resequencing data[5] revealed 13 SNPs located in *NR6A1* and *PLAG1*, most of which belong to breed-specific haplotypes. For each region we selected a tag SNP (Table S1). The insertion variant of *VRTN* was adopted from a previous study.[3] PCR-RFLP genotypes were scored on a 1.5% agarose gel (Table S2). The association between the markers and the number of ribs was tested with a single-factor analysis of variance using R software and with multiple-marker analysis of gene combinations.[6]

The association analysis revealed that SNP chr1:299291323C>T, located in the second intron of *NR6A1*; SNP chr4:82405777G>T, located 5′ upstream of *PLAG1* and the insertion g.20311_20312ins291 in *VRTN* were significantly associated with the number of vertebrae in the Tongcheng × Large White crossbreed population (Table 1), and *NR6A1* TT, *PLAG* GG and *VRTN* insertion genotypes were linked to a higher number of ribs. Joint analysis showed that the three loci act independently, so co-selection of the alleles with the strongest effects in *VRTN* and *NR6A1* may significantly increase the number of ribs (Fig. S1).

Our study confirmed that *NR6A1*, *PLAG1* and *VRTN* are significantly associated with the number of vertebrae and provide candidate SNPs as molecular markers for selection of the rib number trait in Tongcheng × Large White crossbred pigs.

Acknowledgement: This study was supported by the National Key Technology R&D Program (2015BAD03B02).

References

1 Borchers N. *et al.* (2004) *J Anim Breed Genet* **121**, 392–403.
2 Rubin C.-J. *et al.* (2012) *P Natl Acad Sci USA* **109**, 19529–36.
3 Ren D.R. *et al.* (2012) *Anim Genet* **43**, 545–51.
4 Fan Y. *et al.* (2013) *PLoS One* **8**, e62534.
5 Wang C. *et al.* (2015) *Mol Ecol Resour* **15**, 414–24.
6 Jiang Z.H. *et al.* (2009) *Int J Biol Sci* **5**, 528–42.

Correspondence: B. Liu (liubang@mail.hzau.edu.cn)

Supporting information

Additional supporting information may be found online in the supporting information tab for this article:
Figure S1 Co-selection of VRTN and NR6A1 could significantly increase the number of ribs.
Table S1 Allele frequency in Tongcheng and Large White pigs on candidate SNP loci.
Table S2 Polymorphic SNPs markers, PCR primers, restriction enzymes and genotyping.

doi: 10.1111/age.12683

Conserved aspartate-to-glycine mutation in tyrosinase is associated with albino phenotype in domestic guinea pigs (*Cavia porcellus*)

Feng Yu*[1], Shuyu Jiao*[1], Weining Lai*, Zhengxi Liu*, Mingyuan Zhu*, Wanju Zhu†, Chunyan Bai*, Yonghong Zhang*, Jiabao Zhang* and Shouqing Yan*

*College of Animal Science, Jilin University, Changchun 130062, China; †College of Veterinary Medicine, Jilin University, Changchun 130062, China

Accepted for publication 19 April 2018

Coat colours and patterns are highly variable in domestic guinea pigs (*Cavia porcellus*) due to some series of allelomorphic factors.[1] The albino phenotype in guinea pigs, which lack melanin pigment in the skin, hair and eyes, is inherited in an autosomal recessive manner.[2] Here, we analysed the full-length coding sequences of the *tyrosinase* (*TYR*) gene as a strong candidate and identified a missense mutation associated with the albino phenotype in guinea pigs.

Hair samples were collected from a total of 194 guinea pigs including 135 coloured (52 wild type and 83 spotted) and 59 albino individuals (Fig. 1). Genomic DNA was extracted from hair roots using an EasyPure Micro Genomic DNA Kit (TransGen Biotech). The coding and partial flanking sequence of the *TYR* gene from four unrelated albino and four coloured guinea pigs was amplified and directly sequenced using five pairs of primers designed according to GenBank accession no. NT_176379 (Table 1). A missense mutation (c.710A>G, p.Asp237Gly) located in exon 1 was identified between coloured and albino individuals (Fig. S1). All coloured individuals were homozygous AA, whereas the albino guinea pigs were homozygous GG. The data were submitted to the European Variation Archive (accession no. PRJEB26285).

Based on the amplification-created restriction site method,[3] a pair of primers for the PCR-RFLP test was designed to determine the genotype of 154 randomly selected individuals (Table 1, Fig. S2). Genotyping results revealed that 82 out of 114 coloured individuals were homozygous AA and the others were heterozygous AG, whereas all 40 albino individuals were homozygous GG. In addition, genotyping was carried out in 32 F_1 progenies including 17 coloured and 15 albino individuals from four families by mating with the heterozygous AG and homozygous GG individuals. Results revealed that the SNP showed perfect co-segregation with the albino phenotype and supported the autosomal recessive mode of inheritance of albinism in guinea pigs (Fig. S3).

[1]These authors contributed equally to this work.

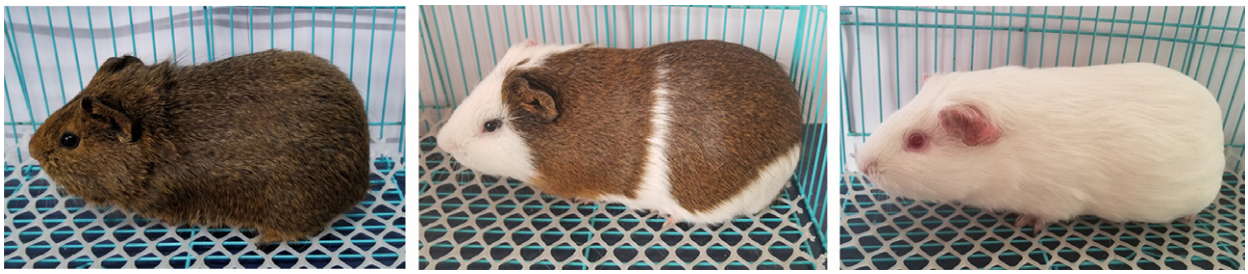

Figure 1 Coat colour phenotypes of adult wild-type (left), spotted (middle) and albino (right) domestic guinea pigs.

Table 1 Primers used for PCR amplification and genotyping of the *TYR* gene in the present study.

Primer name	Primer sequence (5′→ 3′)	Product size (bp)	Application
TYR-Ex1-F	GAAAAAGAAGTCAGCGACTCCAAT	951	Exon 1 amplification
TYR-Ex1-R	GGTAGAGACCTGCCTGAAGAAGTG		
TYR-Ex2-F	GACATTTCCTTCAGAGGTAGGTATTA	586	Exon 2 amplification
TYR-Ex2-R	AATGTGCTGGATTCAGTGTGTTAC		
TYR-Ex3-F	AACACAACTCATTTCCACCAAGAC	523	Exon 3 amplification
TYR-Ex3-R	TTCATGCTGAATCCTACCAACTG		
TYR-Ex4-F	TTCGTGTGTCCTTCAAATGAGTGTA	527	Exon 4 amplification
TYR-Ex4-R	TCAAAGGTTGTACTACACATTCCCAG		
TYR-Ex5-F	CAACCACAGAGTATCATTTTCCT	601	Exon 5 amplification
TYR-Ex5-R	CTGTGTTCTACTTGGGTGTCTGTG		
TYR-Ex1-F2	CTCGGGGGATCTGAAATCTGG	176	PCR-RFLP
TYR-Ex1-R2	CAAATCTCACAGTTTTCCGCGTCCCTCCGG[1]		

[1]The naturally-occurring 'A' nucleotide was substituted with a 'G' nucleotide (underlined) in order to introduce a recognition site for the Hae III restriction endonuclease.

Alignment of amino acid sequences of TYR in guinea pig and 227 other vertebrates available in GenBank revealed that the p.Asp237Gly variant is located in a strictly conserved region, which implies Asp237 may be very crucial for TYR function and that the substitution will disrupt its function (Fig. S4). Missense mutations of *TYR* responsible for albinism have also been identified in other species such as humans, rabbits and donkeys.[4–6] Based on these results, we conclude that the missense mutation (c.710A>G; p.Asp237Gly) in the *TYR* gene is associated with the albino phenotype in guinea pigs.

Acknowledgement: This study was supported by the National Natural Science Foundation of China (No. 31601004).

References

1 Wright S. (1927) *Genetics* **12**, 530–69.
2 Wright S. (1959) *Genetics* **44**, 563–90.
3 Amoli M. *et al.* (2000) *Genes Immun* **1**, 349–50.
4 Balu K. & Purohit R. (2013) *Gene* **513**, 184–95.
5 Aigner B. *et al.* (2000) *Mamm Genome* **11**, 700–2.
6 Utzeri V.J. *et al.* (2016) *Anim Genet* **47**, 120–4.

Correspondence: S. Yan (yansq@jlu.edu.cn)

Supporting information

Additional supporting information may be found online in the supporting information tab for this article:

Figure S1 Electropherogram representing the c.710A>G polymorphism in exon 1 of the *TYR* gene from the coloured (top) and albino (bottom) individuals.

Figure S2 Agarose gel electrophoresis showing the different genotypes detected by PCR-RFLP at the c.710A>G locus. The digestion of the A allele with Hae III yielded an uncut band of 176 bp, whereas the G allele yielded two bands of 146 and 30 bp that were not visible on the gel; M, DL500 molecular marker.

Figure S3 Pedigree analysis of the c.710A>G polymorphism of *TYR* in four families. Coloured animals are shown as solid symbols, albino phenotype individuals as open symbols.

Figure S4 Alignment of the TYR protein sequence containing the p.Asp237Gly substitution in guinea pigs with the corresponding region in other species.