Candidate mutation responsible for inherited skeletal developmental abnormalities in Cheviot sheep

O. Matika[1,2], M. Davey[1,2], J. Del-Pozo[2], H.A. Finlayson[1,2], C. Farquharson[1,2], D.J. Headon[1,2], J.W. Kijas[3], Z.H. Lu[1,2], L. McTeir[1,2], V. Riggio[1,2], J. Schoenebeck[1,2], T. Schwarz[2], K.A. Staines[1,2], J.A. Woolliams[1,2], A.L. Archibald[1,2] and S.C. Bishop[1,2]

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

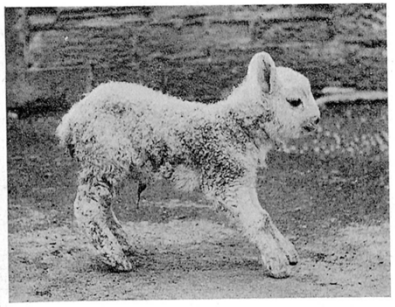


FIG. 1.—Appearance of a lamb showing achondroplastic head, shortened ears and tail.

Introduction

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

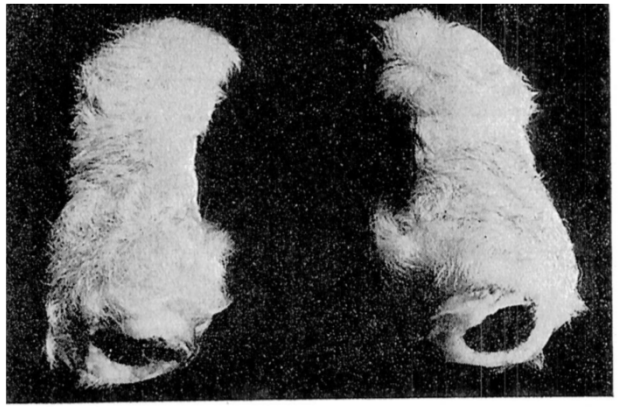


FIG. 2.—Forelimbs showing absence of hooves.

- 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

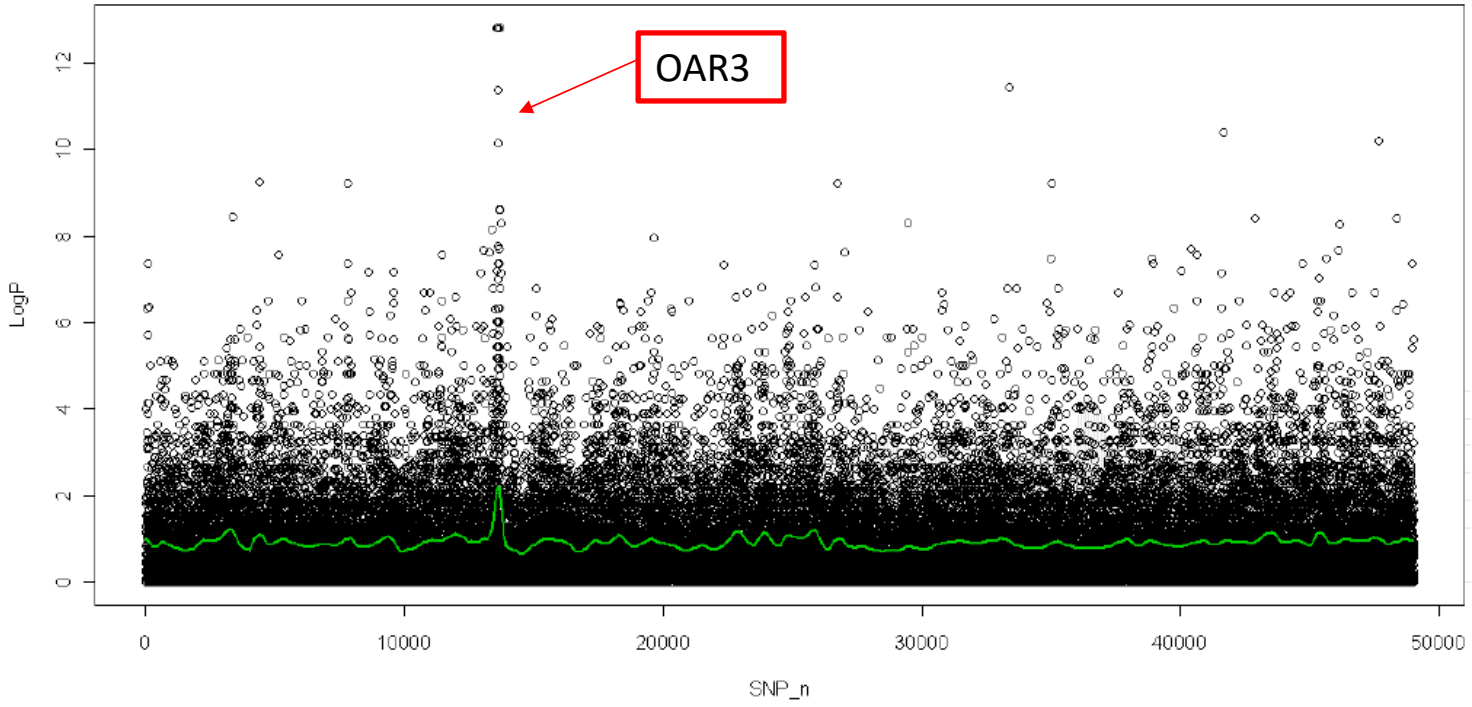
Materials and Methods

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

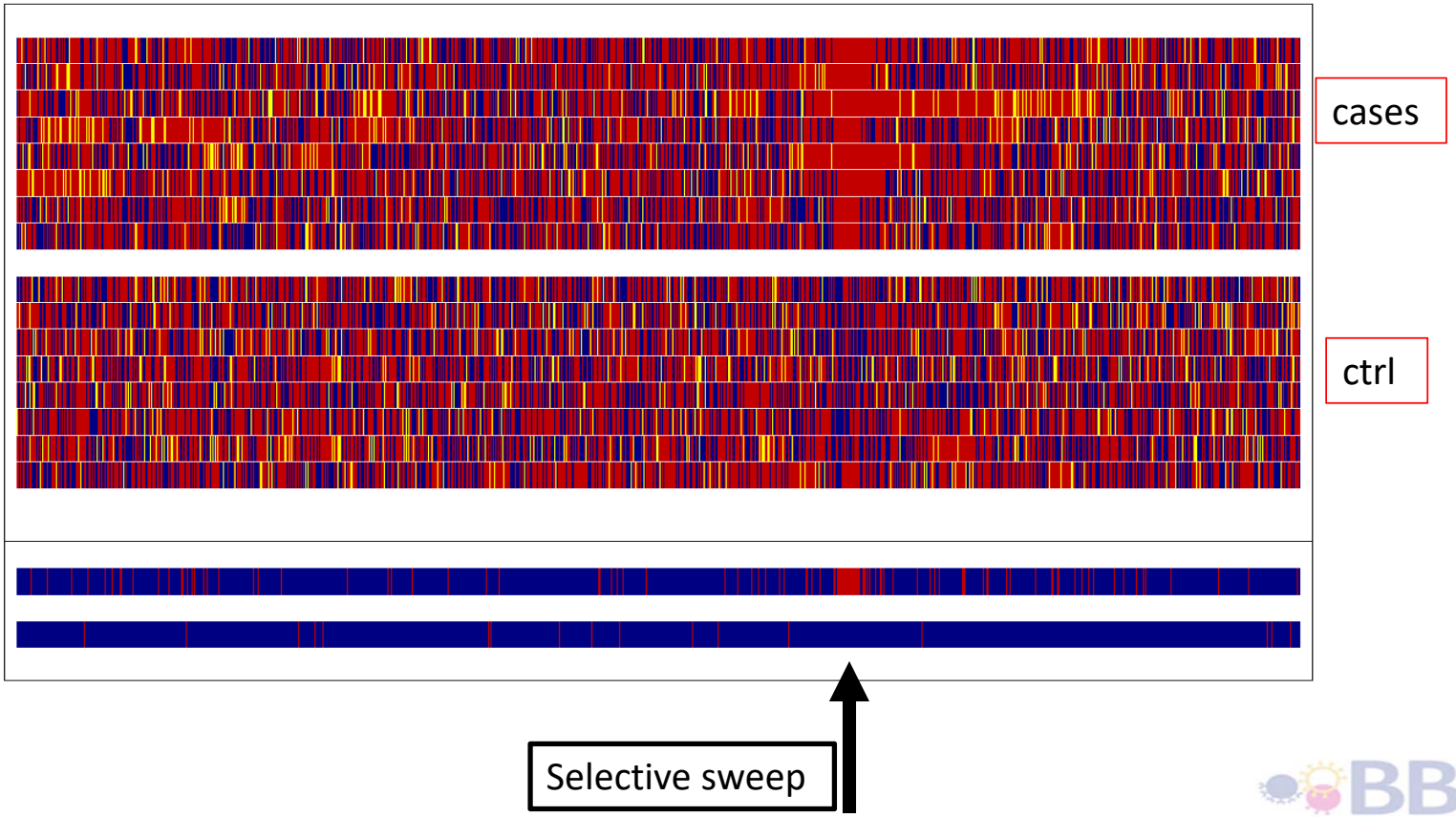
Results

Association study



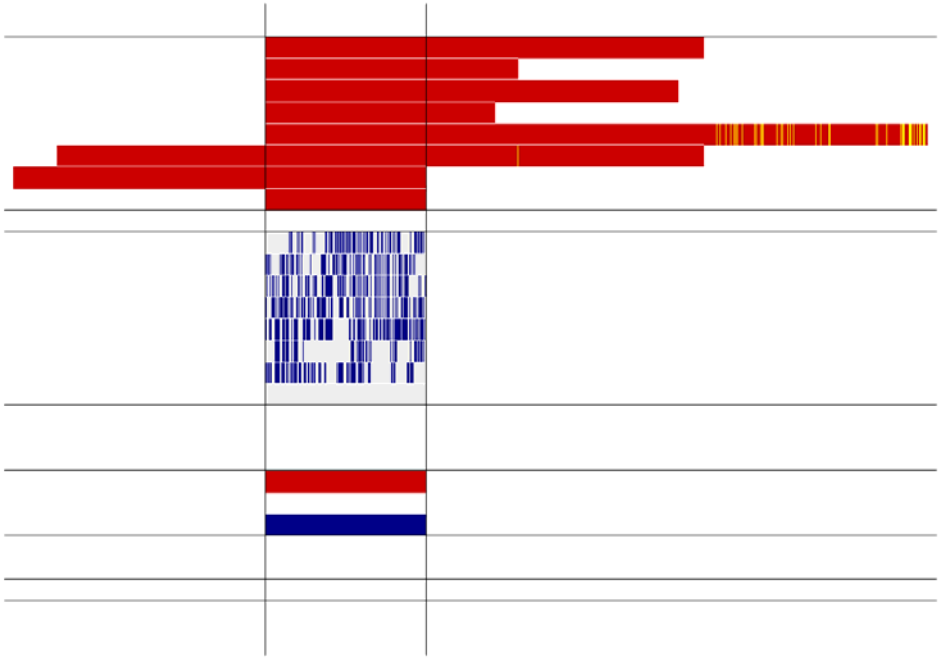
Results

Homozygosity mapping on HD OAR3



Results

Close up on the region on OAR3



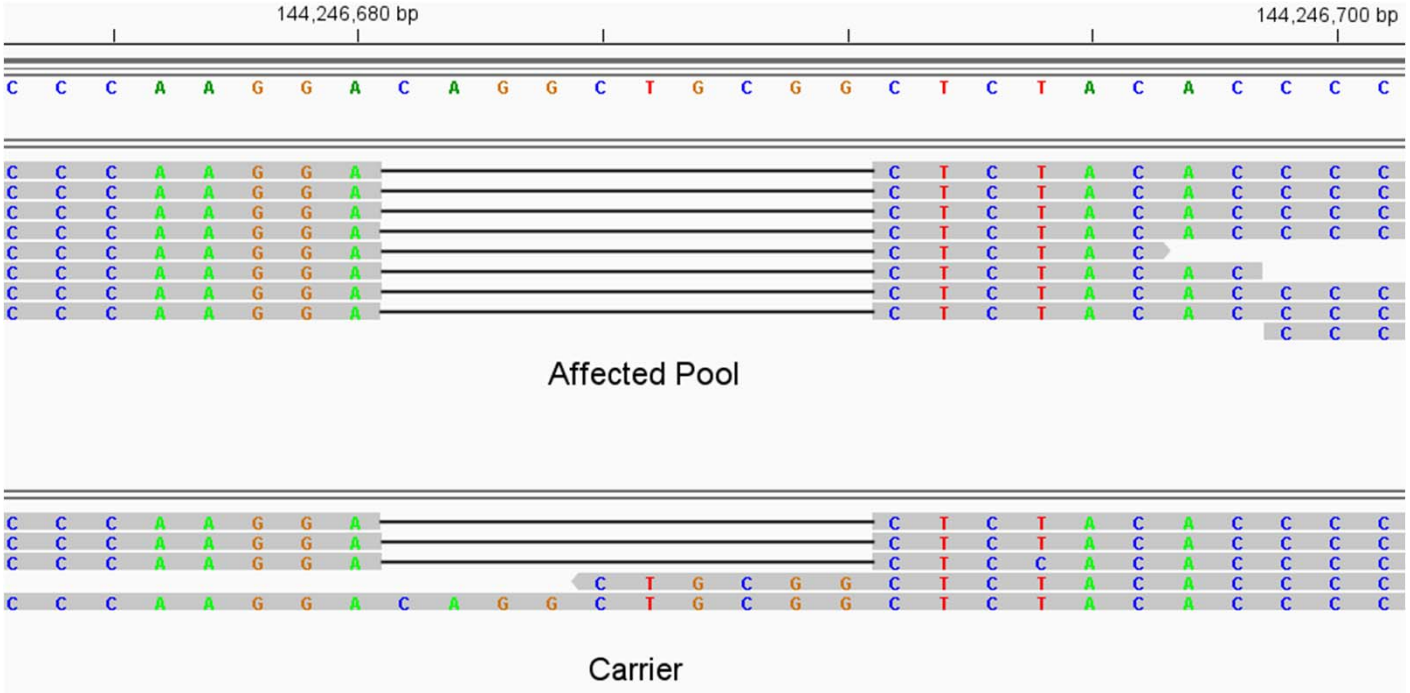
Results

Sequencing

- 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

Results

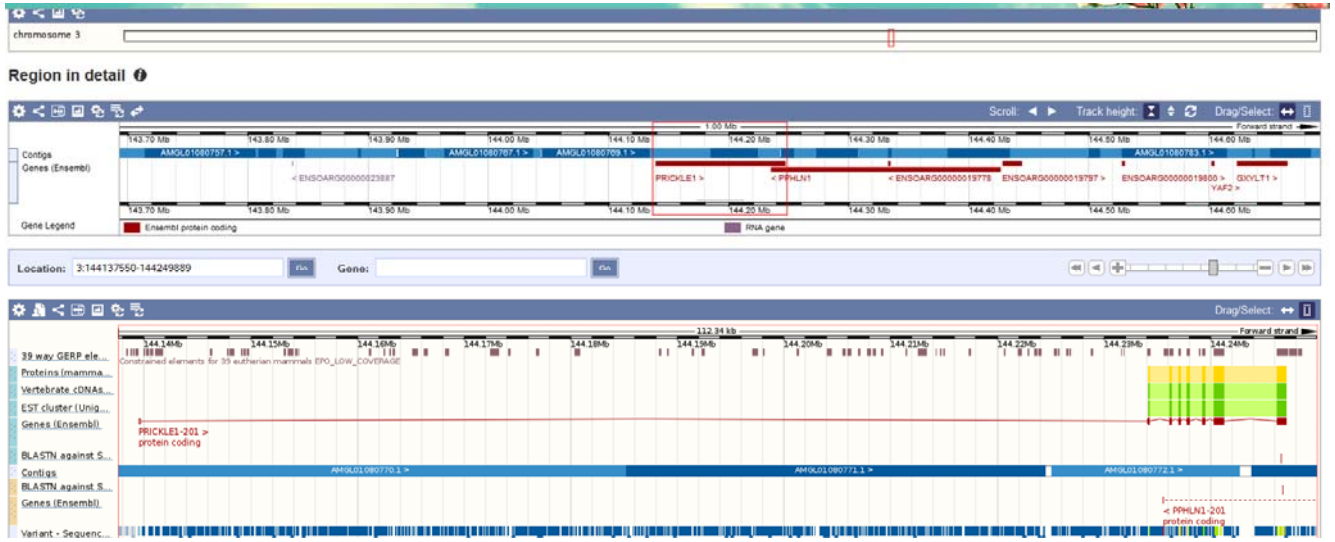
Sequencing



Results

Sequencing

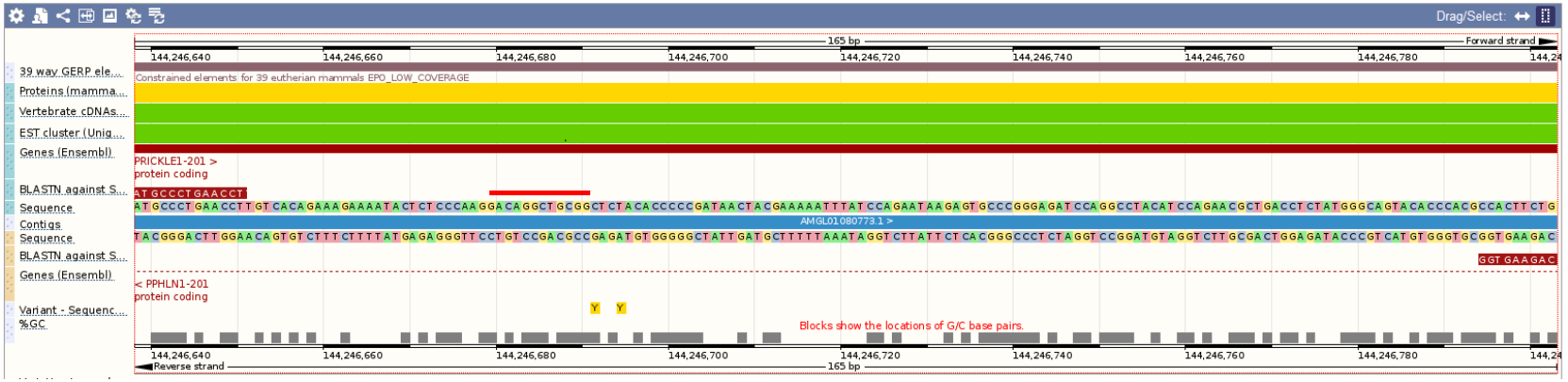
Location	Allele	Gene	Feature	Feature type	Consequence	cDNA position	CDS position	Protein position	Amino acids	Codons	Symbol
3:144246680 -144246690	-	ENSOARG00000019738	ENSOART00000021494	Transcript	frameshift_variant	2276-2285	2103-2112	701-704	DRLR/X	GACAGGCTGCGG/GA	PRICKLE1



Results

Sequencing

Codons	Symbol
GACAGGCTGCGG/GA	PRICKLE1



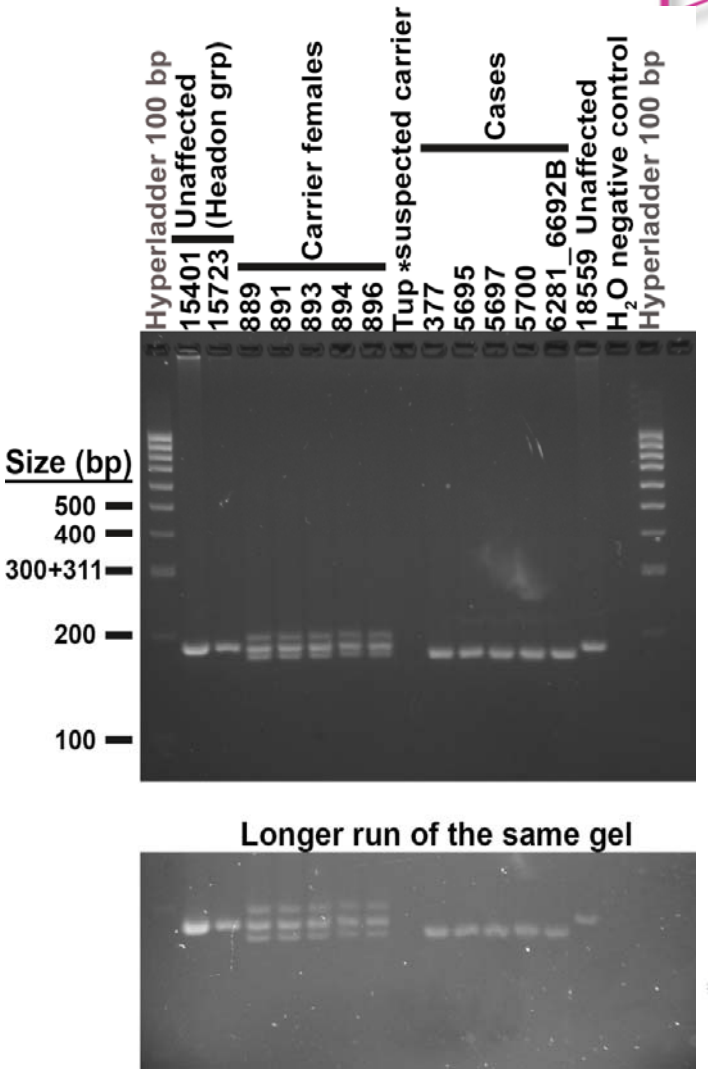
Results

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.



Results

Results

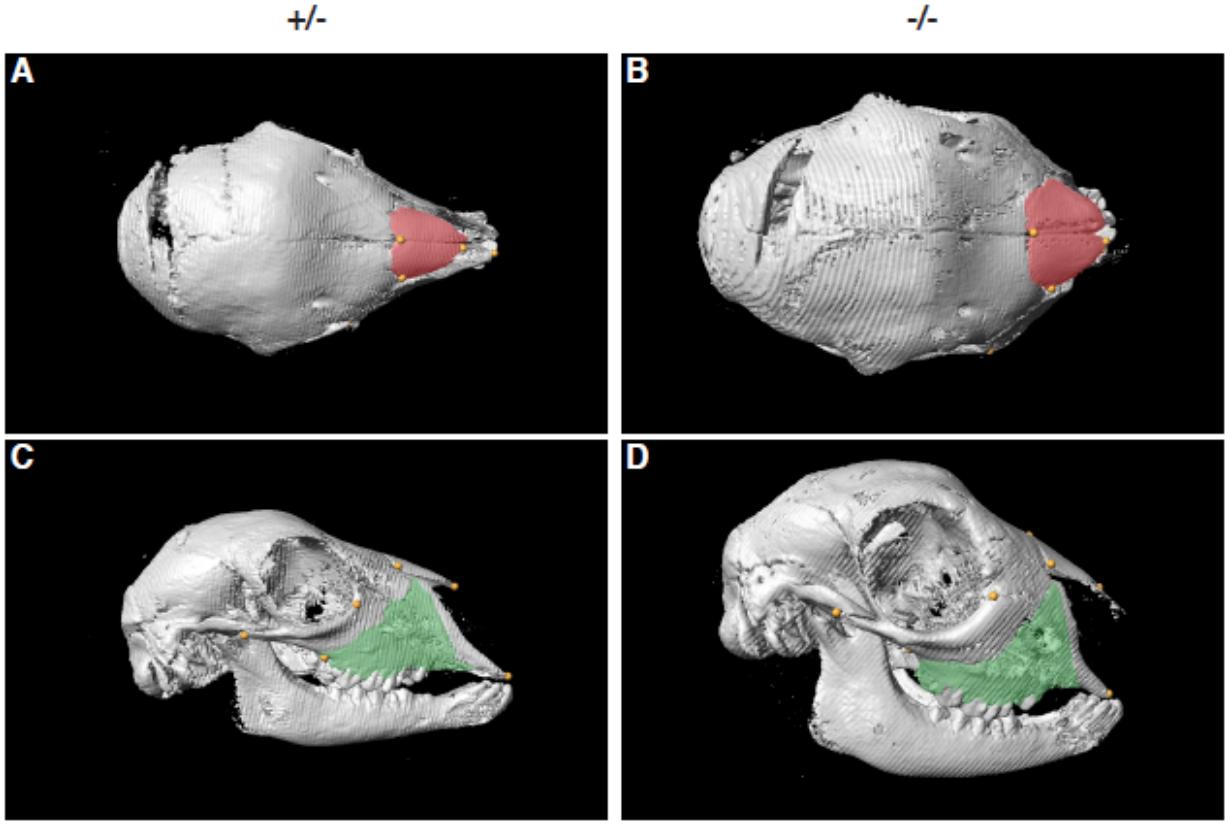
- Borders: normal – affected

Results

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

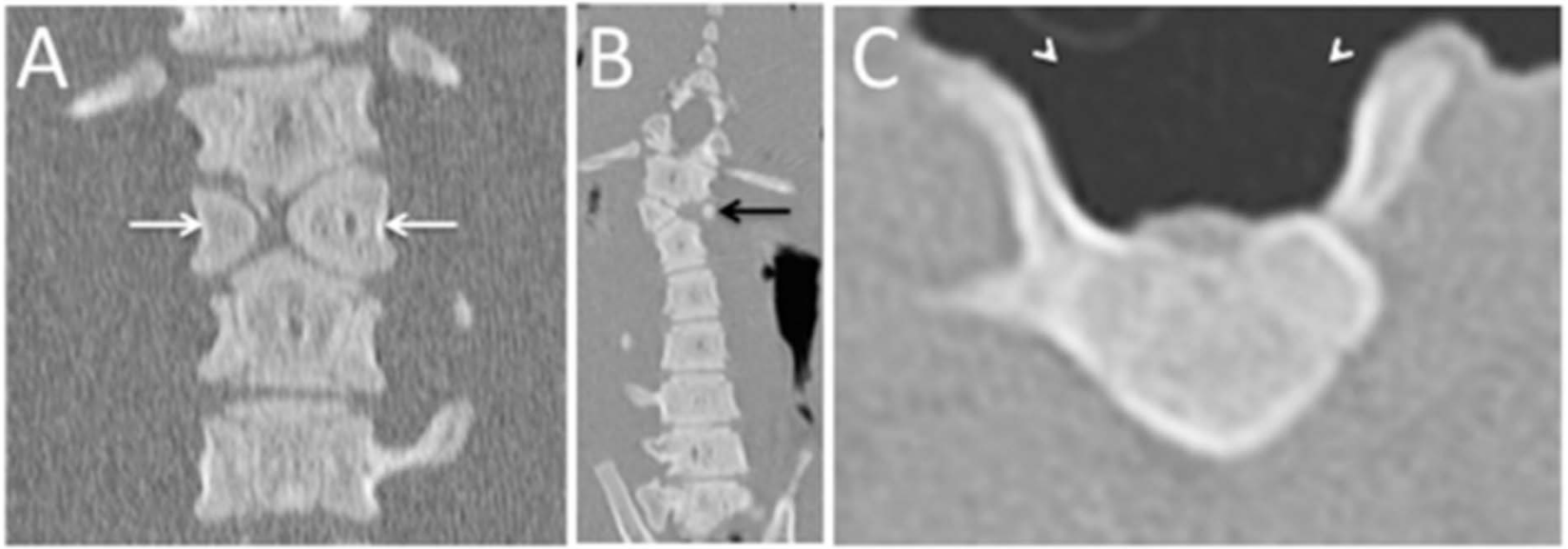
Results



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 (white arrows).

B) Dorsal slice image: lumbar vertebral column showing a missing left vertebral body half (black arrow), 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 (arrowheads).

Discussion

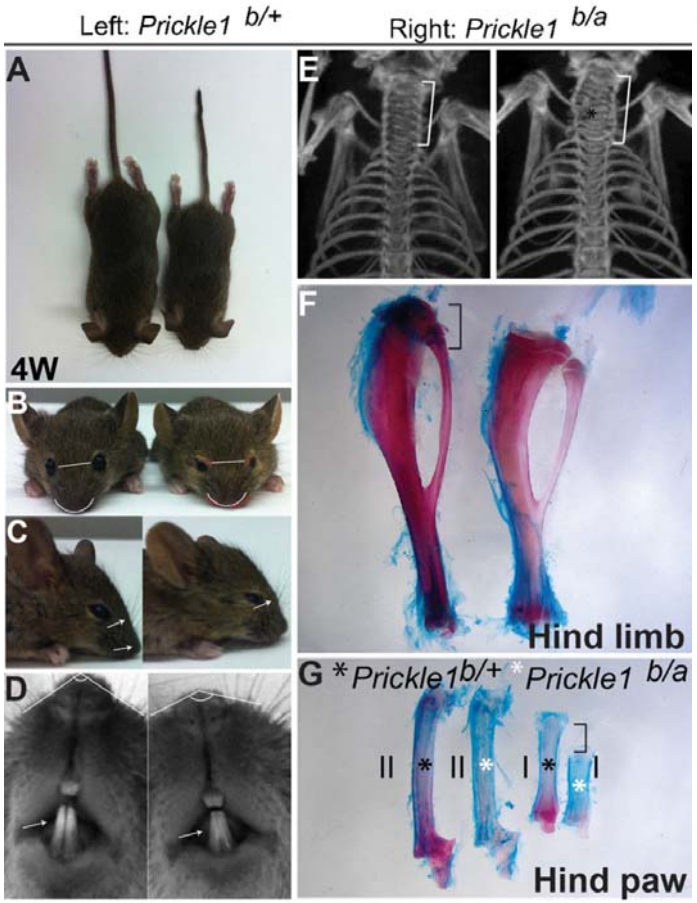


Fig. 9. Robinow syndrome features presented in Prickle1 mutant mice.(A) Top view of short statures of a Prickle1b/a hypomorphic mutant (Prickle1b/a, right) with a kinked tail.

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

ROSLIN

Thank you

for

Listening

THE UNIVERSITY OF EDINBURGH

BBSRC