Acta fytotechn zootechn, 24, 2021(4): 309–314
http://www.acta.fapz.uniag.sk

Review

Candidate genes for congenital malformations in pigs

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Article Details: Received: 2021-06-07 | Accepted: 2021-06-21 | Available online: 2021-12-31

https://doi.org/10.15414/afz.2021.24.04.309-314

Congenital malformations occur in numerous pig breeding programs. Clinical symptoms, etiopathogenesis and candidate genes of the most critical congenital malformations in pigs were briefly overviewed in the study. Based on the recent literature, identifying and evaluating the genomic regions associated with defects, such as splay legs syndrome, hernias, cryptorchidism, atresia ani, kyphosis, intersexuality, and malignant melanoma, can enhance the selection response. As promising genes were published e.g. NREP, FBXO32, and *HOMER1* for splay leg syndrome, SRC, *OSM*, COL family, and CGRP for hernias, *GNRHR*, *GATA2*, and RLF for cryptorchidism, and *GLI2* for atresia ani. Potential candidate genes associated with defects were mainly detected in literature by the genome-wide association approach. Reviewing the studies and following the suggestions in some of papers it is indicated the necessity for molecular and more comprehensive evaluation in terms of the sample standardisation and accurate phenotyping of a broad spectrum of populations and breeds. Moreover, knowledge transmission among all livestock species and humans is recommended in literature to better understand malformation biology.

Keywords: swine, congenital defects, health, association studies, genomic selection

1 Introduction

Breeding in pigs to improve production traits can lead to physiological changes that can deteriorate animal health and negatively affect the pig farm economics (Prunier et al., 2010; Sevillano et al., 2015). Congenital malformations occur in numerous pig breeding programs worldwide (Mattsson, 2011). Splay legs syndrome, hernias, cryptorchidism and other congenital defects (e.g. atresia ani, kyphosis, intersexuality, and malignant melanoma) are defined as the most critical congenital defects. Generally, heritability rate of malformations is of 0.01–0.80 (Mattsson, 2011; Sevillano et al., 2015; Rousseau et al., 2013) and about 1–8% of animals of the given pig category is affected by these defects. A common practice to eliminate malformations is the reproductive exclusion of the affected animals (Mattsson, 2011).

The identification of structural and functional proteins, genes, and quantitative trait loci allowed the study of congenital malformations (i.e. splay legs syndrome, hernias, and cryptorchidism) and other health traits at the genome level (Schumacher et al., 2021). Therefore, the identification and evaluation of genomic regions that control congenital defects was a breakthrough for the breeding programs (Sevillano et al., 2015). Besides, the detailed knowledge of the genome using single nucleotide polymorphism (SNP) markers can be usefully applied to manage the diversity of conventional populations (Moravčíková and Kasarda, 2020).

In the Czech Republic, the negative selection of animals (or entire litters) for malformations has been applied for many years in pig breeding programs. However, the routine phenotype monitoring of malformations is provided in the population (Žáková et al., 2020) and the collection of animal tissues for the genomic evaluation of developmental defects started in accordance with the local breeders' intention to enhance the selection candidates (Krupová et al., 2017). The overall ambition is to identify gene markers and pathways associated with phenotypically measurable traits to enhance the selection response. Therefore, this study aimed to briefly overview the clinical symptoms, etiopathogenesis and candidate genes of the most critical congenital malformations in

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pigs to form the groundwork in the subsequent genomic evaluation of tissue samples.

2 Congenital malformations in pigs

2.1 Splay legs syndrome

The splay legs syndrome (also known as spraddle leg syndrome) is the most frequent lame disorder in newborn piglets and the most observed inherited malformations in pigs. The syndrome has high economic and welfare consequences on the swine industry (Schumacher et al., 2021). The affected piglets are not able to stand or walk due to temporary impairment of the pelvic muscle function that occurs early after birth (Papatsiros, 2012; Hao et al., 2017). Histologically, the syndrome is expressed as myofibrillar hypoplasia and characterised by a higher proportion of less developed and smaller myofibrils (Schumacher et al., 2021). Clinically, the syndrome appears in various forms, from ataxia (sitting like a dog with a possibility to stand up) through the uncoordinated movement of rear legs (piglet is not able to get up without help) or the splaying of rear legs (movement is done just with the front legs) to a severe form, in which both the front and rear legs are splayed (piglet is not able to move). Standard treatments, such as leg binding, vitamin, and selenium intake, and appropriate care, are applied to reduce losses of affected piglets due to malnutrition, hypothermia, or crushing by the sows. Usually, piglets that survive the first week of their life recover completely (Papatsiros, 2012).

The genetic aspects of the syndrome have been thoroughly evaluated in the context of etiopathogenesis (Maak et al., 2009; Hao et al., 2017). An overview of the candidate genes associated with the syndrome is presented in Table 1. The primary pathological mechanism of the syndrome includes atrophy of muscle fibres and delayed skeletal muscle maturation at birth (Schumacher et al., 2021). Therefore, three promising candidate genes, NREP, FBXO32, and *HOMER1*, associated with myogenic differentiation, atrophy, and muscle development should be considered for the study of myofibrillar hypoplasia in pigs. Moreover, muscle sampling should be standardised for improving phenotypic precision.

2.2 Hernias

A hernia is generally characterised as penetration of the intestine, an internal organ, or tissue through the weakened supportive muscles or body wall. The umbilical, inguinal, and scrotal hernias often occur in pigs. Clinical verification is usually needed to diagnose the inguinal and scrotal hernias; therefore, they are frequently evaluated as a single trait (Ding et al., 2009; Grindflek et al., 2006). Inguinal hernias are typically detected in the first week of life at the time of castration, and affected piglets are usually euthanized. Pigs with umbilical hernias do not appear until the nursery or the early fattening stage (Atkinson et al., 2017). Similar to the splay leg syndrome, hernias have economic consequences on the swine industry (Nowacka-Woszuk, 2021) when the feed efficiency and growth ability are reduced in affected animals (Ding et al., 2009).

From the papers intensively focused on the genetic architecture of hernias outcomes their complex background is apparent from the interaction of polygenic heredity and environmental conditions (Nowacka-Woszuk, 2021). The candidate genes associated with hernias are presented in Table 1. The most promising genes are linked to the proper growth and differentiation of cells (SRC and *OSM*), the function of connective tissues (COL family), the gubernacular development and function (INSL3 and MIS), and processus vaginalis (CGRP). The latter gene probably leads to a relationship between the scrotal hernia and cryptorchidism with a correlation of 0.2–0.7 (Mattsson, 2011). Both congenital defects occur often and have a sex-linked expression.

2.3 Cryptorchidism

Cryptorchidism occurs when one or both testicles of male piglets cannot descend from the intra-abdominal placement in the scrotum. The unilateral (left side) cryptorchidism mainly occurs in pigs, and the affected males should be excluded from reproduction (Mattsson, 2011; Mahmud et al., 2015). The malformation is usually diagnosed at birth or early in the postpartum period. Similar to other congenital defects, variability of the malformation prevalence is identified among the evaluated populations, breeds, parities, and litters (Sevillano et al., 2015; Mattsson, 2011).

Cryptorchidism is linked to the animal genome, anatomy, and endocrine system (Mahmud et al., 2015). An overview of the putative candidate genes associated with cryptorchidism are presented in Table 1. The most promising candidate genes are associated with the testes descent (*GNRHR*), the urogenital development and function (*GATA2* and PDGFRA), and the modulation of foetal responses to oestrogens (*AFP*; Sevillano et al., 2015). Besides, *RHOA* that has an indirect impact on scrotal hernia also plays an essential role in cryptorchidism due to the regulation of smooth muscle tissues, which are responsible for the obliteration of processus vaginalis that precedes appropriate testicular descent. Furthermore, a mutation in INSL3 (also known as RLF) probably has an impact on the cryptorchidism incidence in male mice (Tomboc et al., 2000). Therefore, multiple genes are implicated in proper testicular descent and fertility, some of which have a surplus role.

Table 1 Selected candidate genes associated with congenital malformations in pigs

Trait name	Gene symbol	Gene name	Gene function (factor)	SSC1	Reference
Splay leg syndrome	*DDIT4*	DNA-damage-inducible transcript 4	autophagy, apoptosis	14	Maak et al. (2009)
	MAF	MAF bZIP transcription factor	transcription, DNA binding	6	
	SQSTM1	Sequestosome 1	autophagy, protein degradation	2	
	SSRP1	Structure specific recognition protein 1	myogenic differentiation		
	HOMER1	Homer scaffold protein 1	glycogen metabolism, muscle development		Hao et al. (2017), Xu et al. (2018)
	FBXO32 (MAFbx)	F-box protein 32	ubiquitin-proteasome pathway, atrophy, proteolysis	4	Wu et al. (2018)
Umbilical hernia	*SRC (c-Src)*	SRC proto-oncogene, non-receptor tyrosine kinase	collagen deficiency and ventral body wall defects	17	Liao et al. (2015)
	OSM	Oncostatin-M	promotion the cells growth and differentiation, embryogenesis, inflammatory response to injury	14	Grindflek et al. (2018)
	LIF	LIF interleukin 6 family cytokine			
	NUGGC	Nuclear GTPase, germinal center associated	regulation of apoptotic process and nuclear cell cycle DNA replication		Long et al. (2016)
Inguinal/ scrotal hernia	*INSL3 (BLEYI-L, LEY I-L)*	Insulin-like hormone 3	induction of gubernacular development	2	Grindflek et al. (2006)
	MIS (AHM)	Anti-Mullerian hormone	swelling reaction of gubernaculum during the testicular descent; sex differentiation control (reproductive organs development, secondary sex characteristics)		
	CGRP (CALCB)	Calcitonin-related polypeptide beta	impact on processus vaginalis (the fusion induction, transformation of epithelium) and tissue remodelling		
	ELF5	E74-like ETS transcription factor 5	epithelial-mesenchymal transition	2	Du et al (2009)
	KIF18A	Kinesin family member 18A	pathway regulated by the oestrogen receptors		
	NPTX1	Neuronal pentraxin 1		12	
	COL23A1	Collagen type XXIII alpha 1 chain	proper function of connective tissue	2	
	RHOA	Ras homolog family member A	contraction and shortening of smooth muscle tissue	13	Sevillano et al. (2015)
	EGF	Epidermal growth factor	connective tissue problems	8	
	LEF1	Lymphoid enhancer binding factor 1	correct testicular descent through the beta-catenin pathway		
	EIF4E	Eukaryotic translation initiation factor 4E	regulation of the MID1 (midline 1) gene expression, associated with malformations (like is umbilical and inguinal hernia)		Xu et al. (2019)

Continuation of table 1

Trait name	Gene symbol	Gene name	Gene function (factor)	SSC1	Reference
Cryptorchidism	*GNRHR*	Gonadotropin releasing hormone receptor	descent of testes	8	Sevillano et al. (2015)
	AFP	Alpha fetoprotein	interaction with oestrogens, modulation of foetal responses to oestrogens		
	PDGFRA (CD140A, PDGFR-2, RHEPDGFRA)	Platelet-derived growth factor receptor-alpha like	development and function of male gonads		
	v-KIT (MGF)	Tyrosine protein kinase			
	GATA2	GATA binding protein 2	urogenital development	13	
Atresia ani	*GLI2*	GLI family zinc finger 2	embryonic digestive tract development, hindgut morphogenesis	15	Wiedemann et al. (2005), Cassini et al. (2005)
Kyphosis	*PLOD1*	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1	enzyme forming the hydroxylysine on collagen	6	Lindholm-Perry et al. (2010)
	ADAMTS18	ADAM metallopeptidase with thrombospondin type 1 motif 18	extracellular matrix organisation, negative regulation of platelet aggregation		
	SOX9	SRY-box transcription factor 9	chondrogenesis regulation, sex determination, skeletal development, testis differentiation	12	
Intersexuality	*SOX9* (and its regulatory region TESCO)	SRY-box transcription factor 9 (Testis enhancer sequence core element)	chondrogenesis regulation, sex determination, skeletal development, testis differentiation	12	Brening et al. (2015), Rousseau et al. (2013), Szczerbal et al. (2019)
Malignant melanoma	*PLEKHA5*	Pleckstrin homology domain containing A5	melanoma cells survival, metastatic cells extravasation	5	Bourneuf et el. (2018)
	TRAFD1	TRAF-type zinc finger domain containing 1	tumour necrosis factor receptor binding, regulator of toll-like receptor signalling	14	
	LIMK2	LIM domain kinase 2	kinase involved in keratinocyte adhesion		
	DST	Dystonin	keratinocyte integrity, skin homeostasis, downregulated transcript in ulcerated tumours of melanoma	7	

1 Sus Scrofa Chromosome, number where candidate gene is located by reference or at
ttps://www.ncbi.nlm.nih.gov/genome/?term = txid9823[Organism:noexp

2.4 Other congenital malformations

Atresia ani, kyphosis, congenital tremor syndrome, as well as intersexuality and miscellaneous abnormalities (i.e. anatomic defects of head, heart, and tail) also occur as congenital malformations in pigs (Mattsson, 2011). Of these, the frequency of congenital neoplastic diseases (neoplasms), such as sarcomas, fibro-papillomatosis, hereditary malignant lymphoma, and melanoma, is relatively low (Misdorp, 2003; Morey-Matamalas, et al., 2021). The potential candidate genes for various defects and neoplasms are presented in Table 1. Some candidate genes have been specifically identified for spontaneous melanoma that is frequently observed in Duroc breed and crosses and are related to the occurrence (*PLEKHA5*, *TRAFD1*, and *LIMK2*), clinical ulceration of tumour (DST), and progression through metastasis (SPATA31D1 and EPB41L4A-AS2; Bourneuf et al., 2018).

Atresia ani (AA) is the developmental defect of the missing anus usually accompanied by other anomalies

(Cassini et al., 2005). Morphologically, the porcine AA is characterised by abnormal development of the hindgut, which is probably controlled by an oligogenic or polygenic background (Hori et al., 2001). *GLI2* has been recognised as a major positional candidate gene for AA in pigs (Cassini et al., 2005; Qiushi et al., 2013), whereas significant linkages to AA were obtained for some markers on chromosomes 1, 3, and 12 (Wiedemann et al., 2005). From the last studies dealing with this porcine malformation (Qiushi et al., 2013; Jin et al., 2013) resulted proposal to provide further investigations to explicate the genomic mutations involved in AA.

Kyphosis is a porcine back-curvature defect, which is based on deformation in the hemi-vertebrae. The affected piglets have a humpy back or a dipped shoulder and consequently show a slower growth rate, a lower probability of reaching the slaughter weight (Mattsson, 2011), and a deteriorated carcass value (Lindholm-Perry et al., 2010). Several SNPs and potential mutations in coding regions of candidate genes have been identified (Lindholm-Perry et al., 2010); however, the most promising candidate genes associated with kyphosis are *PLOD1*, *SOX9*, and probably *ADAMTS18*. Besides, the HOX gene family and genes located in the TGF-beta superfamily may control the incidence of kyphosis in pigs (Rohrer et al., 2015).

Congenital tremor syndrome is specified as a rhythmic tremor of newborn piglets caused by the genetic background or environmental conditions during the intrauterine foetus development, leading to myelin deficiency, especially in the spinal cord, and approximately 50% morbidity (Mittsson, 2011). The syndrome appears in two types based on the presence of histopathological lesions in the central nervous system: type A with lesions and type B without lesions (Stenberg et al., 2020). Based on causality, type A can be further divided into five subtypes (A-I to A-V). A-I is characterised by the presence of swine fever virus, whereas A-II by that of atypical porcine pestivirus (APPV) or porcine circovirus-II (PCV-II). Previous studies have primarily focused on A-II to identify and understand the causative agents (Stenberg et al., 2020).

Intersexuality (sex reversal) is a sex congenital disorder characterised by the atypical development of the reproductive system. The "female" gonosomes (XX) in affected animals are considered true hermaphrodites or pseudo-hermaphrodites that vary in the activity and presence of both female and male gonads and genitalia (Mittsson, 2011). Therefore, the chromosomal, somatic, and gonadic sex of defected animals could be different (Rousseau et al., 2013). The economic consequences of intersexuality are related to sterility as well as the higher probability of boar taint and infections. *SOX9* (mentioned above in the context of porcine kyphosis incidence) was recognised to affect sexual and skeleton development in some studies (Rousseau et al., 2013; Brening et al., 2015; Szczerbal et al., 2019).

3 Conclusions

The most commonly occurring congenital malformations in pigs along with all the known associated candidate genes were overviewed in the study to establish a background for genomic evaluation. In compliance with the general recommendation, the affected animals are usually excluded from reproduction. The research already focused on the genomic etiopathology of the congenital malformations in pigs has open space to explore their structural and functional genetic background and environment interaction. Promising findings of the candidate genes associated with malformations, mainly using the genome-wide association approach, come to an agreement that it is necessary to provide further and more comprehensive investigation. The trait (phenotype) accurate definition, measurement and description accompanied by the gene identification for a broader spectrum of populations and breeds should be provided to further gain in selection progress. Overall, knowledge transmission among all livestock species and humans is recommended in literature as critical to better understand malformation biology.

Acknowledgements

The study was supported by Project no. QK1910217 and MZE-RO0718 – V001 of the Czech Republic.

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