeated in 36 cycles with an initial denaturation of DNA for 15 min at 95°C and a final extension step at 72°C for 10 min. A 153-bp fragment was obtained after PCR on bovine genomic DNA. The PCR product was sequenced for controlling sequence similarity with *TPD52* (results not shown). Physical mapping of *TPD52* was performed by PCR typing in a bovine-hamster somatic cell hybrid panel (Womack and Moll, 1986) and in a bovine-hamster 5,000-rad whole genome radiation cell hybrid panel (WGRH₅₀₀₀; Womack et al., 1997) followed by subsequent statistical analysis. PCR typing in the WGRH₅₀₀₀ panel was performed twice and experiments were scored independently to increase the accuracy of results. For identification of synteny, statistical analysis was carried out as described by Chevalet and Corpet (1986). Two-point linkage analysis was done with all markers of the cattle WGRH₅₀₀₀ gene map (Band et al., 2000) using the software RHMAPPER 1.22 (Slonim et al., 1997) to link *TPD52* with a chromosome region specific marker. The calculation tool for linkage analysis of bovine RH mapping data is available at <http://bovid.cvm.tamu.edu/cgi-bin/rhmapper.cgi>.

Results

Somatic hybrid panel

Performing PCR with the *TPD52* primers in the somatic hybrid panel, a bovine-specific band was detected in 15 of the 31 hybrid cell lines. The data vector obtained was: 1111000010100011011111000000011 (1 = present in cell line; 0 = not

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Table 1. Segregation of *TPD52* with BTA14 in somatic cell hybrids

Reference marker/ New marker	Chromosome 14			
	TG <i>TPD52</i>	BSPN	RM180	ILSTS011
+/+	11	11	14	10
-/-	14	11	14	13
+/-	4	4	1	5
-/+	2	5	2	3
% concordant	81	71	90	74
% discordant	19	29	10	26

present in cell line). A concordance value of 0.90 with marker RM180 and higher concordance values with three other markers characterizing BTA14 within the panel (Table 1) in comparison with the remaining chromosome specific markers represented in the somatic hybrid panel (concordance: <0.71) allowed the syntenic assignment of *TPD52* to BTA14.

Radiation hybrid panel

RH mapping confirms the assignment of *TPD52* to BTA14. Double analysis in 90 selected cell lines of the WGRH₅₀₀₀ panel resulted in the vector: 0000000010 0001001000 0001000010 0101000000 1100000000 1100100000 1000100100 0010010010 1000000001. A retention frequency (RF) of 0.22 was calculated based on twenty bovine-specific PCR typing signals. This RF value represents the average calculated for all markers within the first-generation cattle WGRH₅₀₀₀ map and is a little lower than the published RF of 0.25 for BTA14 (Band et al., 2000). Data evaluation linked *TPD52* at a LOD score >12 significantly with marker *IL7* in a distance of about 19 cR₅₀₀₀. The low distance of about 1.2 Mb in the human genome between loci for *TPD52* on HSA8q21 at nucleotide position 32.8 Mb and *IL7* on HSA8q12→q13 at nucleotide position 31.6 Mb as well as loci for both genes in one small conserved segment in mouse on MMU3A1-A2 supported the assignment in cattle. Comparing relevant genetic and physical mapping data in cattle suggested that *TPD52* is cytogenetically located in the distal half of BTA14. The marker *IL7* is located in RH linkage group 3 of the first-generation cattle WGRH₅₀₀₀ map (Band

et al., 2000) and in RH linkage group 4 of the second-generation cattle WGRH₅₀₀₀ map (Everts-van der Wind et al., 2004). With its RH linkage to *IL7*, the assignment of *TPD52* is distal from *TOX* (BTA14q17) and RM192 (65 cM) and proximal from *ODF1* (BTA14q23→q24) and BM2934 (84 cM; www.marc.usda.gov/genome/genome; Ihara et al., 2004). Interestingly, the cytogenetic position of *IL7* is BTA14q24→q26 suggesting a locus for *TPD52* at the telomere region (<http://locus.jouy.inra.fr/cgi-bin/bovmmap/intro.pl/>). Cytogenetic anchoring of *TPD52* on BTA14 and alternatively the shortly available assembled cattle genome shotgun DNA sequence will contribute to the clarification of the accurate locus.

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