

ORIGINAL ARTICLE

A 6-bp deletion in the *TYRP1* gene causes the brown colouration phenotype in Chinese indigenous pigs

J Ren, H Mao, Z Zhang, S Xiao, N Ding and L Huang

Key Laboratory for Animal Biotechnology of Jiangxi Province and the Ministry of Agriculture of China, Jiangxi Agricultural University, Nanchang, PR China

Brown coat colour has been described in Chinese-Tibetan, Kele, and Dahe pigs. Here, we report the identification of a causal mutation underlying the brown colouration. We performed a genome-wide association study (GWAS) on Tibetan and Kele pigs, and found that brown colours in Chinese breeds are controlled by a single locus on pig chromosome 1. By using a haplotype-sharing analysis, we refined the critical region to a 1.5-Mb interval that encompasses only one pigmentation gene: tyrosinase-related protein 1 (*TYRP1*). Mutation screens of sequence variants in the coding region of *TYRP1* revealed a strong candidate causative mutation (c.1484_1489del).

The protein-altering deletion showed complete association with the brown colouration across Chinese-Tibetan, Kele, and Dahe breeds by occurring exclusively in brown pigs ($n=121$) and lacking in all non-brown-coated pigs ($n=745$) from 27 different breeds. The findings provide the compelling evidence that brown colours in Chinese indigenous pigs are caused by the same ancestral mutation in *TYRP1*. To our knowledge, this study gives the first description of GWAS identifying causal mutation for a monogenic trait in the domestic pig.

Heredity (2011) **106**, 862–868; doi:10.1038/hdy.2010.129; published online 27 October 2010

Keywords: causal mutation; coat colour; genome-wide association; pig; *TYRP1*

Introduction

Coat colour is one of the most strikingly variable and visible traits in mammals, making it a convenient tool for genetic analysis from the beginning of the last century (Searle, 1968; Silvers, 1979). In mammals, the first genetic studies were conducted on coat colour genetics. At present, 368 coat colour loci, including 159 cloned genes, have been described in mice (<http://www.espcr.org/micemut/>). The coat colour loci influence the development, differentiation, proliferation, and migration of melanocytes, the construction and transport of melanosomes, as well as the synthesis of melanin. Intriguingly, these colour genes are shared by different species (Jackson, 1994; Barsh, 1996). A notable example is the conserved role of the melanocortin 1 receptor in mammalian pigmentation (Andersson, 2003). Studies of melanocortin 1 receptor have provided valuable insights not only into the biology of pigmentation but also the evolution of domesticated animals (Fang *et al.*, 2009).

The domestic pig displays a bewildering diversity in coat colour, which intrigued scientists for centuries. Two major colour loci and causative mutations have been well characterized in pigs: the *KIT* gene for the dominant white colour (Johansson Moller *et al.*, 1996; Marklund *et al.*, 1998;

Giuffra *et al.*, 2002; Pielberg *et al.*, 2002) and the *MC1R* locus for coloured phenotypes (Kijas *et al.*, 1998, 2001). For other colour phenotypes, such as belt (Giuffra *et al.*, 1999) and black-and-tan (Drögemüller *et al.*, 2006) in Western pigs, the causative mutation(s) has not yet been found.

Selective breeding during the long period has resulted in diverse coat colour phenotypes in Chinese indigenous breeds, including solid black, white (Lai *et al.*, 2007), brown (Mao *et al.*, 2010), belted and spotted (Xu *et al.*, 2006) pigment types. In most breeds, all pigs share the same colour pattern as breed character. However, variable colours are allowed within the standard in a few breeds, such as Tibetan, Kele, and Dahe pigs (Zhang *et al.*, 1986).

Tibetan pigs, renowned for their unique adaptability to the adverse living conditions of the Qinghai–Tibetan plateau, segregate for black, reddish, and silver brown colours (Figure 1). The colour variants fit an autosomal monogenic model of inheritance and the brown colour is a codominant trait. Heterozygous (*B/b*) animals display the reddish brown phenotype and homozygotes show silver brown (*b/b*), or black colours (*B/B*) (Zhang *et al.*, 1986). Kele and Dahe pigs are two local populations from the Yunnan–Guizhou plateau classified as a single breed (namely, Wujin). A range of light to dark brown shades, as well as a black phenotype (Figure 1), has been observed in Kele and Dahe pigs. Different brown shades are caused by epistatic interaction between two loci (Lian and Wu, 1985). So far, the molecular basis of the brown phenotypes of Tibetan, Kele, and Dahe pigs remains elusive. Here, we report a genome-wide association study (GWAS) and haplotype-sharing analyses that

Correspondence: Dr L Huang, Key Laboratory for Animal Biotechnology of Jiangxi Province and the Ministry of Agriculture of China, Jiangxi Agricultural University, Meiling Road, Nanchang 330045, PR China.

E-mail: Lushenghuang@hotmail.com

Received 2 June 2010; revised 19 July 2010; accepted 30 July 2010; published online 27 October 2010



Figure 1 Brown coat colour phenotypes in Chinese indigenous pigs. (a) A reddish brown Tibetan piglet. (b) A flock of Kele piglets with dark brown, pale brown, and black colouration phenotypes. (c) A black brown Dahe sow. (d) A silver brown Dahe sow.

characterize the responsible gene and subsequent detection of a genetic variant that appears to cause the brown colouration in Chinese breeds.

Materials and methods

Sources of animal samples

Tibetan pigs were sampled from a multiplying population with brown colour variants in Guangdong province ($n=127$) and a nucleus herd with the solid black phenotype in the Gongbujiangda county of the Tibetan Autonomous Region ($n=32$). Brown Tibetan pigs are descendants of one brown boar and two brown sows from the Gannan Tibetan Autonomous Prefecture of Gansu Province. A total of 65 Tibetan pigs, including 32 reddish brown, 3 silver brown, and 30 black individuals from the multiplying population, were used for the genome-wide association mapping. Of the 65 pigs, 32 individuals from 4 families, clearly segregating for the brown phenotype (Supplementary Figure S1), were used for a genome-wide linkage analysis.

Black and brown-coated Kele pigs ($n=86$) were collected from the Hezhang county of Guizhou province. Of the 86 pigs, 48 pigs from 8 dam families, including 24 dark brown, 2 pale brown, and 22 black individuals, were used for the genome-wide association mapping. Brown-coated Dahe pigs ($n=36$) were sampled from the Fuyuan county of Guizhou province. A panel of 966 pigs from 27 breeds and wild boars representing a range of distinct coat colours (Table 1) were used for validation of candidate causative mutations. Individual phenotypes of Tibetan, Kele, and Dahe pigs were determined by direct visual inspection and photographs taken outdoors on a sunny day. Coat colour phenotypes of the remaining breeds were obtained directly from the breed registration association. Genomic DNA of all samples was extracted from ear tissues according to standard protocols.

Genome-wide association analysis and haplotype reconstruction

The PorcineSNP60 BeadChips (Illumina, San Diego, CA, USA) were used to genotype the 65 Tibetan and 48 Kele pigs on an Illumina iScan System following the manufacturer's protocol. Bead arrays with call rate $<85\%$ were excluded for further analyses. GWASs were performed in a case-control way under the dominant model on all single-nucleotide polymorphisms (SNPs) with a minor allele frequency >0.05 and call rate $>95\%$ by using PLINK (<http://pngu.mgh.harvard.edu/purcell/plink/>). The genome-wide significance was calculated by phenotype permutation testing ($n=100\,000$). A genome-wide linkage analysis was performed using CRIMAP (available at <http://linkage.rockefeller.edu/soft/crimap/>). We assumed that the brown phenotype was inherited as a biallelic autosomal codominant trait with complete penetrance. Faithful haplotypes in the mapped interval were reconstructed with DualPHASE (Druet and Georges, 2010). The most likely responsible gene (*TYRP1*) was identified by the pig-human comparative map on the basis of the well-characterized coat colour genes.

TYRP1 sequencing

To detect genetic variants in the coding region of the *TYRP1* gene, we designed six intronic primer pairs flanking *TYRP1* exons 2–7 and one primer pair for the 3'-UTR from publicly available sequences (NM_001025226 and NC_010443.1). Primer sequences are listed in Supplementary Table S1. Genomic DNA of 5 black and 6 brown Tibetan pigs, 6 Chinese and European wild boars, 48 pigs from 16 different breeds were amplified with these primers. Amplifications were performed in a 25- μ l reaction containing 0.4 units Taq DNA polymerase (TaKaRa, Dalian, China) and a magnesium concentration of 2.0 mM at optimal annealing temperatures (Supplementary Table S1) using standard protocols. PCR

Table 1 Genotype and allele frequencies of the *TYRP1* c.1484_1489del (p.M495_G496del) mutation in Chinese and European pig breeds

Breed	Coat colour	No.	Genotype			The del frequency
			+/+	+/del	del/del	
<i>Chinese breed</i>						
Tibetan	Reddish brown	51	0	51	0	0.50
	Silver brown	5	0	0	5	1.00
	Black	103	103	0	0	0.00
Kele	Dark brown	27	0	27	0	0.50
	Pale brown	2	0	0	2	1.00
	Black	57	57	0	0	0.00
Dahe	Black brown	10	0	10	0	0.50
	Yellow brown	21	0	21	0	0.50
	Silver brown	5	0	0	5	1.00
Dianan small ear	Black	31	31	0	0	0.00
Erhualian	Black	31	31	0	0	0.00
Hetao large ear	Black	32	32	0	0	0.00
Jinhua	Two-end-black	33	33	0	0	0.00
Laiwu	Black	32	32	0	0	0.00
Luchuan	Black with white belly	34	34	0	0	0.00
New Dahe	Black	44	44	0	0	0.00
Rongchang	White	34	34	0	0	0.00
Yushan	Black	31	31	0	0	0.00
Wuzhishan	Black with white belly	32	32	0	0	0.00
Wild boars	Wild type	72	72	0	0	0.00
<i>European breed</i>						
Berkshire	Black	4	4	0	0	0.00
British Lop	White	1	1	0	0	0.00
Chester White	White	2	2	0	0	0.00
Duroc	Red	67	67	0	0	0.00
Hampshire	White belt and black body	42	42	0	0	0.00
Iberian	Black	10	10	0	0	0.00
Landrace	White	39	39	0	0	0.00
Large Black	Black	2	2	0	0	0.00
Large White	White	64	64	0	0	0.00
Mid White	White	2	2	0	0	0.00
Old Spot	Spotted	2	2	0	0	0.00
Pietrain	Spotted	27	27	0	0	0.00
Saddle Back	White belt and black body	1	1	0	0	0.00
Tamworth	Tan and black	1	1	0	0	0.00
Yorkshire	White	2	2	0	0	0.00
Wild boars	Wild type	13	13	0	0	0.00

products were bidirectionally sequenced with original PCR primers. Sequence data were assembled and aligned with ClustalX (<http://abs.cit.nih.gov/clustalx/>) to reveal genetic variants.

Mutation genotyping

A *KpnI* PCR-RFLP assay was used to genotype the *TYRP1* c.1484_1489del mutation. Genomic DNA was amplified with primers for *TYRP1* exon 8 (Supplementary Table S1) as described above. PCR products were digested with *KpnI* (New England Biolabs, Ipswich, MA, USA) at 37 °C for 4 h. The restriction fragments were separated on 2% agarose gels and genotypes were determined from the resulting band patterns. For genotyping the c.428G>A polymorphism, primers for *TYRP1* exon 3 (Supplementary Table S1) were used to amplify a 736-bp fragment containing the mutation site. The resulting PCR products were subjected to SNaPshot analyses with an extension primer (5'-TTTTTTTTT TTTTACCTAAGTACAGAAGAAAAGAACC-3') and the ABI PRISM SNaPshot Multiplex Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. SNaPshot products (0.5 µl) were mixed with 9 µl

of formamide and 0.5 µl of GeneScan-120 LIZ Size Standard and separated by capillary electrophoresis in a 3130XL Genetic Analyzer (Applied Biosystems). The genotype data were finally recorded with GeneMapper 4.0 software (Applied Biosystems).

Results

Mapping of the responsible gene

Of the 62 163 SNPs on BeadChip (Ramos *et al.*, 2009), 48 174 SNPs in Tibetan samples and 46 508 SNPs in Kele pigs had a call rate >95% and had annotated genomic positions on Build 7.0 of the *Sus scrofa* genome (http://www.sanger.ac.uk/Projects/S_scrofa/). Four Tibetan pigs having an overall call rate <85% were discarded for further analyses. A final panel of 30 342 and 41 347 informative SNPs with minor allele frequency >0.05 was included in the GWAS on 61 and 48 successfully genotyped Tibetan and Kele pigs, respectively. The 30 342 SNPs were also used for a genome-wide linkage mapping analyses in four Tibetan families.

In Tibetan pigs, the GWAS identified the most strongly associated SNP (INRA0006065, nominal *P*-value =

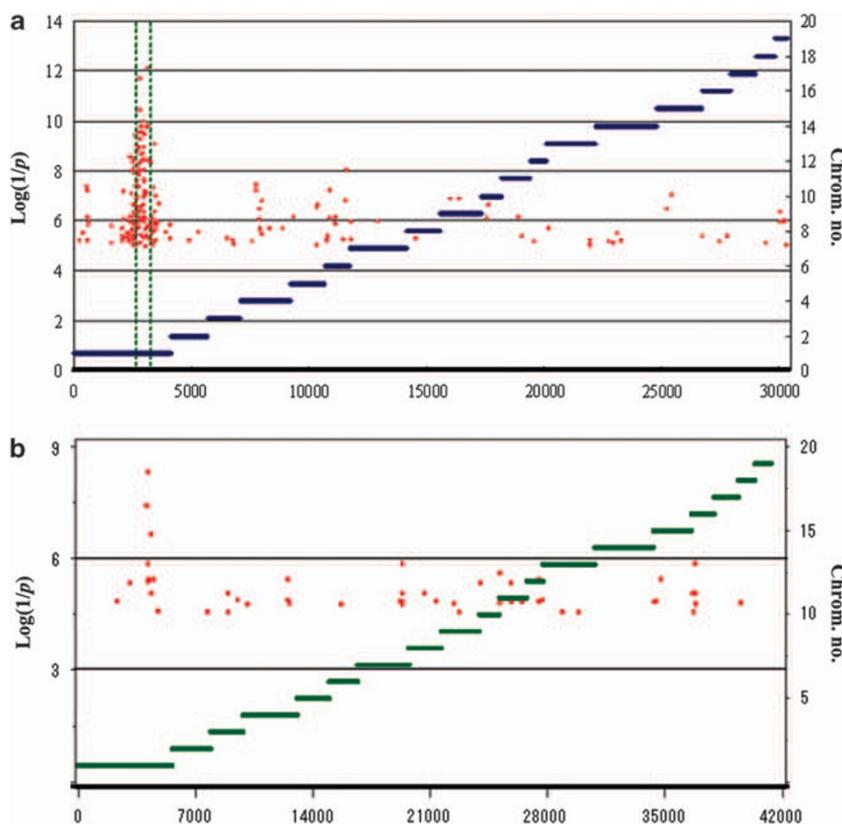


Figure 2 Genome-wide association mapping of the brown coat colour locus in Tibetan (a) and Kele (b) pigs. The black horizontal bars indicate the limits between the 18 autosomes. Evidence for linkage (y axis) is measured as $\log(1/p)$. Only genome-wide significant markers are shown. The genome-wide significant threshold was determined by 100 000 phenotype permutations. The green vertical dashed line represents the non-recombinant region in four Tibetan families.

7.3×10^{-13}) at 194.62 Mb on chromosome 1 (SSC1), followed by SNP (INRA0005584, nominal P -value = 1.9×10^{-12}) at 165.46 Mb on this chromosome. This association is 100-fold stronger than that for any other region in the genome (Figure 2a). By using linkage analyses on four Tibetan families, we observed that three SNPs (INRA0005874 at 182.55 Mb, ALGA0007793 at 184.00 Mb, and INRA0005944 at 188.64 Mb) in the INRA0006065–INRA0005584 region (~ 29 Mb) showed complete linkage disequilibrium with the *brown* locus. These observations strongly support that the responsible gene locates in the 29-Mb region on SSC1.

In Kele pigs, SNP INRA0005944 at 188.64 Mb on SSC1 showed the strongest association (nominal P -value = 4.6×10^{-9}). SNPs ALGA0007745 at 182.29 Mb and DRGA0002004 at 196.38 Mb on SSC1 define the critical interval (~ 14 Mb), as the association of SNPs in the interval is 100-fold stronger than that for any other region in the genome (Figure 2b). The mapped interval perfectly overlaps the 29-Mb region harbouring the Tibetan *brown* locus. This observation supports the assumption that a common mutation underlies the brown phenotypes in Chinese indigenous pigs. We, hence, reconstructed haplotypes around the mapped interval for all tested animals. A 1.5-Mb region from 183.1 to 184.6 Mb on SSC1 appears to be the crucial interval, as it defines the minimum shared haplotype among brown chromosomes from Tibetan and Kele pigs (Figure 3). We conclude that the causal mutation is located in this interval.

As the quality of the pig genome annotation is still far from perfect, we inferred the gene annotation of the critical interval from the corresponding human region. The human orthologous region on HSA9 (assembly GRCh37) contains only three annotated genes, including one well-characterized pigmentation gene: tyrosinase-related protein 1 (*TYRP1*), which is at ~ 200 kb from the homologous sequence of the SNP ALGA0007793 in the 1.5-Mb critical interval. In humans, *TYRP1* mutations lead to oculocutaneous albinism type 3, known as Brown and Rufous OCA (Sarangarajan and Boissy, 2001). Moreover, *TYRP1* plays a well-established and crucial role in mammalian pigmentation and refers to the classic *brown* locus in mice (Jackson *et al.*, 1990; Zdarsky *et al.*, 1990; Javerzat and Jackson, 1998). Mutations in *TYRP1* are repeatedly associated with brownish coat colour in a number of domestic animals, including cat (Lyons *et al.*, 2005; Schmidt-Kuntzel *et al.*, 2005), dog (Schmutz *et al.*, 2002), cattle (Berryere *et al.*, 2003), and sheep (Gratten *et al.*, 2007), as well as quail (Nadeau *et al.*, 2007) and fish (Braasch *et al.*, 2009). We, therefore, selected *TYRP1* as the prime candidate gene and investigated whether mutations in *TYRP1* might be responsible for the brown phenotypes in Chinese pig breeds.

Mutations in *TYRP1*

We designed PCR primers (Supplementary Table S1) to amplify all seven protein-coding exons and exon–intron boundary sites of *TYRP1*. Mutation screening of 5 black

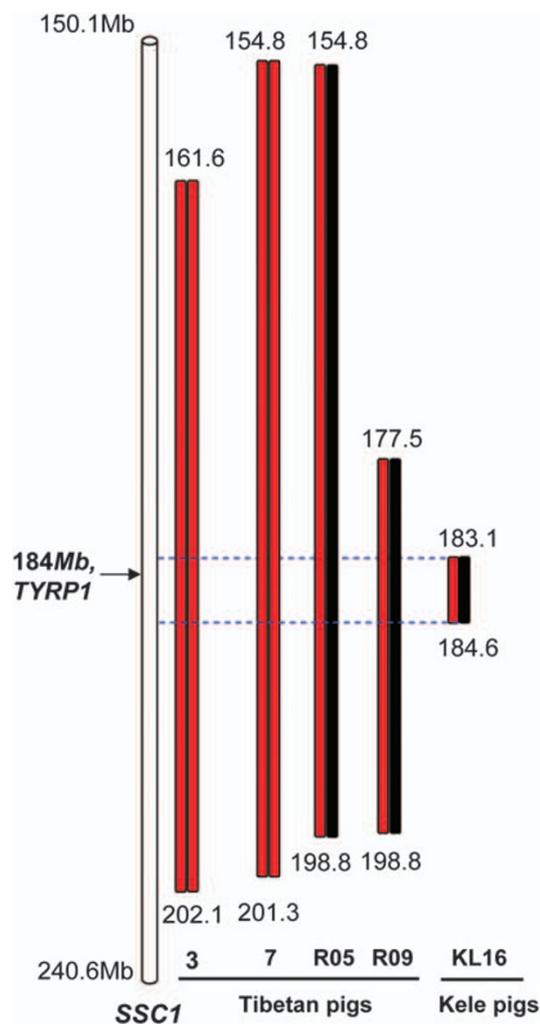


Figure 3 Shared haplotypes in the critical region harbouring the *brown* locus between Tibetan and Kele pigs. Red bars indicate the *brown* haplotype and black bars represent the *non-brown* haplotype. Only haplotypes of five individuals faithfully reconstructed by the hidden Markov model (DualPHASE) on the basis of family information are shown. The boundaries of homozygous haplotype blocks are given in Mb. The *Sus scrofa* genome assembly (Build 7.0, available at http://www.ensembl.org/Sus_scrofa/Info/Index) is used as standard reference. The horizontal dashed green lines indicate the minimum shared haplotype block of 1.5 Mb.

and 6 brown Tibetan samples, as well as 54 animals from 18 breeds, revealed 22 *TYRP1* sequence variants. Of the 22 polymorphisms, 13 SNPs were located in introns and none altered the conserved splice sites. The intronic SNPs occurred in both brown-coloured and non-brown-coated breeds, and were therefore not analysed further. Of the nine mutations within the coding region, seven substitutions also segregated in both brown-coloured and non-brown-coated breeds (Supplementary Table S2), and were apparently excluded as the causative mutation. Two other coding variants result in amino acid changes: c.428G > A and c.1484_1489delITGGGTA (Supplementary Figure S3). The non-synonymous c.428G > A polymorphism at exon 2 leads to a transition from a histidine to arginine at amino acid 143 of the protein (p.143His > Arg). The 6-bp deletion in exon 8, c.1484_1489del, removes two amino acids from protein at positions 495 and 496 (p.M495_G496del), respectively. We

performed alignment of the *TYRP1* protein sequence from 13 vertebrate species and found that the hexanucleotide deletion occurs in a conserved transmembrane domain region predicted by SMART (<http://smart.embl-heidelberg.de/>) (Supplementary Figure S3). The two exonic mutations are likely of functional significance and were hence analysed further. We used *KpnI* PCR-RFLP to genotype the deletion polymorphism using primers for exon 8 (Supplementary Table S1). The wild-type allele is represented by two fragments of 398 and 347 bp and the *del* allele by an uncut amplicon of 745 bp (Supplementary Figure S2). The c.428G > A polymorphism was genotyped by a SNaPshot assay (Supplementary Figure S2).

Association of *TYRP1* mutations with brown phenotypes

We investigated the distribution of genotypes at the c.428G > A and c.1484_1489del sites in 966 pigs from 27 different breeds and wild pigs, representing a wide range of colour phenotypes. The analysis revealed that a majority of the non-brown-coated breeds segregated for the c.428G > A polymorphism (Supplementary Table S3). This excluded this missense mutation as the cause of the brown coat colour phenotype. Intriguingly, we found a perfect association of the c.1484_1489del mutation with the brown phenotype. All 121 brown-coated pigs from Tibetan, Kele, and Dahe pigs were homozygous or heterozygous for the *del* allele (Table 1). None of the 160 black Tibetan and Kele pigs, and 685 pigs from other 27 diverse pig breeds and wild boars had the *del* allele (χ^2 -test, $P = 4.41 \times 10^{-212}$; Table 1). In Tibetan pigs, all the presumed heterozygous animals in the family samples (Supplementary Figure S1) carried the +/*del* genotype. Moreover, all heterozygotes had the reddish brown phenotype, whereas the *del/del* animals uniformly displayed the silver brown colouration, reflecting that this mutation perfectly fitted the proposed codominant model of inheritance for the phenotype. Kele and Dahe animals homozygous for the *del* allele show pale or silver brown colouration, indistinguishable from those observed in Tibetan pigs. However, discordant brown shades are observed among +/*del* animals of Tibetan (reddish brown), Kele (dark brown), and Dahe (black or yellow brown) pigs.

Discussion

Genome-wide association analysis on the basis of large-scale SNP scan is emerging as a new and powerful approach for high resolution genetic analysis in domestic animals (Andersson, 2009), but to our knowledge this approach has not yet been reported in pigs. In this study, we used newly developed PorcineSNP60 BeadChip to perform a genome scan for the *brown* coat colour locus in Chinese pig breeds. The majority of SNPs on BeadChip were originally discovered from western commercial breeds and wild pigs (Ramos *et al.*, 2009). Applications of the chip in Asian breeds are hence cautioned for the ascertainment bias. Nevertheless, 64.1 and 66.5% of the SNPs on the chip are segregating in the Tibetan and Kele breeds with minor allele frequency >0.05, proving the usefulness of BeadChip in Chinese pig populations, although not as powerful as the chip did in western breeds.

The GWAS identified significantly associated regions in Tibetan and Kele pigs that perfectly overlap on SSC1, indicating that the *brown* mutation in the tested breeds is derived from a common ancestor. The mapped intervals are unexpectedly large (~29 and 14 Mb). This is most likely caused by very strong linkage disequilibrium in tested animals. Chinese local breeds, including Tibetan, Kele, and Dahe pigs, have been endangered and their number and size have decreased dramatically in recent years resulting from the wide introduction of international breeds for commercial interests. A high level of inbreeding is hence expected to occur in these threatened breeds, leading to strong linkage disequilibrium and large mapped intervals in the GWAS.

Haplotype-sharing analysis further revealed a critical region of 1.5 Mb on SSC1 that encompasses only one pigmentation gene, *TYRP1*. We, therefore, investigated *TYRP1* variants in a collection of diverse pig breeds and identified a strong candidate causative mutation: c.1484_1489del. The deletion causes a lack of two amino acids in a predicted transmembrane domain of the protein and is likely of functional significance. Of the *TYRP1* functional variants affecting brown phenotypes in vertebrates, none affect the two amino acids. The protein-altering deletion showed complete association with the brown colouration across Tibetan, Kele, and Dahe breeds by occurring exclusively in brown pigs and lacking in all non-brown-coated pigs from 27 different breeds. The perfect association across breeds, together with high-resolution mapping of the responsible locus, positional characterization of the strong candidate gene, protein-altering nature, and concordant inheritance model of the mutation, collectively provide evidence of the causality of *TYRP1* c.1484_1489del for the brown coat colour phenotypes in Chinese breeds.

TYRP1 functional mutations usually show a recessive inheritance in vertebrates except for a dominant mutation causing the white-based brown in mice (Zdarsky *et al.*, 1990). Interestingly, the c.1484_1489del mutation is inherited in a codominant way. One explanation is that the mutation is a dominant-negative mutation, which interferes with the wild allele and consequently leads to a different phenotype in the heterozygote. Alternatively, the brown phenotype might be caused by *TYRP1* haploinsufficiency in Chinese pigs.

The causative mutation is probably of Asian origin and occurred after domestication, as determined by its absence in European breeds and wild pigs. It is not a recent mutation as brown-coated Dahe and Kele pigs have been reported as early as the seventeenth century (Zhang *et al.*, 1986). Black Tibetan pigs and dark brown Kele pigs have always fascinated their human owners, whereas yellow brown-coated Dahe pigs are generally favoured compared with silver brown individuals (Lian and Wu, 1985). A molecular test for the c.1484_1489del mutation will give breeders an efficient and accurate tool to breed for brown phenotypes or to select against them.

More than a single *TYRP1* mutation have been associated with various brown shades in mice (Jackson *et al.*, 1990; Zdarsky *et al.*, 1990; Javerzat and Jackson, 1998), cats (Lyons *et al.*, 2005; Schmidt-Kuntzel *et al.*, 2005), and dogs (Schmutz *et al.*, 2002). We hence expected *TYRP1* allele series for brown variants in Chinese pigs. Nevertheless, we did not identify other equivalently significant variants in the coding region of *TYRP1* gene

except for the common causative variant, c.1484_1489del. There are several possible explanations. The first is the involvement of epistatic effect of other coat colour genes. The interaction between *TYRP1* and melanocortin 1 receptor has been shown to be important in pigmentation in dogs (Schmutz *et al.*, 2002). However, we found that the epistasis interaction for *TYRP1* and melanocortin 1 receptor did not explain the brown shades in Kele and Dahe pigs (Supplementary Table S4). A further investigation on other pigmentation genes, including dilution genes, is worthwhile. The second possible explanation is minor polygenic or modifier gene effects leading to variations in pigment intensity.

In conclusion, we have identified the c.1484_1489del mutation in the *TYRP1* gene as the causative mutation for the brown colouration in Chinese indigenous pigs. This result provides the first direct evidence for the involvement of *TYRP1* in pigmentation of pigs and advances the understanding of the molecular basis of colour variation in pigs. Our study also provides a genetic testing tool to breed for or against brown coat colour in Chinese indigenous pigs.

Accession numbers

The coding sequences of *TYRP1* determined in this study have been submitted to GenBank with accession numbers GU345783—GU345801.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors would like to thank Zhizhong Li (the brown Tibetan pig owner), Professor Linsheng Lian (Yunnan Agricultural University) and Dr Junxun Wang (the Ministry of Agriculture of China) for their kind help in sample collection. The authors also thank Dr Carole Charlier at the University of Liège for valuable comments on positional candidate genes and Dr Leif Andersson at Uppsala University for critical comments on the manuscript. This work was funded by grants from National Natural Science Foundation of China (30425045 and 30871771) to Lusheng Huang and Jun Ren.

References

- Andersson L (2003). Melanocortin receptor variants with phenotypic effects in horse, pig, and chicken. *Ann N Y Acad Sci* **994**: 313–318.
- Andersson L (2009). Genome-wide association analysis in domestic animals: a powerful approach for genetic dissection of trait loci. *Genetica* **136**: 341–349.
- Barsh GS (1996). The genetics of pigmentation: from fancy genes to complex traits. *Trends Genet* **12**: 299–305.
- Berryere TG, Schmutz SM, Schimpf RJ, Cowan CM, Potter J (2003). *TYRP1* is associated with dun coat colour in Dexter cattle or how now brown cow? *Anim Genet* **34**: 169–175.
- Braasch I, Liedtke D, Volf J-N, Schartl M (2009). Pigmentary function and evolution of *tyrp1* gene duplicates in fish. *Pigment Cell Melanoma Res* **22**: 839–850.
- Drögemüller C, Giese A, Martins-Wess F, Wiedemann S, Andersson L, Brenig B *et al.* (2006). The mutation causing the black-and-tan pigmentation phenotype of Mangalitza

- pigs maps to the porcine *ASIP* locus but does not affect its coding sequence. *Mamm Genome* **17**: 58–66.
- Druet T, Georges M (2010). A hidden markov model combining linkage and linkage disequilibrium information for haplotype reconstruction and QTL fine mapping. *Genetics* **184**: 789–798.
- Fang MY, Larson G, Ribeiro HS, Li N, Andersson L (2009). Contrasting mode of evolution at a coat color locus in wild and domestic pigs. *PLoS Genet* **5**: e1000341.
- Giuffra E, Evans G, Tornsten A, Wales R, Day A, Looft H *et al.* (1999). The Belt mutation in pigs is an allele at the Dominant White (*I/KIT*) locus. *Mamm Genome* **10**: 1132–1136.
- Giuffra E, Törnsten A, Marklund S, Bongcam-Rudloff E, Chardon P, Kijas JM *et al.* (2002). A large duplication associated with Dominant White color in pigs originated by homologous recombination between LINE elements flanking *KIT*. *Mamm Genome* **13**: 569–577.
- Gratten J, Beraldi D, Lowder BV, Mcrae AF, Visscher PM, Pemberton JM *et al.* (2007). Compelling evidence that a single nucleotide substitution in *TYRP1* is responsible for coat colour polymorphism in a free-living population of Soay sheep. *Proc Biol Sci B* **274**: 619–626.
- Jackson IJ, Chambers D, Rinchik EM, Bennett DC (1990). Characterization of *TRP-1* mRNA levels in dominant and recessive mutations at the mouse brown (*b*) locus. *Genetics* **126**: 45–459.
- Jackson IJ (1994). Molecular and developmental genetics of mouse coat color. *Annu Rev Genet* **28**: 189–217.
- Javerzat S, Jackson IJ (1998). White-based brown (*Tyrrp1^{B-w}*) is a dominant mutation causing reduced hair pigmentation owing to a chromosomal inversion. *Mamm Genome* **9**: 469–471.
- Johansson Moller M, Chaudhary R, Hellmen E, Hoyheim B, Chowdhary B, Andersson L (1996). Pigs with the dominant white coat color phenotype carry a duplication of the *KIT* gene encoding the mast/stem cell growth factor receptor. *Mamm Genome* **7**: 822–830.
- Kijas JM, Wales R, Tornsten A, Chardon P, Moller M, Andersson L (1998). Melanocortin receptor 1 (*MC1R*) mutations and coat color in pigs. *Genetics* **150**: 1177–1185.
- Kijas JM, Moller M, Plastow G, Andersson L (2001). A frameshift mutation in *MC1R* and a high frequency of somatic reversions cause black spotting in pigs. *Genetics* **158**: 779–785.
- Lai F, Ren J, Ai H, Ding N, Ma J, Zeng DQ *et al.* (2007). Chinese white Rongchang pig does not have the dominant white allele of *KIT* but has the dominant black allele of *MC1R*. *J Hered* **98**: 84–87.
- Lian LL, Wu ZD (1985). A study on the coat color inheritance of Dehe pig. *Chinese J Anim Sci* **21**: 10–12.
- Lyons LA, Foe IT, Rah HC, Grahn RA (2005). Chocolate coated cats: *TYRP1* mutations for brown color in domestic cats. *Mamm Genome* **16**: 356–366.
- Mao HR, Ren J, Ding NS, Xiao SJ, Huang LS (2010). Genetic variation within coat color genes of *MC1R* and *ASIP* in Chinese brownish red Tibetan pigs. *Anim Sci J* (e-pub ahead of print; doi:10.1111/j.1740-0929.2010.00789.x).
- Marklund S, Kijas J, Rodriguez-Martinez H, Ronnstrand L, Funa K, Moller M *et al.* (1998). Molecular basis for the dominant white phenotype in the domestic pig. *Genome Res* **8**: 826–833.
- Nadeau NJ, Mundy NI, Gourichon D, Minvielle F (2007). Association of a single-nucleotide substitution in *TYRP1* with roux in Japanese quail (*Coturnix japonica*). *Anim Genet* **38**: 609–613.
- Pielberg G, Olsson C, Syvanen AC, Andersson L (2002). Unexpectedly high allelic diversity at the *KIT* locus causing dominant white color in the domestic pig. *Genetics* **160**: 305–311.
- Ramos AM, Crooijmans RPMA, Affara NA, Amaral AJ, Archibald AL, Beever JE *et al.* (2009). Design of a high density SNP genotyping assay in the pig using SNPs identified and characterized by next generation sequencing technology. *PLoS ONE* **4**: e6524.
- Sarangarajan R, Boissy RE (2001). *Tyrrp1* and oculocutaneous albinism type 3. *Pigment. Cell Res* **14**: 437–444.
- Schmidt-Kuntzel A, Eizirik E, O'Brien SJ, Menotti-Raymond M (2005). *Tyrosinase* and *tyrosinase related protein 1* alleles specify domestic cat coat color phenotypes of the *albino* and *brown* loci. *J Hered* **96**: 289–301.
- Schmutz SM, Berryere TG, Goldfinch AD (2002). *TYRP1* and *MC1R* genotypes and their effects on coat color in dogs. *Mamm Genome* **13**: 380–387.
- Searle AG (1968). *Comparative Genetics of Coat Colour in Mammals*. Academic Press: New York.
- Silvers WK (1979). *The Coat Colors of Mice: A Model for Mammalian Gene Action and Interaction*. Springer-Verlag: New York.
- Xu GL, Ren J, Ding NS, Ai HS, Guo YM, Chen CY *et al.* (2006). Genetic analysis of the *KIT* and *MC1R* genes in Chinese indigenous pigs with belt-like coat color phenotypes. *Anim Genet* **37**: 518–519.
- Zdarsky E, Favor J, Jackson IJ (1990). The molecular basis of brown, an old mouse mutation, and of an induced revertant to wild type. *Genetics* **126**: 443–449.
- Zhang ZG, Li BT, Chen XH (1986). *Pig Breeds in China*. Shanghai Scientific and Technical Publisher: Shanghai, China.

Supplementary Information accompanies the paper on Heredity website (<http://www.nature.com/hdy>)