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Limited polymorphisms of two Y-chromosomal SNPs in Chinese and Iranian sheep

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Source/description: Genetic variation present at the male-specific region of the *Y*-chromosome provides crucial complementary information in understanding male-mediated events and their impact during livestock domestication and recent breed development.[1] In sheep (*Ovis aries*), one single nucleotide polymorphism (SNP) at g.88A>G was detected in the 5′-promoter region of the *sex-determining region Y* (*SRY*) gene.[2] Genotyping of this SNP together with a compound microsatellite (*SRYM18*) established 11 haplotypes in 519 domestic and wild sheep. Seven of these haplotypes were present in domestic sheep.[3] An additional seven SNPs were identified in the same *SRY* region through resequencing of 19 individuals of six wild sheep species and eight domestic sheep; however, none of these SNPs was polymorphic in domestic sheep.[4]

Samples: A total of 378 male sheep sampled from 18 Chinese and two Iranian indigenous sheep breeds as well as four commercial breeds were used in this study (for details see Fig. 1 and Table S1). All males were selected to be as unrelated as possible.

Resequencing the SRY region: The polymerase chain reaction (PCR) and resequencing of the same region of the *SRY* gene were performed according to Meadows *et al.*[2] A total of 152 individuals were directly sequenced, including 129 Chinese sheep (33 TIB, 21 TAN, 23 ZT, 12 HZK, 12 TSK, 15 YC, 6 KRK, 3 JZ and 4 HAN sheep) and 23 Iranian sheep (16 ZEL and 7 GHR sheep). Apart from the g.88A>G SNP, the sequencing data revealed a novel transversion SNP at g.460G>T. The new sequence has been deposited in the GenBank (accession no. HQ840956). The G allele of g.88A>G was very rare and present once each in ZT and TSK sheep. The T allele of g.460G>T was present only twice, exclusively in TAN sheep.

PCR–RFLP analyses of g.88A>G and g.460G>T: The two SNPs were genotyped separately in the remaining 226 sheep, using PCR–RFLP analyses of the same *SRY* region using BbvI or DpnII restriction enzymes. For g.88A>G, the G allele was fixed in Suffolk, Texel and Polled Dorset, but absent in Mutton Merino and the remaining 193 Chinese sheep. For g.460G>T,

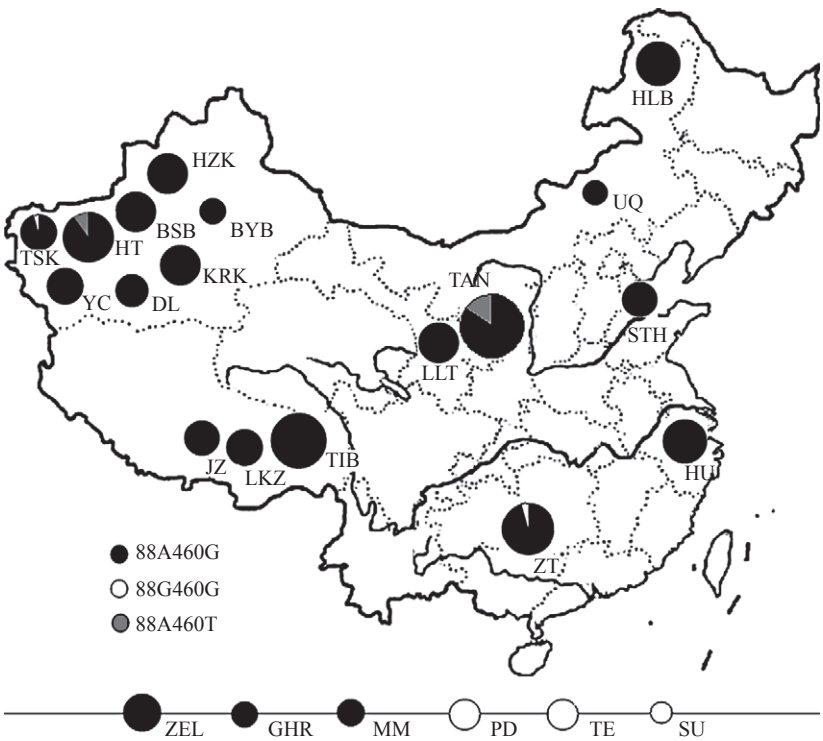

Figure 1 Geographic distribution and *SRY* haplotype frequency of Chinese sheep breeds included in the study (six foreign breeds are shown outside the map).

the T allele was observed in five individuals in another TAN sheep population and two individuals of HT sheep. Further, resequencing of the same *SRY* region[2] and microsatellite *SRYM18* locus[3] of these seven individuals confirmed the PCR–RFLP results and detected a repeat array of ($[TTTTG]_3G[TG]_{15}$).

Comments: Three haplotypes were identified as 88A460G, 88G460G and 88A460T. The first haplotype, present in 342 samples, most likely corresponded to the haplotypes H4 and H6; the second haplotype, present in 27 samples, probably matched haplotype H5 of Meadows *et al.*[3,4] By significantly improving sampling coverage from 22 samples of two breeds[3] up to 322 samples of 18 major indigenous Chinese sheep breeds, and by employing new information on indigenous Iranian sheep, the results not only confirmed previous observations on the four commercial breeds[3] but also strengthened the view that domestic sheep are free from signatures of wild sheep introgression.[4] The third haplotype, however, was distinct, present in nine samples of two breeds distributed in Northwestern China. Several Argali subspecies are found in this region,[5] and their rams are used to hybridize domestic ewes for better performance.[6] Further investigation of *Y*-chromosomal polymorphism of these wild sheep is, therefore, warranted.

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6 Aniwashi J. *et al.* (2007) *Xinjiang Agri Sci* **44**, 702–5.

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Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Distribution of the Y-chromosomal haplotypes in 24 sheep breeds.

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Polymorphic variants within a putative upstream open reading frame of the *MC4R* gene do not affect body weight of farmed red foxes

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Source/description: The *MC4R* gene controls feed intake and energy balance. Body mass is an important breeding goal in fur-bearing animals because it is correlated with pelt size,[1] and thus, the *MC4R* can be considered as a candidate gene. In the *MC4R* of the red fox[2] and dog,[3] several SNPs were identified, but until now no association studies have been performed. We describe novel variants, including an 11 bp indel, in the upstream open reading frame (uORF) of the *MC4R* in relation to body weight of farmed red foxes.

Sample: Samples were obtained from male red foxes ($N = 381$, progeny of 180 females), slaughtered on a local fur-bearing animal farm. All animals were weighed *post-mortem*, and mean age at slaughter was 259 days (SD = 17).

Polymorphism detections: Genomic DNA was extracted from muscle tissue samples. The following primer pairs were used for PCR amplification: pair 1 (375 bp), F: 5′ GATCGGAGCTG TACCTGGAAGACA, R: 5′ AGCAAGCTTATGACCCCCAGAGTC; pair 2 (542 bp), F: 5′ TCAGCAGCAGCCACTAACAC, R: 5′ CGGTTCCAGAAGTGGAGAGA. A polymorphism study was carried out by direct DNA sequencing (ABI 3130) of a 415 bp fragment of the 5′UTR sequence (GenBank: JF826017).

Detected polymorphisms: In the analysed sequence, three already known SNPs (c.-62C>G, c.-65A>G, c.-73A>G),[2] two novel indels (c-129_139delTGGACGGGGCA and c.-270_271delG) and three novel SNPs (c.-124G>A, c.-154C>T, c.-276G>A) were identified (Fig. S1). The 11 bp indel and four SNPs (c.-62C>G, c.-65A>G, c.-73A>G and c.-124G>A) segregated as two haplotypes (H): H-ins (CAAGins) and H-del (GGGAdel). The genotype frequencies were 0.543 (H-ins/H-ins), 0.391 (H-ins/H-del) and 0.066 (H-del/H-del). The comparative *in silico* search for functional sequences in the studied fragment revealed two uORFs (Fig. S1). Within one of them, an 11 bp indel was identified. The deletion variant missed one uORF because of frameshift causing disappearance of the STOP codon.

Association study: An association study between the haplotypes and body weight was performed with the use of the MIXED procedure of SAS statistical package. Four genetic models were considered: general, additive, dominance and recessive. A linear model included a fixed effect of the genotype group, a random effect of the maternal group and the age at slaughter as a

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Table S1 Distribution of the Y-chromosomal haplotypes in 24 sheep breeds

Breed code	Breed name	Sampling location	No. Samples	Haplotypes		
				88A460G	88A460T	88G460G
HZK	Kazakh	Xinjiang, China	17	17		
BSB	Bashibai	Xinjiang, China	18	18		
BYB	Bayinbuluke	Xinjiang, China	6	6		
HT	Hetian	Xinjiang, China	20	18	2	
DL	Duolang	Xinjiang, China	11	11		
KRK	Chinese Karakul	Xinjiang, China	18	18		
TSK	Tashikuergan	Xinjiang, China	12	11		1
YC	Yecheng	Xinjiang, China	15	15		
JZ	Jiangzi	Tibet, China	12	12		
LKZ	Langkazi	Tibet, China	14	14		
TIB	Tibetan	Tibet, China	33	33		
HLB	Hulunbeier	Inner Mongolia, China	23	23		
UQ	Ujumqin	Inner Mongolia, China	6	6		
TAN	Tan	Ningxia, China	45	38	7	
LLT	Lanzhou Large Tailed	Gansu, China	17	17		
STH	Small Tailed Han	Shandong, China	12	12		
HU	Hu	Zhejiang, China	20	20		
ZT	Zhaotong	Yunnan, China	23	22		1
ZEL	Zel	Caspian, northern Iran	16	16		
GHR	Iranian Karakul (Gharegol)	Northeastern Iran	7	7		
MM	Mutton Merino	Sampled in Shanxi, China	8	8		
TE	Texel	Sampled in Beijing, China	10			10
SU	Suffolk	Sampled in Beijing, China	5			5
PD	Polled Dorset	Sampled in Beijing, China	10			10