Assignment¹ of the fibroblast growth factor 10 (*FGF10*) gene to bovine chromosome 20q16→q17 by fluorescence in situ hybridization and somatic cell panel analysis

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

Rationale and significance

Tetradymelia is an inherited defect observed in Holstein cattle (Kühn et al., 2002) characterized by complete lack or extremely severe reduction of all limb constituents distal of scapula and pelvic girdle. Due to the marked similarity in phenotype between fibroblast growth factor (*Fgf10*)^{-/-} knockout mice (Min et al., 1998) and tetradymelia calves, *FGF10* was a strong candidate gene for the bovine defect. In humans *FGF10* is localized on HSA5p13→p12 in close vicinity to the growth hormone receptor (*GHR*) gene. Bovine *GHR* was genetically mapped to the middle part of BTA20 (Barendse et al., 1997). Comparative mapping (Everts-van der Wind et al., 2004) confirmed the chromosomal homology between HSA5 and the centromeric and middle part of BTA20, indicating that *FGF10* should be located in the middle part of BTA20. Multipoint likelihood homozygosity mapping, however, excluded the middle part of BTA20 as locus for tetradymelia (Kühn et al., 2002). Proof of the assumed genomic localization of *FGF10* in the

middle part of BTA20 unambiguously excludes *FGF10* as the causal gene underlying the genetic defect tetradymelia in cattle despite its strong candidacy.

Materials and methods

Isolation of a specific bovine *FGF10* fragment

After alignment of the human, mouse, and rat *FGF10* sequence the heterologous primers FGF10F1 5' TTTGTGCTGTTCTTGGTGTC 3' and FGF10R2: 5' TCTTGGTCCCGCTGACCTTC 3' were designed for exon 1, which represented the largest exon of the *FGF10* genes, to amplify a specific *FGF10* fragment in bovine genomic DNA. Sequencing of the generated fragment (AY736184) revealed a total agreement with a partial bovine cDNA sequence (AY183659.1), which had been entered into the NCBI data base (<http://www.ncbi.nlm.nih.gov/>) during isolation of the bovine genomic fragment. Homology search indicated 98.4, 89.7, 88.3, and 86.8% sequence homology across the entire sequence to the respective region of sheep, rat, human, and mouse *FGF10* cDNA, respectively.

Isolation of bovine BAC clones

A bovine BAC library (Zhu et al., 1999) was screened by PCR with the primers FGF10F1/FGF10R2 identifying two BAC clones BBI_B750O1689 and BBI_B750P1689, respectively. The presence of the *FGF10* gene within the clones was confirmed by PCR with species-specific primers and sequencing of the generated fragments.

Fluorescence in situ hybridization

Fluorescence in situ hybridization was carried out on GTG-banded cattle chromosome preparations, as described previously by Goldammer et al. (1999).

Somatic cell mapping

Chromosomal assignment of *FGF10* has been performed by synteny mapping using a previously characterized hamster/bovine somatic cell panel (Guerin et al., 1994; Laurent et al., 2000). PCR reactions amplifying a 254-bp fragment were carried out in a final volume of 10 µl with 100 ng DNA,

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Fig. 1. Assignment of *FGF10* to BTA20q16→q17; GTG-banded metaphase (left); metaphase with FISH of the bovine BAC clone BBI_B750O1689 containing the *FGF10* gene (middle), ideogram of BTA20 (right).

10 pmol of each primer (FGF10F1, FGF10R2), 200 μ M dNTPs, 0.15 U *Taq* Polymerase (Promega) and standard buffer conditions including 1.5 μ M $MgCl_2$. Samples were denatured at 94 °C for 3 min followed by a touch down procedure. Each PCR cycle consisted of denaturation at 95 °C for 15 s, annealing at 58 °C for 1 min and extension at 72 °C for 15 s. 35 cycles were performed followed by a final elongation step for 7 min at 72 °C. PCR products were separated by electrophoresis on agarose gels stained with ethidium bromide.

Probe name: BBI_B750O1689
 Probe type: Bovine genomic BAC clone
 Insert size: ~ 100 kb
 Vector: pBAC3.6e
 Proof of authenticity: DNA sequencing
 Gene reference: AY736184

Results

Mapping data

Most precise location: BTA20q16→q17

The nucleotide position of *FGF10* in the human chromosome 5 reference sequence is 44.3506 Mb – 44.4343 Mb.

No. of cells examined: 12

Number of cells with specific signal: 1 (0), 2 (0), 3 (3), 4 (9) chromatids per cell

Mapping by FL:

Number of chromosomes examined: 24

Mean location: 0.58

Bands encompassed: BTA20q17

Range: BTA20q16→q17

Standard deviation: ± 0.027

Location of background signals: none observed

The data vector obtained in the 38 hybrid cell lines was 0 0 0 0 0 0 1 1 0 0 0 1 0 0 0 1 0 0 0 1 1 0 0 1 0 0 1 0 0 1 1 0 0 1 0 1.

FGF10 was assigned to bovine chromosome 20 with a correlation coefficient of 0.87 with D20S3 (INRA36), which confirmed the results from in situ hybridization (Fig. 1).

Mapping of *FGF10* to bovine chromosome 20 is in agreement with comparative mapping data between cattle and human, because the centromeric and middle part of BTA20 corresponds to parts of HSA5. *Fgf10* in mice is the most telomeric gene on MMU18 (117.5645–117.6416 Mb), thus delimiting the conserved chromosomal segments between MMU18 and BTA20.

References

- Barendse W, Vaiman D, Kemp SJ, Sugimoto Y, Armitage SM, Williams JL, Sun HS, Eggen A, et al: A medium-density genetic linkage map of the bovine genome. *Mamm Genome* 8:21–28 (1997).
- Everts-van der Wind A, Kata SR, Band MR, Rebeiz M, Larkin DM, Everts RE, Green CA, Liu L, Natarajan S, Goldammer T, Lee JH, McKay S, Womack JE, Lewin HA: A 1463 gene cattle-human comparative map with anchor points defined by human genome sequence coordinates. *Genome Res* 14:1424–1437 (2004).
- Goldammer T, Brunner RM, Vanselow J, Zsolnai A, Furbass R, Schwerin M: Assignment of the bovine aromatase encoding gene *CYP19* to 10q26 in goat and 7q24→q31 in sheep. *Cytogenet Cell Genet* 85:258–259 (1999).
- Guerin G, Eggen A, Vaiman D, Nocart M, Laurent P, Bechet D, Ferrara M: Further characterization of a somatic-cell hybrid panel – 10 new assignments to the bovine genome. *Anim Genet* 25:31–35 (1994).
- Kühn Ch, Weikard R, Panicke L, Schwerin M: Fibroblast growth factor 10: A potential candidate gene for bovine developmental defect Tetradymelia? 7th World Congr Genet Appl Livest Prod, Montpellier, 379–382 (2002).
- Laurent P, Elduque C, Hayes H, Saunier K, Eggen A, Leveziel H: Assignment of 60 human ESTs in cattle. *Mamm Genome* 11:748–754 (2000).
- Min H, Danilenko DM, Scully SA, Bolon B, Ring BD, Tarpley JE, DeRose M, Simonet WS: *Fgf-10* is required for both limb and lung development and exhibits striking functional similarity to *Drosophila* branchless. *Genes Dev* 12:3156–3161 (1998).
- Zhu BL, Smith JA, Tracey SM, Konfortov BA, Welzel K, Schalkwyk LC, Lehrach H, Kollers S, Masabanda J, Buitkamp J, Fries R, Williams JL, Miller JR: A 5' genome coverage bovine BAC library: production, characterization, and distribution. *Mamm Genome* 10:706–709 (1999).