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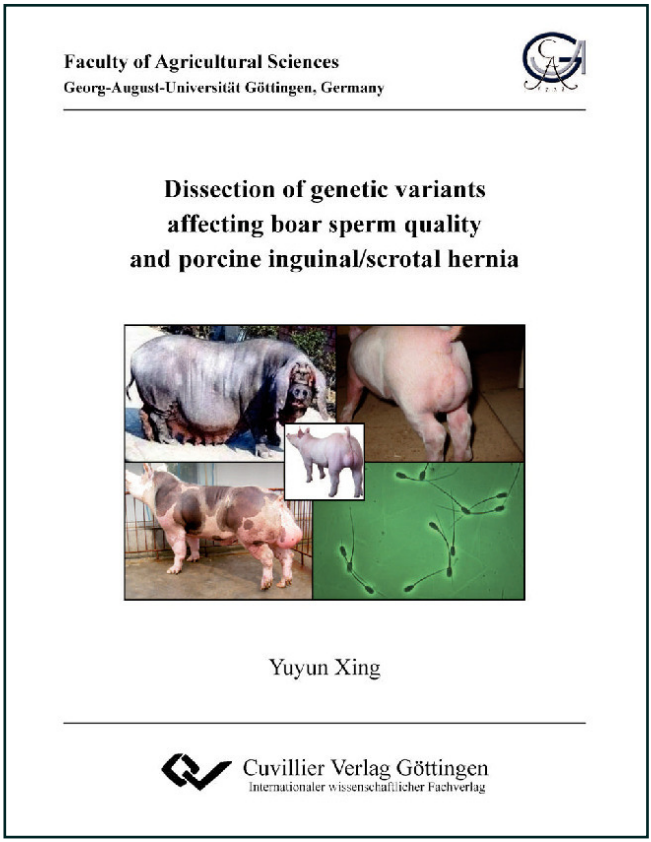
Dissection of genetic variants affecting boar sperm quality and porcine inguinal/scrotal hernia



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1 Literature review

1.1 Brief introduction

Pork occupies about 40% of red meat production worldwide and reproductive traits are undoubtedly important for swine production. In recent decades, artificial insemination (AI) has been extensively applied in the pig industry and has had a significant influence on the swine production. AI reduces the number of boars and demands that boars supply good quality semen for improving production efficiency. However, it is difficult to perform direct selection for semen quality traits due to the low heritability and sex-limited nature (Rothschild & Bidanel, 1998). In addition, human infertility is a social public health problem affecting up to about 15% of couples, and men take account for about 50% of these cases. Pig is a good choice as animal model for human disease, and the genetic research for boar semen quality would provide valuable references to human infertility studies.

Hernia is one of the most common congenital and developmental undesirable defects in pigs. Inguinal/scrotal and umbilical/abdominal hernias are the two main types of hernia in pigs. Pig inguinal/scrotal hernia often cause animal welfare problems and large economic loss. Inguinal/scrotal hernia is a complex disease trait that is affected by both environmental and genetic factors.

Quantitative trait loci (QTL) scanning and candidate gene approach are two main strategies to dissect complex traits. In the present thesis, we first performed a whole genome scan on traits related to sperm quality and ejaculation in pigs in a White Duroc × Erhualian pig resource population. Moreover, based on the QTL mapping and fine mapping results for porcine inguinal/scrotal hernia investigated at the Institute of Veterinary Medicine (Knorr et al., 2006; Germerodt et al., 2008), we then chose the sex-determining region on the Y chromosome (*SRY*)-

related HMG (high-mobility group) box 9 (*SOX9*) that is involved in diverse development processes as candidate gene for porcine inguinal/scrotal hernia. The molecular structure of porcine *SOX9* was characterized and its association with inguinal/scrotal hernia was analyzed.

1.2 QTL mapping for traits related to boar sperm quality and ejaculation

1.2.1 AI and pork production

Pork is one of the most commonly and widely consumed meats in the world. It occupies about 40% of all meat consumed worldwide (Table 1.1). From 1998 to 2008, pork production increased from 88.7 to 103.2 million metric tons according to FAO data (http://faostat.fao.org). It is predicted that the pork production will increase to 122 million tons by 2020 (Table 1.1).

Table 1.1 Projected trends in the food consumption of various livestock products, 1993-2020 (Delgado et al., 1999)

Region/Product	Projected annual growth of total consumption, 1993 – 2020	Total consumption		Annual per capita consumption	
		1993	2020	1993	2020
	(%)	Million metric tons		Kilograms	
Developed world					
Beef	0.4	32	36	25	26
Pork	0.3	36	41	28	29
Poultry	1.0	26	34	20	25
Meat	0.6	97	115	76	83
Developing world					
Beef	2.8	22	47	5	7
Pork	2.8	38	81	9	13
Poultry	3.1	21	49	5	8
Meat	2.8	88	188	21	30

Since AI was first performed in Russia at the beginning of 20th century (Ivanow, 1907), this technology has been widely used in pigs, cattle and horses. Boar-to-sow ratio of natural mating

is 1:15-1:25 (average 1:17 or 18) whereas it can be increased to 1:150-1:250 when using AI mating (Cynthia & Scott, 2008). AI is a useful tool to transfer excellent genetic material to pig offspring, with less risk of diseases and saving a lot on boar feeding costs. In some European countries, AI accounts for more than 90% of the reproductive gilts/sows (Lumb, 2007); in the Netherlands, this rate is 98% (Feitsma, 2009). As a result of high boar-to-sow ratio and the wide application of AI, boars' semen quality profoundly affects sows' reproductive efficiency. AI requires boars with excellent semen quality to improve meat productivity.

1.2.2 Boar selection

Boar selection is becoming increasingly important with the more and more wide use of AI. The earliest selecting method was visual appraisal on the basis of the confirmation, structural soundness and correctness, body length, etc. Until now, visual appraisal is still used in combination with other methods. From the middle of the 20th century, test stations have been built to measure individual boar performance such as birth weight, backfat thickness, growth rate and feed efficiency. Along with the computer technology applied in pig production, estimated breeding values (EBV) and expected progeny difference (EPD) based on best linear unbiased predictors (BLUP) are used to evaluate individual boars (Safranski, 2008). Pig production has achieved significant genetic improvements from these technologies. In comparison, semen quality traits are subordinate considerations. The AI stud, particularly the pig farms usually only check the sperm motility and concentration. Because of the low heritabilities (Table 1.2), direct selection on semen quality traits is comparatively ineffective. With the development of molecular biology technologies, marker-assisted selection (MAS) could be an effective way to improve male reproductive traits (Spötter & Distl, 2006). There are two approaches of detecting genetic markers for MAS. One is QTL strategy, which involves the scanning of the whole

genome using markers combined with phenotypic data. Another way is the investigation of candidate genes based on their possible role in the physiology of the trait.

Table 1.2 Heritability estimates for semen traits (See, 2002)

	Number of estimates	Mean h^2	Range
Total sperm number	3	0.37	0.31-0.42
Sperm motility	7	0.20	0.05-0.55
Semen volume	4	0.21	0.14-0.29
Sperm concentration	4	0.19	0.01-0.26
Sperm morphology	3	0.31	0.05-0.62

1.2.3 QTL mapping for porcine reproductive traits

Reproductive traits are economically important traits in pork production. The goal for pig industry is to produce pork of required quality with minimal cost. Since the first QTL was reported (Andersson et al., 1994), QTL detection in pigs has progressed rapidly. Until March 2010, 5621 pig QTL from 237 publications corresponding to 546 different traits were reported (http://www.animalgenome.org/cgi-bin/QTLdb/SS/index). The traits were assigned to exterior, health, meat quality, production and reproduction performances. Compared with 3985 QTL for meat quality traits, only 262 QTL for reproductive traits were identified.

Of the 262 QTL for reproductive traits, about 100 genome-wide significant ($P < 0.05$) QTL affecting at least 17 female reproductive traits have been identified across the pig genome (Table 1.3). By contrast, only 25 more or less genome-wide significant ($P < 0.05$) QTL for male reproductive traits including testis weight (size), seminal vesicles weight, epididymis weight, length of bulbo-urethral gland and FSH have been reported (Table 1.4). Prior to this study, QTL for traits related to semen quality and ejaculation had remained unexplored in pigs.

Table 1.3 QTL (significant level: $P < 0.05$) for female reproductive traits in pigs

Population	Trait	SSC:Position (cM)	References
Line I based on index of OR × Line II selected at random (114 F_2 animals)	OR	8:105; 13:0; 15:51	Rathje et al., 1997
Meishan × White composite (110 F_4 animals)	OR	3:36	Rohrer et al., 1999
Meishan × Yorkshire 122 F_2 animal	NSB	4:1	Wilkie et al., 1999
	CL	8:101	
	GL	9:135	
Meishan × Göttingen cross (265 F_2 animals)	TN	1: *; 7: *	Wada et al., 2000
Low-indexing × High-indexing (295-428 F_2 animals)	OR	9:1	Cassady et al., 2001
	FF	11:52	
	NSB	5:131; 13:101	
	TN	8:19; 11:46	
	AP	7:1; 8:172	
Meishan × Dutch pig lines (1173 F_2 animals)	TN	2:2; 10:107; 12:80	Hirooka et al., 2001
Meishan × Large White (573 F_2 animals)	AP	13:46	Bidanel et al., 2001
	UHL	1:79; 6:63; 6:121; 7:73; 13:82	
	UHWT	5:41; 7:85; 9:109; 13:70; X:75	
	OVWT	9:53; 13:82; 15:99	
	OR	4:74	
	IGF	7:39; 7:95	
	TRTWT	9:105	
	ES	9:36	
	FSH90	14:100	
Iberian × Meishan 272 F_2 animals	TN	5:29; 10:71; 12:67	Rodríguez et al., 2005
	NSB	13:100	
Low indexing × High indexing (295-428 F_2 animals)	OR	9:1	Holl et al., 2004
	TNB	11:52	
	NUM	6:81; 12:70	
	TN	6:85; 8:20; 11:47; 15:109	
	AP	7:1; 8:172; 15:98	
Meishan × Duroc (CL: 234 F_2 animals) (TN, PN: 801 F_2 animals)	CL	3:37	Sato et al., 2006
	TN	3:117; 7:97; 8:30; 8:63; 12:41	
	PN	2:109; 3:115; 16:39	
Large White × French Landrace (239 F_1 animals)	NSB	6:88; 11:66; 14:28	Tribout et al., 2008
	NBA	7:20; 16:9; 18:1	
White Duroc × Erhulian (299 F_2 animals)	NBA	15:88	Li et al., 2009
White Duroc × Erhulian (1899 F_2 animals)	LTN	1:142; 3:46; 3:119; 4:40; 5:74; 6:125; 7:59;7:95; 8:80; 12:79	Ding et al., 2009a
	RTN	1:143; 3:56; 4:23; 5:77; 7:60; 7:93; 12:58	
	TTN	1:86; 1:142; 3:56; 3:124; 4:39; 5:75; 6:107; 7:59; 7:94; 8:80; 12:82	

OR: ovulation rate; NSB: number of stillborn piglets; CL: corpora lutea; GL: gestation length; TN: teat number; FF: number of fully formed; AP: age at puberty; UHL: length of uterine horns; UHWT: weight of uterine horns; OVWT: weight of ovaries; TRTWT: weight of the reproductive tract; ES: embryo survival; FSH90: FSH concentration at 90 days of age; TNB: total number of born piglets; NUM: number of mummified pigs; PN: pin nipples; NBA: number of born alive piglets; LTN: teat number on the left side; RTN: teat number on the right side; TTN: total teat number; *: unknown data.

Table 1.4 QTL (significant level: $P < 0.05$) for male reproductive traits in pigs

Population	Trait	SSC:Position (cM)	References
Meishan × White Composite (436 boars)	Plasma FSH	3:49; 10:101; X:80	Rohrer et al., 2001
Meishan × Large White (530 F_2 animals)	SVWT	1:162; 3:59; 4:75; X:88	Bidanel et al., 2001
	EPWT	4:75; 7:143; 10:137	
	LBUG	7:86	
	BUGWT	7:36	
	TW	7:66; 10:140; 13:45; X:89	
Meishan × Duroc (450 F_2 animals)	TW	3:47; X:78	Sato et al., 2003
White Duroc × Erhualian (347 F_2 animals)	TW90	X:56	Ren et al., 2009
	TW300	1:86; X:56	
	EW300	7:59	
	STD90	X:56	
	STD300	16:43	
	STC300	7:71	

SVWT: seminal vesicles weight; EPWT: epididymis weight; LBUG: length of bulbo-urethral gland; BUGWT: weight of bulbo-urethral gland; TW: testicular weight; TW90: testicular weight at 90 days; TW300: testicular weight at 300 days; EW300: epididymis weight at 300 days; STD90: seminiferous tubular diameter at 90 days; STD 300: seminiferous tubular diameter at 300 days; STC300: serum testosterone concentration at 300 days.

1.2.4 Candidate genes study for semen quality in boars

To date, the reports of candidate genes for semen quality are still limited, partly due to the comparatively high costs and the difficulty of measuring. In addition, these studies have shown population-specific significant results occasionally.

Urban & Kuciel (2001) reported that boars with homozygous NN genotypes of ryanodine receptor 1 (*RYR1*) gene showed significantly better parameters of semen volume ($P \leq 0.05$), sperm motility ($P \leq 0.001$), number of abnormal sperm ($P \leq 0.01$) and number of AI doses ($P \leq 0.001$) than heterozygous (Nn) ones. A SNP in the testis-specific phosphoglycerate kinase 2 (*PGK2*) gene has positive significant effect on semen volume in the Pietrain breed ($P = 0.08$) (Chen et al., 2004). 13 candidate genes were chosen to study the association between the genetic variants of these genes and boar sperm quality and fertility in 224 Pietrain and 112 Pietrain × Hampshire AI boars. The γ-actin (*ACTG2*) genotypes were significantly associated with semen volume ($P < 0.01$) in Pietrain boars and with sperm motility ($P < 0.05$) in Pietrain × Hampshire

boars (Wimmers et al., 2005). Statistical analysis in the whole population revealed the significant association of gonadotropin releasing hormone receptor (*GNRHR*) variants with motility (P = 0.016), plasma droplets rate (P = 0.005) and abnormal sperm rate (P = 0.020), the significant association of inhibin beta A (*INHBA*) with plasma droplets rate (P = 0.032) and abnormal sperm rate (P = 0.020) and significant association of inhibin beta B (*INHBB*) with sperm concentration (P = 0.036) (Lin et al., 2006a). Haplotypes of β-actin (*ACTB*) affected the variation of the traits motility and abnormal sperm rate in Pietrain and Pietrain × Hampshire boars (Lin et al., 2006b). Another work has shown that boars carrying different estrogen receptor 1 (*ESR1*) and *ESR2* genotypes have significant ($P < 0.01$) differences in semen traits including total sperm number per ejaculate and sperm motility (Terman et al., 2006). A study from Ren et al. (2006) revealed that the C678A polymorphism of *SERPINA7* gene is significantly associated with total sperm per ejaculate ($P < 0.01$) and semen volume ($P < 0.05$) of 110 mature boars from the same White Duroc × Erhualian population that was used in this thesis.

1.2.5 Pig is an ideal biomedical model for male infertility

Deciphering the genetic basis of porcine male fertility is not only beneficial for the pig industry but also human medicine. Human infertility is a major reproductive health problem. Currently, about 15% of couples are infertile, and among these couples, male factors are responsible for approximately half of sterility cases. Oligozoospermia, asthenozoospermia, teratozoospermia and azoospermia are the main reasons of male infertility and account for 20-25% of cases (Poongothai et al., 2009). Genetic abnormalities implicated in male infertility include chromosomal abnormality, Y chromosome microdeletion (McLachlan et al., 1998), mitochondrial DNA mutation (Díez-Sánchez et al., 2003), gene mutation (Foresta et al., 2005),

etc. Genetic factors account for 10-15% of severe male infertility (Ferlin et al., 2006) though the incidences of genetic defects in different male infertility cases are quite diverse.

Because of small family size and lack of information transmission from infertile men, man is not an ideal experimental object for infertility genetic dissection. Alternatively, animal models such as mice, rabbit, and pig are very important tools for biomedical research. Pig has been a well-recognized experimental animal in extensive human biomedical research (Table 1.5). It has similar size, physiology, anatomy, organ development, disease progress and also high genome homology with human. Moreover, using pigs as an animal model, we can artificially design the family structure, get a large family size, collect repeated peripheral samples, obtain diverse tissue samples after the slaughter, etc.

Table 1.5 Summary of swine biomedical models (Lunney, 2007)

Heart physiology	**Reproductive function**	**Skin physiology**
Stent design	Maternal-fetal interactions	Percutaneous permeation
Atherosclerosis	Embryo development	Contact dermatitis
Myocardial infarction	Sperm	Skin equivalent culture model
Ex vivo heart model	**Transplantation**	Melanoma
Emergency procedures	Cell and organ transplants	**Tissue engineering**
Gut physiology and nutrition	Xenotransplantation	Cartilage repair
Gut structure and intestinal metabolism	Drug therapies and biotherapeutics	Lens capsule epithelial cells for cataract repairs
Obesity	**Brain**	Spinal fusion
Probiotics and gut physiology	Stroke-focal cerebral ischemia	Organ specific gene delivery
Biologic and immunological basisi of food allergies	Drug binding sites and interactions	Tooth development-dental enamel
Infectious disease models	AIDS dementia	Polymer scaffolds
Therapeutics	**Biomechanical models**	**Respiratory function**
Developmental interactions	Response to injury	Neonatal respiratory distress
Mucosal tissue responses	Imaging techniques	Thoracic artificial lung
Genomics of host responses	Bone density analyses	Asthma