
1 **Geographic Distribution of a Missense Mutation in the *KRT38***
2 **Gene and Its Association with Heat Tolerance in Chinese Indigenous**
3 **Cattle Breeds**

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16
17 **ABSTRACT**

18 **Context.** China has a vast area across many temperature zones and a variety of cattle
19 breeds. These cattle resources are ideal models to research their adaptability to the
20 environment. The *KRT38* gene is an acidic protein, and its coding product can be used
21 as a component of hair production. **Aims.** The objective of this study was to
22 investigate the diversity of the *KRT38* gene in Chinese local cattle and the association
23 of different genotypes with mean temperature (T), relative humidity (RH) and
24 temperature humidity index (THI). **Methods.** A missense mutation g.41650738 A > G
25 in the *KRT38* gene was screened from the database of bovine genomic variation
26 (BGVD), was genotyped in a total of 246 samples from 15 local cattle breeds in China
27 by PCR amplification and sequencing. Finally, the correlation between the locus and
28 the three climatic factors was analysed. **Key results.** We successfully obtained the
29 frequency of this SNP in three groups of cattle in northern, central and southern China.
30 The frequency of allele A gradually declined from north to south, while the frequency
31 of allele G showed the opposite trend with a clear geographic distribution.
32 **Conclusions.** Our results indicate that *KRT38* variation in Chinese indigenous cattle
33 might be linked to heat tolerance. **Implications.** Our analysis may support in finding
34 out its importance as a genetic signal for heat tolerance in cattle reproduction and
35 genetics.

36 **Keywords:** Cattle; *KRT38*; Climatic; Missense mutation; Single nucleotide
37 polymorphism (SNP); Geographical distribution; Heat tolerance; Breeding

38
39 **Introduction**

40 Based on their geographical location and physical characteristics, the Chinese genetic
41 resources for cattle can be divided into three main groups, which consist of 53
42 recognised native cattle breeds in the north, centre and south of China (Chen *et al.*
43 2018). In previous studies, indicine cattle are the descendants of the same ancestor as
44 European taurine and African cattle, but they have experienced completely different
45 evolutionary processes over the millennia (Verdugo *et al.* 2019). Natural selection has
46 led to the evolution of heat resistance genes and stronger tolerance to warmer
47 environments.

48 The global annual average temperature has risen gradually due to the the
49 greenhouse effect, and the duration and intensity of high temperatures have also
50 increased significantly. Temperature change can enhance heat stress on livestock,
51 which results in a decrease in animal performance, such as the feed intake, milk
52 production (West 2003) and reproductive performance (Hansen 2007). Animal heat
53 tolerance is a quantitative trait (Gaughan *et al.* 2010; Li *et al.* 2011; Chang *et al.*
54 2012), and recent advancements in molecular genetic methodologies have facilitated
55 the identification of the correlation between genetic variation at specific loci and their
56 associated traits. Among these adaptive responses, the coat characteristics play a
57 pivotal role in influencing the heat tolerance of animals under high ambient
58 temperature and humidity conditions (Sarlo Davila *et al.* 2020).

59 Animal-derived filaments and hair fibers are composed of proteins, such as
60 keratin and keratin-associated proteins (KAPs) (Gong *et al.* 2016), and also contain
61 lipids and carbohydrates (Masukawa *et al.* 2005). *KRT38*, a gene belonging to the
62 type II epithelial-keratin gene family, significantly influences the coat production of
63 animals. Previous studies have revealed that several proteins in the keratin family
64 affect the wool composition and structure (Yu *et al.* 2009; Li *et al.* 2018; Sulayman *et al.*
65 2018). In addition, keratins play a role in the formation of the hair shaft (Wu *et al.*
66 2008). Skin color and the thickness of the hair directly influence the thermotolerance
67 of cattle that live in the tropics (Mattioli *et al.* 2000). *Bos indicus* has a smoother and
68 shoter hair coat than *Bos taurus*. Due to these characteristics, *Bos indicus* regulates its
69 body temperature and maintains cellular functions more efficiently during heat
70 (Muchenje *et al.* 2008; Muchenje *et al.* 2009). *KRT38* is a potential candidate gene for
71 wool traits (Sulayman *et al.* 2018), and genetic variation in the keratin and
72 keratin-associated protein (KRATP) genes has been reported in many studies,
73 indicating that the mutation contributes to phenotypic differences in wool (Langbein
74 *et al.* 2007; Zeng *et al.* 2018; Zhang *et al.* 2018).

75 In contrast, there has been no research conducted regarding the correlation
76 between temperature tolerance and genetic variation in the *KRT38* gene among
77 indigenous Chinese cattle breeds. The purpose of this study was to evaluate the
78 *KRT38* gene as a genetic marker for heat tolerance for cattle breeding and genetics, by
79 investigating the diversity of the *KRT38* gene in Chinese native cattle and the
80 relationship between various genotypes and mean temperature (T), relative humidity
81 (RH) and temperature humidity index (THI).

82 **Materials and methods**

83 **Ethical statement**

84 The animal experimentation methods employed in this study were authorized by the
85 Animal Care and Use Committee of the Institute of Animal Science, Northwest A&F
86 University, Shaanxi, China, following the guidelines set forth in the field of biological
87 sciences (Protocol number, WAFAC1008). During the sampling process, all samples
88 collected in this study were examined with the consent of their owners and were
89 standardized as far as possible.

90 **Animal Tissue DNA Extraction and Data Collection**

91 A total of 246 ear tissues from 15 different native Chinese cattle breeds were collected
92 from state-owned farms for this study (Table S1). Genomic DNA was extracted using
93 the phenol-chloroform method (Xu *et al.* 2020). DNA samples were diluted to
94 standard concentrations ($50 \text{ ng } \mu\text{L}^{-1}$) and stored at -80°C . Two environmental
95 parameters (T and RH) over the last 30 years for the sampling sites of the 15
96 indigenous cattle breeds were collected from the Chinese Central Meteorological
97 Office and were used to estimate heat tolerance traits (<https://data.cma.cn/>). The THI
98 was calculated separately by the researchers based on the T and RH data. Based on
99 nucleotide analysis of 15 indigenous cattle breeds, genotype and allele frequencies
100 were determined. THI assesses the combined effects of T and RH to ascertain the heat
101 load intensity of thermal climatic conditions, and it is a useful and simple
102 measurement (Pinto *et al.* 2020). Next, it was calculated using the formula of National
103 Oceanic and Atmospheric Administration:

$$104 \quad \text{THI} = (1.8T + 32) - (0.55 - 0.0055RH)(1.8T - 26)$$

105 where T is the temperature in degrees Celsius and RH is the relative humidity as a
106 percentage (Blanpain and Fuchs 2009).

107 Three climate variables with two genotypes each were subjected to environmental
108 association studies, which used the general linear model (GLM) in SPSS 18.0.
109 (Duricki *et al.* 2016). As Eckert suggested, we treated the environment variable as a
110 phenotype. The statistical equation was:

$$111 \quad \text{Climatic variable} = \text{Marker effect} + \text{Residual}$$

112 where climatic variables were the T, RH, and THI values between 1951 and 1980;
113 marker effect was the fixed impact of the genotypes; and residual was the random
114 element of the residual effect. Differences were considered significant at $P < 0.01$.

115 **Primer design, amplification, and novel SNP identification**

116 The polymerase chain reaction (PCR) primer was designed based on the bovine
117 *KRT38* sequence (GenBank accession rs210366642) by Primer Premier 5.0. The
118 forward primer was 5'-AGGCTGGTCTCCAGTGTCAA-3', and the reverse primer
119 was 5'-CCTCAGTCCACCATGACTTCC-3', which were designed to amplify a

120 114-bp product. The PCR amplification system consisted of 50 ng of genomic DNA,
121 10 μ L of 2x PCR mix, 0.5 μ M of each primer, and 8 μ L of ddH₂O (Jia *et al.* 2019).
122 The following steps were involved in the cycling procedure: 5 min of 95°C
123 denaturation; 35 cycles of 94°C for 30 s; 51°C for 30 s; and 72°C for 30 s primer
124 extension; followed by an 8-min final extension at 72°C. The PCR products were
125 identified directly at Shanghai Sangon Biotech Company, Shanghai, China, by
126 electrophoresis on a 1%-agarose gel stained with ethidium bromide. SEQMAN TMIIv
127 6.1 was used to examine the sequencing outcomes.

128 **Statistical Analysis**

129 The correlation between the three environmental factors (T, RH and THI) at the
130 sampling sites was analyzed using SPSS software. This analysis employed a statistical
131 linear regression model:

$$132 Y_i = \mu + G_i + e_i$$

133 where Y_i is the value of T, RH and THI from 1950 to 1981; μ is the overall mean
134 value; G_i = the fixed genotype effect; and e_i = the random residual effect. Differences
135 were considered significant at $P < 0.05$.

136 Genotypic and allelic frequencies were estimated based on the observed genotypes in
137 the examined breeds. Population indices such as homozygosity (H_o), heterozygosity
138 (H_e), effective allele numbers (N_e) and polymorphism information content (PIC)
139 were calculated. The THI, which considers the combined effects of T and RH, can
140 serve as an indicator for assessing the intensity of heat load under thermal climatic
141 conditions. This enables one to determine the heat load intensity more accurately.

142 Protein sequences and alignments for various species were obtained from the
143 NCBI database (<https://www.ncbi.nlm.nih.gov/>). MEGA software (Kumar *et al.* 2018)
144 was utilized to collect protein sequence similarities. Homology modelling of proteins
145 before and after missense mutation was performed based on the bovine KRT38
146 protein sequence (NP_001070385.1) by the Amino Acid Explore website
147 (www.ncbi.nlm.nih.gov).

148 **Results**

149 **Novel missense mutation in the Chinese cattle *KRT38* gene discovered**

150 In the current study, a novel A-to-G mutation (NC_037346.1 g.41650738 A > G) was
151 identified in exon 1 of KRT38, resulting in the alteration of isoleucine at position
152 p.I17T. This mutation was discovered in the Bovine Genome Variation Database and
153 Selective Signatures (BGVD) available at
154 (<http://animal.nwsuaf.edu.cn/code/index.php/BosVar>). Based on the sequence
155 chromatograms, 26 Chinese cattle breeds exhibited three genotypes: AA (116), AG
156 (72), and GG (51) (Fig. 1 and Table S1). In the present study, we assessed the
157 genotypic and allelic frequencies of this gene in the cattle population (see to Table S2).
158 The three genotypes exhibited relative frequencies of 0.4894, 0.3055, and 0.1850,

159 respectively. The proportions of the A and G alleles were determined to be 0.7205 and
160 0.2795, respectively. A discontinuous distribution was also shown by the frequency of
161 the allele G in *Bos taurus* and *Bos indicus*. From the geographical distribution of
162 sampling sites, allele A in northern, central and southern Chinese cattle reached
163 0.9104, 0.6786 and 0.6031, respectively, and gradually diminished in Chinese
164 indigenous cattle from south to north (Fig. 2).

165 **Genetic parameter analysis of the *KRT38* gene**

166 The genetic indices of Ho, He, Ne, and PIC were calculated and presented in Table S3.
167 Ho, which assesses genetic diversity and historical information of a population,
168 ranged from 0.50 to 1, indicating increased heterozygosity. He values ranged between
169 0 and 0.50, while Ne values ranged between 1 and 2. The PIC values varied from zero
170 to 0.375. The northern populations exhibited predominantly low or intermediate
171 polymorphism, whereas the central herds and southern populations displayed mainly
172 low or intermediate polymorphism at the locus, respectively, based on the PIC
173 classification (PIC < 0.25, low polymorphism; 0.26 < PIC value < 0.5, intermediate
174 polymorphism; and PIC value > 0.5, high polymorphism).

175 **Correlation Analysis of the *KRT38* Gene for Heat Tolerance**

176 The correlation analysis results between the genotype g.41650738 A > G and three
177 environmental parameters (T, RH and THI) were obtained from sample regions and
178 genotypes of 246 Chinese cattle breeds. Table 1 presents the findings of this analysis.
179 The genotypes AA and GG differed significantly ($P < 0.01$) from each other. Our data
180 suggested that more individuals with the GG genotype than the AA genotype were
181 present in hot, humid areas, indicating a relationship between allele G and heat
182 tolerance in Chinese cattle. Based on the subject impact test on the *KRT38* genotypes
183 (Table S4), RH and genotypes were found to be significantly related.

184 **Mutation Analysis**

185 Mammals possess complete coats that endure throughout their lifetimes and exhibit
186 the ability to regulate their body temperature. Animals carrying the I17T mutation
187 demonstrate enhanced heat tolerance compared to non-mutated animals, potentially
188 attributable to functional modifications in *KRT38* caused by sequence variations. The
189 alteration at Ile17 in the *KRT38* gene observed in cattle is identical to that found in
190 zebu, yak, sheep and goats. Consequently, we proceeded to compare the protein
191 sequences of KRT38 with those of other species (Fig. 3A). Our evolutionary analysis
192 indicates that the I17T variation, identified in cattle, is rare among other mammalian
193 species. Furthermore, we investigated the genetic pattern of this substitution (c.50 A >
194 G, p.I17T) in cattle genomes from various geographical regions and observed a higher
195 frequency of the mutation (c.50 A > G) in cattle residing in hot and warm climates,
196 which aligns with the trend observed in China (Fig. 3B). The bovine KRT38 protein
197 sequence was used to predict the protein three-dimensional structure by the

198 homologous modelling method with the Amino Acid Explore website
199 (www.ncbi.nlm.nih.gov). The missense mutation at g.41650738 A > G leads to a
200 change in the amino acid encoding from isoleucine to threonine, which may lead to a
201 change in protein properties (Fig. 4). Thus, we hypothesized that the substitution of
202 residue 17 in the *KRT38* gene is probably related to heat tolerance, and this locus is
203 likely to play a role in heat tolerance.

204 **Discussion**

205 Due to climate change and the imbalance between heat production and dissipation in
206 some animals, the challenging tropical production environment could have
207 determined more favorable bovine genotypes (Wolfenson and Roth 2019). Hair on
208 mammalian skin serves multiple functions in thermogenesis, encompassing
209 thermoregulation, sensory perception, and environmental defense (Blanpain and
210 Fuchs 2009). A slick coat can be considered an indicative or indirect phenotype of
211 various significant production qualities in tropical regions, as it is consistently
212 associated with enhanced thermotolerance and higher milk yield in crossbreeds
213 grazing under such conditions. This trait is regarded as a potential marker for the
214 aforementioned production traits in tropical settings (Carabano *et al.* 2017). The
215 performance of livestock under heat stress needs to be improved, and since thermal
216 stress also affects animal productivity and genetic diversity among cow breeds
217 negatively, it is essential to examine new methods and strategies. However, most
218 economically important traits are quantitative by nature, meaning they are under the
219 control of hundreds or thousands of genes (Meuwissen *et al.* 2001), with very few
220 major genes having been identified as having an impact on these types of traits. In
221 those cases, a genomic selection approach is the best method of genetic improvement.
222 Identifying causal mutations for economically important traits would be beneficial for
223 improving genomic selection, and hence, Meuwissen *et al.* (2022) have recently
224 suggested new approaches to identify likely causal variants for complex traits by
225 compensating for the very small effects with significantly larger sample sizes.

226 During the development of the epidermis, keratins play a crucial role as the
227 predominant structural constituents (Wang *et al.* 2007). While a definitive correlation
228 between the *KRT38* gene and heat stress effects in cattle is yet to be established, the
229 site analysis and comparison of three environmental parameters (A and G) conducted
230 in this study may imply a potential association between the mutation site of the
231 *KRT38* gene and the heat tolerance of Chinese cattle. This observation aligns with the
232 geographical distribution of hot and humid climates across China (Supplementary
233 Table S2). The frequency of mutations at this SNP is higher in Indian cattle
234 bloodlines than in Chinese taurine cattle bloodlines, according to the results of the
235 association study. In Fig. 1, regions with more intense heat stress or greater light
236 intensity had higher percentages of individuals with the GG or AG genotype, and
237 these regions are also where the majority of the indigenous cattle with Indian cattle
238 blood can be found. We speculate that this SNP is related to heat stress as the

239 mutation site shows a correlation with temperature and humidity.

240 *Bos taurus* cattle are mainly found in northern China, while *Bos indicus* cattle are
241 mainly found in southern China. *Bos taurus* and *Bos indicus* are both prevalent in
242 central Chinese cattle due to trade and population mobility (Cai *et al.* 2007; Chen *et al.*
243 2018). While northern cattle breeds are often cold-tolerant, southern cattle breeds are
244 typically resistant to thermal stress, as a prior study revealed. (Yudin *et al.* 2021). The
245 examination of genotypic and allelic frequencies for the SNP (NC_037346.1
246 g.41650738 A > G) revealed that there was a significant difference in the regional
247 distribution of the *KRT38* gene variation among the native Chinese cow breeds. In
248 contrast to the A allele, which had a different pattern and corresponded with the
249 distribution of indicine and taurine cattle in China, the frequency of the mutant G
250 allele increased progressively from the southern region to northern China. This
251 indicated how southern and northern breeds were affected by the introduction of the
252 heat-resistant *Bos indicus* and the heat-sensitive *Bos taurus*, respectively. The unique
253 geographical features of the Qinghai-Tibet Plateau have given rise to very distinctive
254 breeds of cattle. In our study, we classified Tibetan cattle and Rikaze cattle from the
255 Tibet region as special groups. Fig 2 shows that Tibetan cattle share most of their
256 haplotypes with northern cattle. Interestingly, we found that the mutation G in Rikaze
257 is consistent with the pattern of *Bos indicus*, which is consistent with previous
258 research results that the Shigatse Humped have a pedigree of *Bos indicus* (Xia *et al.*
259 2019). The haplotype distribution of the special group indicates that the Qinghai-Tibet
260 Plateau region has haplotype types of both *Bos taurus* and *Bos indicus*.

261 The GG genotype was more frequent in areas with higher T, RH and THI, as
262 shown by the correlation between the novel SNP (NC_037346.1 g.41650738 A > G)
263 and the three environmental parameters (T, RH, and THI). The GG genotype may be
264 favored by the hot and humid climate in southern China, while the north may favor
265 the AA genotype. Our research indicates that variations in bovine *KRT38* may have an
266 effect on temperature tolerance. However, further testing is needed to testing different
267 genotypes of animals and observe their physiological performance and response.

268 Furthermore, various studies have supported the notion that marked introgression
269 between the bovine species yak, gayal, gaur, and banteng may help in adaptation to
270 local conditions (Chen *et al.* 2018; Wu *et al.* 2018). China has been identified as a
271 region where interbreeding between *Bos taurus* and *Bos indicus* has occurred,
272 particularly in the central plains (Yue *et al.* 2014). In our research, the *KRT38* gene
273 mutation stands as a unique exemplification of an amino acid residue convergent
274 alteration that is shared by at least of 5 animal species. Thus, it is speculated that the
275 cattle breeds in the central area carry allele G due to the occurrence of genetic mixing.
276 The *KRT38* variant was the only cattle-specific missense mutation that was not
277 observed in other closely related species or the Bovinae species in our study. We
278 hypothesize that this genetic variation is exclusive to cattle. Our phylogenetic analysis
279 reveals that the I17T mutation discovered in cattle is infrequent in northern China but
280 has a higher likelihood of occurrence there. We propose that the *KRT38* variation in

281 cattle potentially exerts an influence on their skin pigmentation, hair length, and
282 thickness, thus playing a pivotal role in enhancing their capacity to adapt to extreme
283 ambient temperatures.

284 **Conclusions**

285 Our findings have indicated that mutations within the *KRT38* gene have expanded the
286 characterisation of genetic variations and their potential may be association with heat
287 tolerance in indigenous Chinese cattle. However, it is important to note that the
288 majority of adaptive traits related to environmental conditions are quantitative in
289 nature and exhibit limited heritability. Furthermore, environmental adaptation is
290 influenced by a multitude of genes. Therefore, it is crucial to continue identifying
291 significant genes or loci that can enhance cattle productivity. These findings
292 significantly contribute to our evolving comprehension of the adaptive genetic
293 differences observed in cattle and other livestock species inhabiting diverse climatic
294 regions.
295

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411 indigenous cattle. *BMC Genet* **19**, 114.
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414 **Table 1.** Least squares mean and standard error for the temperature (T), relative
415 humidity (RH) and temperature–humidity index (THI) of different genotypes of
416 the single-nucleotide polymorphism (SNP) rs210366642 (NC_037346.1
417 g.41650738 A > G) in the *KRT38* gene.

418

SNP	Genotype (n)	Temperature (°C) (LSM ± SE)	Relative Humidity (%) (LSM ± SE)	Temperature–Humidity Index (LSM ± SE)
rs210366642: (g.41650738 A > G)	AA (116)	10.156±0.501 ^A	62.095 ^A ±1.353 ^A	52.492 ^A ±0.717 ^A
	AG (79)	13.087±0.710 ^B	70.481 ^B ±1.119 ^B	56.513±1.053 ^B
	GG (51)	13.230 ^B ±0.877 ^B	71.059 ^B ±1.255 ^B	56.689±1.267 ^B

419 LSM ± SE, the least square means with standard errors for diverse genotypes and
420 environmental parameters. Means in the same column and locus with difference
421 capital superscripts, A and B, are different at $P < 0.01$.

422

423

424

425 **Fig. 1.** Identification of rs210366642 (NC_037346.1 g.41650738 A > G) mutations in
426 the *KRT38* gene of bovines.

427

428 **Fig. 2.** Geographical distribution of the A and G alleles of the locus rs210366642
429 (NC_037346.1 g.41650738 A > G) of the keratin 38 (*KRT38*) gene among 15 cattle
430 breeds in China. Circled areas are proportional to the sample size. Blue and purple
431 represent genotypes A and G, respectively. BH, Bohai Black; JX, Jiaxian red; LX,
432 Luxi; MG, Mongolian; QC, Qinchuan; XZ, Tibetan; WN, Wannan; WS, Wenshan;
433 YB, Yanbian; YL, Yunling; CDM, Chaidamu; HN, Hainan; WZ, Weizhou; RKZ,
434 Rikaze; KZ, Kazakh.

435

436 **Fig. 3.** Genetic polymorphism of *KRT38* across five mammals.

437 A: Graph depicting the bovine *KRT38* gene structure, showing the location of
438 rs210366642 (NC_037346.1 g.41650738. A > G) mutation. Local alignment of exon 1
439 of the *KRT38* protein showing the p. I17T mutation and adjacent amino acids in the
440 five mammals. B: Genetic pattern of *KRT38* rs210366642 (NC_037346.1 g.41650738
441 A > G) in cattle genomes worldwide.

442 **Fig. 4.** The tertiary structure prediction of *KRT38* protein. A: wild type (Isoleucine) B:
443 mutant type (Threonine).

444

445 **Data availability.** Data availability is not applicable to this article, as no new data
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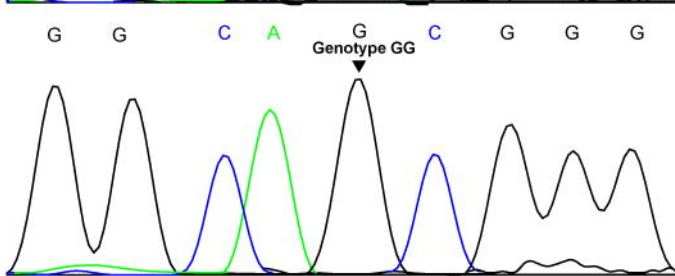
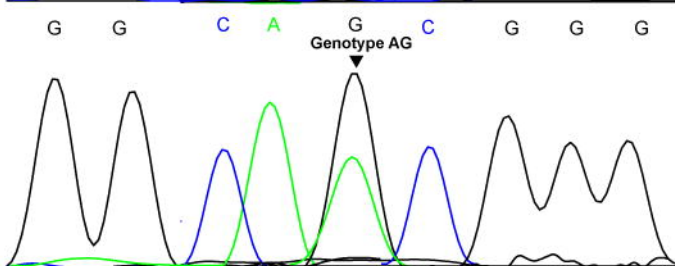
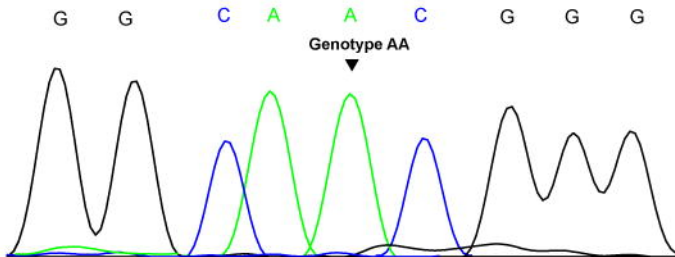
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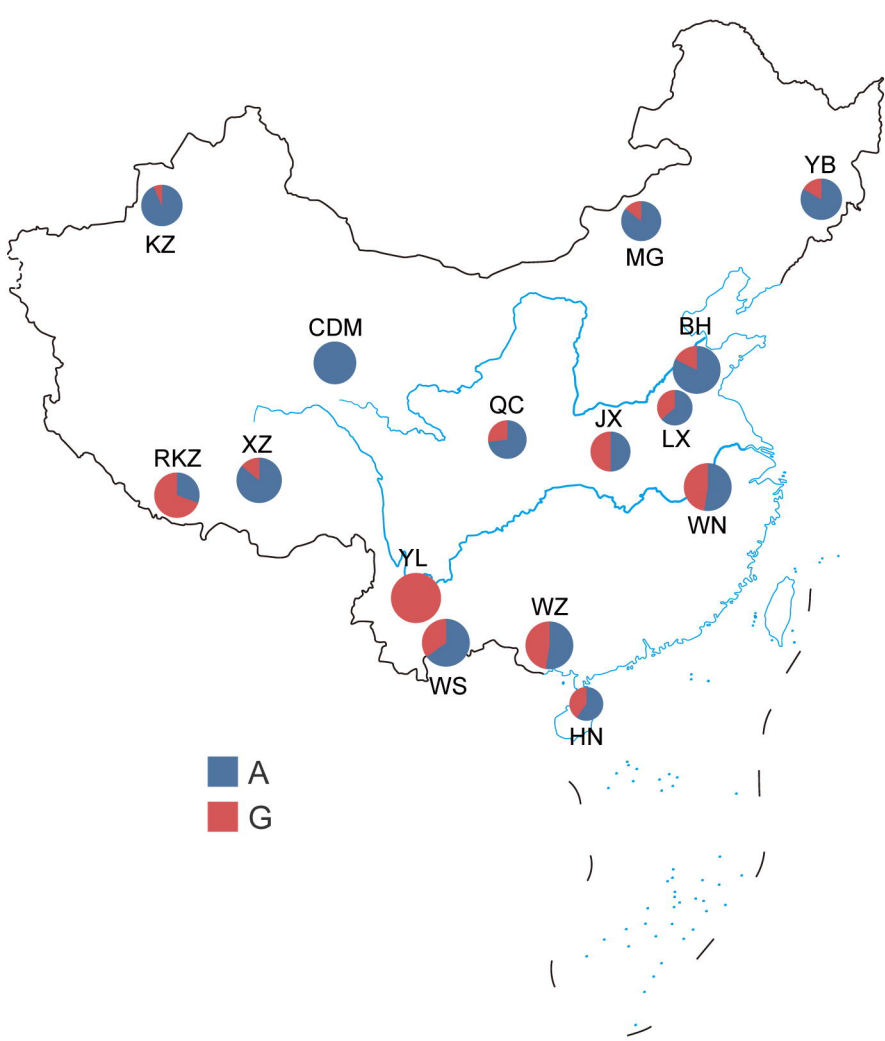
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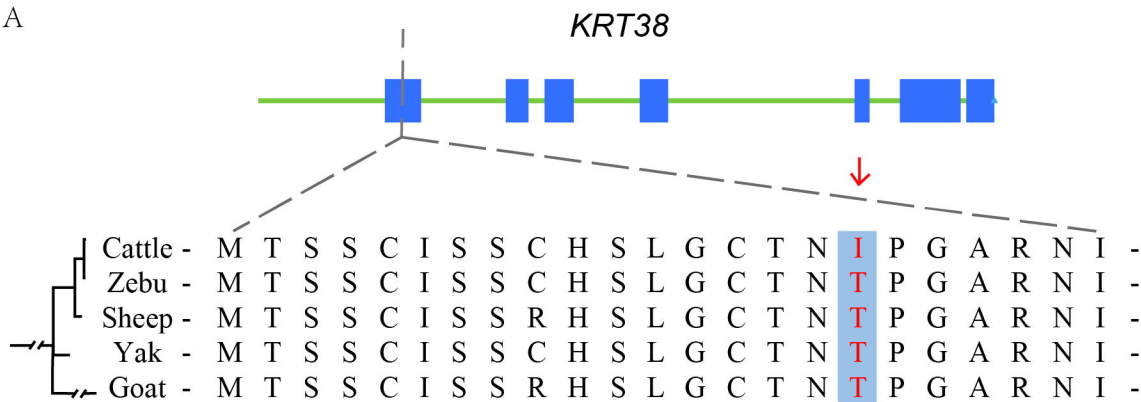
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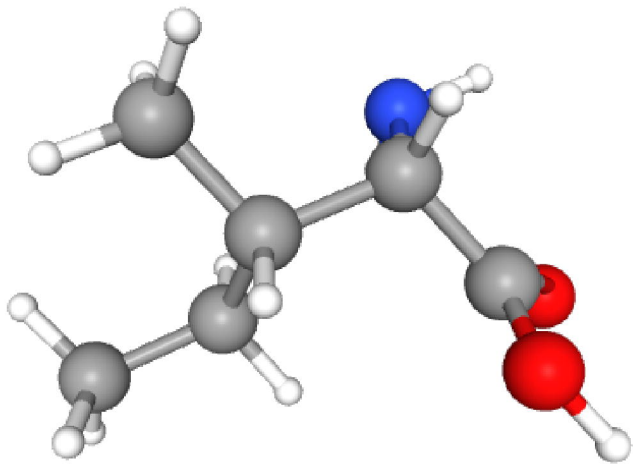
A



B

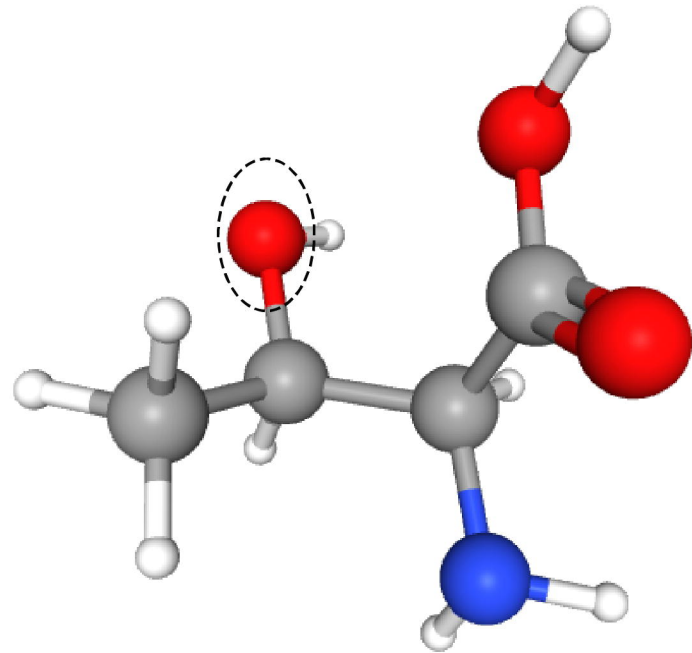


A



L-Isoleucine

B



T-Threonine