

Candidate mutation responsible for inherited skeletal developmental abnormalities in Cheviot sheep

O. Matika^{1,2}, M. Davey^{1,2}, J. Del-Pozo², H.A. Finlayson^{1,2}, C. Farquharson^{1,2}, D.J. Headon^{1,2}, J.W. Kijas³, Z.H. Lu^{1,2}, L. McTeir^{1,2}, V. Riggio^{1,2}, J. Schoenebeck^{1,2}, T. Schwarz², K.A. Staines^{1,2}, J.A. Woolliams^{1,2}, A.L. Archibald^{1,2} and S.C. Bishop^{1,2}

¹The Roslin Institute and ²Royal (Dick) School of Veterinary Studies, University of Edinburgh, U.K., ³CSIRO Agriculture, Australia





Introduction



 First described in 1971 from researchers in Edinburgh

An Achondroplastic Syndrome in South Country Cheviot Sheep

February 19th, 1971.

C. WRAY,
A. O. MATHIESON.

Veterinary Investigation Laboratory,
East of Scotland College of Agriculture,
Greycrook, St. Boswells, Roxburghshire.
A. N. COPLAND.

Department of Veterinary Anatomy,
Royal (Dick) School of Veterinary Studies,
Summerhall, Edinburgh.



Fig. 1.—Appearance of a lamb showing achondroplastic head, shortened ears and tail.





Introduction



- The condition was characterised by:
 - Abnormalities of the head
 - with protruding eyes
 - Short ears and tail
 - Shortened limbs
 - Sometimes with no hooves

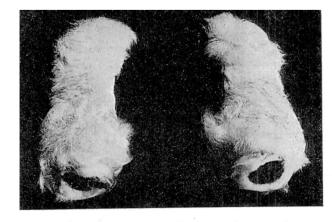


Fig. 2.—Forelimbs showing absence of hooves.

Condition observed only in certain families





Introduction



- Condition observed only in certain families
 - Specific rams and ewes produce affected progeny
 - Cases can be co-twinned to normal lambs
 - Farmers still observing the problem
 - No farmers openly admit to the problem
 - Indicative of a recessive and genetic effects





Objectives



- Test if achondroplasia in Cheviots was genetic
- Identify underlying "causal" variants using homozygosity mapping and whole genome sequencing





Materials and Methods



Data available

- Cheviots sampled:
 - in 2009
 - 9 cases and 27 controls
 - Genotyped with OvineSNP50 BeadChip
 - in 2014
 - 8 cases and 8 controls
 - Genotyped with HD BeadChip (~800k)





Materials and Methods



Data available

- Next generation sequencing:
 - in 2014
 - 5 cases pooled and 5 control
- Additional samples collected for validation





Materials and Methods



Analyses

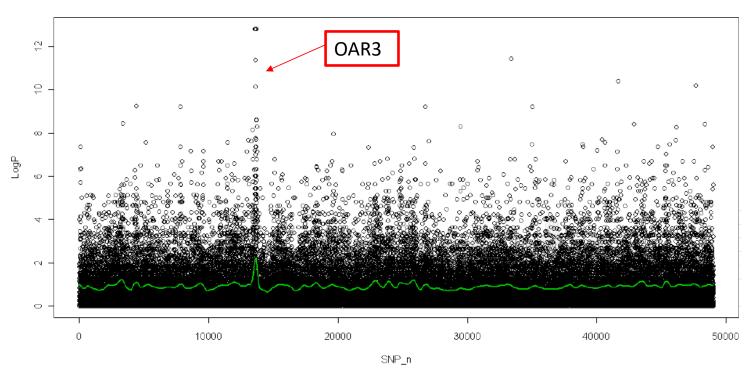
- Association study
- Homozygosity mapping
- Confirmation and validation work in the lab
 - About 20 cases and some controls
 - Phenotyping by pathologists and post mortem exams
 - Bone mineralisation
 - CT scans of Skull and selected skeletal parts







Association study

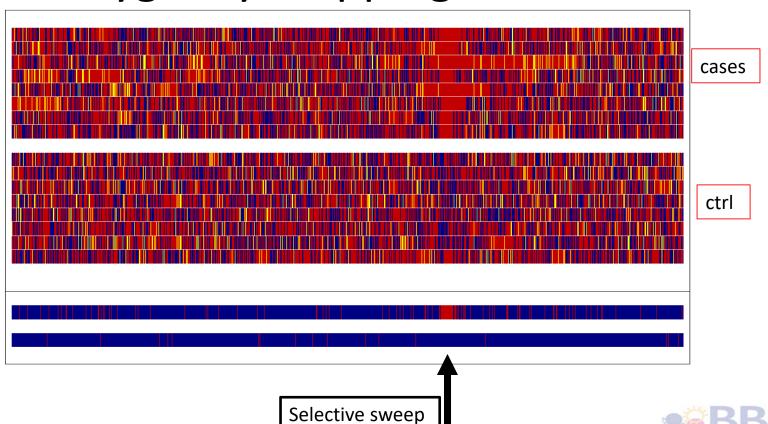








Homozygosity mapping on HD OAR3

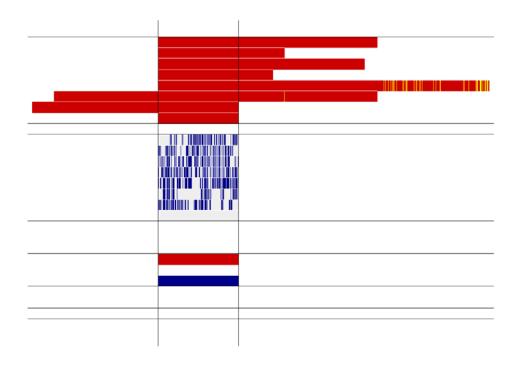








Close up on the region on OAR3







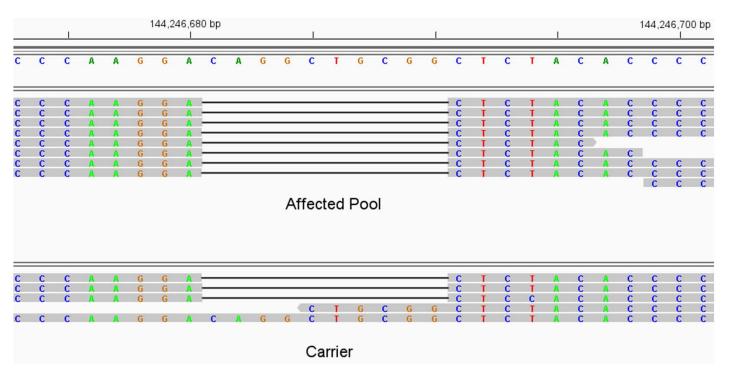


- Identified putative genes
 - ADAMT20 and PRICKLE1 genes (prickle planar cell polarity protein 1)
- We identified 10bp deletion in *Prickle1* gene
 - Plays a critical role in tissue morphogenesis.
 - Open reading frame
 - Reported to cause systemic tissue outgrowth defects, aberrant cell organization and disruption of polarity machinery















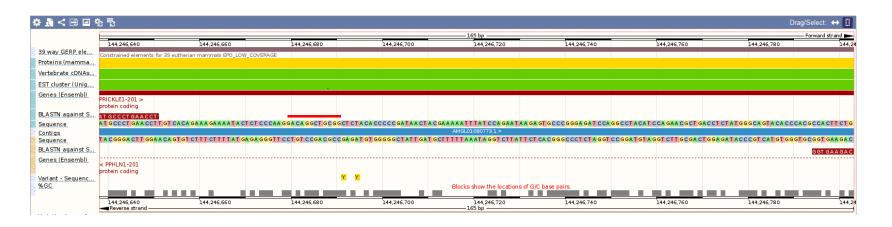
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Codons	Symbol
GACAGGCTGCGG/GA	PRICKLE1









PCR primers

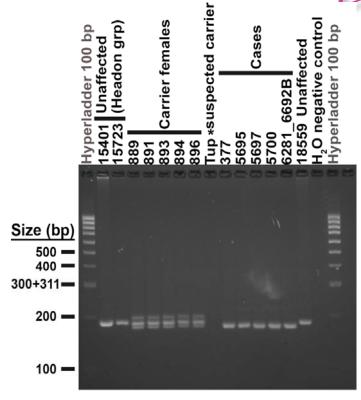
sPRICKLEdelF:

TCAGACAATGCCCTGAACCT

sPRICKLEdelR:

CAGGGCGTAGTCAGAAGTGG

Predicted PCR product size from the reference genome is 183 bp. The mutant allele detected by sequencing should be 10 bp smaller.



Longer run of the same gel

















• Borders: normal – affected









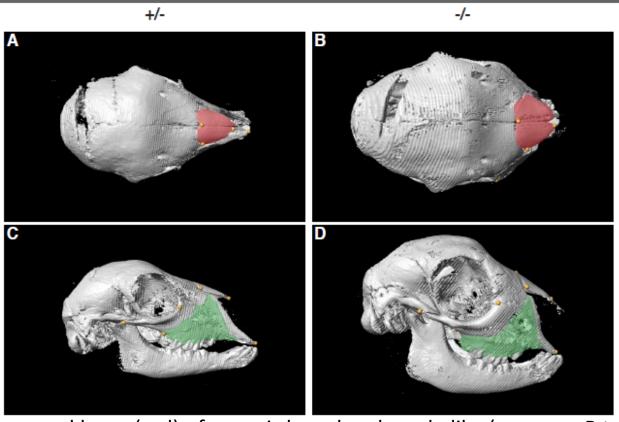
Abnormities from CT scans

- T13 transitional vertebra
- deviation from normal vertebral distribution of 13 thoracic and 6 lumbar vertebrae
- sarcal and/or coccygeal spina bifida
- L1 cleft vertebra
- hemivertebrae









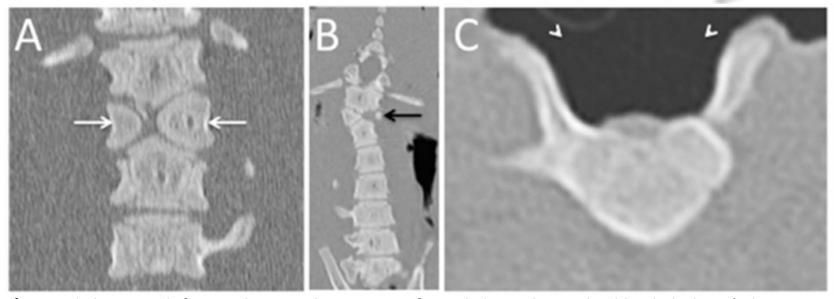
Dorsal view: nasal bone (red) of cases is broad and spade like (compare B to A)



Lateral view: much of cases' caudal maxilla (green) tucked under orbits (compare D to C)

CT images of vertebral malformations





- **A)** Dorsal slice: L1 cleft vertebra resulting in two funnel shaped vertebral body halves (<u>white arrows</u>).
- **B)** Dorsal slice image: lumbar vertebral column showing a missing left vertebral body half (<u>black arrow</u>), an opposing wedge-shaped right vertebral body half and secondary vertebral curvature deformation (scoliosis)
- **C)** Transverse slice: L2 spina bifida malformation in which the dorsal arch is missing (<u>arrowheads</u>).

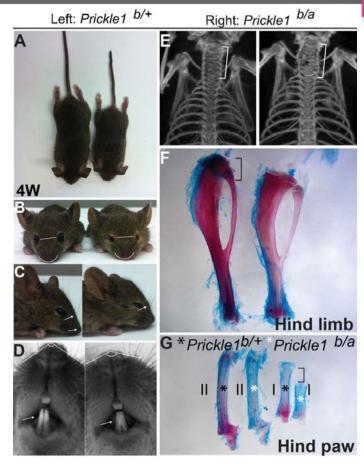




Discussion

PROSLIN

Fig. 9. Robinow syndrome features presented in Prickle1 mutant mice.(A) Top view of short statures of a Prickle1b/a hypomorphic mutant (Prickle1b/a, right) with a kinked tail.



Chunqiao Liu et al. Biology Open 2014;bio.20148375





Conclusion



- We validated the mutation on over 20 samples using Sanger sequencing
- We have identified a putative causative region
- We have a test available to be used as possible control measure





Acknowledgement



- Special mention to Steve Bishop
- RIDGENE/KTN



- BBSRC
- Ricardo Pong-Wong- homozygosity mapping software
- Cheviot farmers







Thank you for Listening



