Analysis of the KIT gene in a Sicilian pig population and identification of the Id allele at the Dominant white locus

Fontanesi L., D'Alessandro E., Scotti E., Liotta L., Chiofalo V., Russo V.

in

De Pedro E.J. (ed.), Cabezas A.B. (ed.).
7th International Symposium on the Mediterranean Pig

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 101

2012
pages 25-29

To cite this article / Pour citer cet article

Fontanesi L., D'Alessandro E., Scotti E., Liotta L., Chiofalo V., Russo V. **Analysis of the KIT gene in a Sicilian pig population and identification of the Id allele at the Dominant white locus.** In : De Pedro E.J. (ed.), Cabezas A.B. (ed.). *7th International Symposium on the Mediterranean Pig.* Zaragoza : CIHEAM, 2012. p. 25-29 (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 101)

Analysis of the *KIT* gene in a Sicilian pig population and identification of the *I^d* allele at the *Dominant white* locus

L. Fontanesi*, E. D'Alessandro, E. Scotti*, L. Liotta**, V. Chiofalo** and V. Russo***
*Dipartimento di Protezione e Valorizzazione Agroalimentare, Sezione di Allevamenti Zootecnici, University of Bologna (Italy)
**Dipartimento di Morfologia, Biochimica, Fisiologia e Produzioni Animali, Sezione di Zootecnica e Nutrizione Animale, University of Messina (Italy)

Abstract. Mutations in the porcine *KIT* gene (*Dominant white* locus) have been shown to affect coat colors and color distribution in pigs. Classical genetic studies have indicated that the gray/roan coat colour observed in different pigs is determined by an allele (I^d) at the *Dominant white* locus. In a survey of the pig populations of the Nebrodi mountain area (Sicily), we identified a few animals showing morphological characteristics resembling the Nero Siciliano genetic type but with gray/roan (determined by a close intermingling of white and black hairs) instead of black hair, having, in several cases, depigmented skin areas. According to the data we obtained on crosses between gray/roan and black pigs, the mode of inheritance of the roan phenotype is due to a dominant allele over black. All 21 exons and intronic regions of the *KIT* gene were sequenced in gray/roan and in several other pigs. The gray/roan haplotype carried a 4 bp deletion in intron 18 but did not include any duplication of the *KIT* gene. Analysing polymorphic site co-segregation with the gray/roan phenotype provided evidence that this *KIT* allele may identify the I^d allele described in the early genetic literature.

Keywords. Coat colour – *Dominant white* locus – KIT – DNA polymorphisms – Roan.

Analyse du gène KIT et identification de l'allèle I^d du locus blanc dominant au sein de la population porcine en Sicile

***Résumé**. Les mutations au niveau du gène KIT (locus blanc dominant) affectent la couleur de la robe et la répartition des pigments chez le porc. Les études de la génétique classique suggèrent que le phénotype gris/rouan de la robe chez le porc est déterminé par un allèle (I^d) du locus blanc dominant. Lors d'une évaluation de ce patron de coloration au sein de populations porcines dans les régions montagneuses de Nebrodi (Sicile) nous avons observé que certains animaux présentant des caractéristiques morphologiques similaires à ceux de la race Nero Siciliano possèdent une robe grise/rouanne (mixture de poils blancs et noirs) plutôt qu'une robe noire. Par ailleurs, certains d'entre eux présentent des régions cutanées dépigmentées. Le croisement entre des porcs gris/rouans et des porcs noirs révèle que l'allèle rouan est dominant sur l'allèle noir. Nous avons séquencé les 21 exons et les régions introniques du gène KIT chez plusieurs animaux gris/rouans mais aussi chez d'autres animaux porcins. Les animaux de phénotype gris/rouan présentent une délétion de 4 pb au niveau de l'intron 18 sans aucune duplication du gène KIT. Cet allèle du gène KIT co-ségrège avec le phénotype gris/rouan et, par conséquent, correspondrait à l'allèle I^d précédemment décrit par l'analyse génétique.*

***Mots-clés.** Couleur de la robe – Locus* Dominant white *– KIT – Polymorphismes de l'ADN – Rouan.*

I – Introduction

Several studies on coat colour genetics in pigs have identified that mutations in the melanocortin 1 receptor (*MC1R*) and v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (*KIT*) genes are the major determinants of the variability of this phenotypic trait among and within different pig breeds (Kijas *et al.*, 1998; Marklund *et al.*, 1998; Giuffra *et al.*, 1999;

Kijas *et al.*, 2001; Johansson *et al.*, 2005; Fang *et al.*, 2009; Fontanesi *et al.*, 2010a). Epistatic interactions between these two loci have been reported in QTL analysis for this trait (Hirooka *et al.*, 2002). The *KIT* gene encodes the mast/stem cell growth factor receptor that is involved in driving the melanocyte migration from the neural crest along the dorsolateral pathway to colonize the final destination in the skin (Besmer *et al.*, 1993).

A large number of *KIT* mutations have been reported to cause pigmentation anomalies in several species (i.e. Jackson, 1997). In cattle, selection signature at the *KIT* gene is evident in a few breeds in which polymorphisms are associated with the spotting phenotype (Fontanesi *et al.*, *2*010b). In pigs, the *KIT* gene maps on chromosome 8 and causes the *Dominant white* (*I*) locus (Johansson *et al.*, 1992). An unusual large number of alleles, each with different or partially overlapping phenotypic effects, have been characterized at this locus (Johansson Moller *et al.* 1996; Marklund *et al.*, 1998; Giuffra *et al.*, 1999; Giuffra *et al.*, 2002; Pielberg *et al.*, 2002; Johansson *et al.*, 2005): the recessive wild type allele (*i*) is usually associated with a solid or wild type coat color; the patch allele (I^P) causes the presence of colored patches on a white background (Johansson Moller *et al.*, 1996); the *Belt* allele (I^{Be}) determines the white belt observed in the Hampshire and Cinta Senese breeds (Giuffra *et al.*, 1999; Fontanesi *et al.*, 2010a); four *Dominant white* alleles (I^1, I^2, I^3 and I^L) determine white uniform colored pigs, even if in few cases black spots have been reported in animals carrying some of these forms (Pielberg *et al.*, 2002; Johansson *et al.*, 2005). A few of these alleles (*i*, I^{Be} and I^L) have a single copy of the *KIT* gene whereas all other alleles are determined by copy number variation (CNV) of this gene with a duplication or triplication of a region including the whole coding sequence, encompassing 21 exons, and flanking regions (Giuffra *et al.*, 2002; Pielberg *et al.*, 2002). In addition to the gene duplication, the I^1 allele carries a splice mutation in one of the two copies at the level of intron 17 (causing the skipping of exon 17, predicted to impair an important function of the coded protein) in linkage disequilibrium with a 4 bp deletion in intron 18 (*intron18-g.29_32delAGTT*) (Marklund *et al.*, 1998; Giuffra *et al.*, 2002). In the I^2 and I^3 alleles the splice mutation is in one or two copies of the three-copies gene forms, respectively (Pielberg *et al.*, 2002).

It seems possible that other alleles with a larger number of copies and combinations with the splice mutation are present in white pig populations (Johansson *et al.* 2005; our unpublished data). The I^P allele lacks the splice mutation present in the other duplicated alleles (Johansson Moller *et al.*, 1996). The I^L allele contains a single mutated copy of the *KIT* gene (with the splice mutation) and might be lethal if homozygous (Pielberg *et al.*, 2002; Johansson *et al.*, 2005). The I^{Be} allele is probably caused by a not yet characterized regulatory mutation (Giuffra *et al.*, 1999). In addition to this complex allele series, earlier studies on pig coat color indicated the presence of an additional allele (I^d), not characterized yet at the molecular level, giving a gray-roan phenotype and dominant over the *i* allele (Hetzer, 1948; Lauvergne and Canope, 1979).

Nero Siciliano breed (Sicilian Black) is reared mainly under extensive management in the Nebrodi and Madonie mountains of the provinces of Messina and Palermo on the island of Sicily. This is a local population whose genetic pool has been shaped by subsequent introduction of blood from other local or cosmopolitan populations and breeds. In addition to the completely black animals, Nero Siciliano population includes pigs with white portions, mainly in the head and legs. Recently, in a survey of the pig populations of the Nebrodi mountain, we identified several animals showing morphological characteristics resembling the Nero Siciliano genetic type but with gray/roan instead of black hair, having almost always some pigmented skin areas and dark/black hair in the head. This coat color phenotype resembles the gray/roan coat color described in the early literature (McLean, 1914; Hetzer, 1948; Lauvergne and Canope, 1979), assumed to be caused by the I^d allele at the *Dominant white* locus.

In this study we characterized by sequencing and/or genotyping the *KIT* and *MC1R* genes of these pigs, compared these data with those we obtained for other breeds and provided evidences that the *KIT* allele in the gray/roan animals should correspond to the I^d allele.

II – Materials and methods

Animals investigated in this study were 42 gray/roan pigs sampled from 2 different farms in Sicily (14 and 28 respectively) (D'Alessandro *et al.*, 2007; Fontanesi *et al.*, 2010). Twenty-one of these gray pigs, derived from one farm, were obtained by crossing 7 Nero Siciliano black sows with one gray boar. Eighteen completely black pigs were obtained from the same litters. For the other gray pigs pedigree information was not available. Of the 42 gray hair pigs, almost all (38) presented pigmented skin areas of variable extension. Blood or hair root samples were collected from these pigs and DNA was extracted using standard protocols. DNA samples were also available for other pigs used for sequencing (see below) (D'Alessandro *et al.*, 2007; Fontanesi *et al.*, 2010).

Mutations in the *MC1R* and *KIT* genes were analysed by PCR-RFLP or amplified fragment analysis as previously reported (Kijas *et al.*, 1998; Marklund *et al.*, 1998; Giuffra *et al.*, 2002; Kijas *et al.*, 2002; D'Alessandro *et al.*, 2007; Fontanesi *et al.*, 2010a).

The complete coding region (spanning 21 exons) and partial upstream and downstream intronic regions of the *KIT* gene was sequenced in pigs of different breeds and coat colour (Fontanesi *et al.*, 2010a): 10 Nero Siciliano pigs, 4 with completely black coat color, 4 with white spots but without duplication of the *KIT* gene, 2 with white spots but with the duplication of the *KIT* gene; 3 gray animals (one was homozygous for intron 18 deletion and two were heterozygous at this polymorphic site); 6 Cinta Senese; 4 Hampshire; 4 Duroc, 2 Italian Large White, one carrying the splice mutation and one not carrying this mutation; 2 Pietrain, one positive and one negative at the duplication test; 2 Meishan; 2 wild boars. Polymorphisms were detected using CodonCode Aligner (http://www.codoncode.com/aligner) with the AC141857 sequence (pig genomic sequence derived from clone RP44-473N18 containing the 21 *KIT* exons) included as a reference (Fontanesi *et al.*, 2010a).

Calculation of the *LOD* score in the half sibling family structure was obtained with the LODS program (Linkage Utility Programs, Rockefeller University). Polymorphisms determined by sequencing of the *KIT* gene were used to reconstruct haplotypes with PHASE program v. 2.1.1 (Stephens *et al.*, 2001). Phylogenetic analyses of the identified haplotypes of the *KIT* gene were conducted with MEGA4 software v. 4.0.2 (Tamura *et al.*, 2007) with Kimura's two parameter model and Neighbour Joining (NJ) trees using sequence information and polymorphisms identified in the coding regions including also other porcine *KIT* cDNA sequences available in GenBank database. Standard errors were obtained from 1000 bootstrap replicates.

III – Results and discussion

Genotyping the grey/roan pigs for two polymorphisms of the *KIT* gene (*intron18-g.29_32delAGTT* and duplication test; Johansson Moller *et al.*, 1996; Giuffra *et al.*, 2002) unusual results were obtained. Interestingly, all these animals carried at least one *KIT* allele with the deletion of 4 bp in intron 18 (*intron18-g.29_32delAGTT*), typical of one of the two (or three) *KIT* copies of the I^1, I^2 or I^3 alleles of the *Dominant white* locus. However, no duplication of the *KIT* gene was observed in these animals, that means CNV was not present in the *KIT* alleles of these pigs. Pedigree relationships recorded in one of the two farms demonstrated that 21 gray/roan pigs (heterozygous for the 4 bp deletion of intron 18) derived from 7 litters obtained crossing a gray/roan boar (heterozygous for the *KIT intron18-g.29_32delAGTT* allele) with 7 different black Nero Siciliano sows (that did not carry the 4 bp deletion of intron 18)

(Fontanesi *et al.*, 2010a). Eighteen other pigs of the same litters were completely black. These black piglets did not carry the 4 bp deleted allele. Considering all these litters, no deviation from the classical 1:1 Mendelian ratio of the two coat color phenotypes (gray/roan and black) was evident, suggesting dominance of the gray/roan phenotype (associated with the *KIT intron18-g.29_32delAGTT* allele) over the colored (wild type) *KIT* allele(s) of the black sows. Association between the *KIT intron18-g.29_32delAGTT* allele (in heterozygous condition) and gray coat color in these 7 litters was highly significant (θ = 0.00; *LOD* = 11.74). All these animals were also genotyped for mutations in the *MC1R* gene. Gray/roan piglets of these litters had several genotypes (E^{D2}/E^{D2}, E^{D2}/E^{P}, or E^{P}/E^{P}) indicating that the genotype at the *MC1R* locus did not affect coat color of the gray animals (skin areas were pigmented in all three genotypes). It is also worth to mention that the wild boar *MC1R* allele (E^{+}), that should give a wild gray coat color, was not observed in these animals (Fontanesi *et al.*, 2010a).

A total of 6.7 kb of the *KIT* gene, sequenced in 35 pigs from 9 breeds and populations, provided a total of 69 polymorphisms, including 65 single nucleotide polymorphisms (SNPs) and 4 indels (Fontanesi *et al.*, 2010a). These data were used for phylogenetic analysis of the reconstructed haplotypes obtained for the different sequenced pigs (data not shown). Results indicated that the gray/roan haplotype was very close to the wild boar haplotype, suggesting that it might have been originated from a wild type sequence.

IV – Conclusions

This study provided evidences that the *KIT intron18-g.29_32delAGTT* mutation (but in a single copy *KIT* gene) characterizes the I^{d} allele at the *Dominant white* locus that was already described in the early pig coat color genetic literature. Considering the structure of the gray/roan *KIT* haplotype and that of the other *Dominant white KIT* alleles, it could be possible to reconstruct the mutational events that contributed to create the large heterogeneity observed at the *Dominant white* locus. The I^{d} allele might be one of the two copies of the I^{P} allele that was originated from a first ancestral duplication at this locus. Then, another mutational event at the level of intron 17 (splice site mutation) occurring at this putative I^{d} form in a duplicated allele could have created the I^{1} allele. Subsequent unequal crossing over events might have originated other alleles with more that two duplicated *KIT* genes. The example of the pig *Dominant white* locus could indicate that CNVs might be important source of genetic variability in livestock affecting phenotypic and , possibly, production traits.

Acknowledgments

This work was supported by MiPAAF Selmol (LF), University of Bologna RFO (LF, VR) and Regione Sicilia (VC) funds.

References

Besmer P., Manova K., Duttlinger R., Huang E.J., Packer A., Gyssler C. and Bachvarova R.F., 1993. The kit-ligand (steel factor) and its receptor c-kit/W: pleiotropic roles in gametogenesis and melanogenesis. In *Development,* (S1): 125-137.

D'Alessandro E., Fontanesi L., Liotta L., Davoli R., Chiofalo V. and Russo V., 2007. Analysis of the *MC1R* gene in the Nero Siciliano pig breed and usefulness of this locus for breed traceability. In *Veterinary Research Communication*, 31 (S1): 389-392.

Fang M., Larson G., Ribeiro H.S., Li N. and Andersson L., 2009. Contrasting mode of evolution at a coat color locus in wild and domestic pigs. In *PLoS Genetics,* 5, e1000341.

Fontanesi L., D'Alessandro E., Scotti E., Liotta L., Crovetti A., Chiofalo V. and Russo V., 2010a. Genetic heterogeneity and selection signature at the *KIT* gene in pigs showing different coat colours and patterns. In: Animal Genetics, 41, 478-492.

Fontanesi L., Tazzoli M., Russo V. ans Beever J., 2010b. Genetic heterogeneity at the bovine *KIT* gene in cattle breeds carrying different putative alleles at the *spotting* locus. In Animal Genetics, 41: 295-303.

Giuffra E., Evans G., Törnsten A., Wales R., Day A., Looft H., Plastow G. and Andersson L., 1999. The *Belt* mutation in pigs is an allele at the *Dominant white (I/KIT)* locus. In *Mammalian Genome*, 10: 1132-1136.

Giuffra E., Törnsten A., Marklund S., Bongcam-Rudloff E., Chardon P., Kijas J.M., Anderson S.I., Archibald A.L. and Andersson L., 2002. A large duplication associated with dominant white color in pigs originated by homologous recombination between LINE elements flanking *KIT*. In *Mammalian Genome*, 13: 569-577.

Hetzer H.O., 1948. Inheritance of coat color in swine. VII. Results of Landrace by Hampshire crosses. In *Journal of Heredity*, 39: 123-128.

Hirooka H., de Koning D.J., van Arendonk J.A.M., Harlizius B., de Groot P.N. and Bovenhuis H., 2002. Genome scan reveals new coat color loci in exotic pig cross. In *Journal of Heredity,* 93: 1-8.

Jackson I.J., 1997. Homologous pigmentation mutations in human, mouse and other model organisms. In *Human Molecular Genetics*, 6: 1613-1624.

Johansson A., Pielberg G., Andersson L. and Edfors-Lilja I., 2005. Polymorphism at the porcine *Dominant white/KIT* locus influence coat colour and peripheral blood cell measures. In *Animal Genetics,* 36: 288-296.

Johansson M., Ellegren H., Marklund L., Gustavsson U., Ringmar-Cederberg E., Andersson K., Edfors-Lilja I. and Andersson L., 1992. The gene for dominant white color in the pig is closely linked to *ALB* and *PDGRFRA* on chromosome 8. In *Genomics,* 14: 965-969.

Johansson Moller M., Chaudhary R., Hellmén E., Höyheim B., Chowdhary B. and 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. In *Mammalian Genome,* 7: 822-830.

Kijas J.M., Moller M., Plastow G. and Andersson L., 2001. A frameshift mutation in *MC1R* and a high frequency of somatic reversions cause black spotting in pigs. In *Genetics,* 158: 779-785.

Kijas J.M., Wales R., Törnsten A., Chardon P., Moller M. and Andersson L., 1998. Melanocortin receptor 1 (*MC1R*) mutations and coat color in pigs. In *Genetics,* 150: 1177-1185.

Lauvergne J.J. and Canope I., 1979. Etude de quelques variants colorés du porc Créole de la Guadeloupe. In *Annales Génétiqué Sélection Animale*, 11: 381-390.

Marklund S., Kijas J., Rodriguez-Martinez H., Rönnstrand L., Funa K., Moller M., Lange D., Edfors-Lilja I. and Andersson L., 1998. Molecular basis for the dominant white phenotype in the domestic pig. In *Genome Research*, 8: 826-833.

McLean J.A., 1914. The shapphire hog. In Journal *of Heredity,* 5: 301-304.

Pielberg G., Olsson C., Syvänen A.C., Andersson L., 2002. Unexpectedly high allelic diversity at the *KIT* locus causing dominant white color in the domestic pig. In *Genetics,* 160: 305-311.

Stephens M., Smith N.J. and Donnelly P., 2001. A new statistical method for haplotype reconstruction from population data. In *American Journal of Human Genetics*, 68: 978-989.

TamuraK., Dudley J., Nei M. and Kumar S., 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. In *Molecular Biology Evolution*, 24: 1596-1599.