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Genome-wide association analysis reveals insights into the genetic architecture of mesenteric torsion in pigs

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Mesenteric torsion (MT) is a condition that affects several animal species and can lead to the animals' death. However, little is known about its etiology. Therefore, this study aimed to identify genomic regions and candidate genes associated with MT. Phenotypic and genotypic data from 405 pigs, including MT records and genealogy were used. In the model, contemporary group (sex, year, and week of weaning) was considered fixed effect, the linear effect of weaning weight as a covariate, while direct additive genetic effect was random. In the genome-wide association study, genomic windows explaining more than 0.3% of the genetic variance were considered significant. Fifty-two significant windows were identified, covering 299 genes located on 15 chromosomes. The *HSD17B4*, *TNFAIP8*, *TENM4*, *CHD2*, *RGMA*, *OPRM1*, *PPARGC1A*, *CHIA*, *KCNJ2*, *KCNJ16*, *KCNJ15*, *ELN*, *SGO1*, *IL17A*, *IL17F*, *GATA4*, *OVOL2*, *GLI3*, and *RAP1A* genes were considered candidates to MT since they are related to intestinal morphogenesis, feeding behavior, intestinal barrier, digestion, and intestinal motility. These processes could induce intestinal malformations, dysbiosis, excessive fermentation, delay intestinal transit, and obstruction. Our findings contribute to understanding the mechanisms involved in the occurrence of MT in pigs and may help to elucidate the etiology of intestinal torsion/volvulus in other mammals, including humans.

Mesenteric torsion (MT) is characterized by the rotation of intestinal loops around the mesenteric axis, resulting in the occlusion of the cranial mesenteric artery, interruption of blood flow, and mechanical obstruction of the intestines, which can lead to the death of animals and humans[1]. Due to the complexity of its diagnose and clinical-pathological signs, this condition can be designated with different terminologies, depending on the species, among them: Hemorrhagic Bowel Syndrome and Intestinal Volvulus[2–5].

MT is a serious concern since it affects welfare and is related to high mortality rates. In humans, the mortality rate can vary from 9 to 35% depending on the method of diagnosis used[4]. In dogs and horses, the mortality rate is approximately 16% and 42%, respectively[5,6], while in cattle, 55.2 to 77% die or are euthanized due to complications that occurred during or after surgery[4,7,8]. In pig production, MT is one of the most common reasons for sudden death in the growing and finishing phases, affecting animals at the end of the production cycle and leading to increased economic losses[9,10]. In growing- and finishing phase, the occurrence of the MT ranges between 0.9% and 3.64%[11,12], and in a nucleus farm, it was around 2.5%, with 100% mortality[13].

Despite the knowledge of several risk factors, the etiology and pathogenesis of MT remain insufficiently elucidated[14]. In pigs, it is believed that the unique characteristics of the pig's digestive system, feeding behavior, housing, management, breed, and other factors contribute to the occurrence of the pathology[2,15]. Moreover, genetic factors may influence MT, suggesting the potential for breeding selection to reduce its incidence in

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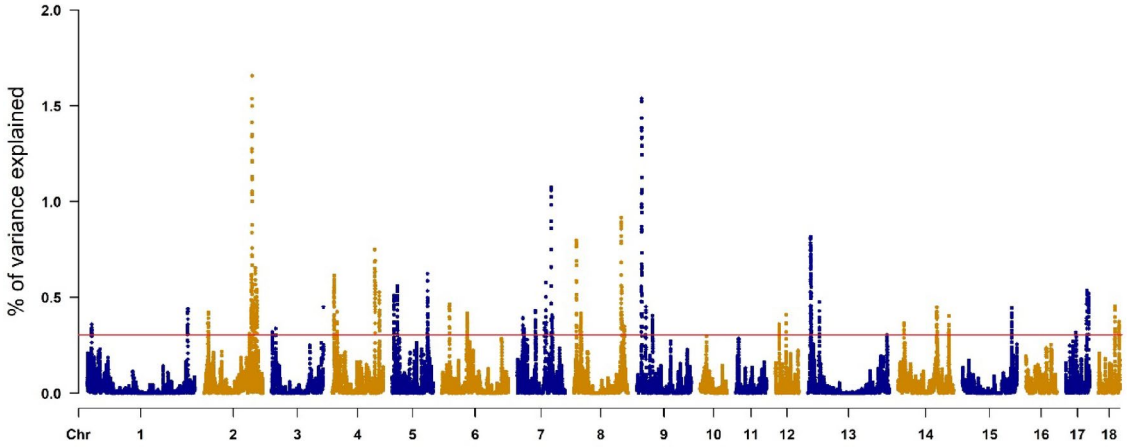

Fig. 1. Manhattan plot of the percentage of genetic variation explained by 1-Mb windows for mesenteric torsion in pigs. The X axis represents the chromosomes and the Y axis shows the percentage of genetic variance explained by each window. The red line indicates the significance threshold (0.3% of the genetic variance explained, i.e., five times more than expected). The Manhattan plot was constructed with R software version 4.2.2 [95] using the results from the POSTGSF90 package of the BLUPF90 program (Misztal et al.[88]).

Chromosome	Genomic window		Explained genetic variance (%)	Genes located in the genomic windows
	Initial position	Final position		
2	123,177,514	124,163,901	1.66	*FAM170A*, *HSD17B4* and *TNFAIP8*
9	12,338,078	13,310,672	1.54	*ALG8*, *GAB2*, *USP35*, *NARS2*, *TENM4*, *KCTD21*, *THRSP* and *NDUFC2*
7	85,640,036	86,623,643	1.07	*FAM174B*, *CHD2*, *RGMA* and *ST8SIA2*

Table 1. Characterization of the genomic windows that explained more than 1% of the genetic variance for mesenteric torsion in pigs.

herds. Nevertheless, traditional selection methods may not efficiently reduce the incidence of MT due to its low heritability[13], polygenic nature, and the challenge of measurement, as accurate diagnosis is possible only post-mortem. Therefore, the application of genomics presents a viable alternative to enhance effectiveness in selecting for this trait[16].

In genome-wide association studies (GWAS), individuals' genomes are examined to identify genetic variants associated with the studied trait. Thus, each single nucleotide polymorphism (SNP) undergoes statistical testing for its significance of association with the trait/phenotype of interest[17,18]. This approach aids the understanding of the genetic architecture of complex traits, such as diseases and syndromes, and facilitates the discovery of variants and candidate genes[18] to try to reduce their occurrence. Therefore, although MT is an important pathology causing several problems in different species, there is a dearth on how genetic mechanisms may predispose the occurrence of this pathology. For this reason, GWAS could contribute significantly to elucidate the etiology and to clarify the biological factors involved in the development of MT in pigs and, consequently, in other species. Hence, the objective of this study was to identify genomic regions and candidate genes associated with MT in pigs through a genome-wide association study.

Results

From the 405 genotyped pigs with the GGP Porcine 50K BeadChip, eight were removed due to quality control before GWAS analysis. From the 50,697 SNPs originally available on the chip, 36,438 SNPs were kept for further analysis. In the GWAS, conducted in the BLUPF90 program, a total of 52 1 Mb genomic windows explained more than 0.3% of genetic variance for MT (five times more than expected) and, therefore, were considered significantly associated with MT. They were located on 15 porcine chromosomes (SSC): 1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 13, 14, 15, 17, and 18 (Fig. 1). These windows collectively accounted for 27.56% of the total additive genetic variance for MT and encompassed 299 positional candidate genes for this condition (Supplementary Table S1). The variance explained by significant windows ranged from 0.3 to 1.66%.

To identify key genes involved with MT, the genes located in the windows explaining the greatest proportion of genetic variance (windows above 1%, a total of 15 genes) were selected and submitted to functional annotation and investigated for their possible relationship with MT (Table 1).

Evaluating the ontologies of the 299 candidate genes located in all the significant windows in the Panther database[19], it was possible to observe that 18 of them were in biological processes (BP) that have already been related to feeding behavior, digestion and intestinal diseases (Table 2) and could contribute to the occurrence of MT in pigs. Furthermore, those 299 genes were also submitted to a statistical overrepresentation analysis in the

Chromosome	Gene	Biological process
1	*OPRM1*	Feeding behavior
2	*MCC*	Intestinal diseases and alterations
3	*ELN*	Digestion and absorption of proteins
4	*PGB*	Digestion
4	*CHIA*	Digestion, Digestion of dietary carbohydrates, Digestion and absorption
6	*GOT2*	Digestion and absorption of lipids
7	*IL17A*	Maintenance of intestinal epithelial structure, Intestinal diseases
7	*IL17F*	Inflammatory bowel disease
8	*MTTP*	Digestion and absorption of lipids
8	*PPARGC1A*	Response to excess feed intake
12	*KCNJ2*	Gastric acid secretion
12	*KCNJ16*	Gastric acid secretion
13	*SGO1*	Intestinal diseases, Chronic Intestinal Dysrhythmia
13	*KCNJ15*	Gastric acid secretion
14	*GATA4*	Differentiation of intestinal epithelial cells, Embryonic morphogenesis of the anterior intestine
17	*OVOL2*	Embryonic morphogenesis of the digestive tract
18	*ADCYAP1R1*	Activity of the receptor for vasoactive intestinal polypeptide
18	*GLI3*	Embryonic morphogenesis of the digestive tract, Morphogenesis of the posterior intestine

Table 2. Genes and biological processes that may be related to the development of mesenteric torsion in pigs, according to the functional annotation in the Pantherdb database.

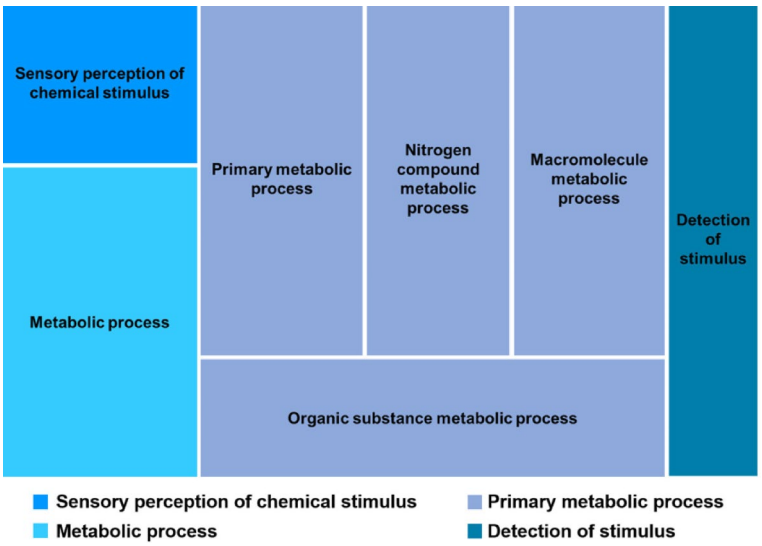

Fig. 2. Superclusters of significant biological processes related to genes in the genomic windows associated with mesenteric torsion in pigs. The figure was constructed using REVIGO tool (Supek et al.[20]).

Panther database[19] and seven BP were enriched (Supplementary Table S2; $p < 0.05$), comprising 135 genes. These BP were grouped into four superclusters using the Revigo tool[20]: metabolic process, primary metabolic process, sensory perception of chemical stimulus and detection of stimulus (Fig. 2).

A gene network (Fig. 3) was constructed in the Network Analyst platform[21] using the 73 genes located on the first ten windows with the highest genetic variances. This analysis revealed interactions between candidate genes and their interactions with other genes (for example, *RAP1A* with *NTRK2*, *PTPN11* and *BRAF*), suggesting potential interference of candidate genes with other genes that may play an important role in the phenotypic expression of MT. This hypothesis could be reaffirmed by exploring the biological functions from this gene network. The key genes responsible for gastrointestinal hemorrhage, atresia, and intestinal obstruction were highlighted (in blue, Fig. 3) and, in humans, these conditions can predispose to intestinal volvulus. Given the similarities between pigs and humans in anatomical, physiological, and genetic terms[22], there is a possibility that genes associated with anomalies related to intestinal volvulus in humans may also be linked to the initiation of MT in pigs.

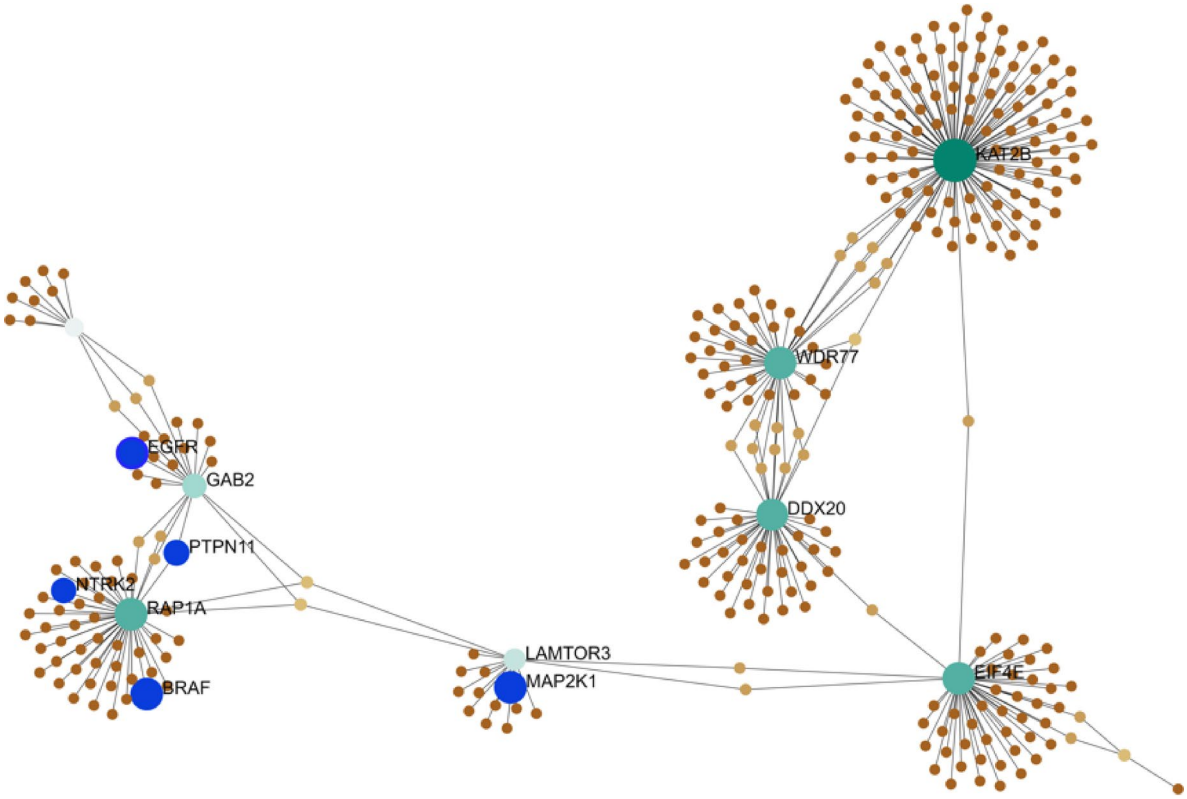

Fig. 3. Gene network constructed with genes located on the first ten genomic windows with the highest association with mesenteric torsion in pigs. Colored circles represent genes and lines represent the predicted interactions between genes. In blue, genes related to intestinal abnormalities that can trigger intestinal volvulus in humans. The gene network was constructed using Network Analyst platform (Zhou et al.[21]).

Discussion

The MT is a common cause of sudden death in pigs, and it is characterized by the torsion of the intestinal loops through the mesentery. This pathology is triggered by several environmental conditions; however, Padilha et al.[13] verified the existence of additive genetic variance and estimated a heritability of 0.12 ± 0.02 to this trait. Similar to most complex traits, MT is polygenic, i.e., affected by thousands of genetic variants with small effects[23]. Here, we found that MT is influenced by several genomic regions (windows) situated on different chromosomes (Fig. 1) that explained 27.56% of the total additive genetic variance for MT, encompassing 299 genes (Supplementary Table S1). Hence, it is anticipated that, by examining the genetic architecture of this pathology, we were able to elucidate some biological mechanisms contributing to the onset of MT in pigs, even using a limited sample size.

The genes located in the most relevant windows were investigated for their relationship with mesenteric torsion (MT). However, for some of those genes, the literature poorly describes their biological functions and the mechanisms through which they act and, in some cases, there are no studies relating them with intestinal diseases. Therefore, those were not discussed in the current study. Nevertheless, several genes that may predispose pigs to MT were identified, including hydroxysteroid 17-beta dehydrogenase 4 (*HSD17B4*), TNF alpha-induced protein 8 (*TNFAIP8*), teneurin transmembrane protein 4 (*TENM4*), chromodomain helicase DNA binding protein 2 (*CHD2*), repulsive guidance molecule BMP co-receptor A (*RGMA*), opioid mu receptor 1 (*OPRM1*), *PPARG* coactivator 1 alpha (*PPARGC1A*), chitinase acidic (*CHIA*), potassium inwardly rectifying channel subfamily J member 2, 16 and 15 (*KCNJ2*, *KCNJ16*, and *KCNJ15*), elastin (*ELN*), shugoshin 1 (*SGO1*), interleukin 17 A (*IL17A*) and interleukin 17 F (*IL17F*), GATA binding protein 4 (*GATA4*), ovo-like zinc finger 2 (*OVOL2*), GLI family zinc finger 3 (*GLI3*), and member of RAS oncogene Family (*RAP1A*) (Supplementary Table S1).

Three genomic windows that explained more than 1% (Table 1) of the genetic variance for mesenteric torsion were mapped on chromosomes 2, 7 and 9, which were fully investigated for their association with MT. In the genomic window with the highest genetic variance explained for MT (1.66%) on SSC2, three genes were found: *FAM170A*, *HSD17B4* and *TNFAIP8*. The *HSD17B4* encodes a protein known as Peroxisomal D-bifunctional, a key enzyme in fatty acid oxidation metabolism[24]. This gene was upregulated in late-preterm (113 days) pigs and was enriched in the fatty acid biosynthesis pathway in the colon tissue[25]. Fatty acids influence digestion and intestinal absorption, and many fatty acids are produced through microbial fermentation[26,27]. Thus, mutations in this gene could affect its function related to the digestion process, resulting in increased intestinal fermentation and gas production. The *TNFAIP8* gene plays a major role in the composition and modulation of intestinal epithelial cell differentiation[28] and its deficiency exacerbates the inflammatory response, allowing greater bacterial invasion causing intestinal dysbiosis[29,30]. Consequently, the imbalance of the intestinal microbiota

could cause increased intestinal fermentation and heightened gas production. As a result, intestinal dilation due to gas production could lead to compression of the mesenteric vein, initiating a cascade of events that predisposes to intestinal torsion[2].

In the second window with the highest genetic variance explained (1.54%), located on SSC9 (Table 1), eight genes were mapped: *ALG8*, *GAB2*, *USP35*, *NARS2*, *TENM4*, *KCTD21*, *THRSP* and *NDUFC2*. However, there are few studies relating the function of these genes in the intestine. The *TENM4* was upregulated in situation of intestinal dysbiosis induced by antibiotic[31]. On SSC7, the third highest explained variance window (1.07%) (Table 1), four positional genes were mapped: *FAM174B*, *CHD2*, *RGMA* and *ST8SIA2*. The *CHD2* encodes a protein that regulates the structure and expression of DNA and belongs to the family of helicases, enzymes crucial for unwinding and manipulating DNA[32]. According to Chénier et al.[33], mutations in this gene are associated with epilepsy syndrome and developmental delay. Berg et al.[34] reported that children with epileptic and developmental encephalopathies associated with the *CHD2* gene also exhibited high rates of constipation and gastric dysmotility. Hence, mutations in this gene could lead to a delay in the propulsion of intestinal contents and increased fermentation. In addition, the *RGMA* gene (Table 1) exerts an inhibitory effect on the growth of neurites from neuronal progenitor cells and is involved in the differentiation process of enteric neurons in the intestine[35]. According to Furness[36], the enteric nervous system (ENS) controls motility, fluid secretion, and vasodilation in the intestines. Consequently, defects in the development of the ENS can lead to intestinal motility disorders[37]. Thus, mutations in the *CHD2* and *RGMA* genes could potentially alter intestinal motility and the proper functioning of the intestines, resulting in intestinal obstruction and predisposing pigs to mesenteric torsion. The presence of intestinal obstruction increases the possibility of small bowel volvulus[38], a similar condition of MT in humans.

Some other genes found in chromosomes 1, 2, 3, 4, 6, 7, 8, 12, 13, 14, 17 and 18 in our study were in biological processes related to feeding behavior, digestion, intestinal diseases, and morphogenesis of the intestine (Table 2) and were also investigated for their association with MT in pigs. The *OPRM1* gene is located on SSC1 and codes for an opioid receptor, also known as MOR, which has been linked to binge-eating disorder and eating behavior in humans. Binge-eating disorder has been associated with a variant of the *OPRM1* gene (*A118G*), suggesting that individuals with this mutation have a genetic predisposition to this condition due to hyper-reactivity to the hedonic properties of foods[39]. In fact, opioid signaling regulates the hedonic impact of food, and the *A118G* variant has also been linked to the consumption of high-calorie foods (sweet and fatty) and the behavior of overeating[40,41]. Elevated levels of hedonic response to food can promote increased intake, leading to binge eating[42]. Moreover, MOR receptors expressed in the mesenteric-portal area control a gut-brain neural circuit that regulates intestinal gluconeogenesis. In this way, they indirectly control feed intake and feelings of satiety[43]. Therefore, it is hypothesized that mutations in the *OPRM1* gene can alter the feeding behavior of pigs, resulting in increased feed intake. Consequently, an animal that excessively increases its feed intake in a short time is at a greater risk of excessive fermentation in the intestines, thus being more likely to develop MT.

Located on SSC8, the *PPARGC1A* or *PGC-1α* gene is associated with the biological process of responding to excess feed intake (Table 2). It codes for a transcriptional coactivator that controls the expression of several genes involved in glucose and fatty acid metabolism[44] and, in the current study, several metabolic processes were significant (Fig. 2). However, in porcine intestinal epithelial cells, activation of the *SIRT1/PGC-1α* pathway contributes to increased autophagy/mitophagy activities, reducing oxidative injury, and maintaining the integrity of the intestinal barrier[45]. Mutations in the *PPARGC1A/PGC-1α* gene could affect luminal digestion and the intestinal barrier, increasing the inflammatory response and compromising functions such as permeability and transit. This may lead to issues such as intestinal dysbiosis, high fermentation, and intestinal obstruction, contributing to the development of MT.

In our study, ontological terms related to "metabolic processes" were significant (Fig. 2), and some genes were associated with digestion (Table 2), a process involving various metabolic pathways. The *CHIA* gene, located on SSC4, encodes chitinase A, which, like other chitinases, catalyzes the breakdown of chitin, producing more digestible fragments serving as sources of carbon, energy, and nitrogen[46]. As noted by Ohno et al.[47], the *CHIA* gene is expressed in the stomach of humans and other animal species, including mice, chickens, monkeys, and pigs. Since *CHIA* is involved in digestion, mutations could compromise nutrient metabolism. Still in a sense of digestion, the *KCNJ2* and *KCNJ16* genes, located on SSC12, and *KCNJ15* on SSC13, code for rectifying potassium (K+) channels conducting K + ions into cells[48]. Expressed in parietal cells, these genes may be involved in gastric acid secretion[49–51]. Therefore, given their relationship with digestion, mutations in these genes could potentially compromise the digestion process, resulting in a delay in the propulsion of intestinal content, leading to constipation, increased intestinal fermentation, and a predisposition to developing MT.

The *ELN* gene, located on SSC3, although involved in biological process of digestion, encodes elastin, a component of the extracellular matrix, previously related to diverticular colon disease[52], a clinical condition characterized by small pouches in the intestinal wall that can lead to abnormalities in the colon wall structure and disordered intestinal motility[53]. According to Böttner et al.[54], as the disease affects neurotransmitter receptors, it can lead to dysregulation of intestinal motility. Furthermore, in humans, diverticulum can cause intestinal obstruction and volvulus[38,55]. In the same sense, the *SGO1* gene, found on SSC13, is associated with chronic atrial and intestinal dysrhythmia syndrome in humans (Table 2)[56]. Dysrhythmias linked to *SGO1* mutations can result in severe disorders in intestinal motility, characterized by the ineffective propulsion of intestinal contents[57]. Thus, mutations in these genes could potentially alter intestinal motility, leading to intestinal obstruction and predisposing pigs to mesenteric torsion.

The *IL17A* and *IL17F* genes, located on SSC7, encode interleukins 17 A and 17 F, respectively, and have been related to biological processes that maintain intestinal epithelial structure and combat intestinal diseases (Table 2). Interleukins are proteins primarily produced by leukocytes, playing a key role in activating or suppressing the immune system. *IL17A* and *IL17F* are linked to TH17 cells, crucial for maintaining mucosal

barriers and eliminating pathogens from mucosal surfaces[58]. *IL17A* orchestrates antimicrobial peptides and neutrophils, while *IL17F* contributes to mucosal immunity against pathogens[59]. Kiliç et al.[60] reported that rabbits experiencing mesenteric ischemia (an interruption of intestinal blood flow secondary to issues like embolism or thrombosis) exhibited a higher release of inflammation mediators, including interleukins. Additionally, mice with testicular torsion (a condition involving the rotation and strangulation of the testicles' blood supply) also showed elevated interleukin levels[61]. As these genes influence the immunity of mucous barriers, alterations in the intestinal barrier could initiate inflammation due to bacterial activity and intestinal hyperemia. This, when coupled with a compromised immune response, might facilitate the overgrowth of certain bacteria, leading to heightened intestinal fermentation and an increased predisposition to MT.

In the current study, the *GATA4*, *OVOL2* and *GLI3* genes were also enriched in biological process of morphogenesis of the intestine (Table 2). In humans, intestinal malformations have been associated with the volvulus[38,62,63]. The *GATA4* gene, located on SSC14, encodes a transcription factor that operates in the expression of the definitive endoderm during the embryonic development of the intestine, giving rise to the primitive intestinal tube. Additionally, this transcription factor acts as a regulator in the proliferation of intestinal epithelial cells, influencing the length of the intestine and participating in villous morphogenesis[64,65]. The *GATA4*, together with *GATA6*, contribute to maintaining the intestinal epithelial structure, regulating cytodifferentiation. Moreover, these genes can repress the differentiation of goblet cells, promoting the differentiation of enterocytes[66]. In adulthood, *GATA4* is responsible for regulating the expression of intestinal epithelial genes[67], establishing jejunal-ileal identities, as an example. Consequently, alterations in the expression of specific genes in the ileum can modify the ileal transcriptome, making it more akin to the duodenum and jejunum. Similarly, changes in gene expression in the jejunum may lead to loss of jejunal function, compromising the absorption of fat and cholesterol[68,69]. Moreover, *GATA4* induces morphological differentiation in intestinal cells, resulting in the development of functional characteristics like microvilli, and acts as a transcriptional regulator for maintaining the integrity of the intestinal epithelial barrier[70,71]. Mutations in the *GATA4* gene can impact intestinal morphogenesis and the differentiation of intestinal cells, compromising functions such as digestion, nutrient absorption, and the integrity of the intestinal epithelial barrier. These changes may contribute to intestinal malformations and hinder proper intestinal function, increasing the likelihood of developing MT.

The *OVOL2* gene, identified on SSC17, codes for a crucial evolutionarily conserved regulator that determines and differentiates the epithelial lineage during embryogenesis, particularly in the development of various tissues, including the intestinal tube[72,73]. Studies by MacKay et al.[73] revealed that mice lacking expression of this gene in the endoderm exhibited abnormal intestinal morphology and a less developed intestinal epithelium. The *GLI3* gene, situated on SSC18, encodes a transcription factor that is a member of the Hedgehog signaling pathway (HH), capable of acting as an activator (Gli3-FL), regulating genes involved in HH, or as a repressor (Gli3-R), inhibiting HH functions[74]. The HH signaling pathway plays a crucial role in mesenchymal growth and smooth muscle differentiation during embryonic gut morphogenesis. However, in adults, it influences intestinal epithelial homeostasis, controlling cell migration from the crypt to the villi and increasing apoptosis[75,76]. Mutations in the *GLI3* gene and alterations in the HH pathway have been associated with various diseases and birth defects. Mice with mutations in this gene exhibited anal stenosis, ectopic anus, and abnormalities in the embryonic cloaca (which later gives rise to the urinary and digestive system), indicating that mutations affecting HH signaling can disrupt the normal development of the small intestine and result in malformations, including anorectal malformation[77,78]. Moreover, the negative expression of *GLI3* can lead to the absence of neurons in certain areas of the small intestine and colon, causing intestinal dilation resembling Hirschsprung's disease[79], which is characterized by the lack of enteric neurons, leading to intestinal obstruction. Furthermore, Curry-Jones syndrome, characterized by multiple malformations, including digestive hemorrhage, intestinal malrotation, dysmotility, and intestinal obstruction, may be linked to a series of mutations involving HH signaling. For example, mutations in the *SUFU* (negative regulator of hedgehog signaling) gene, which codes for a protein that normally binds to *GLI3* and promotes its repressive form (Gli3-R), could contribute to this syndrome[80,81]. Considering this, mutations in the *OVOL2* and *GLI3* genes might impair the embryonic development of the intestine, causing dysmotility and intestinal obstruction, besides impairing intestinal functions, and contribute to the emergence of malformations, including intestinal malrotation, which in humans predisposes to intestinal volvulus and, in pigs, could predispose to MT.

The *RAP1A* gene, located on SSC4, exhibits interactions with genes implicated in disorders and malformations in humans, potentially predisposing to intestinal volvulus (Fig. 3). This gene encodes a protein from the Ras family of small GTPases capable of performing various functions in the organism. *RAP1A* may play a crucial role in two metabolic pathways (cAMP/Epac and cAMP/PKA) that mediate the release of neurotensin, an intestinal peptide responsible for gastrointestinal secretion, motility, inflammation, and the growth of intestinal tissues[82]. Additionally, this gene may be involved in the secretion of pancreatic amylase[83], an enzyme produced by the acinar cells of the pancreas and necessary for digestion that occurs in the intestinal lumen[84]. Moreover, the activation of *RAP1A* stabilizes β1 integrin levels and regulates cell migration, a fundamental process for the maintenance and repair of the intestinal epithelial barrier[85]. Elevated levels of *RAP1A* inhibit the activity of the RhoA protein, leading to the relaxation of intestinal smooth muscle[86] and triggering a dependent pathway that regulates intestinal fluid transport[87]. Consequently, mutations in this gene could impact digestion, intestinal motility, and the integrity of the epithelial barrier, resulting in delayed intestinal transit, excessive fermentation, and other issues that collectively may contribute to the onset of MT.

Therefore, genetic variants associated with mesenteric torsion are dispersed among genes linked to biological processes such as embryonic morphogenesis of the intestine, epithelial differentiation of intestinal cells, maintenance of the intestinal barrier, feeding behavior, and functions such as digestion, permeability, and intestinal motility. These processes have the potential to induce intestinal malformations, bacterial infection, intestinal dysbiosis, excessive fermentation, delayed intestinal transit, and obstruction, hampering the proper

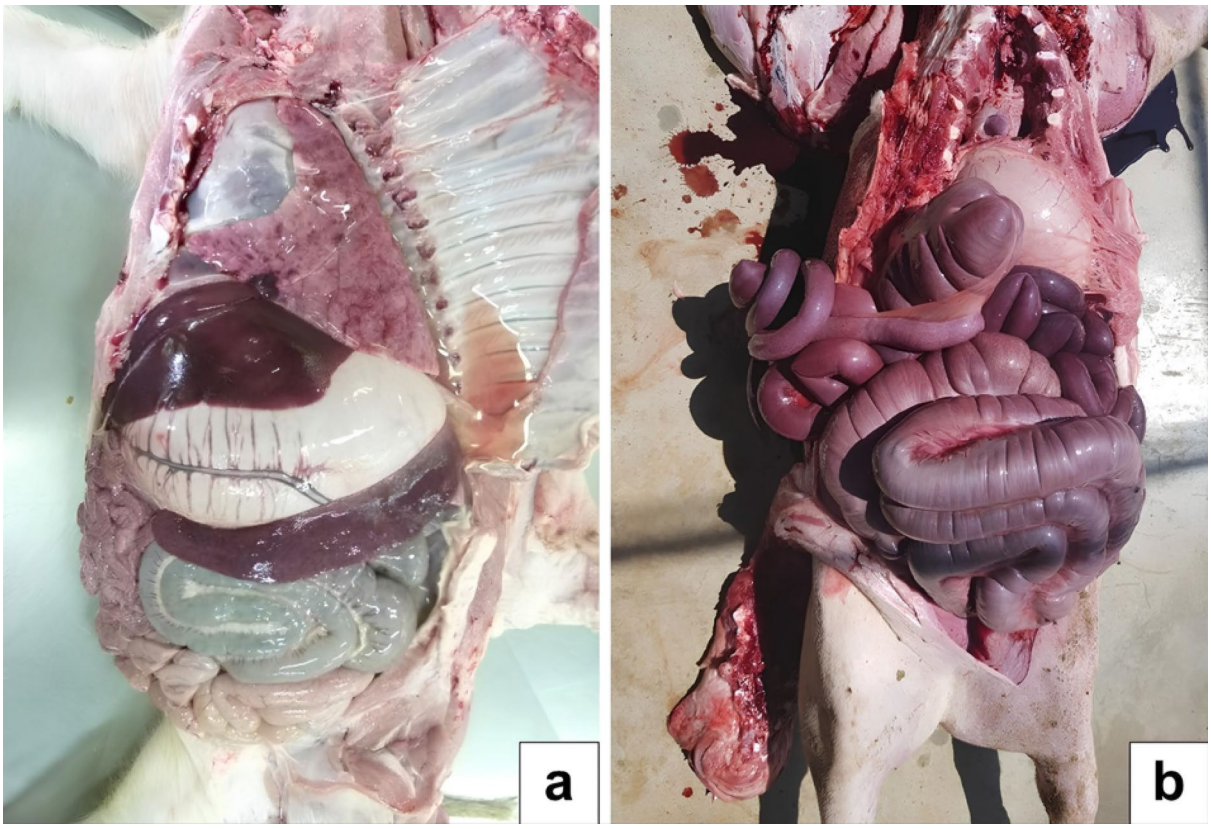

Fig. 4. Abdominal cavity of unaffected pig (**a**) and pig with mesenteric torsion (**b**).

functioning of the intestines and predisposing pigs to mesenteric torsion. These findings contribute to a better understanding of the genetic mechanisms involved in the occurrence of this metabolic problem, which causes significant economic losses in pig production and impacts animal welfare. The variants and genes identified in this study are potential markers to be used in genetic selection to reduce the incidence of mesenteric torsion in commercial swine herds after appropriate validation. Furthermore, the results of this study may contribute to elucidate the etiology and pathogenesis of intestinal torsion/volvulus in other mammals, including humans.

Methods

Ethics statement

All experimental procedures were conducted in conformity with the guidelines of the Ethics Committee for Animal Use (CEUA) from the Embrapa Swine and Poultry National Research Center, with approval protocol number 002/2016, in agreement with the rules of the National Council of Animal Experimentation Control (CONCEA) to ensure compliance with international guidelines for animal welfare.

Animals and data

This study used data from a Large White maternal line of the BRF S.A. nucleus breeding farm, located at the Santa Catarina State, in the southern region of Brazil. The dataset comprised 405 animals (121 males and 284 females) born between 2019 and 2022, with information on genealogy (animal, sire, and dam), contemporary groups (CG: sex, year, and week of weaning), weaning weight (WW), and mesenteric torsion (MT), classified as 0 for healthy and 1 for affected animals. Animals that died with suspected MT (139) underwent necropsy to confirm the pathology. Necropsies were conducted with the animal in dorsal decubitus by opening the abdominal wall in the midline surrounding the costal arch to visualize the positioning of the viscera, as depicted in Fig. 4. Furthermore, a histopathological analysis was carried out to discard other conditions, such as hemorrhagic enteritis that could be mistaken as MT. The genotype file included the same 405 animals, with 266 normal (Fig. 4A) and 139 affected (Fig. 4B), i.e., those that died due to MT. Animals classified as normal showed no signs of the pathology, were contemporaries of the affected animals, and originated from families with no MT history in the last two generations. Pigs from the normal group were not necropsied, because they were healthy and kept alive in the nucleus farm, as they were candidates for selection to produce the next generation. All genotyped samples were sourced from the BRF Tissue Bank, with DNA extracted from pig tail tissue. Genotyping was carried out using the GGP Porcine 50K BeadChip (Neogen Genomics in Lincoln, NE, USA).

Genotype quality control

Quality control procedures for samples and SNPs were performed using the PREGSF90 package from the BLUPF90 programs[88]. Samples and SNPs were filtered when call rate of < 0.90, SNPs were monomorphic, and with a minor allele frequency (MAF) < 0.05. Non-autosomal SNPs, duplicates, and SNPs with an unknown position in the genome were also removed. Animals showing divergence in the relationship matrix were subsequently excluded. After the quality control process, the dataset retained 397 samples and 36,438 SNPs.

Genome-wide association study (GWAS)

GWAS analysis was conducted using the Bayesian approach and the GBLUP methodology within the BLUPF90 family programs[88]. The genetic and residual variance components utilized for GWAS were estimated by Padilha et al.[13]. The PREGSF90 package[89] was employed to construct the genomic relationship matrix (G). Subsequently, BLUPF90+[88] was applied to solve the mixed model equations, and the POSTGSF90 package[89] was used for the GWAS analysis. The window size (1 Mb) was defined based on the density of the SNP panel used and on the literature[90–93].

The model used to analyze MT in the matrix form can be represented as follows:

$$y = Xb + Zu + e$$

where:

y = vector of phenotypic observations (MT; 0 or 1);
β = vector of fixed effects (GC and linear WW covariate effect);
u = vector of additive genetic effects of markers;
X and Z = incidence matrices associated with each effect (β and u, respectively);
e = vector of residual effects.

The SNP effects were estimated as follows: $\hat{m} = DM' \left[MDM'\right]^{-1} \hat{a}_g$, where $\hat{m}$ is the vector of SNP marker effects, D is a diagonal matrix containing weights of SNPs, M is a matrix containing the genotypes of each locus, and $\hat{a}_g$ is the vector of estimated genomic breeding values[94]. In our study, SNPs were given equal weights, and the percentages of genetic variance explained by each 1-Mb window were computed using the POSTGSF90 package[89] as described below:

$$\frac{var\left(u_i\right)}{\sigma_u^2} \times 100 = \frac{var(\sum_{j=1}^{N} M_j m_j)}{\sigma_u^2} \times 100$$

where:

u_i = breeding value of genomic region i under consideration;
N = total number of adjacent SNPs within the 1-Mb genomic region;
m_j = effect of the SNP marker j within region i.

After the GWAS analysis, to define the threshold, the genome was divided into 1,640 non-overlapping windows of 1 Mb each. Each window is expected to explain 0.06% of the genetic variance (100%/1,640), and windows that explained five times more than the expected (0.3%), were considered significant[92,95,96] and were used to identify candidate genes. The Manhattan plot was constructed with R software version 4.2.2[97] using the results from the POSTGSF90 package[89].

Identification of candidate genes and enrichment analysis

A list of genes was retrieved based on the initial and final positions of each genomic window associated with MT using the Ensembl Genes 109 database available in the Ensembl BioMart tool[98]. Subsequently, to elucidate the biological significance of the identified genes, functional annotation was conducted using the Pantherdb database[19], providing insights into the biological processes involving the candidate genes. A statistical overrepresentation analysis was performed in Panther database[19] using the Fisher's Exact test with FDR correction (p adjusted < 0.05). The REVIGO tool[20] was then used to summarize and enhance the visualization of significant biological processes. To obtain the predicted interactions among protein-protein genes, a network with genes located in the top ten windows with the highest genetic variances was constructed using the information from human annotation in the String database through the Network Analyst platform[21]. To verify the gene function regulation related to possible diseases/conditions, the DisGeNet database was used with human data, since there is no swine information in this database. Moreover, a search at public databases (NCBI, GeneCards, and OMIM) and relevant literature was undertaken to identify genes located in windows explaining a greater proportion of the MT genetic variance and those involved in biological processes potentially associated with MT.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

A.M.G.I., J.O.P., L.O.D.C., and M.C.L. conceived and designed the experiment. A.M.G.I., J.O.P., M.C.L., S.F.P., R.M., and M.E.C performed data analysis and curation. L.O.D.C., M.S.F., and J.S.L. performed data collection. L.M.H. and M.A.Z.M. were responsible for image evaluation and histopathological analysis. M.E.C, R.M., R.A.T., L.O.D.C., M.S.F., and J.S.L assisted in results interpretation. A.M.G.I., J.O.P., M.C.L. and L.T.D. supervised the research, monitored data analysis procedures and results interpretation. S.F.P, A.M.G.I., J.O.P., M.C.L., prepared the original draft manuscript. M.C.L. was responsible for funding acquisition. All authors have read and approved the final manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Ethics

The protocols and the use of animals for this research were approved by the Ethics Committee on Animal Use (CEUA) from the Embrapa Swine and Poultry National Research Center under the protocol # 002/2016. This study was carried out in compliance with the ARRIVE guidelines (https://arriveguidelines.org).

Additional information

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