Proc. Assoc. Advmt. Anim. Breed. Genet. 19:215-218

MAPPING OF CONGENITAL CONTRACTURAL ARACHNODACTYLY IN CATTLE

I. Tammen, M.S. Khatkar, J.A.L. Cavanagh, P.A. Windsor, P.C. Thomson and H.W. Raadsma

ReproGen Animal Bioscience group. Faculty of Veterinary Science,
University of Sydney, Camden, NSW 2570

SUMMARY

In the beef cattle industry, congenital contractural arachnodactyly (CA) formerly known as "fawn calf syndrome" (FCS) was recently acknowledged as a non-lethal genetic defect in Angus cattle. The paper describes the identification and fine-mapping of a genomic region carrying the CA locus based on a staged full genome screen with high-density SNP marker panels. The coding region of a possible candidate gene was sequenced without the identification of any obvious disease causing mutations. The causal relationship between underlying gene(s) and the biological relationship to other growth and development traits remains unclear.

INTRODUCTION

Congenital contractural arachnodactyly (CA) or 'fawn calf syndrome' (FCS) (OMIA Phene ID 2983, http://omia.angis.org.au/) is an inherited 'conformational' non-lethal defect in newborn Angus calves, first recognized in Australia in 1998 (Windsor and Tammen 2001). A preliminary research collaboration between Angus Australia and scientists from the University of New England, the University of Sydney and NSW Agriculture showed that CA-affected calves were descendents of a single US Angus cow born in 1978, Freestate Barbara 871 of Kaf (Bruce Tier, pers. communication) and that CA was most likely inherited as a single-locus recessive disorder.

Clinical signs are described in detail by Windsor *et al.* (2009) and include congenital contractures of the spine (kyphosis and in some cases scoliosis) and proximal joints (most prominent in the hindlimb joints), congenital generalized joint hyperlaxity (hyperextensibility of the distal joints of limbs and occasionally patellar subluxation), dolichostenomelia (elongated and gracile long bones) and arachnodactyly (elongated digits). The contractures and distal joint hyperextensibility improve after birth if the calf is ambulatory. Although CA is in most cases not a lethal genetic defect, affected calves appear taller and poorly muscled when compared with their unaffected siblings and remain so into adulthood and some residual joint hyperlaxity normally remains, predisposing CA animals to the premature onset of degenerative arthritis and thus affected animals are often culled.

After sample collection commenced in 2001 (Winsor and Tammen 2001), in 2007 the mapping of CA was initiated with support from Angus Australia to develop a diagnostic test for CA. Recent developments in high density genome scans have allowed the rapid mapping of monogenetic defects and the development of indirect DNA tests. However, the development of direct DNA tests, which require the identification of causative genes and mutations can be more difficult, especially if the phenotype is poorly defined or if there is a lack of obvious positional candidate genes as is the case in CA.

In 2010, a DNA test for CA was developed in the USA and is now commercially available. The disease causing mutation is presumably a deletion of approximately 54 kb (Beever 2010).

MATERIALS AND METHODS

Animals. This study used bovine hair, EDTA-blood and semen samples provided by farmers from Australia, Argentina and the USA in response to a request for sample submission (Windsor and

Tammen 2001) either directly to ReproGen or to the Angus Australia. CA status was in most cases assessed by the farmer. Additional DNA samples were obtained from a family of known and confirmed CA created by mating known carrier bulls to known affected dams in a breeding study at EMAI (NSW Industry & Investment). Pedigree information was provided by the owners of the animals and supplemented using the online resources of the Australian, American and Argentinean breed organisations. DNA was extracted using standard extraction protocols and if required amplified using the Qiagen REPLI-g whole genome amplification kit.

Whole genome scan. Forty-five animals were selected and consisted of 20 affected animals from Australia and Argentina, 15 obligate carrier animals, and 10 control animals with no known history of CA in the recent pedigree. DNA was sent to Affymetrix USA (http://www.affymetrix.com/estore/index.jsp) for genotyping with their Bovine Mapping 25k SNP Array. Association and homozygosity analysis (Charlier *et al.* 2008) was conducted using single marker tests and sliding windows of 8 and 4 markers. A 3x3 Chi-square was calculated for the frequency of the three genotype classes (AA, Aa and aa) in three groups (Affected, Carrier and Control) for all 29 autosomes. A homozygosity index (p_affect-0.5 p_carrier- p_control, where p is the proportion of animals with homozygosity) was computed on a 4 SNP sliding window. An arbitrary homozygosity index threshold of equal to the top one percentile was chosen to identify the regions in which affected animals showed higher degrees of homozygosity compared with carrier and control animals.

Fine-mapping. A set of 144 animals was used for fine-mapping and consisted of 44 affected animals, 55 carrier animals and 23 animals predicted to be unaffected and 22 animals in which status of CA could not be ascertained or was in doubt. DNA was sent to Sequenom (http://www.sequenom.com) for genotyping with a panel of 401 custom designed SNP. Target SNPs in a 5 Mb region of interest on chromosome 21 were identified using the Interactive Bovine In Silico SNP (IBISS) database (http://www.livestockgenomics.csiro.au/ibiss/). All SNPs were positioned on the Btau 4th assembly by the International Bovine Genome Sequencing Consortium (IBGSC) (http://www.hgsc.bcm.tmc.edu/projects/bovine/) and a subset of 401 SNP was selected for genotyping. For all 250 SNP which met quality control standards, animals pedigree status was checked with marker inheritance. After animals with low call rates, ambiguous CA phenotype status or SNP genotypes that were not consistent with pedigree information were excluded 32 confirmed affected animals, 45 carrier animals and 23 control animals remained for further analysis. Visual homozygosity analysis was conducted to compare the expected increased homozygosity in affected animals over carrier and random control animals. A chi-square test on a 3×3 contingency table was calculated for the frequency of the three genotype classes (AA, Aa and aa) in three groups (Affected, Carrier and Control) and the homozygosity index calculated as described above. SNP with significant associations were then used in a diagnostic panel to best separate Affected, Carrier and Control animals (data not shown).

RESULTS AND DISCUSSION

Whole Genome Scan. From the 25k Affymetrix genome scan 25,340 SNP were genotyped of which 23,520 SNP yielded assay results. On average one SNP was placed every 100 kb across all autosomes. The mean minor allele frequency (MAF) for all the polymorphic SNPs across all samples was 0.24 (with 0.11 and 0.37 being first and third quartile range respectively). MAF greater than 1%, 5% and 10% yielded 20,218, 17,617 and 15,770 SNP respectively. Only 18,627 SNP unambiguously positioned on the bovine genome (Btau4.0) were taken further in the

homozygosity mapping analyses.

The homozygosity analysis identified 1821 SNP markers exceeding P < 0.05 and 514 markers exceeding P < 0.01. Although markers with significant associations were identified on most of the chromosomes, strongest statistical support was for markers predominantly on chromosome 21. In particular on chromosome 21 affected animals showed long stretches of homozygosity in the same chromosomal region. In order to minimize the identification of single markers being spuriously associated with CA, markers were ordered in windows of 8 consecutive SNPs to detect larger regions of homozygosity. The homozygosity index confirmed a region of homozygosity in affected animals of approximately 5 Mb on chromosome 21 at position 23,596,278 bp – 28,411,725 bp.

Fine-mapping. A total of 5,000 potential SNP markers were identified in the 5 Mb target region on chromosome 21, from which a panel of 401 SNP was selected for fine-mapping. Priority in SNP selection was given to SNP that had been previously analysed on the Affymetrix 25k SNP chip (n = 26), that were included on the Illumina 54k bovine SNP chip (n = 38, indicating potential for higher than average call success rate), or that had been identified in an IBISS interbreed panel of animals for SNP mining (n = 21). The genotyping was performed in two panels of 227 and 174 SNP. The second panel was selected to cover regions in which SNP in the first panel did not yield results, or identified further sub-regions of interest.

From the target SNP panel of 401 SNP, a total of 350 SNP were identified which had a call rate > 50% in 142 DNA samples. Two samples (1 affected and 1 carrier) failed to yield acceptable call rates and were omitted from further analyses. Of the 350 SNP, 95 SNP were monomorphic and 5 SNP showed inconsistent genotype calls and were removed from further analysis. This resulted in a final panel of 250 SNP for the mapping analysis. On average one SNP was placed every 12,000 bp across the target region. The minor allele frequency of informative SNP across all samples was in the range of 0.01to 0.50 (mean 0.20).

The distribution of *P*-values from the chi-square association for each SNP and position in the target region is shown in Figure 1. Results for the initial panel and subsequent back-up panel are shown in red and blue respectively. Results show a strong and significant association between SNP in region 23,500,000 and 26,400,000 bp on chromosome 21 confirming the initial region of interest from the whole genome association with the 25k Affymetrix array. A panel of 85 markers was identified via preliminary discriminate analysis (data not shown) as a test panel for an indirect DNA test.

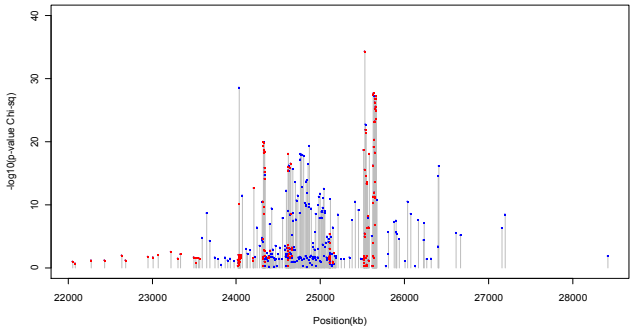

Figure 1. Single point SNP association for each SNP in a panel of high utility SNP. Red points denote results for initial 227 SNP panel and blue for a subsequent panel of 173 SNP. All monomorphic and poorly performing SNP have been removed.

Visual analysis was conducted to examine boundary intervals in the region of homozygosity linked to CA status by inspection of recombination events in samples from affected animals.

Based on recombination events in affected animals, the outer limits of the CA region were set at between 23,069,201 bp and 26,401,500 bp on BTA 21.

A preliminary positional candidate gene analysis identified 17 genes / 25 transcripts in the refined region of interest. The selection of a positional candidate gene was complicated by the fact that the phenotype was poorly defined and information on function for most of the positional genes/transcripts is limited. A literature review suggested BTBD1 as a possible candidate gene as it has been suggested to be required for normal muscle cell differentiation and is highly expressed in skeletal muscle (Pisani *et al.* 2004, 2007). The coding sequence was sequenced in 2 affected and 2 normal animals and two SNPs were identified (data not shown). However, these did not segregate with the predicted disease genotypes.

CONCLUSIONS

A major locus most likely responsible for CA was mapped to BTA 21 in a target region of ~3.4 Mb. Association analysis identified a panel of 85 markers as a test panel for an indirect DNA test which could ascertain normal, carrier and affected status with high accuracy but would require independent confirmation. Ongoing research for a direct DNA based test for CA would be deemed feasible. The management of mono-genic inherited disorders ought to be considered a routine inclusion in breeding programs as it is relatively straightforward to develop indirect and in some cases direct tests for such conditions. The need for high quality disease phenotypes, pedigree information and rapid translation from problem identification to applied diagnostic tests remain obstacles for using such advanced breeding tools.

ACKNOWLWDGEMENTS

We are grateful for the support of farmers who provided samples and pedigree information and this project received funding from Angus Australia, Te Mania Australia, and MLA Donor Company. Bruce Tier (AGBU) conducted the initial pedigree analysis. Peter and Julie Healy and Brandon O'Rourke (EMAI, NSW Industry & Investment) and James Reecy (Iowa State University) provided DNA samples. Laurence Denholm and Patrick Staples (EMAI, NSW Industry & Investment) assisted in the characterisation of clinical signs. The technical support and input by Ms Marilyn Jones in sample and DNA preparation is gratefully acknowledged.

REFERENCES

Beever J. (2010) available at: http://www.angus.org/pub/CA/CA_Summary.pdf

Charlier C., Coppieters W., Rollin F., Desmecht D., Agerholm J.S., Cambisano N., Carta E., Dardano S., Dive M., Fasquelle C., Frennet J.C., Hanset R., Hubin X., Jorgensen C., Karim L., Kent M., Harvey K., Pearce B.R., Simon P., Tama N., Nie H., Vandeputte S., Lien S., Longeri M., Fredholm M., Harvey R.J., Georges M. (2008) *Nat. Genet.* **40**:449-454.

Pisani D.F., Cabane C., Derijard B., Dechesne C.A. (2004) *Cell Death Differ*. **11**:1157-65.

Pisani D.F., Coldefy A.S., Elabd C., Cabane C., Salles J., Le Cunff M., Derijard B., Amri E.Z., Dani C., Leger J.J., Dechesne C.A. (2007) *Exp. Cell. Res*. **313**:2417-26.

Windsor P.A. and Tammen I. (2001) *J. Aust. Assoc. Cattle. Vets.***18**:36.

Windsor P.A., Denholm L.J. and Tammen I. (2009) *AVA Annual Conference,* available at http://www.angusaustralia.com.au/Breeding/FCS180510-Emerging-inherited-diseases-of-cattle-in-Australia.pdf