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Research Report

Variations of Melanocortin Receptor 1 (*MC1R*) Gene in Three Pig Breeds

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Abstract: Variations of Melanocortin Receptor 1 (*MC1R*) were investigated using sequencing, PCR-RFLP and PCR-SSCP, in three pig breeds, Landrace, Yorkshire, and Duroc. Five polymorphic sites were found, in which 668G→C occurred within 5′ UTR, nt894insCC in coding region resulting in a premature stop at codon 56, and 1318C→T, 1554G→A, 1197G→A in coding region resulting in Ala164Val, Ala243Thr, and Asp124Asn respectively. All individuals in Landrace and Yorkshire present homozygous 668*GG*, 1197*AA*, 1318*CC*, and 1554*GG*, and have CC insertions at the 894 site, whereas the individuals in Duroc present a contrast homozygous 668*CC*, 1197*GG*, 1318*TT*, and 1554*AA*, and have no CC insertions at the corresponding site. No heterozygote has been found at these mutation sites. Presumably, 668G→C, 1318C→T, and 1554G→A may be associated with the recessive red color in the Duroc breed, and nt894insCC making 1197G→A nonsense may be associated with the white color in Landrace and Yorkshire breeds.

Keywords: pig; *MC1R* gene; variation; coat color

Melanocortin Receptor 1 (*MC1R*) encoded by *Extension* (*E*) coat color locus is a G-protein coupled receptor, and MC1R signaling determines that melanocytes produce black eumelanin or red/yellow pheomelanin. Thus, *MC1R* gene is considered as one of the animals' candidate genes for the research of coat, hair, and skin color variation. It has been reported that *MC1R* mutations alter pigment synthesis and are associated with hair, skin, and coat color in some species [1−12]. Highly polymorphic sites have been observed and over 30 variant alleles have been found in Caucasian populations from the British Isles, Holland, and Australia [1−3]. In these populations, Arg151Cys, Arg160Trp, and Asp294His (*RHC* alleles) cause loss of MC1R function and are associated with red hair and fair skin, whereas, few *MC1R* coding region variations are observed in African population [4, 5]. In mice, four mutant alleles associated with coat color have been found, including Ser69Leu ($MC1R^{tob}$), Glu92Lys ($MC1R^{so-3J}$), and Leu98Pro ($MC1R^{so}$), resulting in dominant black, and the deletion of one nucleotide at codon 183 (frameshift mutation), resulting in recessive yellow (*RHC*) [6]. Dominant black coat color in sheep is predicted to be caused by an allele E^D (Asp121Asn) of *MC1R* [7] and the substitution of Lys226Glu of the goat *MC1R* gene is associated with the red head and neck of the Boer goat [8]. Meanwhile, the dominant extension allele E^D [9] and the recessive red allele e [10, 11] in cattle and a single missense mutation (Ser83Phe) in the *MC1R* allele associated with the chestnut color in horses [12] have also been investigated.

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A series of alleles of the *MC1R* gene have been found in pigs. Kijas *et al.*[13, 14] reported that six allelic variants corresponded to five different *E* alleles, wild boars (E^+/E^+) corresponding to *MC1R*1* or *MC1R*5*, Large Black and Black Meishan pig (E^{D1}/E^{D1}) corresponding to *MC1R*2* (Leu99Pro and Val91His), Hampshire (E^{D2}/E^{D2}) corresponding to *MC1R*3* (Asp121Asn), Landrace, Yorkshire, and Pietrain (E^P/E^P) corresponding to *MC1R*6* (nt67insCC) shared together with Asp124Asn, Duroc (*e/e*) corresponding to *MC1R*4* (Ala160Val and Ala240Thr). Deng *et al.*[15] analyzed genotypes at the *MC1R* locus among individuals from 16 full-sib pedigrees and six Chinese native breeds. It is reported that the Chinese native pig breeds carry a dominant black allele at *MC1R* at a high frequency and the result of pedigree analysis has reconfirmed that E^{D1} is dominant over E^P and *e*, whereas, E^P is incompletely dominant over *e* [15].

However, there are some different reports about E^P and *e* alleles in some pig breeds [13]. Landrace and Yorkshire breeds (AY365253 and AY365255) present nt67insCCC rather than nt67insCC. Duroc (AY916524) does not have Ala160Val and Duroc (AY916523) does not have Ala240Thr. In this study, variations of nt67insCC, Ala160Val, and Ala240Thr correspond to nt894insCC, Ala164Val, and Ala243Thr, respectively. Given these different reports, three pig breeds, Landrace, Yorkshire, and Duroc were selected to screen the polymorphism of the coding region and 5′UTR of the *MC1R* gene, to explore new variation sites and better evaluate the relationship between the *MC1R* gene variations and pig coat color.

1 Materials and Methods

1.1 Samples and DNA extraction

A total of 152 pig samples including 60 Landrace, 60 Yorkshire, and 32 Duroc from Tianjin and Shijiazhuang Pig Breeding Farm were investigated. DNA was extracted from the ears using the phenol extraction method.

1.2 Primer design, PCR amplification and sequencing

Five pairs of primers targeting 5′ UTR and the encoding region were designed, using Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) according to pig AF326520 (Table 1).

PCR reactions were carried out in a Biometra personal PCR instrument with a total volume of 20 μL containing 100 ng of pig genomic DNA, 2.0 μL of 10×PCR standard reaction buffer, 0.2 mmol/L dNTPs, 0.4 mmol/L of each primer, 0.5 units *Taq* DNA polymerase (Tiangen Biotechnology Co. Ltd., Beijing, China), and 13.6 μL distilled water. Five percent formamide and dimethylsulfoxide (DMSO) were added into the reaction solution of Primers 1 and 2 respectively, to achieve a better outcome. After pre-denaturation for 6 min at 94℃, the PCR profile consisted of a denaturation step at 94℃ for 30 s, an annealing step at Ta in Table 1 for different pairs of

Table 1 Primers used to amplify regions of pig *MC1R* gene and corresponding analysis methods

Primer	Primer sequences (5′→3′)	Length and region	Ta (℃)	Methods
Primer 1	Pf : GCAGGGGTGTCTCTGTGTC Pr: GAGTGCAGGTTGCGGTTCT	803 bp (246 – 1048)	60	*Bbv*12Ⅰ (668G→C)
Primer 2	Pf: GGCTGCTGGCTTCCCTCA Pr : GGTTGCGGTTCTTGGCGA	189 bp (853 – 1041)	60	PCR-SSCP (nt894insCC)
Primer 3	Pf : TCGCCAAGAACCGCAACC Pr : GCGCAGCGCGATGAAGAT	266 bp (1024 – 1289)	57	*Pag*Ⅰ (1197G→A)
Primer 4	Pf : GACCGCTACGTGTCCATCTT Pr: TGTGGTGGTAGTAGGCGATG	136 bp (1257 – 1392)	58	PCR-SSCP (1318C→T)
Primer 5	Pf : ACCCTCTTCATCGCCTAC Pr: AGAGGTGCAGGAAGAAGG	256 bp (1365 – 1620)	56	*Bst*FNⅠ (1554G→A)

Pf: the forward primer; Pr: the reverse primer.

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