

# Population Variation Revealed High-Altitude Adaptation of Tibetan Mastiffs

Yan Li,<sup>1,2</sup> Dong-Dong Wu,<sup>\*,1,2</sup> Adam R. Boyko,<sup>3</sup> Guo-Dong Wang,<sup>1,2</sup> Shi-Fang Wu,<sup>1</sup> David M. Irwin,<sup>1,4,5</sup> and Ya-Ping Zhang<sup>\*,1,2,6</sup>

<sup>1</sup>State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China

<sup>2</sup>Kunming College of Life Science, University of Chinese Academy of Sciences, Kunming, China

<sup>3</sup>Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University

<sup>4</sup>Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada

<sup>5</sup>Banting and Best Diabetes Centre, University of Toronto, Toronto, Ontario, Canada

<sup>6</sup>Laboratory for Conservation and Utilization of Bio-resource, Yunnan University, Kunming, China

\*Corresponding author: E-mail: wudongdong@mail.kiz.ac.cn; zhangyp@mail.kiz.ac.cn.

Associate editor: Anna Di Rienzo

## Abstract

With the assistance of their human companions, dogs have dispersed into new environments during the expansion of human civilization. Tibetan Mastiff (TM), a native of the Tibetan Plateau, was derived from the domesticated Chinese native dog and, like Tibetans, has adapted to the extreme environment of high altitude. Here, we genotyped genome-wide single-nucleotide polymorphisms (SNPs) from 32 TMs and compared them with SNPs from 20 Chinese native dogs and 14 gray wolves (*Canis lupus*). We identified 16 genes with signals of positive selection in the TM, with 12 of these candidate genes associated with functions that have roles in adaptation to high-altitude adaptation, such as *EPAS1*, *SIRT7*, *PLXNA4*, and *MAFG* that have roles in responses to hypoxia. This study provides important information on the genetic diversity of the TM and potential mechanisms for adaptation to hypoxia.

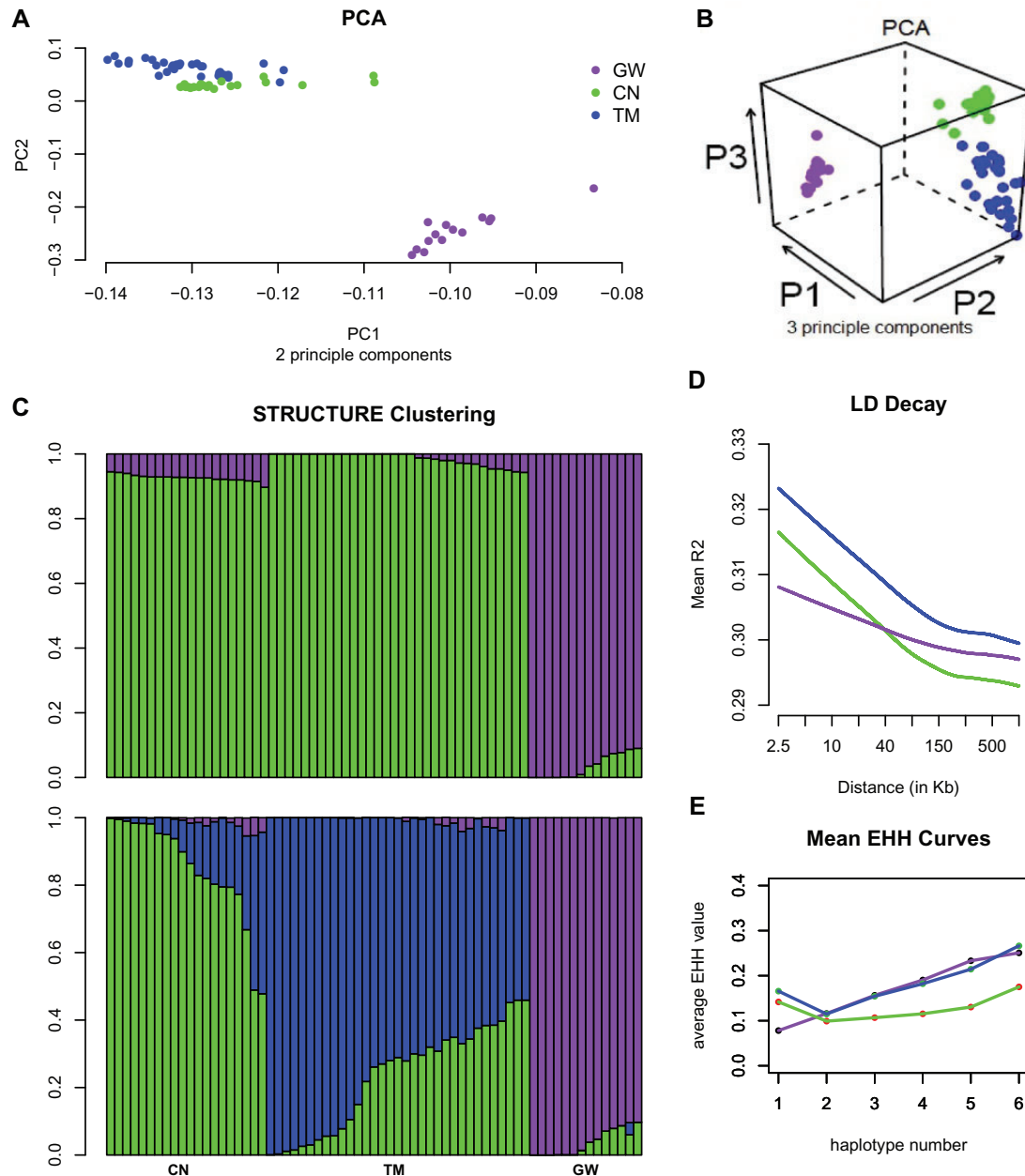
**Key words:** high-altitude adaptation, Tibetan Mastiff, domestication.

With the assistance of their human companions, dogs have dispersed into new environments as human civilization expanded during the Paleolithic (Germonpre et al. 2009). Among these dogs was the Tibetan Mastiff (TM), which has adapted to the extremely high altitude of the Tibetan Plateau (typically 4,500 m) (Messerschmidt 1983). As a guardian of property, the TM has acquired enhancements to its metabolic capacity to maintain a powerful body and physiological adaptations for persistence at high elevations that compensate for hypoxia (Cui and Guo 2010). Research on multiple species has revealed the identity of various candidate genes that may contribute to high-altitude adaptation, including studies on humans (Bigham et al. 2009; Beall et al. 2010; Simonson et al. 2010; Yi et al. 2010; Peng et al. 2011; Xu et al. 2011; Scheinfeldt et al. 2012; Xiang et al. 2013), deer mice (Storz et al. 2007; Cheviron et al. 2012), Tibetan antelope (Ge et al. 2013), some birds (Cai et al. 2013; Qu et al. 2013), and yak (Qiu et al. 2012). The TM is derived from the Chinese Native dog (CN), which resides at lower altitudes (Li and Zhang 2012), and have been modified by intentional selection over a relatively short period of time to adapt to high altitudes. Possible adaptations include a thicker coat and lower hemoglobin levels (Wen and Yuan 1998; Cui and Guo 2010). The genetic mechanism underlying the adaptation of the TM to the Tibetan Plateau is unknown and possibly differs from that of wild animals due to artificial selection.

Analysis of mitochondrial DNA across a variety of breeds of domestic dog has shown that the TM is an archaic lineage that is genetically more similar to the CNs from Southeast Asia than to any other dog breed (Li and Zhang 2012). To address the issue of divergence, we take advantage of this close genetic similarity and use the CNs as a powerful control for dissecting the biological basis of high-altitude adaptation in TMs. Here, we genotyped more than 120,000 single-nucleotide polymorphisms (SNPs) across the genome in 32 TMs, and integrated data from 20 CNs and 14 gray wolves from our previous analysis (Li et al. 2013), to study the adaptive evolution of TMs.

## Analyses of Population Structure and Variation

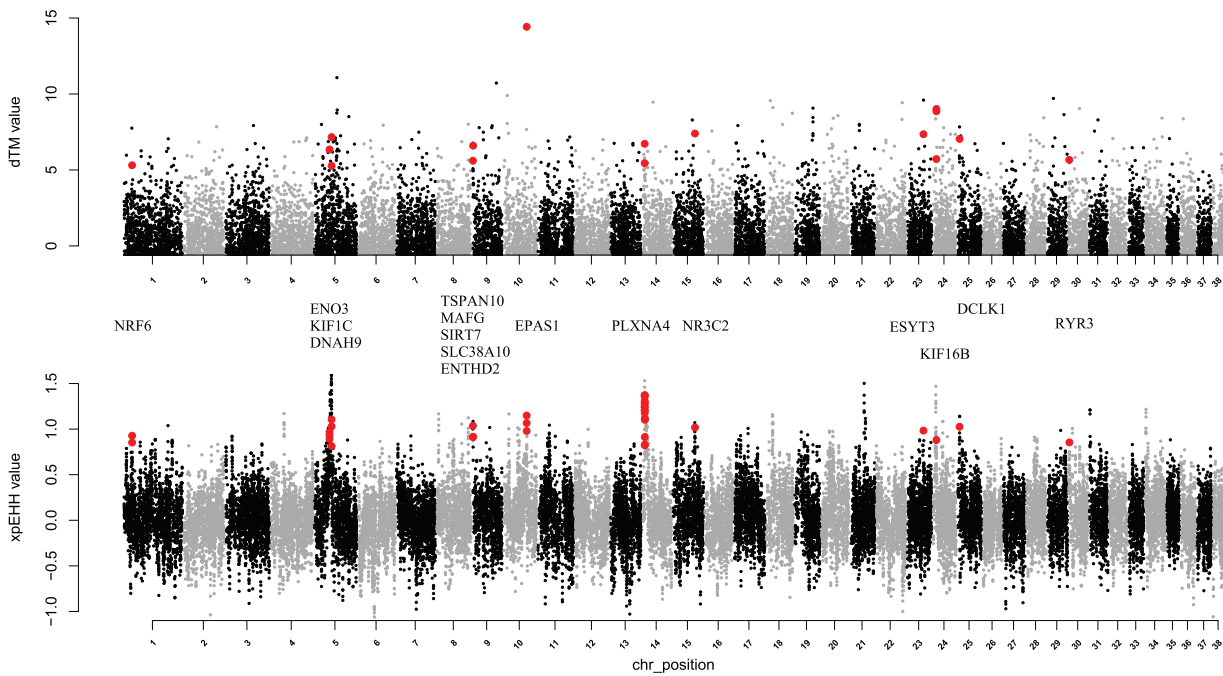
We first used principal component analysis (PCA) to examine the SNP data. The first two axes in an initial clustering analysis using PCA identified two distinct groups, one being the wild relatives (wolves) and the second cluster consisting of the domesticated dogs (fig. 1A). The third axis of the PCA separated the TMs from the CNs (fig. 1B). Phylogenetic trees constructed using the weighted neighbor-joining method (Bruno et al. 2000) with pairwise  $F_{ST}$  values among the different breed populations, including wolves, red wolves, and coyotes, supported a close relationship between CNs and TMs,



**Fig. 1.** Population genetic analysis of the TM, CN, and Chinese gray wolf (GW). Blue denotes TM, green denotes CN, and purple denotes GW. (A–B) PCA analysis of the 32 unrelated TMs, 20 CNs, and 14 GWs; (C) population structure results for  $K = 2/3$  analysis; (D) LD decay analysis; (E) plot of the haplotype number at the core region with the average EHH value at 500 kb from the core region.

and other ancient dogs, indicating that the TMs and CNs are ancient breeds (supplementary data S1, Supplementary Material online). To verify the PCA-based inferences of the genetic structure, we next implemented a Bayesian model-based approach to assess the population relationship of our samples of TMs, CNs, and gray wolves using the program STRUCTURE (Pritchard, Stephens, Rosenberg, et al. 2000). Domesticated dogs are clearly distinguished from the wild gray wolves, assuming two ancestral populations ( $K = 2$ ), a result that corroborates previous studies (vonHoldt et al. 2010, 2011). TMs and CNs are marginally separated when  $K = 3$  (fig. 1C), suggesting a much closer relationship between these two populations.

Next, we analyzed the population variation. Observed heterozygosity was highest in the TMs ( $H_o = 29.93\%$ ), with the CNs showing slightly lower levels (29.23%), with the wolf being lowest (24.4%). Similar inbreeding coefficients are observed in the CNs and TMs (0.18 and 0.16, respectively). Gray wolves captured from the wild presented a relatively high inbreeding coefficient ( $F = 0.32$ ) and low level of heterozygotes (24.4%), which are consistent with previous reports on runs of homozygosity in wolves (Boyko et al. 2010). A possible explanation for this observation may be due to the extinction of wolves from eastern and southern Asia. Alternatively, SNP ascertainment may have overestimated the number of derived polymorphisms in dogs and



**Fig. 2.** Genome-wide distribution of the  $d_i$  and XP-EHH values in the TM. Red large dots represent sites showing signatures of positive selection, with the identity of the potentially selected genes indicated.

underestimated the variation within wolves. To better understand population variation, haplotype-based analyses were performed. Linkage disequilibrium (LD) decay analysis revealed that longer blocks exist in the TM compared with CNs (fig. 1D). In addition, average EHH values within 500-kb windows across the genome did not change much among different core regions with different number of core haplotypes in CNs (EHH range from 0.099 to 0.175), TMs (EHH range from 0.115 to 0.266), and wolves (EHH range from 0.078 to 0.251) (fig. 1E). However, this pattern differs from that seen in breed dog, where an apparent negative relationship was observed from 0.872 to 0.541 (Li et al. 2013).

### Genome-Wide Mapping of Selected Genes in the TM Genome

Compared with the CNs in highland, TMs have lower hemoglobin levels in the blood (Wen and Yuan 1998) but have excellent endurance in their locomotory abilities in highland (Kunming Police Dog Base, Ministry of Public Security, Kunming, China, personal communication), which illustrates potential adaptations for the hypoxia found at high altitudes. To attempt to identify potential mechanisms for the adaptation in Tibet Plateau, we searched for loci that show evidence of selection specific to the TM using the statistic  $d_i$  (Akey et al. 2010), which evaluates the population differentiation of TM from the other two populations. A total of 353 SNPs, which were within the top 1% of the distribution ( $d_i > 5.117$ ), were identified and the 139 genes near these sites (supplementary table S1, Supplementary Material online). In addition, we calculated pairwise XP-EHH values, a haplotype-based parameter that is robust to ascertainment bias (Sabeti et al. 2006), for each SNP between the TMs and CNs and identified 115 genes that were near the 378 SNPs within the 99% percentile

(XP-EHH  $> 0.806$ ) (supplementary table S2, Supplementary Material online). We merged the SNP lists generated by these two approaches to identify a subset of 33 SNPs that showed the strongest signature of selection by displaying both high  $d_i$  and XP-EHH values, and these SNPs mapped to 11 genes (fig. 2). Of these 11 candidate genes showing strong signatures of selection, 9 are illustrated in figure 3 as having plausible biological functions associated with high-altitude adaptation and should be targets for further functional studies. These genes are *EPAS1*, *SIRT7*, *PLXNA4*, *MAFG*, *ENO3*, *DNAH9*, *KIF1C*, *KIF16B*, and *MR* (fig. 3).

Hypoxia-inducible factors (HIFs) are a group of transcription factors that regulate cellular adaptation to hypoxia and accumulate under hypoxic conditions (fig. 3). *EPAS1*, also known as HIF2 $\alpha$ , is one of these HIFs and has previously been implicated in hypoxia adaptation in Tibetans through its association with hemoglobin concentration (Beall et al. 2010; Yi et al. 2010; Peng et al. 2011; Xu et al. 2011). In TMs, the mean hemoglobin concentration is lower than that in CNs in highland (Wen and Yuan 1998), thus is similar to the pattern seen in Tibetans. This observation supports the possibility that convergent evolution in the mechanisms in dealing with hypoxia has occurred in dogs and humans. A second candidate selected gene in TMs, *SIRT7*, negatively regulate HIF-1 $\alpha$  and HIF-2 $\alpha$  (Hubbi et al. 2013) and has important functions in the homeostasis of cardiomyocytes (Vakhrusheva et al. 2008).

Angiogenesis, which is largely initiated by the HIF pathway (Rey and Semenza 2010), is a crucial response to hypoxia. Indeed, candidate genes that contribute to angiogenesis and/or vasculogenesis were universally detected in species living at high altitudes, although there was little overlap in specific genes among species. *NOS3* was detected in the



possibility that one gene in each cluster was adaptively selected, while the other genes were carried by the selective sweep. Which was selected and which was swept cannot be distinguished with our data. A second caveat is the ascertainment bias of the genome-wide SNP data. Indeed, a significant difference between the frequency spectrum of the actual genotyped SNPs and the calculated corrected frequency spectrum was found (data not shown); however, the long-range haplotype test is relatively robust to SNP ascertainment bias (Sabeti et al. 2006). Both population differentiation and long-range haplotype tests were used to detect signatures for positively selected sites, increasing the likelihood that we identified true targets. Obtaining whole-genome sequences from multiple TM individuals would be necessary for validating potential selective targets, which should also lead to the identification of additional key genes responsible for the adaptation of TMs to their home on the Tibetan plateau. In addition, these candidate genes need additional functional analysis to validate the potential roles of the selected alleles for high-altitude adaptation.

## Materials and Methods

SNPs from 40 TM individuals were genotyped using the Affymetrix v2 Canine array based on the CanFam 2.0 assembly. SNPs in the CNs and the wild gray wolves were described in our previous study (Li et al. 2013). A platinum library file (DogSNPs520431P) and the MAGIC (Multidimensional Analysis for Genotype Intensity Clustering) calling algorithm (Boyko et al. 2010) were used to ensure the reliability and robustness of the SNP annotation and the accuracy of the genotyping, yielding a total of 48,445 high-quality genotyped SNPs. We discarded SNPs that had > 10% missing data or that failed the Hardy–Weinberg equilibrium test ( $P < 0.001$ ) within each population using PLINK (Purcell et al. 2007), resulting in a retained data set of 35,266 SNPs for the downstream analyses. Further, to assess recent relationships among samples, following previous methods (vonHoldt et al. 2010), we used known parental relationships to calibrate identity-by-state (IBS) scores (0.84). Of the 40 original samples, 8 individuals were excluded due to their high pairwise genetic similarity with another one (IBS > 0.84, suggesting close relationship), leaving 32 individuals as unrelated samples used in the downstream analyses. PCA was performed using R ([www.r-project.org](http://www.r-project.org), last accessed February 21, 2014), based on the genotype data of each individual at each allele. Population structure was evaluated using STRUCTURE software (Pritchard, Stephens, and Donnelly 2000). Heterozygosity, inbreeding coefficient, and decay of LD were calculated by PLINK (Purcell et al. 2007). The haplotype of each chromosome was inferred by fastPHASE (Scheet and Stephens 2006). EHH and REHH values were calculated for each core haplotype using SWEEP software ([www.broadinstitute.org/mpg/sweep](http://www.broadinstitute.org/mpg/sweep), last accessed February 21, 2014). Pairwise per-SNP genetic differentiation ( $F_{ST}$ ) values between breeds and  $d_i$  were calculated as described by Akey et al. (2002, 2010) to evaluate population differentiation between TM, CNs, and gray wolves. XP-EHH between TMs and CNs was calculated by a program from <http://hgdp.uchicago.edu/Software/> (last

accessed February 21, 2014). Candidate genes were identified if they were located within 5,000 bp of a SNP having a selective signature (Li et al. 2013). For gene identification, we chose two approaches. First, we identified SNPs within the top 1% of  $d_i$  and XP-EHH values. These SNPs were mapped to 11 genes. Then, we identified SNPs within the top 1% of  $d_i$  values, which map to 139 genes, and SNPs with top 1% XP-EHH values, which map to 115 genes. The overlap between these two sets was 16 genes having SNPs with high  $d_i$  values and having SNPs with high XP-EHH values.

## Supplementary Material

Supplementary data S1 and tables S1 and S2 are available at *Molecular Biology and Evolution* online (<http://www.mbe.oxfordjournals.org/>).

## Acknowledgments

The authors thank Drs Bridgett M. vonHoldt and Robert K. Wayne for their suggestions and comments on the manuscript. They thank Bing Liu from CAS Tibetan Mastiff Co., Ltd for his help with sampling the Tibetan Mastiff. This work was supported by grants from the National Natural Science Foundation of China (31321002, 91231108, 31123005), and the 973 program (2013CB835200).

## References

- Akey JM, Ruhe AL, Akey DT, Wong AK, Connelly CF, Madeoy J, Nicholas TJ, Neff MW. 2010. Tracking footprints of artificial selection in the dog genome. *Proc Natl Acad Sci U S A*. 107:1160–1165.
- Akey JM, Zhang G, Zhang K, Jin L, Shriver MD. 2002. Interrogating a high-density SNP map for signatures of natural selection. *Genome Res*. 12:1805–1814.
- Bassett JHD, Gogakos A, White JK, Evans H, Jacques RM, van der Spek AH, Ramirez-Solis R, Ryder E, Sunter D, Boyde A, et al. 2012. Rapid-throughput skeletal phenotyping of 100 knockout mice identifies 9 new genes that determine bone strength. *PLoS Genet*. 8:e1002858.
- Beall CM, Cavalleri GL, Deng L, Elston RC, Gao Y, Knight J, Li C, Li JC, Liang Y, McCormack M, et al. 2010. Natural selection on EPAS1 (HIF2 $\alpha$ ) associated with low hemoglobin concentration in Tibetan highlanders. *Proc Natl Acad Sci U S A*. 107:11459–11464.
- Bigham A, Bauchet M, Pinto D, Mao X, Akey JM, Mei R, Scherer SW, Julian CG, Wilson MJ, Lopez Herraez D, et al. 2010. Identifying signatures of natural selection in Tibetan and Andean populations using dense genome scan data. *PLoS Genet*. 6:e1001116.
- Bigham AW, Mao X, Mei R, Brutsaert T, Wilson MJ, Julian CG, Parra EJ, Akey JM, Moore LG, Shriver MD. 2009. Identifying positive selection candidate loci for high-altitude adaptation in Andean populations. *Hum Genomics*. 4:79–90.
- Blank V, Kim MJ, Andrews NC. 1997. Human MafG is a functional partner for p45 NF-E2 in activating globin gene expression. *Blood*. 89:3925–3935.
- Boyko AR, Quignon P, Li L, Schoenebeck JJ, Degenhardt JD, Lohmueller KE, Zhao K, Brisbin A, Parker HG. 2010. A simple genetic architecture underlies morphological variation in dogs. *PLoS Biol*. 8:e1000451.
- Bruno WJ, Succi ND, Halpern AL. 2000. Weighted neighbor joining: a likelihood-based approach to distance-based phylogeny reconstruction. *Mol Biol Evol*. 17:189–197.
- Cai Q, Qian X, Lang Y, Luo Y, Pan S, Hui Y, Gou C, Cai Y, Hao M, Zhao J, et al. 2013. The genome sequence of the ground tit *Pseudopodoces humilis* provides insights into its adaptation to high altitude. *Genome Biol*. 14:R29.
- Carbonaro M, Escuin D, O'Brate A, Thadani-Mulero M, Giannakakou P. 2012. Microtubules regulate hypoxia-inducible factor-1 $\alpha$  protein

- trafficking and activity: implications for taxane therapy. *J Biol Chem.* 287:11859–11869.
- Cheviron ZA, Bachman GC, Connaty AD, McClelland GB, Storz JF. 2012. Regulatory changes contribute to the adaptive enhancement of thermogenic capacity in high-altitude deer mice. *Proc Natl Acad Sci U S A.* 109:8635–8640.
- Cui TB, Guo X. 2010. A monograph of Tibetan Mastiff in China. Shanghai (China): Shanghai Scientific & Technical Publishers.
- Gardner DS, Fletcher AJW, Fowden AL, Giussani DA. 2001. Plasma adrenocorticotropin and cortisol concentrations during acute hypoxemia after a reversible period of adverse intrauterine conditions in the ovine fetus during late gestation. *Endocrinology* 142: 589–598.
- Ge R-L, Cai Q, Shen Y-Y, San A, Ma L, Zhang Y, Yi X, Chen Y, Yang L, Huang Y, et al. 2013. Draft genome sequence of the Tibetan antelope. *Nat Commun.* 4:1858.
- Germonpre M, Sablin MV, Stevens RE, Hedges REM, Hofreiter M, Stiller M, Despres VR. 2009. Fossil dogs and wolves from Palaeolithic sites in Belgium, the Ukraine and Russia: osteometry, ancient DNA and stable isotopes. *J Archaeol Sci.* 36:473–490.
- Heiniger CD, Kostadinova RM, Roachat MK, Serra A, Ferrari P, Dick B, Frey BM, Frey FJ. 2003. Hypoxia causes down-regulation of 11 beta-hydroxysteroid dehydrogenase type 2 by induction of Egr-1. *FASEB J.* 17:917–919.
- Hubbi ME, Hu H, Gilkes DM, Semenza GL. 2013. Sirtuin-7 Inhibits the Activity of Hypoxia-Inducible Factors. *J Biol Chem.* 288: 20768–20775.
- Jiang F, Dong Y, Wu C, Yang X, Zhao L, Guo J, Li Y, Dong J, Zheng G-Y, Cao H, et al. 2011. Fine mapping of chromosome 3q22.3 identifies two haplotype blocks in ESYT3 associated with coronary artery disease in female Han Chinese. *Atherosclerosis* 218:397–403.
- Kigel B, Rabinowicz N, Varshavsky A, Kessler O, Neufeld G. 2012. Plexin-A4 promotes tumor progression and tumor angiogenesis by enhancement of VEGF and bFGF signaling. *Blood* 118:4285–4296.
- Kodama T, Shimizu N, Yoshikawa N, Makino Y, Ouchida R, Okamoto K, Hisada T, Nakamura H, Morimoto C, Tanaka H. 2003. Role of the glucocorticoid receptor for regulation of hypoxia-dependent gene expression. *J Biol Chem.* 278:33384–33391.
- Li Y, vonHoldt BM, Reynolds A, Boyko AR, Wayne RK, Wu D-D, Zhang Y-P. 2013. Artificial selection on brain-expressed genes during the domestication of dog. *Mol Biol Evol.* 30:1867–1876.
- Li Y, Zhang Y. 2012. High genetic diversity of Tibetan Mastiffs revealed by mtDNA sequences. *Chin Sci Bull.* 57:1483–1487.
- Maher JT, Jones LG, Hartley LH, Williams GH, Rose LI. 1975. Aldosterone dynamics during graded exercise at sea level and high altitude. *J Appl Physiol.* 39:18–22.
- Messerschmidt DA. 1983. The Tibetan Mastiff: canine sentinels of the range. *Rangelands* 5:172–174.
- Moncloa F, Donayre J, Sobrevilla LA, Guerra-Garcia R. 1965. Endocrine studies at high altitude. II. Adrenal cortical function in sea level natives exposed to high altitudes (4300 meters) for two weeks. *J Clin Endocrinol Metab.* 25:1640–1642.
- Peng Y, Yang Z, Zhang H, Cui C, Qi X, Luo X, Tao X, Wu T, Chen H, Shi H, et al. 2011. Genetic variations in Tibetan populations and high-altitude adaptation at the Himalayas. *Mol Biol Evol.* 28:1075–1081.
- Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155:945–959.
- Pritchard JK, Stephens M, Rosenberg NA, Donnelly P. 2000. Association mapping in structured populations. *Am J Hum Genet.* 67:170–181.
- Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, Maller J, Sklar P, De Bakker PIW, Daly MJ. 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet.* 81:559–575.
- Qiu Q, Zhang G, Ma T, Qian W, Wang J, Ye Z, Cao C, Hu Q, Kim J, Larkin DM, et al. 2012. The yak genome and adaptation to life at high altitude. *Nat Genet.* 44:946–949.
- Qu Y, Zhao H, Han N, Zhou G, Song G, Gao B, Tian S, Zhang J, Zhang R, Meng X, et al. 2013. Ground tit genome reveals avian adaptation to living at high altitudes in the Tibetan plateau. *Nat Commun.* 4:2071.
- Rey S, Semenza GL. 2010. Hypoxia-inducible factor-1-dependent mechanisms of vascularization and vascular remodelling. *Cardiovasc Res.* 86:236–242.
- Sabeti PC, Schaffner SF, Fry B, Lohmueller J, Varilly P, Shamovsky O, Palma A, Mikkelsen TS, Altshuler D, Lander ES. 2006. Positive natural selection in the human lineage. *Science* 312:1614–1620.
- Sawhney RC, Malhotra AS, Singh T. 1991. Glucoregulatory hormones in man at high altitude. *Eur J Appl Physiol Occup Physiol.* 62:286–291.
- Scheet P, Stephens M. 2006. A fast and flexible statistical model for large-scale population genotype data: applications to inferring missing genotypes and haplotypic phase. *Am J Hum Genet.* 78:629–644.
- Scheinfeldt LB, Soi S, Thompson S, Ranciaro A, Woldemeskel D, Beggs W, Lambert C, Jarvis JP, Abate D, Belay G. 2012. Genetic adaptation to high altitude in the Ethiopian highlands. *Genome Biol.* 13:R1.
- Semenza GL. 2011. Oxygen sensing, homeostasis, and disease. *N Engl J Med.* 365:537–547.
- Simonson TS, Yang Y, Huff CD, Yun H, Qin G, Witherspoon DJ, Bai Z, Lorenzo FR, Xing J, Jorde LB. 2010. Genetic evidence for high-altitude adaptation in Tibet. *Science* 329:72.
- Storz JF, Moriyama H. 2008. Mechanisms of hemoglobin adaptation to high altitude hypoxia. *High Alt Med Biol.* 9:148–157.
- Storz JF, Sabatino SJ, Hoffmann FG, Gering EJ, Moriyama H, Ferrand N, Monteiro B, Nachman MW. 2007. The molecular basis of high-altitude adaptation in deer mice. *PLoS Genet.* 3:e45.
- Vakhrusheva A, Smolka C, Gajawada P, Kostin S, Boettger T, Kubin T, Braun T, Bober E. 2008. Sirt7 increases stress resistance of cardiomyocytes and prevents apoptosis and inflammatory cardiomyopathy in mice. *Circ Res.* 102:703–710.
- vonHoldt BM, Pollinger JP, Earl DA, Knowles JC, Boyko AR, Parker H, Geffen E, Pilot M, Jedrzejewski W, Jedrzejewska B, et al. 2011. A genome-wide perspective on the evolutionary history of enigmatic wolf-like canids. *Genome Res.* 21:1294–1305.
- vonHoldt BM, Pollinger JP, Lohmueller KE, Han E, Parker HG, Quignon P, Degenhardt JD, Boyko AR, Earl DA, Auton A, et al. 2010. Genome-wide SNP and haplotype analyses reveal a rich history underlying dog domestication. *Nature* 464:898–902.
- Wang Y-X, Zheng Y-M. 2010. ROS-dependent signaling mechanisms for hypoxic Ca<sup>2+</sup> responses in pulmonary artery myocytes. *Antioxid Redox Signal.* 12:611–623.
- Wen J, Yuan N. 1998. Analysis on the parameters of blood in healthy Tibetan Mastiffs. *J Qinghai Medical College.* 20:7–9.
- Xiang K, Ouzhuluobu, Peng Y, Yang Z, Zhang X, Cui C, Zhang H, Li M, Zhang Y, Bianba, et al. 2013. Identification of a Tibetan-specific mutation in the hypoxic gene EGLN1 and its contribution to high-altitude adaptation. *Mol Biol Evol.* 30:1889–1898.
- Xu S, Li S, Yang Y, Tan J, Lou H, Jin W, Yang L, Pan X, Wang J, Shen Y, et al. 2011. A genome-wide search for signals of high-altitude adaptation in Tibetans. *Mol Biol Evol.* 28:1003–1011.
- Yi X, Liang Y, Huerta-Sanchez E, Jin X, Cuo ZXP, Pool JE, Xu X, Jiang H, Vinckenbosch N, Korneliussen TS, et al. 2010. Sequencing of 50 human exomes reveals adaptation to high altitude. *Science* 329: 75–78.