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Exploiting phenotype diversity in a local animal genetic resource: Identification of a single nucleotide polymorphism associated with the tail shape phenotype in the autochthonous Casertana pig breed

This is the submitted version (pre peer-review, preprint) of the following publication:

*Published Version:*

Exploiting phenotype diversity in a local animal genetic resource: Identification of a single nucleotide polymorphism associated with the tail shape phenotype in the autochthonous Casertana pig breed / Bertolini, Francesca; Schiavo, Giuseppina; Tinarelli, Silvia; Santoro, Laura; Utzeri, Valerio Joe; Dall'Olio, Stefania; Nanni Costa, Leonardo; Gallo, Maurizio; Fontanesi, Luca\*. - In: LIVESTOCK SCIENCE. - ISSN 1871-1413. - ELETTRONICO. - 216:(2018), pp. 148-152. [10.1016/j.livsci.2018.08.007]

This version is available at: <https://hdl.handle.net/11585/661002> since: 2019-02-06

*Published:*

DOI: <http://doi.org/10.1016/j.livsci.2018.08.007>

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1 *This is the pre-peer reviewed version of the following article: “Exploiting phenotype diversity in a*  
2 *local animal genetic resource: identification of a single nucleotide polymorphism associated with*  
3 *the tail shape phenotype in the autochthonous Casertana pig breed” by Francesca Bertolini,*  
4 *Giuseppina Schiavo, Silvia Tinarelli, Laura Santoro, Valerio Joe Utzeri, Stefania Dall’Olio,*  
5 *Leonardo Nanni Costa, Maurizio Gallo and Luca Fontanesi, which has been published in final form*  
6 *at 10.1016/j.livsci.2018.08.007. This article may be used for non-commercial purposes in*  
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8

9 **Exploiting phenotype diversity in a local animal genetic resource: identification of a single**  
10 **nucleotide polymorphism associated with the tail shape phenotype in the autochthonous**  
11 **Casertana pig breed**

12

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26

27 **Running title:** SNPs and tail shape phenotype in pigs

28 **Highlights**

- 29       • Casertana is an autochthonous pig genetic resource reared in Central-South of Italy.
- 30       • Tail shape phenotype variability in the breed was investigated in a GWAS.
- 31       • A single nucleotide polymorphism on porcine chromosome 12 was associated with this trait.
- 32       • This marker is close to the *SRY-box 9 (SOX9)* gene that is essential in skeletogenesis.

33 **Abstract**

34 Casertana is a local pig breed mainly raised in Central-South regions of Italy. Pigs of this breed  
35 are considered the descendants of the ancient Neapolitan population that largely influenced the  
36 constitution of the modern commercial pigs. The pigs of this breed are usually curly-tailed, like  
37 several other domestic pig populations. However, Casertana population shows some variability for  
38 this trait, including animals having strait tail as observed in wild boars. In this study, we run, for the  
39 first time, a genome wide association study (GWAS) comparing the curly-tailed (no. = 53) and strait-  
40 tailed (no. = 19) Casertana pigs to identify genomic regions associated with the tail shape phenotype  
41 in *Sus scrofa*. All animals were genotyped with the Illumina PorcineSNP60 BeadChip v.2. GEMMA  
42 software was used in the GWAS for which we were able to correct for stratification in the analysed  
43 cohort. A single nucleotide polymorphism (rs81439488), located on porcine chromosome 12, was  
44 significantly associated with the investigated trait. This marker is close to the *SRY-box 9 (SOX9)* gene  
45 that encodes for a transcription factor that is required during sequential steps of the chondrocyte  
46 differentiation pathway, notochord maintenance and skeletogenesis. As the shape of the tail could be  
47 important in relation to the problem of tail biting in pigs, the obtained results might open new  
48 perspectives for defining selection programs answering indirectly animal welfare issues. This work  
49 demonstrated that autochthonous animal genetic resources might be used to disclose genetic factors  
50 affecting peculiar traits by exploiting segregating phenotypes and genetic variability.

51

52 **Keywords:** Animal genetic resource; autochthonous breed; GWAS; morphological trait; SNP; *Sus*  
53 *scrofa*.

54 **1. Introduction**

55 Conservation of animal genetic resources is mainly aimed to preserve genetic diversity and  
56 associated inheritable phenotypes characterizing different populations that might be interesting for  
57 current or future purposes, including potential use in breeding programs. These resources can be also  
58 useful to understand biological mechanisms determining unique phenotypes derived by diversity in  
59 selection pressures or as result of adaptation to environmental and production conditions (Leroy et  
60 al., 2016).

61 Casertana pigs constitute a local breed mainly raised in Central-South regions of Italy. Pigs of  
62 this breed are considered the descendants of the ancient Neapolitan pig population that largely  
63 influenced the constitution of the modern commercial pig breeds through introgression of blood into  
64 British pig populations during the 19<sup>th</sup> century (Porter, 1993). Neapolitan pigs were, in turn,  
65 influenced by Asian blood in the late 18<sup>th</sup> century (Porter, 1993). Casertana is enlisted among the  
66 endangered animal genetic resources as the herd book of this breed accounts for about 100 boars and  
67 sows currently registered (ANAS, 2016). Animals are mainly raised in extensive or semi-extensive  
68 production systems with possible contacts and crossbreeding with European wild boars that could  
69 have contributed, at least in part, to shape their morphological characteristics. Casertana pigs have a  
70 black or grey coat colour, wrinkled skin, forward ears, and usually a typical hairless phenotype. The  
71 pigs of this breed are usually curly-tailed, like several other domestic pig populations. However,  
72 Casertana population shows some variability for this trait, including animals having strait and wavy  
73 tail as in a few other pig breeds and in wild boars.

74 Domestication in mammals has been a complex and continuous process associated with a series  
75 of changes in the domesticated animals compared to the wild counterparts, derived by selective  
76 breeding of animals showing favourable production and reproduction performances, and increased  
77 docility that indirectly shaped the genome of domesticated populations (Wiener and Wilkinson, 2011;  
78 Larson and Burger, 2013; Carneiro et al., 2014; Wang et al., 2014; Wilkins et al., 2014). Several  
79 morphological features have been also directly or indirectly selected and, in most cases, fixed in

80 domesticated populations as result of the domestication process (Darwin, 1868). Coat colour is one  
81 of the most common phenotypic traits that has been modified as result of reduced selective pressure  
82 against colours with low fitness in the wild and of aesthetic preferences of the breeders, sometimes  
83 associated with higher production performances (Clutton-Brock, 1999). Among several other  
84 morphological characters, curliness of the tail and shape has been associated with domestication in  
85 mammals (Trut et al., 2009).

86 The tail is considered an extension of the spinal column usually composed of specifically  
87 shaped vertebrae. Spontaneous curly tail phenotypes in mice have been the matter of studies that  
88 investigated the role of embryonic development in this morphological anomaly (Copp et al., 1988;  
89 van Straaten and Copp, 2001; Ohnishi et al., 2017). Curly tail is also commonly observed in many  
90 dog breeds. Vaysse et al. (2011) compared the genome of dog breeds having curly tails with that of  
91 breeds with straight tails using single nucleotide polymorphisms (SNPs) chip data and identified a  
92 genomic region on chromosome 1 significantly associated with these alternative tail shapes.

93 In pigs, few studies have been reported on the genetic factors affecting tail shape. A putative  
94 recessive genetic defect known as kinky tail (or flexed or screw tail), derived by fused caudal  
95 vertebrae associated in some cases with other defects, has been described in the mid of the last century  
96 (Nordby, 1934; Donald, 1949; Brooksbank, 1958). It is not known if this defect could be, in some  
97 way, related or not to the normal curling of the tail that is common in domestic pigs. This signature  
98 of domestication, however, seems not fixed in all pig breeds (Porter, 1993) but no systematic study  
99 has been conducted so far, probably because the difficulties in retrieving phenotype information due  
100 to the usual practice of tail docking in most herds.

101 In this study, we took advantage from the variability of the shape of the tail that we recorded in  
102 the Casertana pig population and run a genome wide association study (GWAS) comparing the  
103 genome of curly-tailed and strait-tailed animals to identify genomic regions associated with the tail  
104 shape phenotype in *Sus scrofa*.

105

## 106 **2. Materials and methods**

### 107 **2.1. Animals**

108 A total of 101 Casertana pigs (of about 7 to 20 months old) from six different farms were  
109 evaluated. Photographic records of each animal were obtained to capture information on the tail shape  
110 in standardized restraining conditions (including a direct evaluation of the personnel on this  
111 phenotype during this phase for the biological sampling) for all animals and after release (Figure 1).  
112 Pigs were classified as follows: 53 (25 males and 28 females) showed the curly tail phenotype; 19  
113 (five males and 14 females) showed the strait-tail phenotype; 29 were not classified and excluded  
114 from the study as tail docking, that was practised by the farmers as routine before weaning of the  
115 piglets, prevented the recording of any tail phenotype.

116

### 117 **2.2. Genotyping**

118 Hairs (with roots) were collected from the investigated pigs. DNA extraction was carried out  
119 using the Wizard® Genomic DNA Purification kit (Promega Corporation, Madison, WI, USA)  
120 following the manufacturer's instructions. Genotyping of the extracted DNA was obtained with the  
121 Illumina PorcineSNP60 BeadChip v.2 (Illumina, Inc., San Diego, CA, USA) that interrogated 61,565  
122 SNPs. Single nucleotide polymorphisms were assigned to the Sscrofa11.1 genome version, as  
123 previously described (Fontanesi et al., 2012). PLINK 1.9 software (Chang et al., 2015) was used to  
124 filter SNPs and genotyping data using the following criteria already used in a similar study (Schiavo  
125 et al., 2018): genotyping call rate >0.9, minor allele frequency >0.01 and Hardy-Weinberg  
126 equilibrium  $P > 0.001$ .

127

### 128 **2.3. Data analysis and genome wide association**

129 To evaluate distance relationships among the animals of the investigated cohort,  
130 multidimensional scaling (MDS) was obtained with the PLINK 1.9 software (Chang et al., 2015).  
131 Genome wide association study was carried out by applying the univariate mixed model of GEMMA



132 (Zhou and Stephens, 2012) that can accommodate the centered relatedness matrix calculated from  
133 SNP genotypes to correct for population stratification in a case and control analysis. The model also  
134 included the farm and the sex as fixed effects. To be able to identify associated markers in this  
135 experiment that included a low number of animals (derived by the fact that the analysed pigs were  
136 almost a complete representation of the whole population of the Casertana breed) and that used a SNP  
137 chip that might originally have an ascertain bias (as local breeds were not used for the selection of  
138 the informative SNPs), the significant threshold was defined at the  $P_{nominal\ value} < 5.00E-05$  level,  
139 according to the Wellcome Trust Case Control Consortium (2007) and as also applied in several other  
140 GWAS in livestock (e.g. Fontanesi et al., 2012; Sanchez et al., 2014). Genomic inflation factor ( $\lambda$ )  
141 and quantile–quantile (Q–Q) plot were obtained with GenABEL (Aulchenko et al., 2007). Gene  
142 annotation information was retrieved from the Sscrofa11.1 genome version available at the Ensembl  
143 database ([http://www.ensembl.org/Sus\\_scrofa/Info/Index](http://www.ensembl.org/Sus_scrofa/Info/Index)), release 91.

144

### 145 **3. Results and discussion**

146 A recent phenotypic characterization of the endangered Casertana pig population that we  
147 carried out noted several morphological differences among distinct animals of this autochthonous  
148 breed (data not shown). For example, in addition to the hairless or hypotrichotic condition (that is the  
149 characteristic phenotype of the Casertana animals), we already described the presence of haired pigs  
150 in this population and this morphological variability was used for a GWAS that we have recently  
151 reported (Schiavo et al. 2018). Despite a limited number of animals was included in that study, we  
152 were able to identify genomic regions associated with the hairless phenotype, demonstrating that local  
153 animal genetic resources can be used to genetically describe phenotypic variability of simple traits  
154 (Schiavo et al., 2018).

155 Another morphological trait that is not fixed in this breed is the shape of the tail (Figure 1). Of  
156 the animals for which we could record this phenotype, 26% (19 out of 72) showed a strait tail without  
157 any curls, similarly to the usual shape of wild boars. This shape was clearly different from the curly

158 tails reported in the remaining investigated pigs (74%). There was no effect of the age and the sex did  
159 not exclusively explain the observed phenotype as both sexes were included in the two phenotype  
160 groups. In addition, we could exclude the possible effect of the behavioral change of tail posture on  
161 this phenotype (Zonderland et al., 2009). The recording system was based on standardized conditions  
162 and subsequent photographic records of the animals confirmed their assignment to one or to the other  
163 group of tail shape phenotype. The two groups were observed in animals from all six farms. Limited  
164 pedigree record prevented the possibility to evaluate any potential founder effect.

165 A total of 36,533 autosomal SNPs, mapped to a unique position in the Sscrofa11.1 genome  
166 version, was used for MDS. The obtained MDS plot showed some structures not well defined in the  
167 analysed pigs that however did not clearly separate the curly and strait tailed Casertana pigs (Figure  
168 2). A stratified sample could be a critical point in GWAS in a very small population where, to some  
169 extent, all animals might be related. Figure S1 reports the genomic inflation factor ( $\lambda$ ) and Q-Q plot  
170 that did not show any biased test statistic distribution, suggesting that the investigated cohort was  
171 corrected for a possible stratification effect.

172 Figure 3 reports the Manhattan plot obtained in this GWAS. One significant SNP ( $P=2.3E-05$ )  
173 was identified on porcine chromosome 12 (SSC12). This marker indicated as ALGA0064877  
174 (rs81439488) is located at position 10,301,075 of this chromosome.

175 One of the closest annotated gene in this desert chromosome region is the *SRY-box 9 (SOX9)*  
176 gene (positions 8,641,629-8,647,764, encoded by the -1 strand), that, according to its function, might  
177 be the most plausible candidate gene, explaining the recorded phenotypic variability. It is well  
178 established that the expression of this gene at the embryonal level marks the onset of cartilage  
179 differentiation (Wright et al., 1995; Healy et al., 1996). *SOX9* encodes for a transcription factor that  
180 is required during sequential steps of the chondrocyte differentiation pathway, notochord  
181 maintenance and skeletogenesis (Akiyama et al., 2002; Barrionuevo et al., 2006; Montero et al.,  
182 2017). Continued expression of *Sox9* in differentiated chondrocytes is essential for subsequent  
183 hypertrophy and sustains chondrocyte-specific survival mechanisms (Ikegami et al., 2011).

184 Heterozygous mutations within and around human *SOX9* cause campomelic dysplasia that is a  
185 malformation syndrome characterized by cartilage derived skeletal structure defects (Foster et al.,  
186 1994; Wagner et al., 1994). These mutations, most of which reduce the level of expression of this  
187 gene, are located upstream spanning a large region (from 50 kb to more than 1 Mb) in which  
188 regulatory elements are present (Wunderle et al., 1998; Bagheri-Fam et al., 2006). Close upstream  
189 mutations produce more severe defects whereas far upstream mutations cause mild defects (Pfeifer  
190 et al., 1999; Velagaleti et al., 2005; Leipoldt et al., 2007).

191 Based on these studies in other species it is tempting to suggest a possible regulatory mechanism  
192 affecting *SOX9* expression in porcine developing chondrocytes that would, in turn, produce a mild  
193 cartilage/skeletal effect determining the shape of the tail. This hypothesis might be worth of further  
194 investigation starting from a precise characterization of the structure and morphology of the pig tail  
195 with different shapes for which, at present, there is no detailed investigation. Our phenotype records  
196 were based only on an external morphological evaluation of the shape of the tail. Furthermore,  
197 analysis of gene expression of *SOX9* at different developmental stages should be also carried out to  
198 evaluate the role of this gene in the phenotype observed in pigs.

199 The results we obtained might have broader impacts than those that would be limited to a simple  
200 morphological characterization. The shape of the tail could be important in relation to the problem of  
201 tail biting in pigs. Tail biting is a widespread behavioral vice with significant animal welfare  
202 implications and economic losses in commercial pig farms (Bracke et al., 2004). A few studies have  
203 established correlations between tail posture and tail biting incidence suggesting limited damages and  
204 related welfare complications with behaviors of the pigs that tended to have a tail posture up that  
205 those with tail posture down (Zonderland et al., 2009; Lahrman et al., 2017). It would be interesting  
206 to evaluate if pigs with genetically determined curly tails (as a possible adaptation derived by the  
207 domestication process) are less affected by tail biting damages than pigs with strait tails.

208

#### 209 **4. Conclusions**

210 This work demonstrated that autochthonous animal genetic resources, even constituted by very  
211 small populations, might be used to disclose genetic factors affecting peculiar traits by exploiting  
212 segregating phenotypes and genetic variability. To our knowledge, this is the first study that reported  
213 a frequency distribution of the tail shape phenotype in a pig population. Our results indicated that this  
214 morphological trait is associated with a marker close to an important gene involved in embryonic  
215 development, opening other hypothesis, worth of further investigations. It will be important to  
216 validate the results we obtained in this GWAS in other breeds and populations, including a more  
217 precise anatomical characterization of this trait, to further extend the impact of the results reported in  
218 Casertana pigs. It would be however first needed to know if diversity for this morphological  
219 characteristic is common in commercial pig populations as at present, there is not information on this  
220 aspect, mainly due to the usual practice of tail docking that prevents the recording of this phenotype.  
221 Considering the potential relationship between tail shape and tail biting damages (that, however,  
222 remains to be formally demonstrated), it could be possible to envisage practical applications of the  
223 identified marker in selection programs aimed to respond to animal welfare issues. Our study  
224 represents one of the few examples of exploitation of animal genetic resources to recover information  
225 that might have potential impacts in commercial populations.

226

## 227 **Acknowledgements**

228 The Authors thank all farmers and personnel that contributed to collect phenotypes and samples.

229

## 230 **Funding**

231 This work has received funding from the Italian Ministry of Agriculture, Food and Forestry  
232 (MiPAAF) under the project INNOVAGEN and from the European Union's Horizon 2020 research  
233 and innovation programme under grant agreement No 634476 (Project acronym: TREASURE). The  
234 content of this paper reflects only the authors' view and the European Union Agency is not

235 responsible for any use that may be made of the information it contains. This study was associated  
236 with the PSRN program (2017-2019).

237

### 238 **Conflict of interest statement**

239 The authors declare that there is no conflict of interest regarding the publication of this paper.

240

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355 **Figure 1.** Tail shape of Casertana pigs: a) curly tail; b) strait tail.

356

**a)**



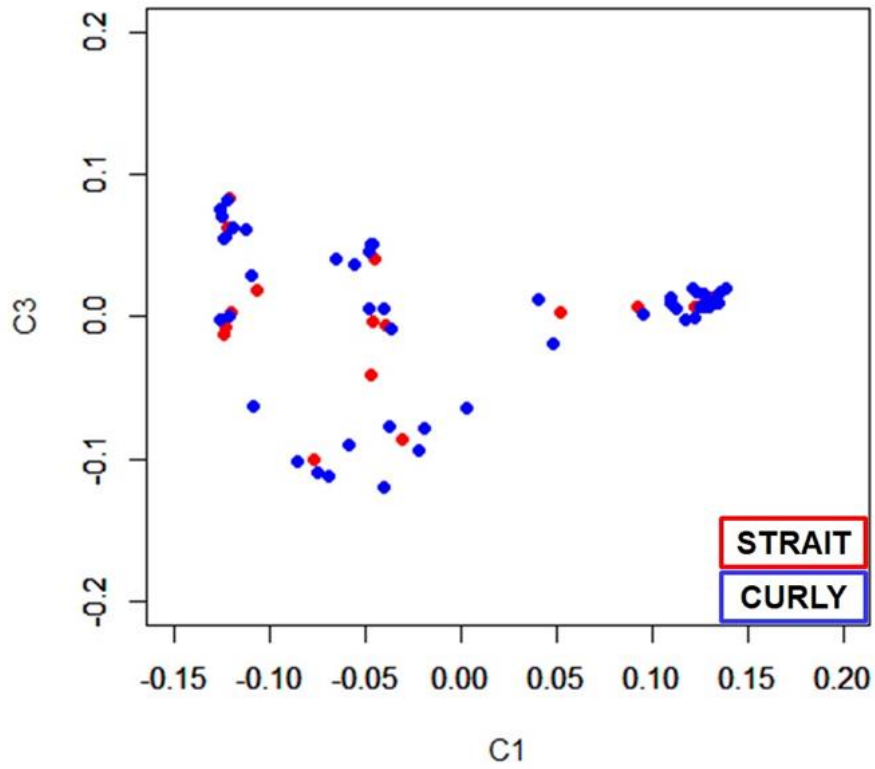
**b)**



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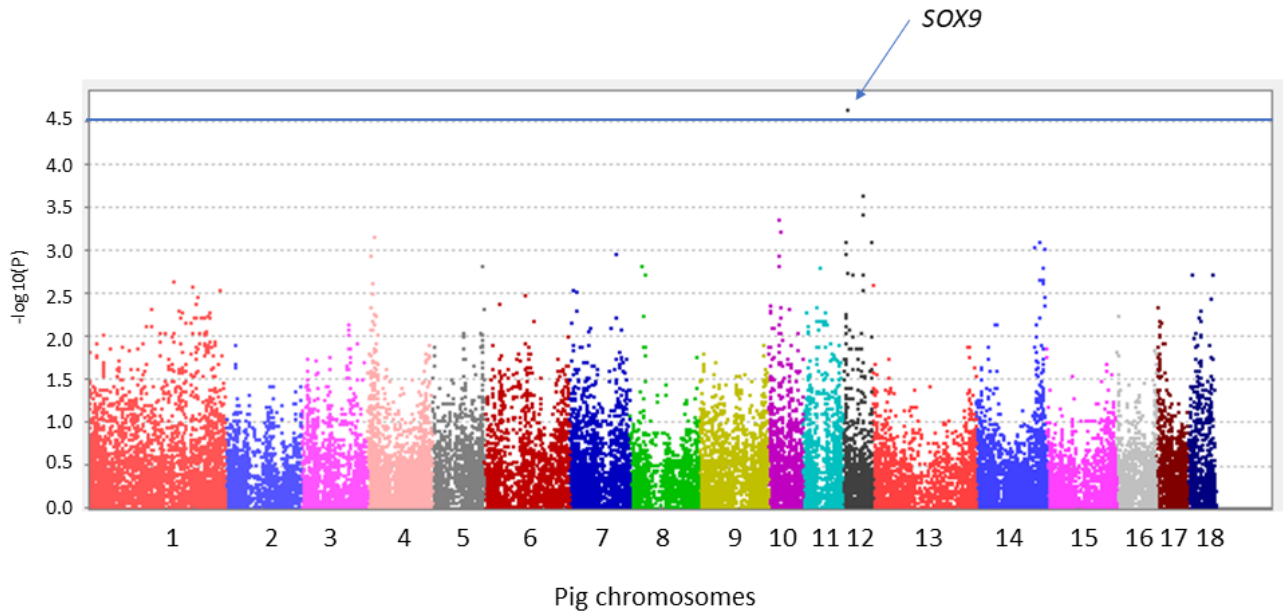
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359 **Figure 2.** Multidimensional scaling (MDS) with represented the pigs (dots) included in this study  
360 divided in the two groups of tail shape.



361

362 **Figure 3.** Manhattan plot obtained for the genome wide association study.

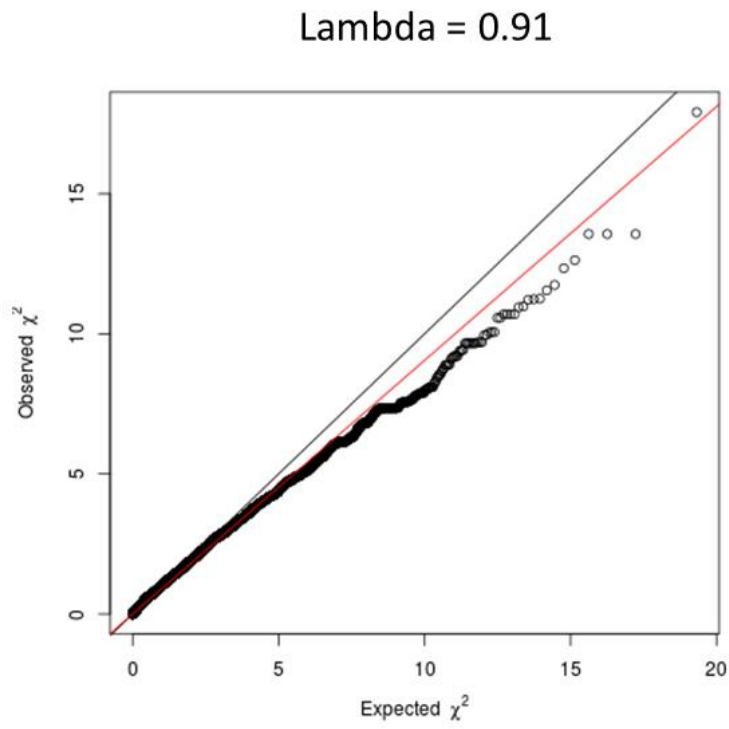


363

364 **Supplementary material**

365

366 **Figure S1.** Quantile–quantile (Q–Q) plot obtained from the genome wide association analysis.



367