FIBROBLAST GROWTH FACTOR 10: A POTENTIAL CANDIDATE GENE FOR BOVINE DEVELOPMENTAL DEFECT *TETRADYSMELIA* ?

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INTRODUCTION

"*Tetradysmelia* is an inherited defect observed in Holstein cattle (Kühn *et al.*, 1998). It is characterised by complete lack or extremely severe reduction of all limb constituents distal of scapula and pelvic girdle. Affected calves are usually delivered in term, but stillborn. In chicken a similar defect "*limbless*" characterised by complete lack of wings and legs and strong reduction of the size of the upper beak had been described (Prahlad *et al.*, 1979). The genetic reasons for these spontaneous extreme reductions in limb development have been detected neither in cattle nor in chicken. Linkage analysis within an inbred backcross Holstein family gave initial indication on a locus responsible for *Tetradysmelia* in the middle part of BTA2 (Kühn *et al*., 1998). However, Engrailed 1 (*EN1*), a putative candidate gene located in this chromosomal area, could be excluded as causal gene for the defect by refined mapping (Kühn *et al*, 1999). No further chromosomal region showed significant linkage to *Tetradysmelia* in the whole genome scan. However, it had to be considered, that due to the fact, that about 35% of the markers in the whole genome scan were homozygous in the founder of the family, there were still several noninformative chromosomal regions.

Developmental studies in vertebrates demonstrated that signalling systems in limb development seem to be highly conserved from Drosophila to mammals. This indicates, that the genetic background of limb malformation may be very similar across species. Therefore, knock-out mouse experiments indicated, that Fibroblast Growth factor (*FGF10*) is a putative candidate gene for *Tetradysmelia*. Homozygous *FGF10*-/- mice are characterised by complete lack of all fore and hind limbs (Min *et al*., 1998, Sekine *et al*, 1999). *FGF10*-/- foetuses are usually carried to term and are severely affected in lung development. In strong contrast to other mouse mutants with complete lack of limb buds *FGF10*-/- mice did not show any further developmental defects. Due to the marked similarity in phenotype between *FGF10*-/- knock out mice and *Tetradysmelia* calves *FGF10* was a strong candidate gene for the bovine defect. *FGF10* was localized on HSA5p13-p12 in close vicinity to *GHR1*. *GHR1* was genetically mapped to the middle part of BTA20 (Barendse *et al*., 1997). Comparative mapping (Solinas-Toldo *et al*., 1995) confirmed the chromosomal homology between HSA5 and the centromeric and middle part of BTA20. Therefore, we concluded, that *FGF10* should be located in the middle part of BTA20. Detailed data analysis of our first genome scan revealed an uninformative marker interval of 40 cM in the middle part of BTA20. Thus, we increased the marker density in the respective chromosomal region in an inbreeding resource family to identify, if *FGF10* may be genetic cause of *Tetradysmelia* in cattle.

MATERIAL AND METHODS

Segregation of defect in backcross family: For our study a backcross family was investigated (figure 1). It focussed on a male Holstein founder (F), who was mated by conventional Embryo Transfer (ET) to five of his purebred daughters (I-V). No cases of dysmelia had been reported for the ancestors or offspring of the sire and the daughter's dams before. Recovered embryos were transferred to recipients on four different farms with no farm contacts. Clinical and biochemical investigation of the 14 live born calves showed no abnormality. Two of the daughters (III and IV) generated 6 calves with uniform malformation (figure 1): complete lack or very strong reduction of all four limbs. Both sexes were affected. Necropsy revealed no further strong deformation in these calves.

Genotyping of BTA20 markers. For DNA preparation blood, sperm or tissue samples were taken. Genetic mapping of the observed defect was performed by genotyping 15 bovine microsatellite markers, spanning the whole chromosome 20, within the backcross family. Microsatellite genotypes were determined after PCR by automated fragment analysis (A.L.F., Amersham-Pharmacia) or detection by silver staining (Weikard *et al*., 1997).

Statistical analysis. Multipoint likelihood homozygosity mapping (program package MAPMAKER/HOMOZ, Kruglyak *et al*., 1995) assuming recessive mode of inheritance and full penetrance was performed within the subfamily F- daughter III.

RESULTS AND DISCUSSION

There was no indication on non-genetic reasons for the defect, as malformed foetuses generated from daughters kept on different farms and from different ET flushings of the same daughter. Furthermore, recipients, which gave birth to the defect foetuses, were kept on several farms. The sire F had several thousand offspring in a conventional breeding program with no further reported affected offspring. On the other hand the frequency of affected calves in our resource population was 26% (figure 1). Hence, we assumed a recessive mode of inheritance. Genotyping of the resource family showed, that 11 of the markers were heterozygous in the founder sire. 8 markers yielded unequivocal information about alternative paternal origin of marker alleles in the dams with affected offspring. Haplotype construction within the pedigree showed, that females III and IV inherited alternative paternal chromosomal regions for the middle part of BTA20. However, assuming recessive inheritance and no transmission of the defect from the maternal dams all daughters giving birth to *Tetradysmelia* calves should receive identical paternal chromosomal regions harbouring the defect. From the affected calves only offspring 2 (figure 1) received two paternal haplotypes identical by descent for the middle part of BTA20, while e. g. offspring 15 and 16 received one greatmaternal and alternative paternal haplotypes. Applying statistical threshold of LOD score –2 homozygosity mapping within subfamily F - daughter III showed, that under the given assumptions the middle part of BTA20 can be excluded as locus for *Tetradysmelia* (figure 2). Results from haplotype construction and homozygosity mapping both indicate, that the middle part of BTA20 and thus also *FGF10* can be excluded as locus for the genetic reason of *Tetradysmelia*.

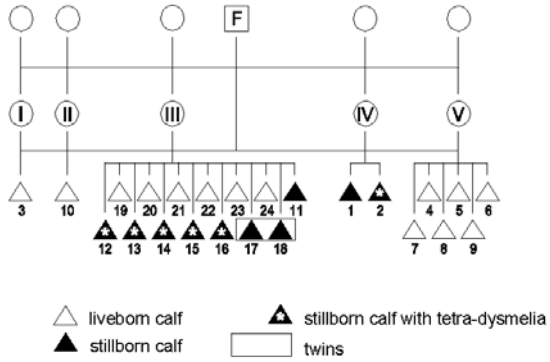

Figure 1. Holstein backcross family segregating for *Tetradysmelia*. F: Founder sire, I – V: daughters of founder sire F, 1-24: backcross offspring

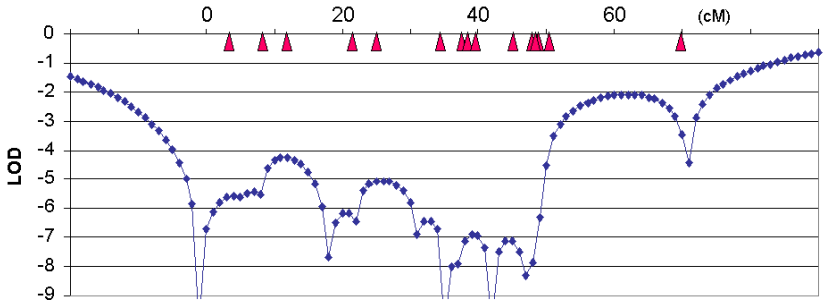

Figure 2. Test statistic on BTA20 for homozygosity mapping within subfamily F - daughter III. Position 0 denotes the centromere of the chromosome. Triangles indicate position of microsatellite markers

CONCLUSION

Inbreeding experiments clearly indicated genetic background of the defect *Tetradysmelia* in Holstein cattle. Although there is a strong phenotypic similarity to *FGF10*-/- knockout mice mapping experiments excluded the chromosomal area, which is assumed to harbour *FGF10* in cattle, as locus for *Tetradysmelia*. Further investigations will be necessary to elucidate the genetic reasons for this defect in order to develop genetic tests suitable for detection of defect carriers in cattle breeding.

REFERENCES

Barendse *et al*. (1997) *Mamm. Genome* **8** : 21-28.

Kruglyak, L., Daly, M.J. and Lander, E.S. (1995) *Am. J. Hum. Genet*. **56** : 519-527

Kühn, Ch., Weikard, R., Panicke, L. and Schwerin, M. (1998) *Anim. Genet*. **29**, Suppl.1, 35

Kühn, Ch., Weikard, R., Panicke, L. and Schwerin, M. (1999) *Proc. Candidate Genes for animal health, FBN-Schriftenreihe***, 12** *:* 63.

Min, H., Danilenko, D.M., Scully, S.A., Bolon, B., Ring, B.D., Tarpley, J.E., DeRose, M. and Simonet, W.S. (1998) *Genes & Development* **12** : 3156-3161.

Prahlad, K.V., Skala, G., Jones, D.G. and Briles, W.E. (1979) *J. Exp. Zool*. **209**, 427-434.

Solinas-Toldo S., Lengauer, C. and Fries, R. (1995) *Genomics* **27** : 489-96

Sekine, K., Ohuchi, H., Fujiwara, M., Yamasaki, M., Yoshizawa, T., Sato, T., Yagishita, N., Matsui, D., Koga, Y., Itoh, N. and Kato, S. (1999) *Nature Genet.* **21** : 138-141.

Weikard, R., Goldammer, T., Kühn, Ch., Barendse, W., and Schwerin, M. (1997) *Mamm. Genome* **8**, 836-840.