0.833, whereas the AA genotype was fixed in cattle breeds outside Tibet (frequency 0.893). The heterozygous GA genotype was found in Tibetan cattle, which most likely have acquired the G allele via admixture with yaks in order to adapt to the extreme cold and hypoxia of the Himalayan plateau. The GA genotype was also detected in the Datong, Sibu and Zhongdian yak breeds, for which hybridization with cattle is a common practice.[3] This study supports the notion that introgression has played an important role in adaptation of Tibetan cattle.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Genotypic and allele frequencies of the *EGLN1* gene across 18 cattle and yak breeds.

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Ruling out *BGN* variants as simple X-linked causative mutations for bilateral corneal stromal loss in Friesian horses

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Description/sample: Bilateral corneal stromal loss (BCSL), a disorder characterized by focal, symmetric thinning of the inferior peripheral corneal stroma unaccompanied by inflammation, was previously documented in Friesian horses.[1] A higher prevalence in males (eight of nine cases) suggested an X-chromosomal inheritance.[1] The corneal stroma is composed of collagen fibers whose assembly is regulated temporally by small leucine-rich repeat proteoglycans (SLRPs) including decorin and biglycan.[2,3] Mouse knockout models have demonstrated that a combined deficiency of biglycan and decorin cause severe phenotypic changes in fibril size and spatial organization in the corneal stroma, whereas milder phenotypic changes are seen for deficiencies in each individual SLRP.[2,3] The genetic background and role of SLRPs in equine BCSL are unknown. To investigate this further, 62 Friesians were included with genomic DNA available from 57. Fifteen Friesians had clinical signs consistent with BCSL, 35 had no corneal lesions and were classified as unaffected and 12 were not bilaterally affected but had other corneal abnormalities (Appendix S1).

Pedigree analysis: In the population assessed, 11 of the 15 cases were male. This supports an X-linked recessive mode of inheritance. On investigation of the pedigrees of affected individuals, a common ancestor was identified that could have propagated an X-linked trait (Figs. 1 & S1).

Candidate gene sequencing: Because the gene encoding biglycan (*BGN*) is located on ECAX, it was a likely functional candidate gene. Sanger sequencing of three BCSL-affected horses and three controls identified 13 variants when compared to EquCab 2.0, none of which were perfectly concordant with BCSL (Tables S1 & S2). Seven of these variants were identified as errors in the reference genome, EquCab2.0, which was previously noted to have errors.[4] Performing BLAST searches against NCBI databases does not support multiple copies of *BGN* in the horse genome. The non-reference allele of the 3′-UTR variant (*BGN*:c.1119+179T>C, rs69537945) was negated as the sole causative variant for BCSL. This variant had a frequency of 0.71 in 63 Thoroughbreds and 0.86 in 57 Quarter Horses but was found in all of the Friesian samples genotyped. There was no predicted effect of the mutation on microRNA binding sites in the 3′-UTR (Appendix S1). It is hypothesized that the disorders seen in Friesians may

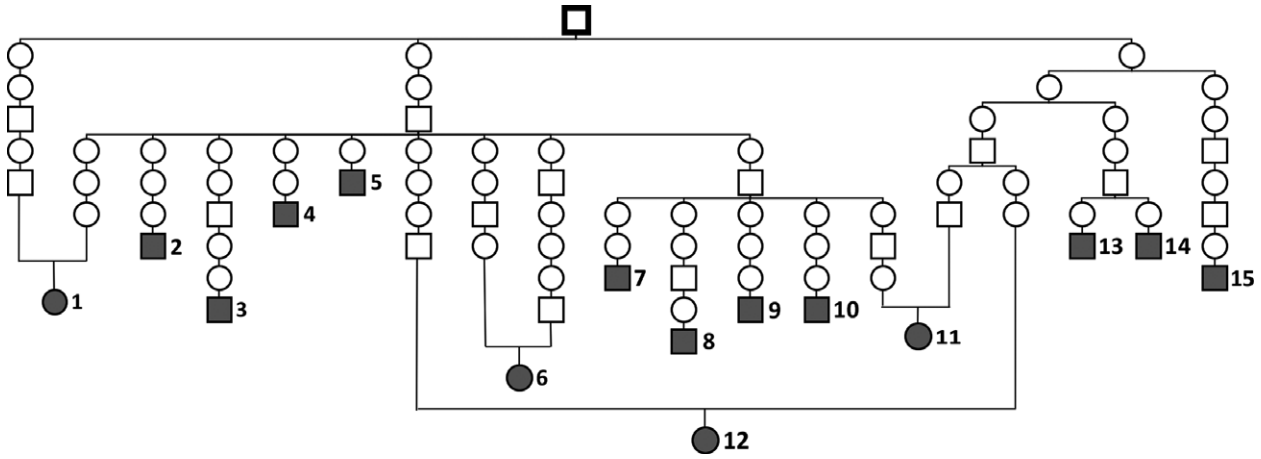

Figure 1 Pedigree of bilateral corneal stromal loss affected Friesians. An X-linked recessive mode of inheritance is supported by a common ancestor (denoted in bold outline) found on the maternal side of all affected males and on both sides for all affected females. BCSL-affected horses are denoted by gray shading. The disease status of all ancestors is unknown, denoted by a lack of shading.

represent a multisystem manifestation of improper collagen formation.[5] Two such disorders have had causal variants reported: dwarfism[6] (*B4GALT7*:c.50G>A) and hydrocephalus[7] (*B3GALNT2*:c.1423C>T). Neither variant was found to be concordant with BCSL ($P = 1.0$, $P = 0.53$ respectively; Tables S3 & S4), thus these variants can be ruled out as digenic contributors to BCSL.

These data rule out *BGN* as a candidate gene for a simple X-linked inheritance for BCSL. However, further research is needed to investigate its effects on all collagen-related disorders in Friesians, as digenic mutation combinations and epistatic interactions have been shown to affect the severity of collagen disorders in humans.[8,9]

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Supporting information

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Figure S1 Pedigree analysis of a common ancestor for bilateral corneal stroma loss (BCSL).

Table S1 Primers and PCR conditions used for sequencing of *biglycan* (*BGN*) and for genotyping *BGN*:c.1119+179T>C and *B3GALNT2*:c.1423C>T.

Table S2 Variants identified from Sanger sequencing of *biglycan* (*BGN*).

Table S3 Dwarfism associated variant (*B4GALT7*:c.50G>A; Leegwater *et al.*, 2016) allelic frequency and genotype counts in 57 Friesian horses.

Table S4 Hydrocephalus-associated variant (*B3GALNT2*:c.1423C>T; Ducro *et al.*, 2015) allelic frequency and genotype counts in 57 Friesian horses.

Appendix S1 Methods.