Assignment¹ of the subunit C of succinate dehydrogenase complex (*SDHC*) gene to bovine chromosome 2 with somatic and radiation hybrid panel mapping

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

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Rationale and significance

Succinate dehydrogenase complex II is an important enzyme complex in the tricarboxylic acid cycle and the aerobic respiratory chains of mitochondria in eukaryotic cells and prokaryotic organisms. Complex II in mitochondria has four subunits encoded by nuclear genes *SDHA*, *SDHB*, *SDHC* and *SDHD*. *SDHA* and *SDHB* encode the flavoprotein and iron-sulfur proteins, respectively, and *SDHC* and *SDHD* encode the two hydrophobic membrane-spanning subunits, which form the large and small subunits of cytochrome b and anchor the catalytic subunits *SDHA* and *SDHB* to the inner mitochondrial membrane (Baysal et al., 2001). The human genes coding for the subunits B and C were assigned to chromosome 1, regions 1p36 and 1q21, respectively

(Leckschat et al., 1993; Hirawake et al., 1997), while *SDHA* was assigned to chromosome 5 (Bourgeron et al., 1995) and *SDHD* to chromosome 11 (Hirawake et al., 1997). At the moment only *SDHD* has been assigned to a bovine chromosome (BTA15; Everts-van der Wind et al., 2004). Defective mitochondrial respiratory enzymes cause myopathy and neurologic diseases in humans, with pathologies that include precocious aging. Results showed by Baysal et al. (2004) confirmed the role of *SDHC* in both familial and sporadic paragangliomas. The physical assignment of bovine *SDHC* by somatic cell and radiation hybrid mapping techniques presented in this report will enhance our knowledge regarding the genomic organization of this important enzyme complex.

Materials and methods

Polymerase chain reaction (PCR) primers were used to amplify a region of about 380 bp of the bovine *SDHC* gene. The primer sequences *SDHC*-F 5'-TGT GAA GTC CCT GTG TTT GG-3' and *SDHC*-R 5'-GAC CCT TTT CCA CCG TTA TG-3' were designed from a *Bos taurus* mRNA sequence (GenBank accession number NM_175814) using Primer3 (http://www-genome.wi.mit.edu/genome_software/other/primer3.html). The PCR reactions were performed in 10-μl reaction mixtures containing 50 ng of template DNA; 1.5 mM MgCl₂; 10 mM Tris-HCl; 50 mM KCl; 0.2 mM dGTP, dTTP, dATP and dCTP; 10 pmol each sense and antisense primer and 0.5 U of Taq DNA polymerase (AmpliTaq Gold; PE Applied Biosystems, Foster City, CA, USA). Reactions

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were conducted in a PTC-200 MJ Research™ thermal cycler with thermal gradient software. PCR conditions included 95°C for 10 min; 35 cycles of 95°C for 30 s, 65°C for 30 s and 72°C for 30 s; with a final extension cycle of 72°C for 7 min. PCR products were visualized on 2% agarose gels in 1.0× TBE buffer and stained with ethidium bromide. Initial bovine chromosomal assignment for the *SDHC* gene was obtained by PCR analysis using a somatic hybrid cell panel of 31 hamster/bovine and mouse/bovine hybrid somatic cell lines described previously (Womack and Moll, 1986). A whole-genome radiation hybrid (WGRH₅₀₀₀) panel (Womack et al., 1997) composed of 90 radiation hybrid cell lines was used to determine the specific chromosomal location for the *SDHC* gene. PCR results were scored as present (1), absent (0), or ambiguous (2). RHMAPPER 1.22 software (Slonim et al., 1997) was utilized in assignment of PCR product to a chromosome region and identification of the nearest marker in the bovine WGRH₅₀₀₀ map (Everts-van der Wind et al., 2004). Minimum LOD score accepted for linkage was 10.

Results

Somatic hybrid panel

A bovine-specific band was detected in six of the 31 hybrid cell lines. The data vector obtained was: 11010 10000 01000 00000 00000 000100 (1 = present in the cell line; 0 = not present in the cell line). The bovine *SDHC* gene was assigned on BTA2 with significant concordant value of 97% to the previously mapped reference marker *ARO28* (Table 1).

Radiation hybrid panel

RH mapping confirms the assignment of *SDHC* to BTA2. All PCRs were performed twice and scored independently resulting in the vector: 00000 00000 00000 00000 00000 00000 00010 00000 00001 10000 00000 00000 00000 00001 00000 00000 11000 00011. A retention frequency (RF) of 0.11 was calculated based on eight bovine-specific PCR bands. This RF value is a little lower than the published RF of 0.18 for BTA2 (Band et al., 2000). Two-point LOD scores showed that *SDHC* is linked to *CTLA4* with a LOD score of 14.0 for a θ value of 0.11. The marker *CTLA4* is located in RH linkage group 4, position 16.3, of the second-generation cattle WGRH₅₀₀₀ map (Everts-van der Wind et al., 2004).

Table 1. Segregation of *SDHC* with bovine chromosome 2 in somatic cell hybrids

Reference marker/ new marker	Chromosome 2	
	BM3627/ <i>SDHC</i>	ARO28/ <i>SDHC</i>
+/+	6	6
-/-	20	24
+/-	0	0
-/+	5	1
% concordant	84	97
% discordant	16	3

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