Exercise-related genes analysis of Mongolian Horse

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Exercise-related genes analysis of Mongolian Horse - Abaga horse and Wushen horse

Keywords: genome, Mongolian horse, Abaga horse, Wushen horse, exercise

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ABSTRACT

The Mongolian horses, as a neglected scientific resource, have excellent endurance and stress resistance to adapt to the cold and harsh plateau conditions. Intraspecific genetic diversity is mainly embodied in various genetic advantages of different branches of Mongolian horse. Abaga horse is better than Wushen horse in running speed, for example. Because people pay progressively attention to the athletic performance of horse, such as horse racing in Mongolia's Naadam festival, we expect to guide the exercise-oriented breeding of horses through genomics research. We obtained the clean data of 630,535,376,400 bp through the entire genome second-generation sequencing for the whole blood of 4 Abaga horses and 10 Wushen horses. Based on the data analysis of single nucleotide polymorphism (SNP), we severally detected that 479 and 943 positively selected genes, particularly exercise-related, were mainly enriched on equine chromosome 4 in Abaga horses and Wushen horses, which implied that the chromosome 4 may be associated with the evolution of the Mongolian horse and athletic performance. Four hundred and forty genes of positive selection were enriched in 12 exercise-related pathways and narrowed in 21 exercise-related genes in Abaga horse, which were distinguished from Wushen horse. So, we speculated that the Abaga horse may have oriented genes for the motorial mechanism and 21 exercise-related genes also provided molecular genetic basis for exercise-directed breeding of Mongolian horse.

INTRODUCTION

As an ancient breed, Mongolian horse has gone through a long breeding period (China

National Commission of Animal Genetic Resources 2011). With a view to research tendentiousness, researchers pay more attention to traits of Thoroughbred horse (Gim *et al.* 2004; Park *et al.* 2012; Capomaccio *et al.* 2013) and Quarter horse (Doan *et al.* 2012; Meira *et al.* 2014), but not the Mongolian horse and its diverse sub-branch. The preeminent endurance and stress-resistance of Mongolia horse are important factors for it to well adapt to the cold and harsh plateau environment (Li *et al.* 2009). Natural factors may have enormous impacts on evolution owing to the rough domestication of Mongolian horse (Hund 2008). Due to various geographic conditions and human necessities, Mongolian horse gradually formed several specific traits. Some horses which adapt to the desert climate have larger feet, for instance, some horses which adjust to mountain road with rocks have supple body and hard hoof; in addition, the features of horse which accommodate to the grassland climate are tall physique and good at running (Elisabeth 2011). Living in the Xilin Gol grassland of Inner Mongolia, Abaga horse belongs to the steppe horse and speeds up to 1600 meters every 91.47 seconds (China National Commission of Animal Genetic Resources 2011). Wushen horse, which is small build and has broad-flat horseshoe, as symbol of the desert horse in the south of Maowusu desert of Ordos City in Inner Mongolia, can hoof steadily in the desert, albeit noffast running speed of 13 to 15 kilometers per hour (Dugarjaviin 2009).

In Mongolia, the herdsmen depend on horses by reason that they are the indispensable sources of pastoral rations, such as meat and dairy products, and used to be one of the means of transport by herders (Hund 2008). Furthermore, Mongolian

71 horse was an essential and distinguished war-horse in history (Article: The Horse in
72 Mongolian Culture 2018). For the Naadam of traditional festivals in Mongolia, horse
73 racing is one of the entertaining activities for herds and regarded as the second most
74 popular sporting events after wrestling (Davis 2010). So, the running speed of
75 Mongolian horses has been one of the focuses of attention. Despite not the fastest
76 horse in the world, people still endeavor to improve running speed of the Mongolian
77 horse through unremitting consideration and breeding.

78 In order to discuss the genetic variation between Abaga horse and Wushen horse
79 in Mongolian horse strains, we planned to analyze data of the entire genome with
80 second-generation sequencing technology to seek out exercise-related single
81 nucleotide polymorphism (SNP) locus of Mongolian horse and offer a reference for
82 identification and improvement of Mongolian horse varieties.

83 **MATERIALS AND METHODS**

84 **Experimental animals and sample preparation**

85 We collected jugular blood of 4 Abaga horses in the Inner Mongolia Abaga County
86 and 10 Wushen horses in the Inner Mongolia Ordos City Wushen County. Adhering to
87 the manufacturer's instructions for the extraction of DNA from the whole blood,
88 genome was extracted by the AxyPrep blood genomic DNA kit. Then we used the
89 NanoDrop ™ 1000 spectrophotometer and polyacrylamide gel electrophoresis to
90 detect the concentration and integrity of the genome. The concentration of extracted
91 DNA was between 26.4 ng/μL and 34.4 ng/μL for subsequent library construction.

92 **Library preparation and whole-genome sequencing**

A TruSeq DNA Sample Prep Kit was used to construct a sequencing library. Whole-genome sequencing of the horses was performed using the Illumina HiSeq X ™ Ten Sequencing System.

Data quality control and comparison to reference genome

The raw data of 639,723,611,100 bp was sequenced from 14 samples. The inferior quality reads which have sequencing adapter, higher than 10% of N (base of uncertainty) content or inferior mass base (Q≤5) content of higher than 50% were filtered out by in-house Perl/Python scripts to achieve clean data of 630,535,376,400 bp. The Q20, Q30, error rate, GC content and other information of these data were counted by in-house Perl/Python scripts (Table 1). The sequencing reads were mapped to reference genomes (Ensembl release 82) by BWAmem (bwa-0.7.8) (Li 2013), which PCR and optical repetition of results were removed by using Picard (Broad Institute 2018). Statistics of mapping rate, average depth and coverage of the data after comparison were computed by in-house Perl/Python scripts (Table 2).

Single nucleotide polymorphism calling and annotation

SNP calling was performed using the GATK HaplotypeCaller (v3.5) (McKenna *et al.* 2010). In order to evaluate the reliability of the detected SNP sites and filter inferior quality SNP, we used SAMTools for SNP detection (Li 2011). Simultaneously, the dbSNP database 5,019,393 SNPs and 670K chip site information were downloaded. The data was used as training set, and the detected SNPs were evaluated and filtered by using the GATK VQSR process. The standard for the retention of the final site is the tranche value of 99 (Ti / Tv=2.02). Finally, the SNPs of equine population were

filtered: GQ> 10, MAF> 0.05, call rate> 0.9 (Figure 1). The variants after filtering were annotated by ANNOVAR (v2016-02-01) (Wang *et al.* 2010) (Table 3).

Selective sweep analysis

To identify potential selective sweeps between Abaga horse (fast) and Wushen horse (slow), Pi log2(slow/fast) and FST was calculated together using VCFtools with a 20kb sliding window and a step size of 10 kb. Windows that contained less than 10 SNPs were excluded from further analysis. The windows that were simultaneously 1) in the top 5% of Z-transformed FST values and 2) in the bottom 5% Pi log2(slow/fast) were considered to be candidate selective regions in Abaga horse (Figure 2A; Table S1 https://doi.org/10.6084/m9.figshare.6289523.v1). The same applies to Wushen horse (Figure 2B; Table S2 https://doi.org/10.6084/m9.figshare.6289532.v1).

Statistic and advanced analysis of positively selected and candidate genes

We annotated the positively selected genes via GO (GOseq) to further screen out the major enriched functions (Young *et al.* 2010). The pathways which included these selected genes were enriched by KEGG (KOBAS) (Xie *et al.* 2011). Many positively selected genes which are in Abaga horse were analyzed further without overlapped genes between Abaga horse and Wushen horse.

RESULTS AND DISCUSSION

Related-clean data stated

We performed the entire genome second-generation sequencing for the whole blood of four Abaga horses (Figure 3A) and ten Wushen horses (Figure 3B) with the Illumina HiSeqX ten sequencing platform. The clean data of 630,535,376,400 bp

(effective rate of data: 98.56%, error rate of data: 0.03%, mean of Q20: 94.95% and mean of Q30: 89.80%) was sequenced by filtration and 41.95G as the mean of clean data was generated in each sample (Table 1). Then, the data was mapped to the reference genome (Ensembl release 82) via BWAmem (Li 2013). Without PCR and optical repetition, the successful mapping rate of data was 98.36%. For 14 samples, the average sequencing depth was 16.75× coverage and average cover degree was 99.55% on reference sequences (Table 2).

Distribution of positive selection genes on chromosomes

Based on the data of SNP following SNP calling (Figure 1), we obtained the genes of significantly genetic differences by using F-statistics between Abaga horses and Wushen horses, and narrowed the above genes down to 479 and 943 positively selected genes combined with SNP polymorphism analysis in Abaga horses and Wushen horses, respectively (Figure 2A, B; Table S1, S2). We discovered that these selected genes were mainly distributed on chromosome 4, 7 and 10 in Abaga horses, and chromosome 1, 4, 8 and 16 in Wushen horses with the analysis of genes distribution on chromosome (Figure 4).

Many genes of the positively selected 479 and 943 genes were enriched on chromosome 4, and the enrichment quantity of positively selected genes was secondary by the chromosome enrichment analysis both in Abaga horse and Wushen horse. In the statement of Schröder (Schröder *et al.* 2011), athletic performance-related genes were significantly enriched on chromosomes 4 and 12 of horses, which coincided with the different traits of running speed in our exploring

direction. Possibly, we will take equine chromosome 4 as the exercise-related emphasis of scientific research.

Gene Ontology, KEGG pathways and exercise-related genes

Above these positively selected genes were functional annotation by Gene Ontology (GO). The selected 479 genes of Abaga horse were mainly enriched in neuron part (GO:0097458), neuron projection (GO:0043005), regulation of membrane potential (GO:0045838), positive regulation of cell projection organization (GO:0031346), neuron-neuron synaptic transmission (GO:0007270), synaptic transmission, glutamatergic (GO:0035249), neurotransmitter secretion (GO:0007269), antigen processing and presentation (GO:0019882), telencephalon cell migration (GO:0022029) and forebrain cell migration (GO:0021885). The selected 943 genes of Wushen horse were mainly enriched in membrane part (GO:0044425), intrinsic component of membrane (GO:0031224), integral component of membrane (GO:0016021), cell projection (GO:0042995), neuron part (GO:0097458), neuron projection (GO:0043005), synapse (GO:0045202), cilium (GO:0005929) and cell projection assembly (GO:0030031) (Figure 5A, B).

The athletic ability of the horse may be influenced not only by physiology, but also thought and motive. According to the previous studies, equine exercise-related genes included *DRD1-5*, *SLC6A4* and *BDNF*, the three genes functions were related to many neurological processes, involving motivation, pleasure, cognition, memory, learning, fine motor control, modulation of neuroendocrine signaling, adaptive ability of controlling emotions, supporting the survival of existing neurons, encouraging the

growth and differentiation of new neurons and synapses (Momozawa *et al.* 2005; Bryan *et al.* 2007; Kulikova *et al.* 2007; Lippi *et al.* 2010). The GO analysis results of Abaga horse were also preferentially enriched in neuronal composition, neurotransmission and brain cell migration. These genes may allow Abaga horse to quickly observe and distinguish the surrounding during moving with high-speed, timely rectify status to respond to the various circumstances.

By pathways enrichment analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) with the positively selected 479 genes in Abaga horse and 943 genes in Wushen horse, the enriched pathways ($P \leq 0.05$) of Abaga horse included Propanoate metabolism, Viral myocarditis, Phototransduction, PI3K-Akt signaling pathway, Glycerolipid metabolism, Morphine addiction and mRNA surveillance pathway in which the pathway with the largest number of enriched genes (13 genes) was PI3K-Akt signaling pathway. As intracellular basal signaling pathways, PI3K-Akt signaling pathway involves lots of vital movement, such as exercise-induced physiologic hypertrophy (Shioi *et al.* 2002; Luo *et al.* 2005; Sagara *et al.* 2012; Song *et al.* 2015) and protecting mitochondria of skeletal muscle by aerobic endurance training (Liu *et al.* 2016), further explaining the excellent athletic performance of Abaga horse. Besides that, the enriched pathways ($P \leq 0.05$) of Wushen horse contained Base excision repair, Glutamatergic synapse, Endometrial cancer, Glycolysis / Gluconeogenesis, Propanoate metabolism and ABC transporters (Figure 6A, B).

Further on SNPs, we analyzed functions of 440 genes of Abaga horse without 39

overlapped genes of positively selected genes between Abaga horse and Wushen horse. We focused on the enriched exercise-related pathways which referred to Metabolic pathways (Hill *et al.* 2010), Ras signaling pathway (Shioi *et al.* 2002; Xie *et al.* 2007), PI3K-Akt signaling pathway (Shioi *et al.* 2002; Luo *et al.* 2005; Sagara *et al.* 2012; Standard *et al.* 2014; Song *et al.* 2015; Liu *et al.* 2016; Vega *et al.* 2017), MAPK signaling pathway (Schröder *et al.* 2011), Hippo signaling pathway (Gabriel *et al.* 2016), Valine, leucine and isoleucine degradation (McGivney *et al.* 2010), Cardiac muscle contraction (Do *et al.* 2015), NF-kappa B signaling pathway (Kramer and Goodyear 2007), Arachidonic acid metabolism (Hill *et al.* 2010), Regulation of actin cytoskeleton (Hill *et al.* 2010; Schröder *et al.* 2011), Insulin signaling pathway (Gim *et al.* 2004; Hill *et al.* 2010) and Fatty acid metabolism (Gim *et al.* 2004; Hill *et al.* 2010) in the 440 positively selected genes of Abaga horse that distinguished from Wushen horse (Figure 7). These enriched pathways comprised some recurrent genes (Figure 7). Taking repeated genes as pivots, we speculated that the synergistic effect of pathways enabled faster running speed of Abaga horse compared with Wushen horse. But, in our studying, the enriched genes of positive selection were different from the previous studied genes in the above exercise-related pathways (Table 4), which indicated species-specific genes of positive selection in Abaga horse compared with other species (human, rat, mouse, leopard, thoroughbred horse etc.).

According to the analysis of GO, KEGG and individual gene function, we subsequently put our interest in exercise-related genes of Abaga horse. Twenty-one genes may involve in exercise of Abaga horse while their functions embodied

vasoconstriction (*HTR2B*) (Launay *et al.* 2002; Bevilacqua *et al.* 2010; Meira *et al.* 2014), angiogenesis (*CDH5*) (Sauteur *et al.* 2014), cardiac contraction (*KCNQ1*) (Jespersen *et al.* 2005; Brown *et al.* 2015; Pedersen *et al.* 2017), cardiac development and muscle structure (*ENAH*) (Franzini-Armstrong 1973; Benz *et al.* 2013), muscle growth (*PIH1D1*, *SMURF1*) (Inoue *et al.* 2010; Ponsuksili *et al.* 2014; Dalbo *et al.* 2013), myogenic differentiation (*UNC13C*) (Meyer *et al.* 2015; Langlois and Cowan 2017), skeletal muscle function (*ATP1A3*) (Aughey *et al.* 2007; Brashear *et al.* 2007), femur strength and bone mineral density (*PPP2R5B*, *PPP6R3*) (Alam *et al.* 2009; Medina-Gomez *et al.* 2017), osteoclast growth (*PTPRE*, *RHOBTB1*) (Chiusaroli *et al.* 2004; Song *et al.* 2014), chondrogenesis (SCFD) (DeLise *et al.* 2000; Hou *et al.* 2017), lipid and carbohydrate metabolism (*PPARD*, *GCG*, *TCF7L2*, *GALNT13*) (Yi *et al.* 2005; Bevilacqua *et al.* 2010; Park *et al.* 2012; Ahmetov and Fedotovskaya 2015; Giordano Attianese and Desvergne 2015; Ropka-Molik *et al.* 2017), exercise stress-induced response (*CD69*, *EIF4G3*) (Testi *et al.* 1989; Gradi *et al.* 1998; Cappelli *et al.* 2007; Morabito *et al.* 2016), exercise coordination (*GRM1*) (Conquet *et al.* 1994; Bossi *et al.* 2017) and height (*VGLL4*) (Gabriel *et al.* 2016). These genes of positive selection were presented simultaneously in Abaga horse, which may be a reason that it runs rapider than Wushen horse.

Counting on exercise-related genes of previous studies, the equine athletic performance is related to glucose metabolism, stress immune response, angiogenesis and muscle supply, insulin signal transduction, fat substrate application, muscle strength and the formation of bones and cartilage with growth (Gim *et al.* 2004; Hill

247 *et al.* 2010; Park *et al.* 2012; Capomaccio *et al.* 2013; Kamm *et al.* 2013). We picked
248 up exercise-related genes as candidate genes in positively selected genes, further,
249 presented enriched KEGG pathways and functions with selected exercise-related
250 genes (Figure 7). *HTR2B* (encoding 5-hydroxytryptamine receptor 2B), has been
251 identified in the genome of Quarter horses of the racing line (Meira *et al.* 2014) and
252 associated with impulsive behavior (Bevilacqua *et al.* 2010) and vasoconstriction
253 (Launay *et al.* 2002). In zebrafish, vascular endothelial cadherin (encoded by *CDH5*)
254 can promote elongation of the endothelial cell interface during angiogenesis (Sauteur
255 *et al.* 2014). *KCNQ1* (encoding KvLQT1, a potassium channel protein) is related to
256 exercise, and mutation of *KCNQ1* and *KCNE1* can casus susceptibility of sudden
257 cardiac death (SCD) for horse (Jespersen *et al.* 2005; Brown *et al.* 2015; Pedersen *et*
258 *al.* 2017). Mena (encoded by *ENAH*) which located in Z line that the borders of the
259 sarcomere, VASP, and αII-Spectrin assemble cardiac multi-protein complexes to
260 regulate cytoplasmic actin networks (Franzini-Armstrong 1973; Benz *et al.* 2013).
261 *PIH1D1* (encoding the components of the apoptotic regulatory complex R2TP) is
262 relevant to muscle mass (Inoue *et al.* 2010; Ponsuksili *et al.* 2014). Because E3
263 ubiquitin-protein ligase SMURF1 (encoded by *SMURF1*) function as negative
264 regulators of myostatin pathway activity and myostatin is negative regulator of
265 skeletal muscle mass, up-regulated expression of *SMURF1* may link to skeletal
266 muscle growth following prolonged training (Dalbo *et al.* 2013). *UNC13C* is
267 connected with differentiation of myoblast while integral myotubes originate in
268 myoblast differentiation and raise the distinct muscle fiber types to build the complex

skeletal muscle architecture for body movement, postural behavior and breathing (Meyer *et al.* 2015; Langlois and Cowan 2017). *ATP1A3* encodes subunit alpha-3 of sodium/potassium-transporting ATPase, which increased expression may be conducive to decrease fatigue after training (Brashear *et al.* 2007; Aughey *et al.* 2007). *PPARD* (encoding peroxisome proliferator-activated receptor delta) participate in regulation of energy metabolism, cell proliferation and differentiation, protection in stress conditions such as oxidative stress and inflammation and other important life activities (Giordano Attianese and Desvergne 2015). The antecedent studies have shown that Arabian horse will change the expression of *PPARD* and other genes of PPAR signaling pathway genes in skeletal muscle during exercise, and improve coefficient of utilization of fatty acids by energy conversion (Ropka-Molik *et al.* 2017). The up-regulated *PPARD* are also found after exercise in Thoroughbred horse (Park KD et al. 2012). So, we speculated that positively selected *PPARD* improved athletic ability by a similar mechanism in Abaga horse. Besides counter-regulatory hormone of insulin, *GCG* (encoding glucagon) is deemed to be involved in adipose metabolism and energy balance (Bevilacqua *et al.* 2010). Transcription factor 7-like 2 (encoded by *TCF7L2*) not only affects the metabolism of adipocytes by DNA methylation, but also activates the corresponding target genes through the Wnt signaling pathway to specifically inhibit glucagon synthesis in enteroendocrine cells (Yi *et al.* 2005). *GALNT13* may be involved in metabolic and energy pathways (Ahmetov and Fedotovskaya 2015).

Exercise has a great influence on the composition of the developing horse joints,

291 the thickness of the hyaline cartilage of the adult horse, the calcified cartilage and
292 subchondral bone (van de Lest *et al.* 2002; Tranquille *et al.* 2009). We found several
293 genes associated with skeleton and cartilage development among candidate genes of
294 Abaga horse. *PPP2R5B* and *PPP6R3* are closely related to femur strength in rats and
295 bone mineral density in humans, respectively (Alam *et al.* 2009; Medina-Gomez *et al.*
296 2017). *PTPRE* encodes receptor-type tyrosine-protein phosphatase epsilon which is a
297 positive regulator of osteoclast function (Chiusaroli *et al.* 2004). *RHOBTB1* is
298 involved in osteoclast-mediated bone absorption activity (Song *et al.* 2014).
299 Chondrogenesis demands transformation of chondrocytes from a simple mesenchymal
300 condensation to cells with a highly enriched extracellular matrix (ECM) in the
301 developing skeleton in which *SCFD1* plays an important role in the secretion of ECM
302 protein during chondrogenesis (DeLise *et al.* 2000; Hou *et al.* 2017). So far there are
303 no studies of association between these genes and the motor function of horses, but
304 these skeleton- and cartilage-related genes provide new inspiration into the
305 correlational research between ossature and exercise.

306 After exercise, the equine stress reaction will involve inflammation, cell
307 signaling, and immune interactions (Capomaccio *et al.* 2013). Cell activation is the
308 first step in the proliferation of immune cells, and CD69 is firstly detected in cell
309 surface glycoproteins after activation (Testi *et al.* 1989). The low- to
310 moderate-intensity aerobic trekking induces activation of CD69 T cell and promotes
311 anti-stress effects on the oxidative balance and the high-altitude-induced injury of the
312 immune responses among women (Morabito *et al.* 2016). *EIF4G3* encodes eukaryotic

translation initiation factor 4 gamma 3 which is indispensable for triggering protein synthesis and is thought to be involved in exercise stress-induced response in horses (Gradi *et al.* 1998; Cappelli *et al.* 2007). We hypothesized that these genes may be involved in the ability of Abaga horses to enhance certain diseases resistance through exercise, but more data and experiments are needed to verify.

GRM1 encodes metabotropic glutamate receptor 1, which deficiency can lead to serious deficits of motor coordination and spatial learning in mice (Conquet *et al.* 1994; Bossi *et al.* 2017). The effectors of Hippo signal pathway regulate several motor-related genes and adaptations while *VGLL4* is Hippo-signal-related to body height (Gabriel *et al.* 2016). These exercise-related genes were positively selected in Abaga horse, indicating that Abaga horse has exercise-related genetic potential compared with Wushen horse.

In conclusion, we analyzed the genomic data of Abaga horse and Wushen horse by sequencing. We uncovered that most of the positively selected genes, particularly exercise-related, of Abaga horse and Wushen horse were concentrated on chromosome 4, which implied that the chromosome 4 may be associated with the evolution of the Mongolian horse, and athletic performance may be the future research direction. The positively selected genes of Abaga horse were enriched in exercise-related pathways that were different from some selected genes of other horses or species, suggesting that the Abaga horse may have exclusively physiological mechanism for the motorial process. Twenty-one exercise-related genes were detected, which provided molecular genetic basis for further research on athletic performance

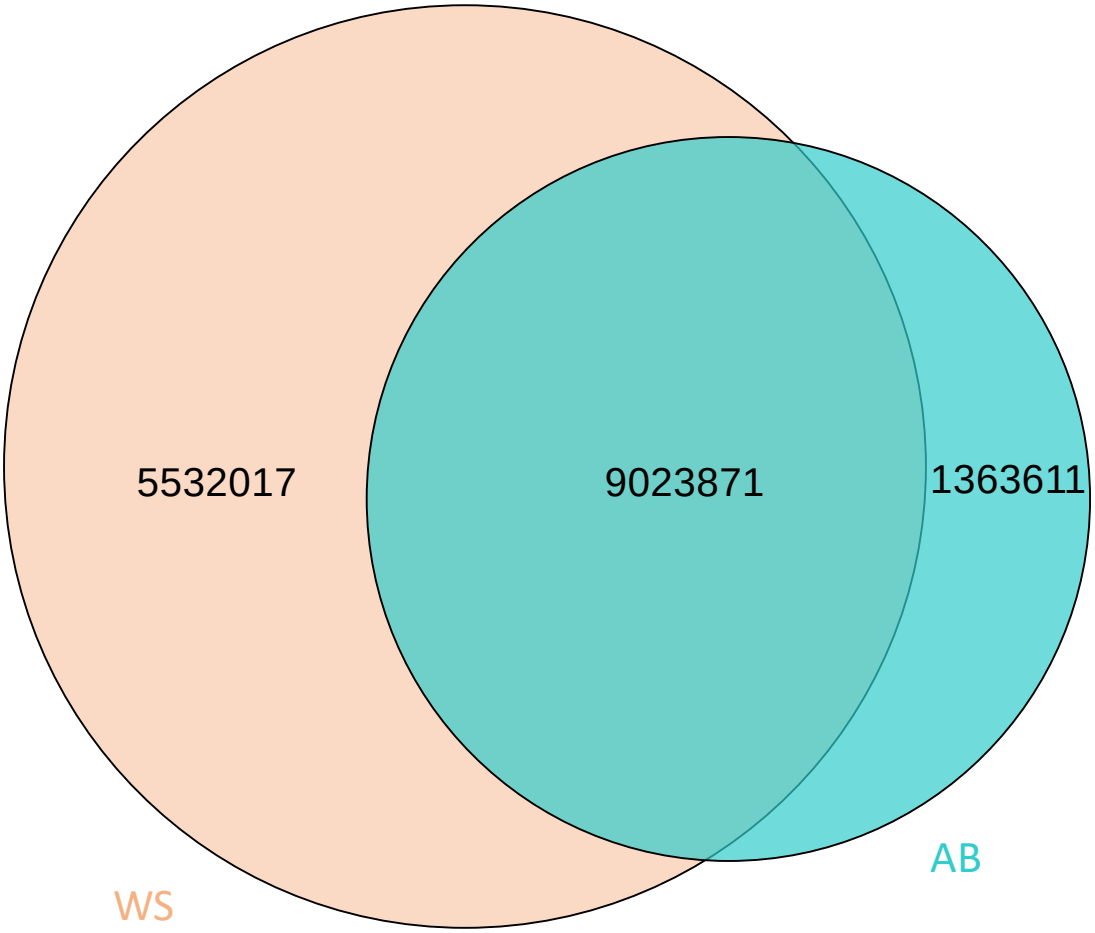
335 and breeding of Mongolian horse.

336 **ACKNOWLEDGMENTS**

337 We thank Y.R. Zhang, K.F. Wu, S.Y. Wang, Z.Z. Liu, W. Wei, P.W. Shi and C.H. Cao

338 for sampling assistance.

339 **FIGURE LEGENDS**



340

341 **Figure 1** SNP following SNP calling. High quality SNPs were evaluated and indentified. AB, WS

342 indicated Abaga horse and Wushen horse, respectively.

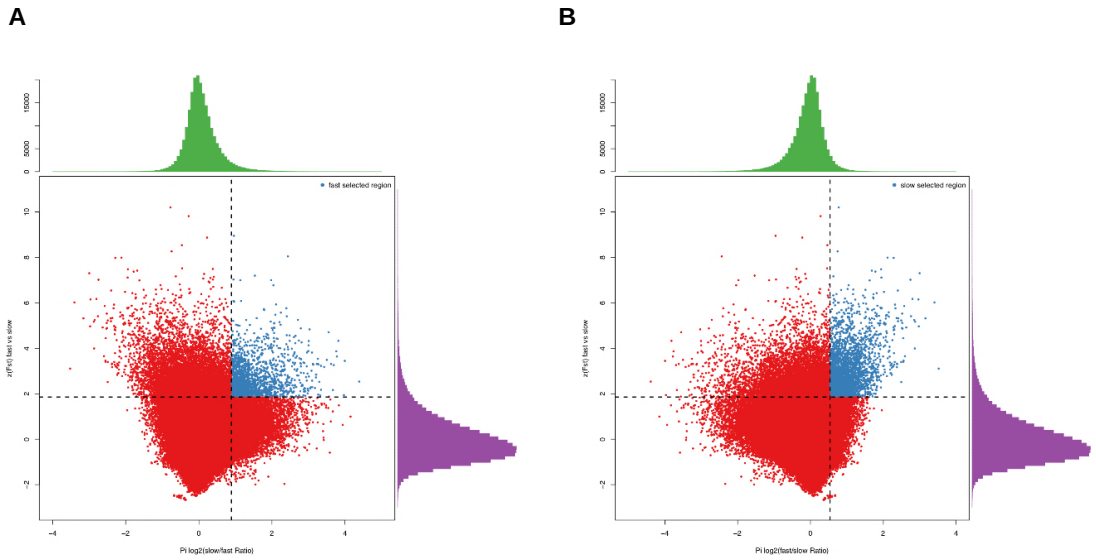


Figure 2 Identification of selected regions in Abaga horse and Wushen horse. To identify potential selective sweeps between Abaga horse (fast) and Wushen horse (slow), log2(πslow/πfast) and FST was calculated together using VCFtools with a 20kb sliding window and a step size of 10 kb. Windows that contained less than 10 SNPs were excluded from further analysis. The windows that were simultaneously 1) in the top 5% of Z-transformed FST values and 2) in the bottom 5% log2(πfast/πslow) were considered to be candidate selective regions in (A) Abaga horse and (B) Wushen horse.

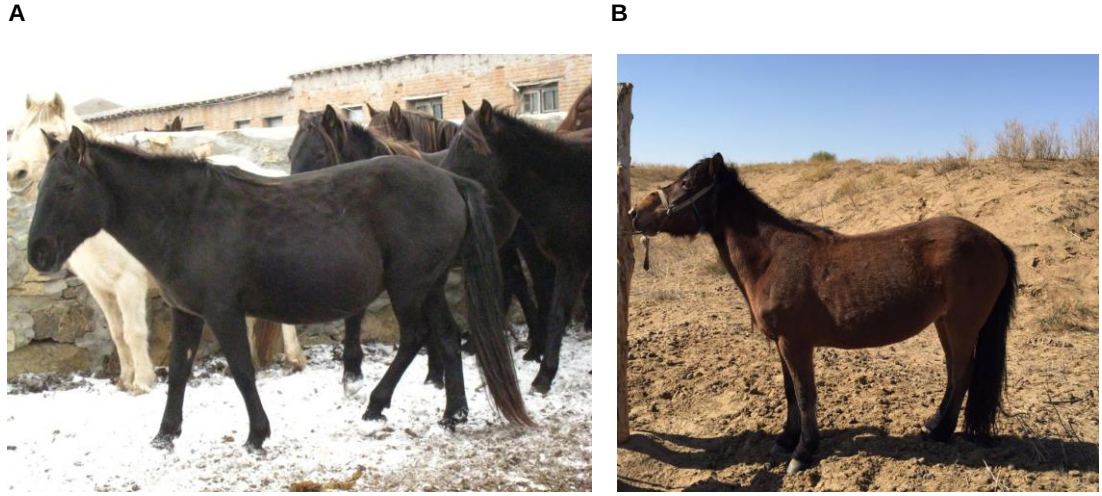
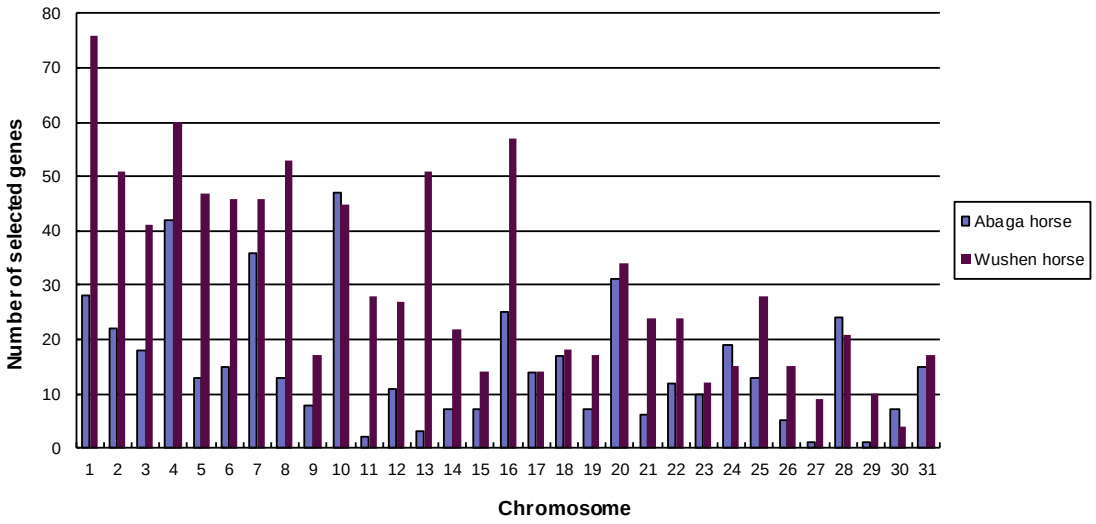


Figure 3 Actual Photos of Mongolian horses, (A) Abaga horse and (B) Wushen horse.



353

354 **Figure 4** Distribution of selected genes on chromosomes.

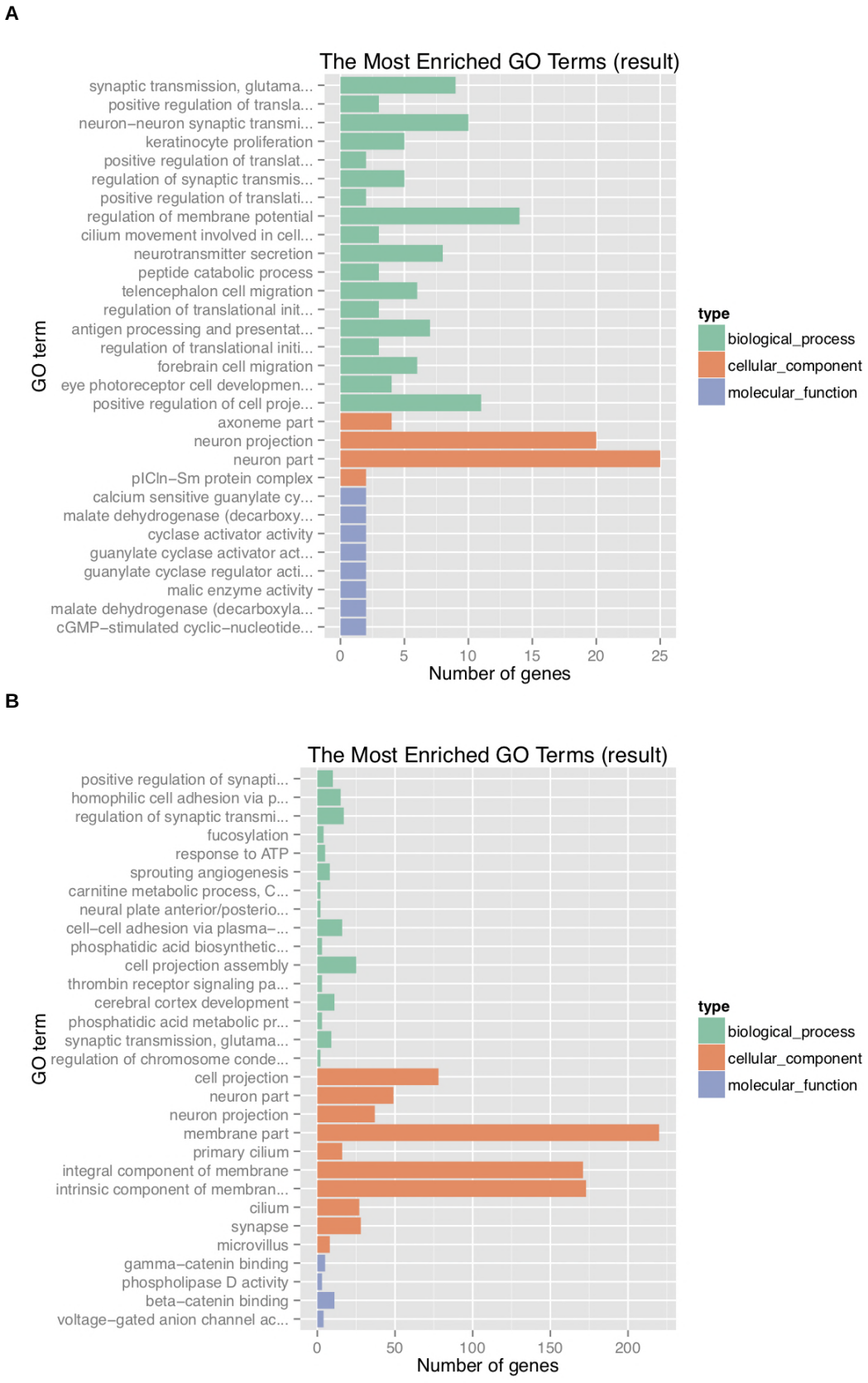
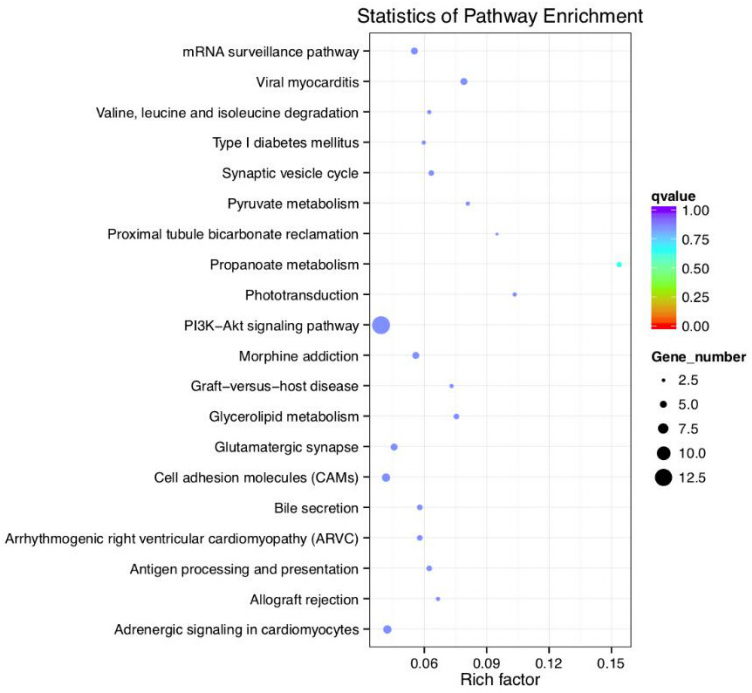


Figure 5 Function analysis based on Gene Ontology. The most enriched GO terms in (A) Abaga horse and (B) Wushen horse.

A



B

Figure 6 The KEGG pathway enrichment analysis. Top 20 of enriched pathways by statistics in (A) Abaga horse and (B) Wushen horse.

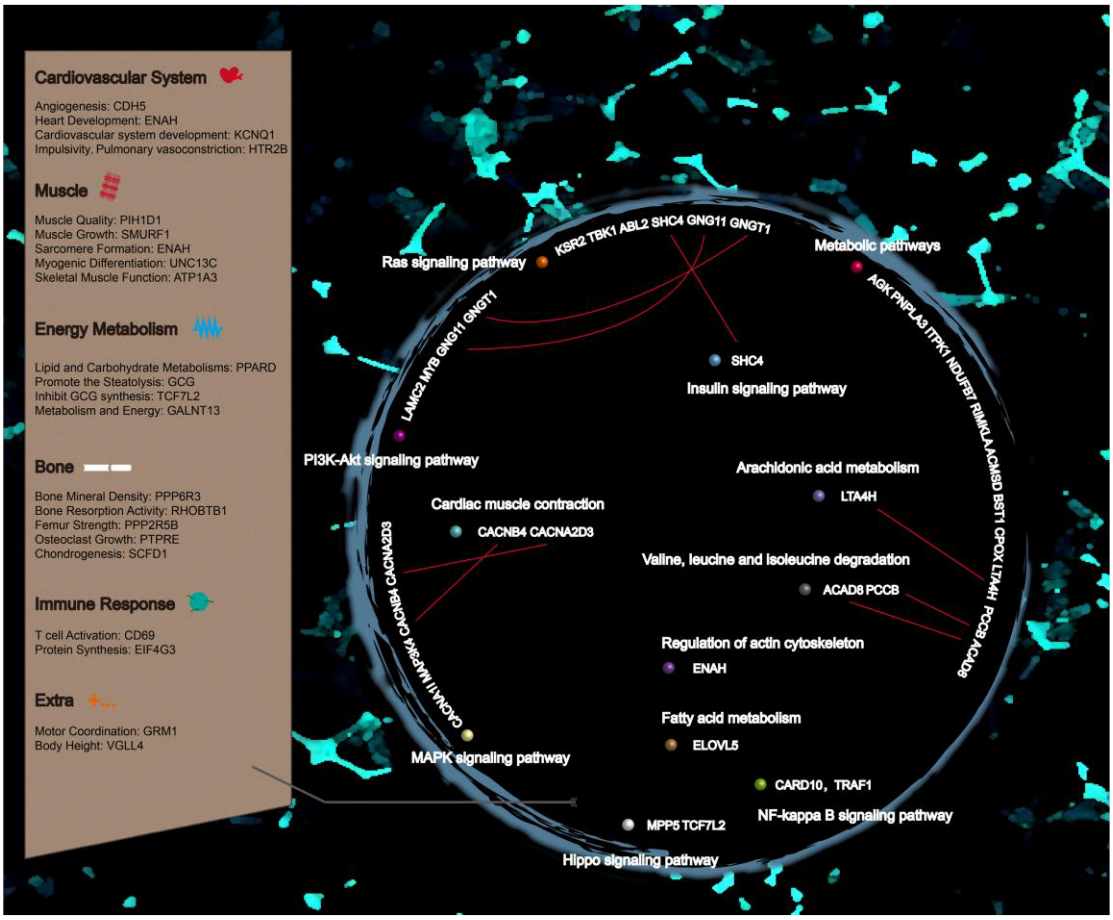


Figure 7 The exercise-related candidate genes and pathways of Abaga horse.

Tables

Table 1 Data volume and quality

Sample	Sample Type	RawData	CleanData	EffectiveRat	ErrorRate	Q20	Q30	GC Content
AB01	Abaga Horse	48,216,007,500	47,152,467,900	97.79	0.03	96.21	92.24	42.89
AB02	Abaga Horse	43,047,445,800	42,340,444,800	98.36	0.03	96.17	92.1	42.65
AB03	Abaga Horse	47,273,694,300	46,714,855,800	98.82	0.03	95.76	91.36	42.55
AB04	Abaga Horse	48,416,284,200	47,906,212,800	98.95	0.03	95.96	91.47	42.13
WS01	Wushen Horse	49,415,626,200	48,784,894,800	98.72	0.03	95.69	91.23	41.97
WS02	Wushen Horse	53,074,008,900	52,417,962,000	98.76	0.03	96.09	92.19	42.35
WS03	Wushen Horse	45,358,642,200	44,789,835,900	98.75	0.03	95.87	91.47	41.97
WS04	Wushen Horse	47,681,665,800	47,139,254,400	98.86	0.03	95.94	91.6	41.66
WS05	Wushen Horse	50,647,742,700	50,012,300,700	98.75	0.03	95.86	91.47	41.84
WS06	Wushen Horse	45,072,226,800	44,633,740,200	99.03	0.03	95.79	91.61	42.19
WS07	Wushen Horse	34,681,049,100	34,040,596,500	98.15	0.05	92.2	84.65	43.07
WS08	Wushen Horse	45,212,322,600	44,350,535,700	98.09	0.04	92.41	84.97	43.02
WS09	Wushen Horse	44,382,563,100	43,593,247,800	98.22	0.04	92.47	85.07	43.23
WS10	Wushen Horse	37,244,331,900	36,659,027,100	98.43	0.04	92.91	85.77	43.12
Average		45,694,543,650	45,038,241,171		0.03	94.95	89.80	42.47
Total		639,723,611,100	630,535,376,400	98.56%				

367 **Table 2** Data comparison

Sample	Total Reads	Mapping Rate(%)	Average Depth	Coverage at least 1X(%)	Coverage at least 4X(%)	Coverage at least 10X(%)
AB01	315216417	98.55	17.46	99.59	99.32	93.57
AB02	282990429	98.49	15.82	99.56	99.24	89.18
AB03	312280472	98.49	16.93	99.52	98.98	90.17
AB04	320340217	98.22	18.25	99.57	99.15	93.1
WS01	326298749	98.6	17.63	99.54	99.25	94.36
WS02	350423462	98.41	19.65	99.57	99.24	94.72
WS03	299507485	98.63	16.45	99.51	99.16	92.2
WS04	315038236	98.52	17.28	99.51	98.98	91.77
WS05	334519623	98.65	18.19	99.54	99.24	95.43
WS06	298331035	98.52	16.88	99.53	99.23	92.89
WS07	227540381	97.88	12.85	99.56	98.73	68.52
WS08	296433759	97.97	16.75	99.61	99.32	90.53
WS09	291390679	98	16.44	99.51	99.18	88.97
WS10	245088985	98.13	13.85	99.6	99.05	76.55
Average	301099994.9	98.36142857	16.745	99.55142857	99.14785714	89.42571429

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Table 3 Single nucleotide polymorphism annotation

sample	total	intergenic	upstream	downstream	upstream;downstream	UTR	intronic	splicing	exonic	ncRNA intronic	ncRNA splicing	ncRNA exonic
AB01	5941934	4312213	53776	48084	1290	5461	1470340	382	44314	0	58	6016
AB02	5980256	4328671	54333	49203	1132	5508	1489605	372	45223	1	57	6151
AB03	5997232	4335631	54186	48901	1150	5562	1499169	370	46143	1	54	6065
AB04	6001989	4357097	53432	49324	1155	5565	1482717	371	46136	0	55	6137
WS01	6064076	4396385	54982	49523	1205	5500	1504242	381	45597	0	58	6203
WS02	6090471	4401130	55567	49597	1208	5739	1523751	398	46839	0	63	6179
WS03	6020664	4356630	53850	48718	1233	5456	1503968	379	44501	0	52	5877
WS04	6085300	4412074	54616	49554	1213	5585	1509129	366	46585	2	57	6119
WS05	6068043	4390157	54591	48846	1243	5508	1516293	385	44908	2	52	6058
WS06	6040564	4375737	54833	49476	1233	5552	1502532	394	44718	1	57	6031
WS07	5768919	4180511	50791	46623	1152	5073	1436192	364	42507	1	54	5651
WS08	6036395	4368807	53612	49117	1212	5410	1506878	378	44892	1	61	6027
WS09	5541660	4011569	49104	45086	973	5031	1382618	358	41503	1	50	5367
WS10	5952039	4316455	52643	47956	1120	5423	1478539	357	43679	0	48	5819

sample	stopgain	stoploss	synonymous SNV	nonsynonymous SNV	non/sys	Het in exon heterozygous	Het rate in exon(‰)	Ts	Tv	Ts/Tv	Het	Het_rate(‰)	Freq(1kb)
AB01	188	16	23564	20497	0.87	28681	0.919	3881220	1930876	2.01	3984470	1.641	2.446
AB02	184	10	23969	21009	0.877	29228	0.936	3907370	1942215	2.012	4032261	1.66	2.462
AB03	206	9	24629	21248	0.863	29837	0.956	3918705	1947553	2.012	3964342	1.632	2.469
AB04	182	13	24558	21354	0.87	30205	0.967	3921646	1948790	2.012	3997852	1.646	2.471
WS01	189	16	24256	21104	0.87	28987	0.928	3965532	1966966	2.016	4014979	1.653	2.497
WS02	204	11	24947	21670	0.869	29835	0.956	3978988	1978429	2.011	3948266	1.626	2.508

WS03	166	11	23690	20583	0.869	27712	0.888	3938680	1952297	2.017	3928887	1.618	2.479
WS04	187	13	24873	21452	0.862	29601	0.948	3980030	1974155	2.016	3960924	1.631	2.506
WS05	181	11	23914	20778	0.869	28242	0.905	3969915	1967229	2.018	4023060	1.656	2.498
WS06	181	12	23838	20644	0.866	27812	0.891	3949609	1959971	2.015	3962792	1.632	2.487
WS07	168	13	22814	19503	0.855	27110	0.868	3777434	1866307	2.024	3850010	1.585	2.375
WS08	193	13	24013	20632	0.859	28452	0.911	3950543	1955509	2.02	4008283	1.65	2.485
WS09	155	10	22172	19118	0.862	21714	0.696	3624488	1796797	2.017	3022587	1.244	2.282
WS10	187	14	23361	20075	0.859	27460	0.88	3895339	1927308	2.021	3905744	1.608	2.451

371 **Table 4** Comparison of enriched genes in candidate pathways in Abaga horse and previous studies

KEGG pathway	ID	Selected genes in Abaga horse	Selected genes or proteins in previous studies
Metabolic pathways	ecb01100	*LTA4H AGK PNPLA3 ITPK1 NDUFB7 RIMKLA PCCB ACAD8 ACMSD BST1 CPOX*	*CYP51A1*
Ras signaling pathway	ecb04014	*SHC4 GNG11 GNGT1 KSR2 TBK1 ABL2*	*APOA1 IGF-1 HRAS*
PI3K-Akt signaling pathway	ecb04151	*LAMC2 GNG11 GNGT1 MYB*	PGC-1a IGF-1 IGF-1R ErbB2 ErbB4
MAPK signaling pathway	ecb04010	*CACNA1I CACNA2D3 CACNB4 MAP3K4*	ERK AP-1
Hippo signaling pathway	ecb04390	*MPP5 TCF7L2*	*WWTR1 LATS2 TEAD YAP1 VGLL2 VGLL3 VGLL4*
Cardiac muscle contraction	ecb04260	*CACNA2D3 CACNB4*	*CK-M*
NF-kappa B signaling pathway	ecb04064	*CARD10 TRAF1*	MnSOD iNOS
Arachidonic acid metabolism	ecb00590	*LTA4H*	*PTGS1*
Regulation of actin cytoskeleton	ecb04810	*ENAH*	*GSN BDKRB2 CHRM MYLK ACTN3*
Insulin signaling pathway	ecb04910	*SHC4*	*GYS1 PPARGC1A*
Fatty acid metabolism	ecb01212	*ELOVL5*	*ADHFE1 SREBP2*

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373 ## SUPPLEMENTAL MATERIALS

374 **Table S1** The genes of the selective regions in Abaga horse.

375 **Table S2** The genes of the selective regions in Wushen horse.

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