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**Publication date:**

2022

**Permanent link:**

<https://doi.org/10.3929/ethz-b-000633563>

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**Originally published in:**

[https://doi.org/10.3920/978-90-8686-940-4\\_187](https://doi.org/10.3920/978-90-8686-940-4_187)

# 187. Markhor-derived introgression of *PAPSS2* confers high-altitude adaptability in Tibetan goats

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## Abstract

Understanding the genetic mechanism of how animals adapt to extreme environments is fundamental to unriddle the relationship between molecular evolution and continuous climate change. After domestication, goats have developed strong adaptation to various environments, including harsh conditions at high-altitudes with low temperature and reduced oxygen concentrations. Here, we utilized 331 genomes of goats living at varying altitudes (high >3,000 m above sea level and low <1,200 m) as well as wild caprid species to precisely dissect genetic determinants underlying the adaptation to high altitudes on the Qinghai-Tibetan Plateau (QTP). Population genomic analyses combined with genome-wide association testing revealed that the *PAPSS2* locus confers adaptability to high altitudes in Tibetan goats. Additionally, this gene harbours significant signatures of interspecies introgression from a wild caprid species, markhor (*Capra falconeri*). We further demonstrated a functional role for *PAPSS2* in response to hypoxia using *PAPSS2*-deficient cells. In conclusion, our results suggest a hitherto unknown contribution of *PAPSS2* to high-altitude adaptation and showed that interspecific introgression contributed to the adaptation of goats to the harsh conditions at the Tibetan Plateau.

## Introduction

Goats (*Capra hircus*) are believed to be one of the first domesticated livestock species, dating back to ~10,000 years ago (Daly *et al.*, 2021; Zeder, 2000). Goats have developed rapid and strong physiological and genetic adaptation to various environments along with human civilization and migration (Daly *et al.*, 2018). This includes adaptation to some challenging agro-ecological conditions, such as high-altitude environments. Adaptive introgressions had occurred in humans (Huerta-Sánchez *et al.*, 2014) and other mammals native to the QTP, such as Tibetan Mastiff (Miao *et al.*, 2017), cattle (Chen *et al.*, 2018) and sheep (Hu *et al.*, 2019). These studies focus on the genetic basis of adaptive traits. However, how Tibetan goats acquired this adaptability is still unknown. In this study, large-scale genomic and transcriptomic data were systematically compared between goats from the Tibetan Plateau and low-altitude regions to provide new insights into the evolutionary origins of high-altitude adaptability in mammals.

## Methods

Blood samples from 91 goats (83 from Tibet, 4 from low altitude, and 4 Siberian ibex) were collected for short-read genome sequencing.

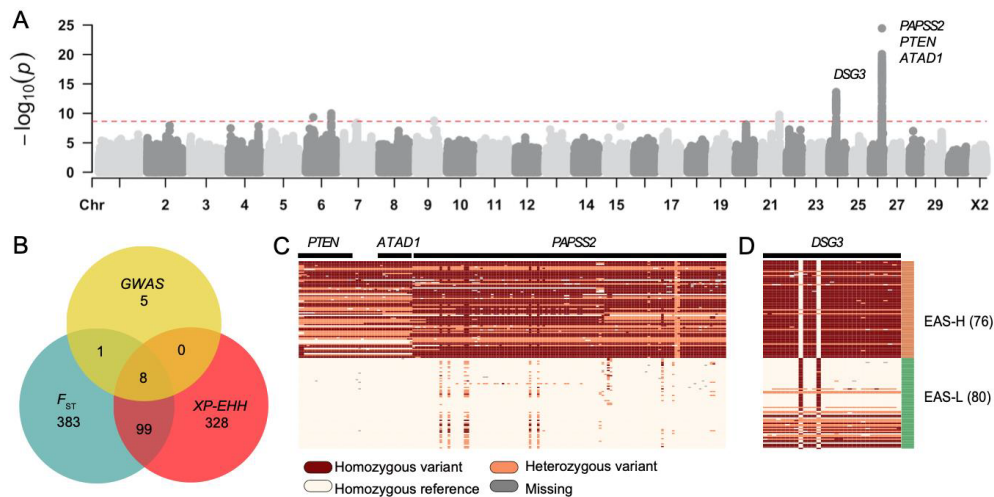
The genomic short-read sequencing data were mapped to the goat reference genome using BWA-MEM (Heng L. 2013), and SNPs were discovered and genotyped using GATK (McKenna *et al.*, 2010). The  $F_{ST}$ , XP-EHH, and GWAS statistics were calculated between 76 high- and 80 low-altitude animals. The  $D$ -statistics (Martin *et al.* 2015) and Q95 (Racimo *et al.* 2017) analyses were used to conduct a genome-wide screening of putative introgression events from the markhor.

We used CRISPR/Cas9 to knock out the murine *papss2* gene in NIH/3T3 cells. The *papss2* mRNA expression in the NIH/3T3 cells was determined by quantitative PCR (qPCR). The *papss2* deficient cells were incubated in a humidified atmosphere under normal (21% O<sub>2</sub>) and hypoxic (3% O<sub>2</sub>) conditions at 37 °C (Thermo Scientific HERAcCell 150i), respectively. Then the expression of *papss2* was measured alongside changes in cell phenotype using quantitative PCR (qPCR). Differentially expressed genes (DEGs) by RNA-sequencing reads were calculated using cufflinks (Trapnell *et al.* 2012).

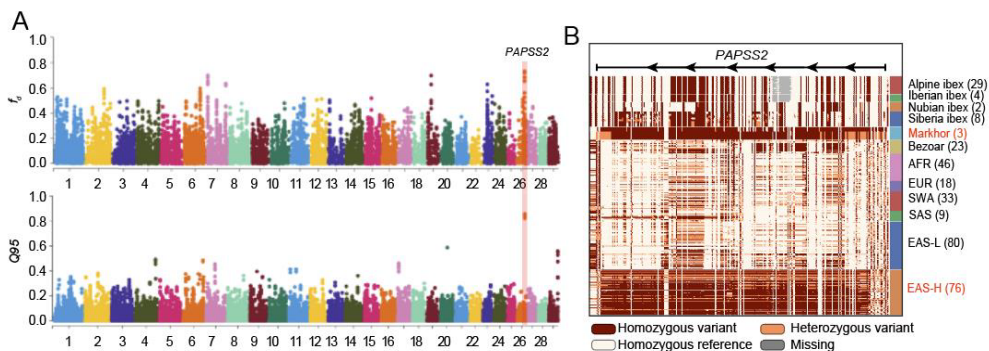
## Results

We used three complementary approaches (GWAS,  $F_{ST}$ , *XP-EHH*) to identify genes potentially involved in high-altitude adaptation (Figure 1A-B). Positively selected haplotypes encompassing *PTEN*, *ATAD1*, *PAPSS2*, and *DSG3* were significantly associated with high-altitude adaptation (Figure 1C). *PTEN* contributes to regulating hematopoiesis and leukemogenesis (Wu *et al.*, 2020). A previous study suggested *PTEN* to likely be under selection in the Tibetan human (Simonson *et al.*, 2010). *ATAD1* plays a critical role in regulating synaptic plasticity, learning, and memory (Ahrens-Nicklas *et al.*, 2017). *PAPSS2* was also previously identified as the major selective gene for high-altitude and cold environment adaptation in horses (Librado *et al.*, 2015; Shi *et al.*, 2019). *DSG3* was previously highlighted as a candidate gene for hypoxia adaptation in Tibetan goats (Kumar *et al.*, 2018). *PAPSS2* has not been implicated in high-altitude adaptability so far.

The *D*-statistic was used to detect the ancestral origin of the selected region and to screen the Tibetan goat genomes for signatures of markhor introgression. The analysis revealed a strong signature of introgression at Chr26:42,070,001-42,120,000 ( $f_d=0.7634$ ; Figure 2A) which overlapped with *PAPSS2* locus and which was also supported by the Q95 (Figure 2A) and a phylogenetic analysis. The genomic region encompassing



**Figure 1.** Gene regions responsible for adaptation to high-altitude in goats. (A) Manhattan plot of genome-wide association results for high-altitude adaptation in goats (n=156). The dotted horizontal line indicates the threshold ( $-\text{Log}_{10}(P)=8.65$ ). (B) The strategy used to identify a list of genes related to high-altitude adaptation. It relies on three sets of genes, derived from the  $F_{ST}$  (blue), *XP-EHH* (red), and GWAS (yellow) analyses.  $F_{ST}$  and *XP-EHH* selection candidate sets include genes in the top 0.5% of the empirical distributions of  $F_{ST}$  and *XP-EHH* results, respectively. (C-D) Comparison of genomic regions identified in selection scans between high- and low-altitude goats. EAS-H, East Asian high-altitude goats, and EAS-L, East Asian low-altitude goats.



**Figure 2.** A genomic region encompassing *PAPSS2* was introgressed into Tibetan goats from markhor. (A) Genome-wide distribution of *D*-statistics (top) and Q95 (bottom) values calculated for 50-kb windows with a 10-kb step across the genome. Each dot represents a 50-kb window. (B) Haplotype structure for *PAPSS2*. Rows represent individuals and columns represent SNPs positions in the genome. AFR, African goats; EUR, European goats; SWA, Southwest Asian goats; SAS, South Asian goats.

*PAPSS2* had a high similarity between Tibetan goats and markhor but was substantially different in other domestic goat populations and wild caprid species (Figure 2B). The functional role of *PAPSS2* in the adaptation to hypoxia was further confirmed with the *papss2*-deficient cells.

## Discussion

In this study, we leveraged a large caprine whole-genome and transcriptome data set encompassing 331 goats and 13 tissues to localize signatures of high-altitude adaptation in Tibetan goats. Collectively, both positively selected haplotypes and expression patterns suggest genomic regions encompassing *PAPSS2* and *DSG3* to be significantly associated with high-altitude adaptation in Tibetan goats.

Although harsh environments may promote the accumulation of adaptive mutations in animals, it is unlikely that many de novo mutations can emerge at the same site during a short period of time (Hu *et al.*, 2019; Jeong *et al.*, 2016; Zhao *et al.*, 2017). Adaptive introgressions from other geographically close species may contribute to the process of adaptation that occurred in humans and many animals native to the QTP. In this study, we revealed that Tibetan goats carry specific adaptive introgressions derived from markhor (*C. falconeri*), a wild goat species native to high Himalayan mountains, including a haplotype encompassing *PAPSS2*. This region showed the strongest signature of both adaptive introgression and selection. A relatively long haplotype (chr26:42,077,488-42,179,191) also indicated that the area was introgressed rather recently.

Several studies have shown that the kidneys and the spleen may play a critical role in human oxygen-sensing (Schodel and Ratcliffe, 2019) and mammalian physical activity (Storz and Cheviron, 2021). Through multi-tissue transcriptome data comparison, we found that the *PAPSS2* gene was differentially expressed in the kidney and spleen between Tibetan and other domestic goats. The functional role of *PAPSS2* in the adaptation to hypoxia was further confirmed by our functional validation assay. Thus, it is plausible that the unique but recent introgression from the markhor may have conferred the adaptability of Tibetan goats to survive over the harsh environment on the QTP.

Our data provide new insights into the role of genetic introgression from wild relatives on environmental adaptation in livestock. We showed that the establishment of the genetic mechanism of plateau adaptation in Tibetan goats had occurred by the combined effects of gene introgression and natural selection. This

enabled Tibetan goats to adapt to the hypoxic environment in a relatively short period of time and continues to strengthen this ability until now.

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