Paper

Genome-wide association study of periweaning failure-to-thrive syndrome (PFTS) in pigs

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Porcine periweaning-failure-to-thrive syndrome (PFTS) is a condition that affects newly weaned piglets. It is characterised by a progressive debilitation leading to death, in the absence of infectious, nutritional, management or environmental factors. In this study, we present the first report of PFTS in South America and the results of a genome-wide association study to identify the genetic markers associated with the appearance of this condition in a crossbred swine population. Four chromosomal regions were associated with PFTS predisposition, one located on *SSCX*, one on *SSC8*, and the two other regions on *SSC14*. Regions on *SSC8* and *SSC14* harbour important functional candidate genes involved in human depression and might have an important role in PFTS. Our findings contribute to the increasing knowledge about this syndrome, which has been investigated since 2007, and to the identification of the aetiology of this disease.

Introduction

Porcine periweaning failure-to-thrive syndrome (PFTS) is a common term used to classify a swine disorder of unknown aetiology characterised by piglets that stop eating after weaning, lose bodyweight, and die (Huang and others 2011). This syndrome was first reported in Canada in 2008 (Dufresne and others 2008), and it has since been described in other countries such as the USA (O'Sullivan and others 2014) and Spain (Segales and others 2012).

In the past, this condition received little attention because its prevalence and economic losses were low. Nowadays, the morbidity and mortality of piglets presenting PFTS has increased, indicating the importance of this emergent swine disease (Dufresne and others 2008). The morbidity and mortality rates are variable, ranging from 1 to 20 per cent of nursery piglets, without necessarily the occurrence of underlying management changes or the presence of infectious pathogens (Huang and others 2012). Although sporadic 'postweaning starve-outs' have occurred in the swine industry over the years, it is important to determine whether the morbidity and mortality rates of these starve-outs in the nursery are now exceeding their historic levels (Huang and Harding 2015b). PFTS generally affects nursery piglets, seven days post weaning, which become anorexic and lethargic with a progressive debilitation within two to three weeks, and sometimes die (Huang and others 2011). Some affected piglets can present abnormal, compulsive oral behaviour such as licking, chewing or chomping (Huang and others 2011). This stereotyped behaviour generally appears when piglets are bored or frustrated, which is an indicator of poor animal welfare (Dantzer 1986), or is possibly caused by a non-suppurative meningoencephalitis (Huang and others 2012).

PFTS has a non-specific lesion characterisation. However, it is common to observe thymic atrophy and an empty gastrointestinal tract, with some piglets presenting mild to severe cranioventral lung consolidation (Huang and others 2011). Microscopic lesions include superficial lymphocytic fundic gastritis, atrophic enteritis, superficial colitis, lymphoid depletion in the thymus cortex, neutrophilic lymphoplasmacytic rhinitis, and mild non-suppurative meningoencephalitis, allowing the differential diagnosis of PFTS with other consumptive problems (Huang and others 2012). No direct transmission of this condition has been observed among piglets, since uninfected age-matched pigs grow and behave normally in contact with affected piglets (Huang and others 2012). Moreover, no relationship has been identified between the most common infectious agents occurring in the swine industry and PFTS (Huang and others 2012), and the disease has not yet been reproduced experimentally (Huang and Harding 2014).

It has been suggested that genetics may play an important role in PFTS, since no infectious pathogens have been detected and its aetiology is still unclear (Huang and Harding 2015a). Furthermore, a recent study has identified the existence of a genetic predisposition to this syndrome in a swine herd (Ramis and others 2015). We present the first report of PFTS in South America and the results of a genome-wide association study (GWAS) on this syndrome.

Material and methods

This study was carried out at Embrapa Swine and Poultry National Research Center, Santa Catarina Brazil. All experimental protocols used were approved by the Institutional Animal Care and Use Committee (CEUA/CNPSA 011/2014).

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Swine population and sample collection

The piglets used in this study were from a commercial swine nursery herd located at the Santa Catarina State, Brazil. The swine nursery had an all-in-all-out production system, with seven days down period, and the piglets were from a single sow operation. For this study, 64 piglets (16 females and 48 males) with an average age of 35 days were chosen based on a case-control design, with 30 piglets presenting suggestive clinical signs of PFTS (cases) along with 34 clinically healthy contemporary controls. Animals were selected from different pens; however, at least one case and one control pig were picked from the same pen.

The piglets originated from a commercial terminal cross of a paternal line *AGPIC337* (PIC) and a maternal line *DB25* (DanBred). *AGPIC337* is a synthetic sire line composed of 40 per cent Pietrain, 30 per cent Duroc, 25 per cent Large White, and 5 per cent Landrace (Joao Donisete do Nascimento, 2016, personal communication). The *DB25* is a grandmother line, which has the Landrace and Large-White breeds in its genetic background.

At weaning (21 days old), piglets were vaccinated for porcine circovirus type 2 (PCV2), *Mycoplasma hyopneumoniae*, and *Haemophilus parasui*s. Brazil is free of porcine reproductive and respiratory syndrome virus (PRRSV), and the farms were located in a Brazilian state free of classical swine fever (CSF), pseudorabies virus (PRV), and foot-and-mouth disease without vaccination. The farm in which the piglets were born is a certified pig farm and is monitored serologically every six months for CSF, PRV, tuberculosis, and brucellosis. The mortality rate and the average daily gain in the previous five months was, respectively, 2.05 per cent and 310.4 g initially, reaching 5.75 per cent and 267 g during the PFTS outbreak, and returning to 2.2 per cent and 303.7 g after the outbreak. At weaning, all piglets were clinically healthy and presented normal performance indicators. The clinical signs suggestive of PFTS began one week after weaning, affecting pigs with an average age of 28 days.

Bodyweight and blood samples collected through jugular puncture were obtained from all 64 piglets used in this study. Ten piglets showing clinical signs of PFTS were randomly selected, and necropsy was performed to characterise the possible lesions associated with this illness. All affected piglets presented anorexia, lethargy, and progressive weight loss, and some of them had abnormal oral behaviour such as repetitive licking, chewing or chomping, as described by Huang and others (2011).

At necropsy following euthanasia, samples of mesenteric and mediastinal lymph nodes, thymus, the fundic region of the stomach, nasal turbinates, lung, liver, kidney, spleen, pancreas, adrenal, heart, small intestine, colon and central nervous system were immediately collected and fixed in 10 per cent formalin for routine histopathology analysis. Lymph node and thymus samples were also analysed by immunohistochemistry (IHC) for PCV2 antigen detection as described by Gava and others (2008). Lung samples were tested for influenza A detection by reverse transcription PCR (RT-PCR) (Fouchier and others 2000).

Genomic DNA extraction and single-nucleotide polymorphism genotyping

DNA was extracted from blood samples using PureLink Genomic DNA Mini Kit (Invitrogen, USA), according to the manufacturer's instructions. The quantity and quality of DNA was verified using Biodrop uLITE (Biodrop Technologies Inc, UK). The 260/280 nm ratio for all samples ranged from 1.8 to 2.0. DNA samples were diluted to a final concentration of 500 ng and genotyped at Deoxi Biotecnologia Brazil using the Illumina PorcineSNP60V2 BeadChip whole-genome single-nucleotide polymorphism (SNP) assay, which contains 61,565 SNPs across the swine genome.

Genotyping quality control

Data quality control was performed using PLINK V1.9 (Purcell and others 2007). SNPs and samples were tested for their quality before the analysis. SNPs were removed if they had a minor allele frequency (MAF) less than 5 per cent, if they failed in more than 10 per cent of the samples, or failed in the Hardy-Weinberg equilibrium test (HWE) with $P<1\times10^{-6}$. Samples were removed from the analysis if they had more than 10 per cent of missing genotypes or an excess of heterozygosity.

To test for population stratification among the piglets used in this study, a multi-dimensional scaling (MDS) plot was constructed using the Identical by State (IBS) information of markers in low linkage disequilibrium (LD; $r^2<0.2$) using PLINK (Purcell and others 2007) and the R statistical environment (R Core Team 2015). From the 61,565 SNPs, 6046 were not in LD and therefore were used for the MDS plot construction. In addition, the degree of relationship between piglets was estimated using the Identical by Descent (IBD) information to verify the possible family relationship between the tested individuals. PLINK was also used to construct the QQ-plot and to compute the genomic inflation factor (λ_{GC}) for the identification of population stratification.

Statistical analysis

A GWAS was conducted to identify genomic regions associated with the clinical presentation of PFTS using PLINK (Purcell and others 2007). A linear model including the fixed effects of SNP and sex, and the residual as a random effect, was applied. Allele frequencies were compared between affected and control groups of piglets using the χ^2 test, and the OR was computed for the favourable allele for each marker. A moderate association was considered if an unadjusted P value was $<5.0\times10^{-5}$ for an individual marker, based on the recommendations of the Wellcome Trust Case and Control Consortium (WTCCC 2007).

Following the single marker association test, a haplotype test using a backward elimination process (Kich and others 2014) was performed using PLINK (Purcell and others 2007) to test the combined effect of suggestive markers in regions with possible involvement with PFTS that failed the WTCCC criteria. In addition, a Cochran-Armitage trend test (CATT) was used to evaluate the effect of the SNP genotypes on the appearance of PFTS, testing for additive, dominance and recessive effects of the alleles, also using PLINK. The physical position of each SNP was expressed relative to the forward strand of the reference genome (Sscrofa 10.2) based on Badke and others (2013). Genes were searched using the UCSC (University of California Santa Cruz) genome browser (https://genome.ucsc.edu/) with the genome assembly SGSC Sscrofa10.2/susScr3, spanning a 100 kb of the associated SNP.

Results

Experimental piglets and pathological findings

There was a significant difference ($P<2.7\times10^{-12}$) between the live weight of healthy (mean=8.71±1.31 kg) and affected piglets (mean=4.20±0.97 kg). No significant difference was identified between the weight of males and females within each group ($P>0.67$).

At necropsy, the main observed gross lesion was severe thymic atrophy (10/10) (Fig 1a). Other findings such as serous heart fat atrophy (9/10), inflammation in the umbilical region (7/10), and mild hypertrophy of the mesenteric lymph nodes (4/10) were also frequently observed. Gastrointestinal tract examination revealed scant ingests and the small intestines were empty or fluid-filled.

The histological findings proposed for the diagnosis of PFTS (Huang and Harding 2015b) and observed in all the affected piglets were: pronounced atrophy of the thymus with reduction in the thickness of the thymic cortex (Fig 1b), mild mononuclear gastritis (Fig 1c), and moderate to severe villus atrophy in the jejunum with superficial mononuclear enteritis (Fig 1d). Other histological findings also included superficial mononuclear colitis, fatty infiltration into the liver and kidney, and discrete rhinitis. Some piglets were also found to have macrophage infiltration and lymphoid depletion in the lymph nodes (4/10), mild

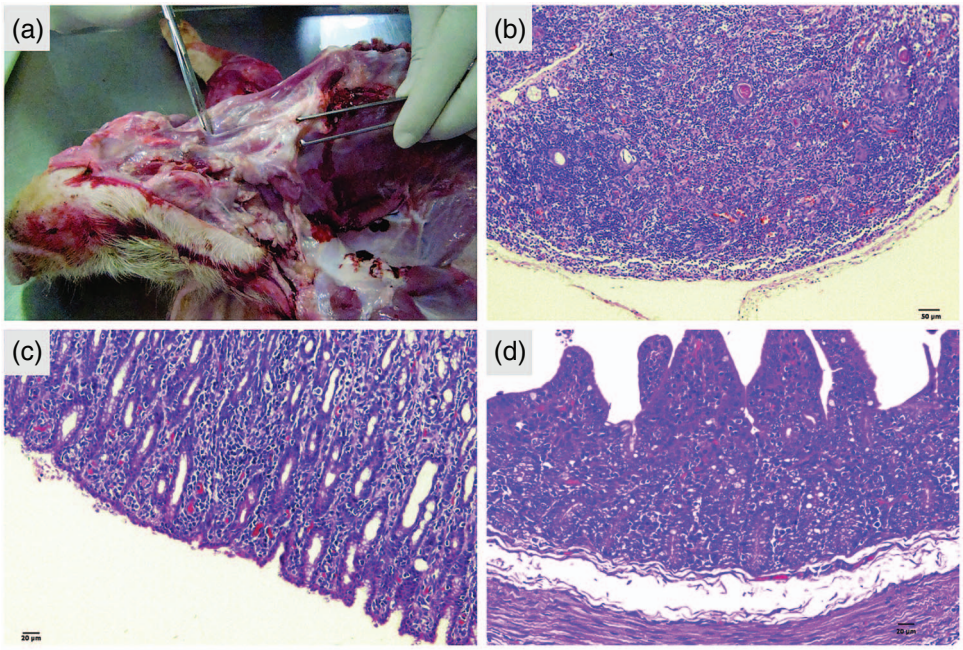

FIG 1: Gross lesion and histological findings observed in piglets affected with periweaning failure-to-thrive syndrome (PFTS), according to the criteria defined by Huang and Harding (2015b). (a) Severe thymus atrophy. (b) Thymus: severe lymphoid depletion in the cortex (haematoxylin and eosin, H&E). (c) Stomach: congestion and diffuse mononuclear infiltration in the mucosa of the fundic area (H&E). (d) Jejunum: villous atrophy and diffuse mononuclear infiltration in the lamina propria (H&E)

pneumonia (3/10), humid dermatitis (2/10), coccidiosis (1/10), and myocardial degeneration (1/10). All lymph nodes and thymus were negative to PCV2 by IHC. All lung samples were negative to influenza A by RT-PCR. All piglets fulfilled the criteria for PFTS diagnosis proposed by Huang and Harding (2015b), presenting with thymus atrophy, gastritis, and enteritis and atrophy of the small intestine villi.

Genotyping quality control

A total of 743 SNPs were removed because they failed in more than 10 per cent of the samples, 8817 SNPs failed in the MAF criteria, and 2413 SNPs failed in both criteria. Six markers were additionally excluded because they failed the HWE test. One piglet was removed from the analysis because it had more than 10 per cent of missing genotype, and 10 piglets (five cases and five controls) were removed because they were from a different genetic background (Fig 2). After quality control, 49,586 SNPs and 53 piglets remained for the association analysis (24 cases and 29 controls). The total genotyping call rate in the remaining individuals was 0.99. The mean IBD probability among all piglets was 0.11±0.07, ranging from 0 to 0.52, indicating a variable level of relationship among the animals. The QQ-plot and

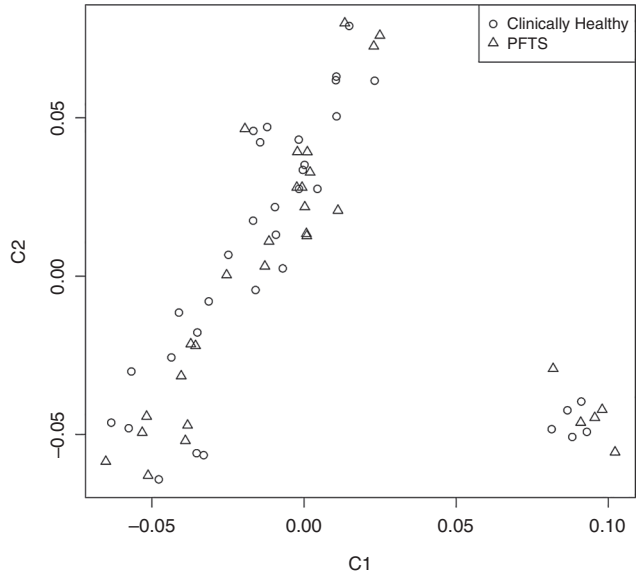

FIG 2: The Multi-Dimensional Scaling (MDS) plot provides a spatial representation of data that can facilitate interpretation and reveal structural relationships in the populations used in this study. Circles represent control piglets and triangles represent piglets affected with periweaning failure-to-thrive syndrome (PFTS). Piglets located in the bottom right corner were considered outliers and therefore were excluded from the analysis

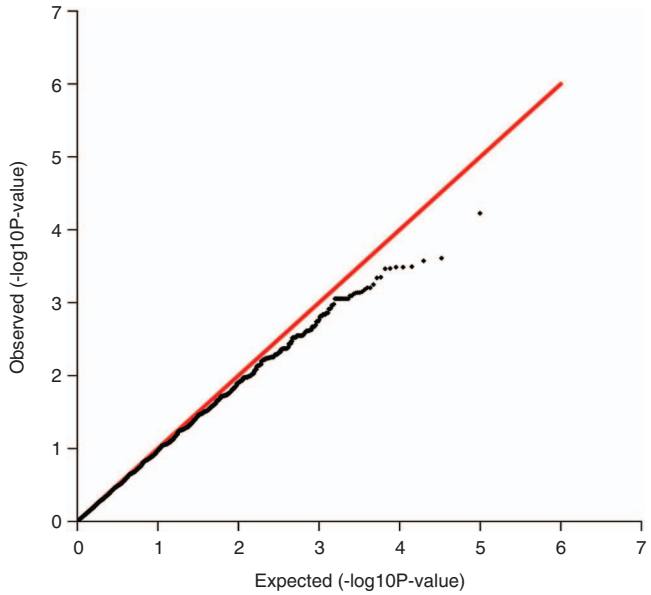

FIG 3: Quantile-quantile (Q-Q) plot for observed P value versus those expected for an association of loci with periweaning failure-to-thrive syndrome (PFTS). The Q-Q plot shows little to no evidence of a deviation from the expected null distribution of P value and therefore shows no evidence of population stratification

TABLE 1: Chromosomal positions with moderate and suggestive association with periweaning failure-to-thrive syndrome (PFTS) using an allelic test (All), a haplotype test (Hap), and the Cochran-Armitage trend test (Catt)

Chr	SNP name	Location (bp)	P value	OR (CI)	Test	Allelic variation	Region
X	ALGA0099945*	112534471	5.95e-05	5.9 (2.4 to 14.7)	All, Catt	A>C	1
14	ASGA0063527+	59414987	0.0002	7.4 (2.3 to 24.0)	All, Catt, Hap	A>C	2
14	ASGA0064155	69709443	0.0008	5.2 (1.9 to 14.5)	All, Catt, Hap	A>G	3
14	ASGA0064190	71670219	0.0008	5.2 (1.9 to 14.5)	All, Catt, Hap	A>G	3
14	INRA0044648	71804184	0.0008	5.2 (1.9 to 14.5)	All, Catt, Hap	A>G	3
14	MARC0046056	72881125	0.0008	5.2 (1.9 to 14.5)	All, Catt, Hap	T>C	3
14	ASGA0064232	73141708	0.0008	5.2 (1.9 to 14.5)	All, Catt, Hap	C>T	3
14	MARC0014474	73202166	0.0008	5.2 (1.9 to 14.5)	All, Catt, Hap	A>G	3
14	ASGA0064233	73300300	0.0008	5.2 (1.9 to 14.5)	All, Catt, Hap	C>T	3
14	ALGA0078575	73811889	0.0008	5.2 (1.9 to 14.5)	All, Catt, Hap	A>T	3
14	ALGA0078658	75973277	0.0008	5.2 (1.9 to 14.5)	All, Catt, Hap	C>G	3
14	ALGA0079529	77974347	0.0008	5.2 (1.9 to 14.5)	All, Catt, Hap	A>G	3
14	ASGA0064326+	78883131	0.0003	7.1 (2.2 to 23.1)	All, Catt, Hap	A>G	3
14	H3GA0040851	79124234	0.0007	5.8 (1.9 to 17.3)	All, Catt, Hap	A>C	3
8	M1GA0011817*	15429369	3.71e-05	-	Catt	C>T	4
8	ASGA0082094*	15464960	3.71e-05	-	Catt	C>T	4
8	ASGA0037908*	15506829	3.71e-05	-	Catt	A>G	4

Makers with *presented a moderate association. Additional markers presented a suggestive association using an allelic test, and when the haplotype was constructed (+) a moderate association was identified. >indicates the allelic change, for example, from A to C (adenine to cytosine).
A, adenine; C, cytosine; G, guanine, SNP, single nucleotide polymorphism; T, thymine.

the genomic inflation factor (λ_{GC}= 1) did not show any evidence of population stratification (Fig 3).

Genome-wide association analysis

Testing the effect of each SNP with PFTS, using a standard χ^2 test, a marker on *SSCX* located at 12,534,471 bp (*ALGA0099945*) was moderately associated ($P=5\times10^{-5}$, OR=5.91, 95 per cent CI 2.38 to 14.68) with the appearance of PFTS (region 1). The frequency of the B allele was 52 per cent in cases of PFTS and 15 per cent in control piglets. No statistical difference between the case and control groups was observed when the sex of the piglets was included in the model, indicating that sex had no influence on the occurrence of PFTS. In addition, a region on *SSC14,* from 59,414,987 to 79,124,234 bp, composed of 13 SNPs showed a suggestive association with PFTS ($P=2\times10^{-4}$, OR=5.2 to 7.4, 95 per cent CI 1.86 to 23.98). Of those 13 SNPs, 12 of them were in high LD ($r^2>0.89$) and were located in a region from 69,709,443 bp to 79,124,234 bp. These results suggest the existence of two important regions on *SSC14* with the appearance of PFTS: one composed of the SNP *ASGA0063527* (region 2), and the second of the other 12 SNPs (region 3), spanning ~9 Mb based on the LD levels (Table 1).

When the haplotype test was carried out with the 13 suggestive markers on *SSC14,* the significance of the region improved to $P=5\times10^{-5}$, confirming our initial assumption related to the importance of this region. The most significant haplotype was composed of two markers: *ASGA0063527,* located at 59,414,987 bp, and *ASGA0064326*, located at 78,883,131 bp, on *SSC14*. The haplotype AB was observed in 25 per cent of piglets with PFTS and it was never observed in healthy piglets.

Using the CATT, three SNPs in high LD ($r^2=1$) located on *SSC8*, from 15,429,369 to 15,506,829 bp, were identified as moderately associated ($P<3\times10^{-5}$) with the clinical-pathologic presentation of PFTS (region 4) (Table 1). In addition, the same region on *SSC14*, from 59,414,987 to 79,124,234 bp, was also identified by this test as being possibly associated with the clinical-pathologic presentation of PFTS ($P=2\times10^{-4}$).

Discussion

In this study, we present the first report of the occurrence of PFTS in South America and the results of a GWAS for the identification of genetic markers associated with the appearance of this pathology in a crossbred swine population from Southern Brazil. A total of four regions were found to be associated with PFTS in three different chromosomes, in which genes previously related to neurological disorders are located.

The PFTS diagnosis was based on the criteria proposed by Huang and Harding (2015b), in which piglets at necropsy presented enteritis and villus atrophy of the small intestine, gastritis, and thymus atrophy. According to Huang and others (2012), the gastrointestinal lesions may explain the clinical presentation of anorexia and wasting. Gastritis, enteritis, and colitis together can cause abdominal discomfort and loss of appetite, and thus refusal to feed. However, it is also possible that the gastrointestinal lesions were secondary to anorexia. The thymic atrophy may be a primary change associated with infection by an immunosuppressive pathogen, such as PCV2 (Harding and others 2008); however, the virus was not identified by IHC. It could also be associated with prolonged sickness or anorexia (Gruver and Sempowski 2008). The pneumonia and humid dermatitis most likely developed secondary to debilitation and was caused by opportunistic bacteria. Most of the studies conducted on the aetiology of PFTS have focused their attention on the association of pathogens, and no conclusive agent has been identified until now. Some predisposing factors such as management, environment and nutrition have been proposed (Huang and others 2011), but they are not able to explain the clinical and pathologic manifestation of the illness. In addition, attempts to reproduce the disease experimentally were also unsuccessful, suggesting that PFTS is not associated with an infectious agent (Huang and Harding 2014).

Genetics involved in the susceptibility (Settles and others 2009) and tolerance to diseases (Zanella and others 2011) have been widely investigated as an additional tool to select animals that will better cope with specific challenges (Boddicker and others 2014, Zanella and others 2015). In this context, the use of high density SNP panels has allowed the identification of genetic markers associated with phenotypes of interest (Settles and others 2009, Wilson and others 2012). Evidence of a genetic predisposition to PFTS has been recently presented (Ramis and others 2015). However, no genomic regions or molecular mechanisms have been identified. Therefore, the importance of studying genetic pathways in the predisposition of PFTS has been reinforced (Huang and Harding 2015a).

In this work, four genomic regions located on three chromosomes were identified, indicating genetic control of PFTS. Using a χ^2 test, an SNP located at 12,534,471 bp on the sexual chromosome was associated with PFTS. Although no annotated genes have been identified in the surrounding area of this SNP (100 kb), the existence of functionally important elements associated with PFTS in swine cannot be excluded. For example,

many quantitative trait loci (QTL) were described in this region on the swine genome, including QTL for feed intake (Cepica and others 2003), mean corpuscular haemoglobin content (Reiner and others 2007a), eosinophil number (Reiner and others 2008), and activity during lying related to the behavioural pattern after challenge with the parasite *Sarcocystis miescheriana* (Reiner and others 2007b), traits that are observed in piglets affected with PFTS.

Using the CATT, a region composed of three SNPs spanning a 100 kb on *SSC8* was associated ($P<3\times10^{-5}$) with PFTS (Table 2). This region was only identified with CATT, possibly due to its property to detect non-additive effects of the SNP alleles. This region identified on *SSC8* harbours several QTL already reported in swine: white blood cell counts (Reiner and others 2007a), bilirubin concentration (Reiner and others 2009), and time spent feeding and lying after infection with *S miescheriana* (Reiner and others 2007b). This region is surrounded by three genes: *SLIT2 (*slit guidance ligand 2), *PACRGL* (PARK2 co-regulated-like), and *KCNIP4* (Kv channel interacting protein 4). In swine no information about these gene functions is available. However, in other species, *SLIT2* and *KCNIP4* have already been associated with neurological disorders (Sokolowski and others 2010, Weissflog and others 2013). In humans, the *SLIT2* gene product is involved in the serotonergic and dopaminergic brain pathways acting on axonal guidance maintenance (Lin and others 2005). These systems have a role in the regulation of behaviour, including stress response (Hood and others 2006), depression (Jasinska and others 2012), and maternal deprivation (Ogawa and others 1994). The same patterns have also been described in rat brain studies (Harro and others 2014). It is important to highlight that weaning is one of the most critical and stressful periods in a piglet's life, where events like maternal deprivation and mood swings are commonly observed. Also, the microRNA miR-218-1 is located in this region and has been previously associated with tumorigenesis (Ran and others 2015), heart development (Chiavacci and others 2012), Parkinson's disease (Chandrasekaran and Bonchev 2013), and motor neuron development (Thiebes and others 2015), among others. Moreover, the miR-218-1 has been found to be developmentally regulated in the hippocampus, contributing to the molecular mechanisms underlying the pathogenesis of mesial temporal lobe epilepsy and hippocampal sclerosis (Kaalund and others 2014). The hippocampal complex has an important role in the pathophysiology of major depressive disorders (MDDs) (Campbell and Macqueen 2004). Metabolic encephalopathy can also underscore the basic neuropathology of depression (Harvey 2008).

TABLE 2: Annotated genes in the associated regions on *SSC8* and *SSC14*

Chr	Gene symbol	Chromosome position (bp)	Gene ID
14	*TSNAX*	63573311-63604624	100156036
14	*C14H1orf124*	63849792-63860814	100155479
14	*GNPAT*	63872123-63904272	100156253
14	*ARV1*	64061498-64073531	100151849
14	*ACTA1*	65236451-65239197	100154254
14	*PHYHIPL*	67475935-67496632	100525186
14	*MRLN*	68099652-68127654	105667210
14	*CDK1*	69262319-69277260	100155762
14	*TMEM26*	69957059-70001294	100157089
14	*mIR9836*	70824287-70824383	104796107
14	*EGR2*	71518195-71521036	100038004
14	*mIR1296*	72055628-72055712	100526395
14	*SIRT1*	77053991-77184717	751859
14	*HNRNPH3*	77540095-77558295	100155513
14	*HK1*	78400222-78521251	100152344
8	*SLIT2*	15354619-15435676	100515495
8	*PACRGL*	15449890-15469991	100515674
8	*mIR-218-1*	15443762-15443845	100526390
8	*KCNIP4*	15470139-16695141	100516499

Using an allelic and the CATTs, a region of 59,414,987 bp to 79,124,234 bp on *SSC14* had a suggestive association with the clinical presentation of PFTS. This region could be divided in two when taking into account the LD between markers. Further investigation of these two regions using a haplotype test with a backward elimination process on *SSC14* reinforced the importance of these genomic regions on the appearance of PFTS. These two regions on *SSC14* harbours 15 positional candidate genes (Table 2), some of them related to neurological disorders in humans, such as *TSNAX* (translin-associated factor X) (Arias and others 2014), *GNPAT* (glyceronephosphate O-acyltransferase) (Buchert and others 2014), *ARV1* (ARV1 homologue) (Alazami and others 2015), *EGR2* (early growth response 2) (Vrebalov Cindro and Vrebalov Cindro 2015), and *SIRT1* (sirtuin 1) (Lo Iacono and others 2015).

The *TSNAX* gene is one of the positional and functional candidate genes located on the region of *SSC14* associated with PFTS. This gene has been previously associated with MDD, acting as a regulator of neurodevelopment, neuroplasticity and neurotransmission (Okuda and others 2010). Besides that, several studies have reported associations of the *GNPAT* gene with neurological disorders in humans, especially with rhizomelic chondrodysplasia punctata (RCDP), a disorder of peroxisome metabolism resulting from a deficiency of plasminogen, a specialised class of membrane phospholipids, causing mental problems (Liu and others 2006). Genes like *ARV1, SIRT1* and *CDK1* (cyclin-dependent kinase 1) have also been identified as being involved in the acute stress response, schizophrenia, and depression in many mammalian species (Kishi and others 2010). Therefore, finding a region associated with PFTS in swine containing genes related to neurological problems, such as depression and other mental disorders in humans, suggests that these genes could be involved in conferring clinical signs of depression observed in affected piglets with PFTS. Furthermore, the *HK1* (hexoquinase 1) gene, also located in this region on chromosome 14, has already been associated with mood and psychotic disorders in humans (Regenold and others 2008). This gene plays a role in brain energy metabolism, especially in the glycolysis pathway. It is already known that these neurological disorders could be caused by an impaired brain energy metabolism, with abnormal glucose metabolism and detachment of hexokinase 1 on the outer mitochondrial membrane (Regenold and others 2012). Also, the previously described miR-218-1 has a sequence target in the *HK1* gene. Therefore, knowing that suboptimal brain energy production could affect neurons and glia, any variation on stress and feeding could trigger this mood symptom in affected piglets with PFTS.

Major depression is generally associated with stressful conditions, where vulnerability to stress will have a direct role in determining the impact of the stressor on the individual's behaviour (Kendler and others 2001). Weaning is considered to be one of the most stressful events in the life of piglets, which can contribute to intestinal and immune system dysfunctions resulting in reduced piglet health and growth, especially during the first week after weaning (Campbell and others 2013). Other studies have also investigated the age of weaning in piglets and its impact on animal behaviour, indicating that piglets weaned at young ages showed more abnormal and aggressive behaviours and cognitive deficits compared to piglets weaned later (Poletto and others 2006). The period of seven days after weaning, where piglets will suffer its effects, concurs with the appearance of clinical signs of PFTS. Therefore, weaning and the piglets' new environment are considered a challenge to the animals, triggering different individual responses. Those variations in the animals' response to the challenge could be partially explained by their genetic makeup, which may determine whether they will be resistant, tolerant or susceptible to a challenge.

Four genomic regions associated with predisposition to PFTS were identified in this study. Two of these regions, one on *SSC8* and the other on *SSC14*, harbour important functional candidate genes associated with personality behaviours in humans,

especially depression. This could indicate that PFTS might be involved in neurological disorders affecting susceptible piglets when challenged to a stressful event such as weaning. Therefore, our findings could help in the identification of susceptible piglets, becoming an important tool to be used in piglet selection to reduce the prevalence of this illness.

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