Research Article

Divergent Evolutional Mode and Purifying Selection of the KIT Gene in European and Asian Domestic Pig Breeds

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The recent geographic expansion of wild boars and the even more recent development of numerous domestic pigs have spurred exploration on pig domestic origins. The porcine KIT gene has been showed to affect pleiotropic effects, blood parameters, and coat colour phenotypes, especially the white colour phenotype formation in European commercial breeds. Here, we described the use of SNPs to identify different selection patterns on the porcine KIT gene and the phylogenetic relationships of the inferred haplotypes. The phylogenetic tree revealed four clades in European and Asian wild and domestic pigs: two major clades with European and Asian origins and one minor clade with Iberian origins as well as the other minor clade in Asia, consistent with the major introgression of domestic Asian pigs in Europe around 18th-19th century. The domestication history of pigs, which occurred in the domestication centers (Europe and Asia), has also been demonstrated by mtDNA analysis. Furthermore, both Asian and European domestic pigs evolved under purifying selection. This study indicated that domestic pigs in Europe and Asia have different lineage origins but the porcine KIT gene was undergoing a purifying selection during their evolutional histories.

1. Introduction

Coat colour variation in animals has been a considerable research hotspot in animal genetics and breeding. Documentation on coat colour can be traced back over 5,000 years ago to Mesopotamia [1]. Numerous mutations accumulated under the effects of natural and human selection, which contributed to the formulation of variations in the coat colour of animal species. Pigmentation in mammals is determined by the amount and distribution of two types of melanin, eumelanin (black/brown), and phaeomelanin (red/yellow), for which production and relative amounts are mainly controlled by the agouti and extension loci [2], which encode the agouti signalling protein (ASIP), and to the melanocortin receptor 1 (MC1R), respectively [3, 4]. However, the most studied and reported target gene is the mast/stem cell growth factor receptor (KIT gene) in porcine coat colour genetics. The KIT gene is located in the Dominant White locus and is confirmed to be responsible for several common colour variants in pigs, such as Dominant White, patch, spotting, belt, and roan [5–12].

The KIT gene encodes the mast/stem cell growth factor receptor, which is a cytokine receptor expressed on the surface of haematopoietic stem cells as well as other cell types [13]. This protein is a type 3 transmembrane receptor for the mast cell growth factor (MGF, also known as the stem cell factor). It contains an extracellular domain composed of five immunoglobulin domains, a single transmembrane domain, a juxtamembrane domain, and an intracellular protein kinase domain that is interrupted by an insertion of approximately 80 amino acids [14]. The protein has the potential to participate in multiple signalling pathways, which accounts for its important role in the control of cell differentiation, survival, and motility and acts as an essential survival factor for the migration and proliferation of melanoblasts [15]. Mutations in the KIT gene are associated with gastrointestinal stromal tumours, mast cell disease, and piebaldism in human [16]. In mice, loss-of-function mutations are associated with limited white spotting in heterozygotes, but the mutations are often lethal or sublethal in the homozygous condition due to their effects on haematopoiesis [17]. Variant coat colour patterns in horses also derive from the KIT gene mutations [18, 19].
Up to now, five alleles have been identified in the KIT gene, the recessive \( i \) allele (wild-type) present in wild boar and coloured pigs (Duroc, Asian black pigs), the \( I^b \) allele for the patch phenotype (Pietrain), the \( I^c \) allele for the belt phenotype (Hampshire), the \( I^{bc} \) allele for the roan/gray coat colour, and the major dominant \( I \) allele (\( I^1 \), \( I^2 \), \( I^3 \), or \( I^4 \)) for the fully dominant white coat colour especially in European commercial breeds such as Landrace and Large White. The origin, domestication, and distribution of the alleles in the porcine KIT gene likely present an independent geographic trend. Subsequently, the European pigs harboured a normal copy number of the KIT gene (\( i \) and \( I^{bc} \) alleles) as well as duplicated copy numbers (\( I^1 \), \( I^2 \), \( I^3 \), and \( I^4 \) alleles), while only the normal copy number was identified in Asian pigs until now. Furthermore, none of the Dominant White alleles were identified in one Chinese white pig breed [20]. However, studies on the KIT gene were limited to only a few of pig breeds [6, 10, 11, 20, 21].

In this study, we aimed to investigate the DNA polymorphisms and haplotype distribution of the porcine KIT gene in 17 Asian wild and domestic breeds, as well as several European wild and domestic breeds. We also compared the divergent evolutionary mode of the KIT gene and addressed which kinds of selection occurred in Asian and European domestic pigs during the process of domestication.

2. Materials and Methods

2.1. Animal Materials. A total of 44 unrelated pigs from 22 domestic breeds were analyzed in this study (15 Asian domestic breeds and 7 European domestic breeds, Supplementary Table 1). In addition, 12 European wild boars, two Chinese wild boars from different regions, and 8 Korean wild boars from 4 subgroups [22] were also included. Genomic DNA was extracted from blood by standard methods or from hair roots by Chelex extraction.

2.2. Detection of Polymorphisms in the Dominant White Locus. A total of 17 PCR primer sets were designed using Primer Premier 5 to amplify the 21 exon regions of the KIT gene. To identify exon boundaries, blast analysis was performed to compare the porcine KIT cDNA sequences (FJ938289) with the working draft sequence of Sus scrofa (ACI418572). The information on primers is listed in Table 1. PCR was carried out in a 25-\( \mu \)L reaction volume containing 40–50 ng of template DNA, 10 \( \times \) PCR buffer, 100 \( \mu \)M of dNTP, 10 pmol of each forward and reverse primers, and 1 unit of Taq DNA polymerase (GenetBio, Korea). The PCR program included an initial denaturation step of 95°C for 5 min followed by 40 cycles of 94°C for 30 s, 50–66°C for 30 s, and 72°C for 40 s, and a final extension step of 72°C for 5 min in a PTC-200 Programmable Thermal Controller (MJ Research, Inc., USA). PCR products were purified with GELase’sTM Agarose Gel-Digesting Preparation kit (Epicerent, USA) and then were directly sequenced using the Big Dye terminator chemistry on the ABI 3130XL DNA Analyzers (PE Applied Biosystems, USA). Ambiguous positions were verified by resequencing. To determine if any contamination was present, blank PCR controls were performed throughout this study.

3. Results and Discussion

3.1. Genetic Characteristics of the Porcine KIT Gene. The polymorphism sites in the whole coding region of the KIT gene were identified by direct sequencing among wild boars and six domestic breeds (Landrace, Large White, Hampshire, Duroc, and Korean native pigs). Subsequently, the variable sites were checked in the other individuals. A total of 28 polymorphisms including one indel event (intron18: g.29,32delAGTT) were obtained by a multiple alignment of the assembled 66 individual sequences using Clustal W [28]. Four novel mutations were revealed in this study, and one of them was missense mutation (c.583 G>A, Val195Met). A small proportion of the SNPs (5 out of 27) were missense mutations, while the others were sense mutations (Supplementary Table 1). A high degree of homozygosity was detected in domestic breeds compared to wild boars originating from either Europe or Asia. Asian domestic pigs also
| Primer Name | Sequence (5’-3’) | Product size (bp) | TM (°C) |
|-------------|------------------|------------------|---------|
| KIT_E1F     | GGAACGTGGAAAGGAGCT | 205               | 66      |
| KIT_E1R     | CAGGCAACCTCTTGACAC | 162               | 55      |
| KIT_E2F     | ACCCTGGAACATATAGGC | 498               | 59      |
| KIT_E2R     | GACTCAGGGCTATGTGC  | 447               | 64      |
| KIT_E3F     | TGATATCTGGTAACACCTGC | 362               | 50      |
| KIT_E3R     | CGAAAGGCCACAGTGAGA | 293               | 54      |
| KIT_E4F     | TGGCTATCTGGATCTA  | 548               | 59      |
| KIT_E4R     | CAGGCAAGGCCTAAACATCC | 385               | 53      |
| KIT_E5F     | TCCCCCGGAGCTGAGATTGTA | 344               | 55      |
| KIT_E5R     | CGCCGATCCTCAACCTACTTCCTAAGTG | 313               | 54      |
| KIT_E6F     | GAAGACATAAGATGGTGATA | 293               | 54      |
| KIT_E6R     | CTGAAAACATATTGGAATTTC | 271               | 63      |
| KIT_E7F     | CCAAGGCAGCGTGTTATTT | 536               | 64      |
| KIT_E7R     | GGTCAAAATATCATGAAAGCAG | 482               | 60      |
| KIT_E8F     | GTCATCTGCAGTCACAAGAAGA | 385               | 53      |
| KIT_E8R     | TGGAAACATATTGGAATTTC | 332               | 50      |
| KIT_E9F     | GGTACCTTGATTGCACACC | 910               | 52      |
| KIT_E9R     | GGTACCTTGATTGCACACC | 325               | 60      |
| KIT_E10-11F | CTAGAACAAAGGAGCTACC | 486               | 60      |
| KIT_E10-11R | GTGCCATGTCCCTTTATTC | 482               | 63      |
| KIT_E12-13F | GCATGTGAATGGCCGTACG | 217               | 63      |
| KIT_E12-13R | ATGCCCTGGATTTGACAC | 271               | 63      |
| KIT_E14F    | GGTGTACACCCAAAGACAG | 271               | 63      |
| KIT_E14R    | CTTGACACTGTCCATTGTA | 271               | 63      |

Table 1: Primer sets used in this study.

It was noted that none of the 27 identified SNPs had given any effect of a camouflaged coat in either Asian or European wild boars. Even 5 of 27 were missense mutations, but Asian and European wild boars did not display any difference in phenotypic or physiological characters. Furthermore, no fixed mutation has been identified as the distinguishing SNP, which could separate the wild boar from domestic breeds.
Table 2: Diversity and neutrality indices of KIT gene in the tested pigs.

| Subgroups       | No. of ns | No. of s | No. of haplotypes | \( \pi^s \) | \( \theta^s \) | Tajima's D | Fu and Li's D* | Fu and Li's F* |
|-----------------|-----------|----------|-------------------|-------------|--------------|------------|----------------|---------------|
| G1 AWB-ADP      | 3         | 14       | 24                | 1.68        | 1.56         | 0.28       | -0.08          | 0.03          |
| G2 EWB-EDP      | 3         | 8        | 7                 | 1.63        | 1.54         | 0.16       | 0.16           | 0.17          |
| G3 AWB-ADPEDP   | 3         | 12       | 9                 | 2.21        | 1.89         | 0.81       | 0.79           | 0.89          |
| G4 East EWB-Iberian | 1     | 4        | 4                 | 0.91        | 0.94         | -0.21      | -0.21          | -0.20         |

Subgroups were identified by network analysis. \( ns \): nsSNP (nonsynonymous single nucleotide polymorphism), \( s \): sSNP (synonymous single nucleotide polymorphism), \( \pi \): nucleotide diversity, and \( \theta \): Watterson's theta estimator; \# per kilobase between sequences, within subgroup, wild boars' haplotypes found in domestic pigs and domestic pigs' haplotypes found in wild boars were excluded from neutrality analysis.

in four exon regions of the KIT gene (exons 5, 6, 19, and 20). It could be explained by the unique breeding history of the European pigs, the majority of which were commercial breeds, except the Iberian pig, which was selected and developed strongly under the effects of human selection. Interestingly, Asian pigs displayed distinct mutations compared with those in European pigs. However, no more novel mutations were detected between wild Asian and domestic pigs, and the polymorphisms displayed a slight decrease in Asian domestic pigs. Aspects of selective pressures acting on the KIT gene were estimated by an analysis of the rates of synonymous, silent \( (d_S) \), and nonsynonymous or nonsilent \( (d_N) \) nucleotide substitutions among wild and domestic pigs from Asia and Europe (Figure 1). All the values of \( d_S \) revealed were well below the values of \( d_N \); furthermore, no obvious differences were detected among wild boars and domestic pigs either in Asia or Europe, which indicated that the porcine KIT gene was undergoing a purifying selection. Coat colour has undergone both natural selection and human selection during evolution. However, the effects of different selections are generally very slight at the level of nucleotide sequence variation. However, it is crucial to point that the polymorphisms analysis was limited by the small sample size in our study.

3.3. Phylogenetic Clustering of Wild Boars and Domestic Breeds. A consensus tree was generated based on the estimated haplotypes of the investigated samples, as well as the one out group sequence (Bos taurus, NM_001166484). Two major and two minor clades were identified in the consensus tree using the mutations of the porcine KIT gene (Figure 2(a)). Majority of the Asian haplotypes clustered to clade 1 (AWB-ADP), except clade 4 (AWB-ADP/EDP) which consisted of five Asian wild boar haplotypes and four domestic haplotypes (BK: DH06-07, VT: DH11 and XZ: DH32). Almost all of the European haplotypes found in wild boar and domestic pigs were grouped together forming clade 3 (EWB-EDP). In addition, a small proportion of European haplotypes (WH04-06 and DH09) were identified as clade 2 (East EWB-Iberian), whereas the clade was clustered with clade 1. Subsequently, a neighbour-joining (NJ) tree was constructed using MEGA under a model of Kimura 2-parameters (Supplementary Figure 1), which gave similar topologies, with minor differences in terms of the different phylogenetic approaches. The values of bootstraps were quite low, which could be caused by low polymorphism among the whole coding region of the KIT gene (only 27 SNPs were detected among 2,919 base pairs).

A parsimony median-joining network was drawn using the haplotypes (Figure 3(a)). The AWB-ADP subgroup contains almost all Chinese domestic pigs except XZ and two KNP. There are a total of 13 different Chinese domestic pigs in this study, and up to 22 haplotypes were inferred from Chinese pigs, which indicated a high genetic diversity in Chinese domestic pigs. There were 7 haplotypes presented in the EWB-EDP branch, which contained all the Landrace, Large White, Hampshire, Pietrain, and Duroc and 12 European wild boars. A unique group was AWB-ADPEDP, which are several "European-like" haplotypes from Chinese and Korean wild boars that contributed to 4 domestic haplotypes, which were mainly found in BK, XZ, and VT. Furthermore, three rare East European boars' haplotypes and an Iberian-specific haplotype were found in the last groups, which were in close proximity to the AWB-ADP group. Four groups were clearly identified and supported the result of phylogenetic tree.
(a) Figure 2: Continued.
Figure 2: A consensus tree reconstructed for the investigated pigs using coding sequence of the KIT gene (a) and D-loop sequence (b).
Figure 3: A parsimony median-joining network for the investigated pigs based on the KIT gene (a) and D-loop sequences (b).
network analyses of the and Asian pigs. As elucidated in phylogenetic trees and even though there were several introgressions in European independent evolution of the domestication events of pigs and were consistent with the nal inheritance in KNP during the early breeding history. Th a p l o t y p e ( H 3 3 ) .Th er e a s o nm i g h tb ef o rE u r o p e a nm a t e r -
Spain, which would thus cause some introgressions with the Vietnam (VT01) and one Korean native pig (KNP01). VT01 Large White (76%) [31]. The European clade was composed of European wild and domestic pigs, one potbelly pig from Vietnam (VT01) and one Korean native pig (KNP01). VT01 was clustered with the European clade, which could be explained by the raising of Vietnamese domestic pigs in Spain, which would thus cause some introgressions with the European samples. Unexpectedly, KNP01 were grouped with three European wild boars, which shared the same mtDNA haplotype (H33). The reason might be for European maternal inheritance in KNP during the early breeding history [32]. The mtDNA distinct clades supported the independent domestication events of pigs and were consistent with the independent evolution of the KIT gene in Asia and Europe, even though there were several introgressions in European and Asian pigs. As elucidated in phylogenetic trees and network analyses of the KIT gene, Landrace, Large White, and Berkshire have a clear Asian genetic background (Figures 2 and 3).

In this study, the Asian wild boars carried two major types of haplotypes (one Asian and one European), while the European wild boars contained one major haplotype and a small portion of other haplotypes (East European type). Since these patterns were revealed within a few wild boars, they may not lead to a scientific conclusion. We confirmed these patterns by a large sample set containing 37 European wild boars and 42 Korean wild boars (Figure 3(b)). The haplotypes of Asian wild boars were classified into two separated parts: the Asian group (WH07, WH08, WH10, WH12, and WH14), which forms the gene pool for Chinese and Korean domestic pigs, and the European group (WH09, WH11, WH13, WH15, and WH16), which contributed to the formation of Large White, Landrace, Pietrain, Hampshire, and Duroc breeds. All the European commercial breeds clustered within the European wild boar group, even though many of them accumulated more mutations. However, one Chinese domestic pig (XZ) clustered with the European pigs, which may have been caused by either of the two major reasons: one possible reason is crossbreeding imported European pigs with domestic Chinese pigs; the other possible cause is random mutations. The phylogenetic tree and network analyses revealed 4 subgroups in the investigated pigs, which was a clear divergence between domestic pigs in Asia and Europe. Both groups were domesticated independently in corresponding locations, even though there were some introgressions in recent history. The result obtained by mtDNA analysis is similar. As the mtDNA data revealed, modern domestic pigs had multiple originations and were subsequently domesticated [29].

3.5. The Relationship between KIT Genotypes and Coat Colour. Up to now, 5 alleles have been identified in the Dominant White locus (I, I, I, I, and I) [7, 11, 33, 34]. Furthermore, a large number of mutations were accumulated during the evolutionary process either in coding regions or noncoding regions (many SNPs were found in intron4 and intron5, in the roan paper) [35]. None of these SNPs were identified as the causative mutation for the corresponding phenotype except the splice mutation in intron7 (G to A substitution), which contributed to the Dominant White phenotype with a normal copy of the KIT gene [5]. A KIT gene duplication mutation associates with a partially dominant phenotype (patch), and one exon skipping leads to the roan phenotype [36], while it is assumed that the basis of the belt phenotype was caused by an unknown regulatory mutation [7].

4. Conclusions

In conclusion, we found that the phylogenetic analysis of the Dominant White locus (KIT) indicated four geographic clusters, which supported the multiorigins of modern pigs. Furthermore, both Asian and European domestic pigs evolved under purifying selection during their evolutionary histories.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

All authors declared that they have no conflicts of interest.

Authors’ Contributions

Lili Niu and KeYu Shi contributed equally to this work.

Acknowledgments

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Supplementary Materials

Supplementary 1. Supplementary Figure 1: a NJ tree constructed for the investigated pigs using 2,919 bp of KIT gene coding sequence. Supplementary 2. Supplementary Table 1: the information about the investigated pigs in this study. (Supplementary Materials)

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