Mamm Genome (2008) 19:127–138
DOI 10.1007/s00335-007-9086-5

QTL for the heritable inverted teat defect in pigs

Elisabeth Jonas · Heinz-Josef Schreinemachers · Tina Kleinwächter · Cemal Ün · Ina Oltmanns · Sylvio Tetzlaff · Danyel Jennen · Dawid Tesfaye · Siriluck Ponsuksili · Eduard Murani · Heinz Juengst · Ernst Tholen · Karl Schellander · Klaus Wimmers

Received: 25 June 2007 / Accepted: 28 November 2007 / Published online: 25 January 2008

Abstract The mothering ability of a sow largely depends on the shape and function of the mammary gland. The aim of this study was to identify QTL for the heritable inverted teat defect, a condition characterized by disturbed development of functional teats. A QTL analysis was conducted in a porcine experimental population based on Duroc and Berlin Miniature pigs (DUMI). The significant QTL were confirmed by linkage analysis in commercial pigs according to the affected sib pair design and refined by family-based association test (FBAT). Nonparametric linkage (NPL) analysis revealed five significant and seven suggestive QTL for the inverted teat defect in the porcine experimental population. In commercial dam lines five significant NPL values were detected. QTL regions in overlapping marker intervals or close proximity in both populations were found on SSC3, SSC4, SSC6, and SSC11. SSC6 revealed QTL in both populations at different positions, indicating the segregation of at least two QTL. The results confirm the previously proposed polygenic inheritance of the inverted teat defect and, for the first time, point to genomic regions harboring relevant genes. The investigation revealed variation of the importance of QTL in the various populations due to either differences in allele frequencies and statistical power or differences in the genetic background that modulates the impact of the liability loci on the expression of the disease. The QTL study enabled us to name a number of plausible positional candidate genes. The correspondence of QTL regions for the inverted teat defect and previously mapped QTL for teat number are in line with the etiologic relationship of these traits.

E. Jonas · H.-J. Schreinemachers · T. Kleinwächter · C. Ün · I. Oltmanns · D. Jennen · D. Tesfaye · H. Juengst · E. Tholen · K. Schellander
Institute of Animal Breeding Science, University of Bonn, Endenicher Allee 15, 53115 Bonn, Germany

S. Tetzlaff · S. Ponsuksili · E. Murani · K. Wimmers (✉)
Research Institute for the Biology of Farm Animals (FBN), Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany
e-mail: wimmers@fbn-dummerstorf.de

Introduction

Inherited disorders significantly affect animal health and welfare issues and economic efficiency of livestock breeding. Although the phenotype itself often suggests monogenic inheritance with the occurrence of either affected or nonaffected individuals, many inherited diseases are liability traits with polygenic background. In pig breeding the number of piglets reared per sow per year is an important characteristic of efficiency. Next to the number of piglets born alive, the mothering ability of the sow, which is related to the number of functional teats, plays an important role in this. The inverted teat defect is the most common and most important disorder of the mammary complex in pig (Brevern et al. 1994; Mayer 1995). This disorder is a condition characterized by the failure of teats to protrude from the udder surface. The teat canal is held inward, forming a small crater so that normal milk flow is prevented, thus limiting the rearing capacity and increasing the risk of mastitis. The inverted teat defect has a polygenic background and heritability estimates range between 0.2 and 0.5 (Brevern et al. 1994; Mayer 1995). It occurs in commercial pig breeds with frequencies between 8% and 30% (Große Beilage et al. 1996; Mayer 1995). In comparison to other hereditary disorders, it is difficult to diagnose during routine selection where young sows are inspected while standing or moving within an

arena (Steffens 1993). Since the mode of inheritance is not fully understood and the number of genes involved is unknown, it is important to get more knowledge of the genetic cause of the defect. Moreover, identification of markers for this liability trait offers perspectives to develop efficient DNA-based selection tools for the improvement of the quality of teats. Therefore, the main objective of this study was to identify chromosomal regions and candidate genes for the inverted teat defect by a QTL scan in an experimental population and to validate these results in a commercial population.

Material and methods

Animals

Analyses were performed on two different populations, an experimental population and animals from commercial dam lines. The German Landrace (DL) and Large White (DE) breeds were chosen for the confirmation studies that are the most commonly used dam lines in Germany. Accordingly, there is selection against the inverted teat defect in these lines.

The experimental population was based on a reciprocal cross between Duroc and Berlin Miniature pigs (DUMI population). For F_1 one boar from the Duroc breed was mated to four Miniature pig sows and one Miniature pig boar was mated to five Duroc sows to produce 43 F_1 dams and five F_1 boars. The F_1 boars and dams were mated to finally produce 905 F_2 animals. At 200 days of age the phenotypes of the teat traits were observed by two investigators of a team of four trained and skilled persons involved in this study. Animals were placed on their backs and teats were evaluated by inspection and palpation. Numbers of functional and inverted teats were recorded. This is similar to the time-point of selection of young sows in breeding companies. At this time the inverted teat defect was detected in 42.2% of the F_2 animals.

For the commercial population, 2160 animals (castrated male pigs) of the German Landrace (DL) and German Large White (DE) breeds and their crosses were used. The animals were kept at performance test stations (*LPA Haus Düsse* and *LPA Frankenforst*) under standardized conditions according to the guidelines of the German performance test (ZDS 2003) and monitored at the slaughter line when 180 days old on average. Each animal was examined by two inspectors by inspection and palpation of teats at a separate slaughter line. Of these, 244 animals were identified with at least one inverted teat. Samples of some of these animals and their affected or nonaffected full-sibs were used for QTL analysis. The samples from the parents of these animals were collected in commercial farms and the artificial insemination station, respectively. Only animals for which samples of parents and at least one full-sib were available were used in this study for the verification of QTL detected in DUMI population. Overall, 119 families could be analyzed from these samples. In addition, 100 animals generating 11 families derived from *LPA Grub* from the *Bayerische Landesanstalt für Landwirtschaft* in Bavaria.

Genotyping and mapping

DNA for genotyping was isolated from sperm, muscle, or tail using phenol-chloroform extraction. Each of the 12.5 µl of PCR reaction contained 50 ng genomic DNA, 0.2 µM of each primer, 50 µM of each dNTP, 0.5 U of *Taq* polymerase, and 1.5 mM $MgCl_2$ in 1× PCR buffer. PCR was performed in the thermal cycler PTC 100 (MJ Research, Waltham, MA) at 94°C for 3 min, followed by 30–40 cycles at 94°C for 1 min, 55–65°C for 1 min, 72°C for 1 min, and final extension at 72°C for 10 min. The annealing temperature was used depending on the optimal temperature for primer. Seventy-two type II markers and 29 type I markers covering the porcine autosomes with a mean interval of 23.1 cM were selected from published linkage maps (USDA-MARC and PiGMaP) and used in the DUMI population. In the commercial population, almost the same set of polymorphic markers was used.

Electrophoresis was performed with the LI-COR model 4200 automated DNA sequencer (LI-COR Biosciences, Lincoln, NE) using 12% SequaGel in 1 × TBE buffer at a power of 1500 V, 50 mA, and 50 W at 50°C. Evaluation of genotypes was done using OneDScan v4.10 (LI-COR Biosciences). Linkage analysis for building of the maps was performed using CRIMAP v2.4 (Green 1992). The genetic map obtained has been described previously (Wimmers et al. 2006). The QTL analysis for the inverted teat quantitative trait was performed with the software package Genehunter v2.0 (Whitehead Institute, Cambridge, MA) (Kruglyak et al. 1996). Genehunter calculated a normalized score $Z(v)$ with a mean of zero and a variance of one under the null hypothesis. The null hypothesis is that there is no linkage between the disease locus and the marker allele (Kruglyak et al. 1996).

$$Z(\bar{x}) = \frac{[S(\bar{x}) - \mu]}{s}$$

$$S(\xi) = S_{\text{pairs}}$$

$$\xi = \text{mean of } S_{\text{pairs}} = p\xi S_{\text{pairs}}$$

$$p = a\ priori \text{ probability}$$

$$s^2 = p\xi(S_{\text{pairs}} - \mu)^2$$

Afterward the null hypothesis is calculated under the normalised test statistic NPL (nonparametric linkage)

$$\mathrm{NPL} = \xi Z(v) \times P$$

P = a posteriori probability

The NPL analysis calculated the part of the shared allele at each loci on the whole chromosome. These alleles are mentioned as $Z0$, $Z1$, and $Z2$ where

$$z_0 = \frac{a_0}{\lambda_s},\ z_1 = a_1,\ z_2 = a_2\frac{(2\lambda - 1)}{\lambda_s}$$

where λ_s is the relative risk for the sibling, $a_0 = ¼$, $a_1 = ½$, and $a_2 = ¼$. Chromosome-wide significance was calculated using Genehunter and transformed to experiment-wide significance levels using a permutation test (Churchill and Doerge 1994). The experiment-wide significance level was calculated by

$$P_{\mathrm{g}} = \frac{1 - (1 - P_{\mathrm{c}})}{r}$$

where r is the length of the specific chromosome divided by the total length of all chromosomes and P_{c} is the chromosome-wide significance level.

Further significant and suggestive QTL were defined (Lander and Kruglyak 1995), whereas in a F_2 population the NPL score of 4.3 is necessary to reach a significance level of 1% using a genome-wide investigation, the significance level of 5% corresponds to a NPL score of approximately 3.0 (Lander and Kruglyak 1995).

The association analysis was performed using the FBAT (family-based association test), a modified test based on TDT (transmission disequilibrium test). In this study the FBAT program (version 1.4) was used to perform the qualitative and quantitative family-based analyses of both populations (http://www.biostat.harvard.edu/~fbat/fbat.htm). For all, the quantitative analyses were done under the condition of an additive model (Horvath et al. 2003). FBAT performed the testing by a two-step procedure. First, the test statistic was defined showing the association between the trait locus and the marker locus. Second, the distribution of the genotype data is tested under the hypothesis. The genotypes of the offspring are treated as random (Rabinowitz and Laird 2000). The general FBAT statistic U is based on a linear combination of the genotypes of offspring and the traits:

$$U = S - E[S]$$

$$S = \sum_{ij} T_{ij}X_{ij}$$

where X_{ij} is a function of the genotype of the jth offspring in family i at the locus tested (depending on the used genetic model) and T_{ij} is a function of the trait (dependent on possible unknown parameters).

$$T_{ij} = Y_{ij} - \mu_{ij}$$

where Y_{ij} is the observed trait of the jth offspring in the family i and μ_{ij} is a random variable. The general FBAT statistic is calculated under the hypothesis that there is "no linkage and no association between marker and gene." This depends on T_{ij} and on the genotypes of the parents. Under this hypothesis, U is such that $E(U) = 0$. With the distribution of the genotypes of offspring (X_{ij} is treated as random and as T_{ij} fixed), $V = \mathrm{Var}(U) = \mathrm{Var}(S)$ can also be calculated under the hypothesis and can be used for the standardization of U. If X_{ij} is the scalar sum of the genotypes of an individual, then the total statistic of the samples is

$$Z = U/\sqrt{V}$$

approximately $N(0,1)$. If X_{ij} is a vector, then

$$\chi^2 = U'V - U$$

an approximately χ^2 distribution with the degrees of freedom similar to V (Horvath et al. 2003). In this study the FBAT was performed under the hypothesis: "no association and no linkage."

Results

In the F_2 of the experimental population, the mean number of teats was 12.9. The occurrence of the inverted teat defect was 42% (380 of 905 animals), with on average 5.2 inverted teats per affected animal at equal frequencies in both sexes. For the commercial breeds, of the 2160 animals monitored in the slaughterhouse, 244 animals (11.3%) were found to have one and more inverted teats (mean = 2.3). In the commercial dam lines, the mean number of teats was 14.6. The incidence of inverted teats was slightly higher in DL than in DE and F_1 animals (12.5%, 10.7%, and 10.5%, respectively).

In the experimental population, QTL were detected on all autosomes except SSC17, reaching 5% chromosome-wide significance. Fourteen QTL reached the experiment-wide significance level. Of these 14, eight were found with highly significant experiment-wide NPL values (Table 1). In the experimental population, 12 suggestive QTL (NPL > 3.0) for the inverted teat defect were detected (Lander and Kruglyak 1995). Of these, five significant QTL (NPL > 4.3) were detected. As in the commercial population, the affected sib-pair design was used and the p values of the estimated NPL values detected by the Genehunter program were used for definition of the significance levels. Five QTL positions were found, with only one NPL value exceeding the 5% experiment-wide

Table 1 Evidence for QTL significant at 5% chromosome-wide[a] and experiment-wide[b] levels for the inverted teat defect in the DUMI population

SSC	NPL value	*p* value[a]	*p* value[b]	Closest marker to QTL	Marker position[c]	Flanking marker	
						Upper	Lower
1	4.43	≤0.01**	0.09	SW1301	141	S0155	–
2	4.56	≤0.01**	0.04*	SW2443	0	–	L144
3	3.9	≤0.01**	≤0.01**	S0164	61	SW72	NTAN1
4	5.2	≤0.01**	≤0.01**	S0097	120	S0214	–
5	3.83	≤0.01**	≤0.01**	SW1482	40	–	SW1134
6	2.81	≤0.01**	0.02*	S0035	7	–	S0087
6	9.25	≤0.01**	≤0.01**	S0220	78	S0300	S0059
7	2.63	≤0.01**	0.04*	S0101	135	S0115	–
8	3.41	≤0.01**	≤0.01**	SW2410	1	–	KIT1
9	2.35	≤0.01**	0.12	SW911	37	SW21	SW54
10	2.88	≤0.01**	0.02*	SW2067	128	ITIH2	–
11	3.35	≤0.01**	≤0.01**	S0386	60	S0071	SW703
12	6.7	≤0.01**	≤0.01**	S0143	6	–	SW874
13	1.92	0.02*	0.35	SW398	79	SW344	IL12A
14	3.78	≤0.01**	≤0.01**	S0007	60	MBL2	VIN
15	2.44	≤0.01**	0.08	SW936	89	SW1111	SW1119
16	3.14	≤0.01**	0.01*	S0061	93	IL12B	–
17	0.64	0.23	0.99	SW2431	94	SW840	–
18	3.23	≤0.01**	0.02*	SWR414	58	SW787	–

* Significant at 5% level; ** significant at 1% level

[a] Chromosome-wide, [b] experiment-wide, [c] position of marker closest to QTL on public linkage map (USDA-MARCv2)

significance threshold (Table 2). QTL regions in overlapping marker intervals or close proximity in both populations were found on SSC3, SSC4, SSC6, and SSC11 (Fig. 1).

The association analysis using the FBAT program revealed more alleles of Duroc origin with a positive influence on the development of normal teats (negative *Z* score). The following alleles of Duroc origin could be shown to be highly associated with normal teat development: on SSC1, allele 165 of marker SW1301 (*Z* score = −3.8); on SSC4, allele 234 of marker S0097 (*Z* score = −7.3); on SSC6, allele 154 of marker S0220 (*Z* score = −4.9); and on SSC14, allele 174 of marker S0007 (*Z* score = −5.1). The allele 161 of marker SW1301 on SSC1 and the allele 286 of marker S0164 on SSC3 originated from the Miniature pig and were shown to be highly associated with the inverted teat defect (*Z* scores = 7.2 and 2.5, respectively). The Miniature pig-originated alleles 207 of marker SW2443 (SSC2), 302 of marker S0164 (SSC3), and 212 of S0097 (SSC4) showed positive impact on teat development (*Z* scores = −2.6, −2.3, and −2.0, respectively). Data of association compared to linkage analysis of both populations are shown in Table 3.

The distal region of SSC1 contained a QTL for the inverted teat defect with NPL value reaching the 1% chromosome-wide significance in the DUMI population (Table 1). No QTL were found on SSC1 in the commercial dam lines. However, the family-based association test revealed significant association and linkage of marker SW1301 in both the experimental and the commercial population (Table 3).

A significant QTL was detected on SSC2 in the telomeric region with a 5% experiment-wide significant NPL value in animals of the experimental population (Table 1). This could be confirmed by association analysis of the marker SW2443 (Table 3). The QTL could not be detected when analyzing all animals of the commercial population. Linkage analysis of only families of crossbreeds (DE × DL, DL × DE) revealed a QTL with a NPL value significant at the 5% chromosome-wide level at marker SW1564, central on SSC2.

The distal region on SSC3 showed a highly significant QTL for the inverted teat defect in the DUMI population (Table 1). The locus was confirmed at 5% chromosome-wide significance in the commercial animals as well as by association analysis (Tables 2 and 3). Two alleles (218, 286) were identified, inherited from Duroc and Miniature

Table 2 Evidence for QTL significant at 5% chromosome-wide[a] and experiment-wide[b] level for the inverted teat defect in the commercial dam lines

SSC	NPL value	Closest marker to QTL	Marker position[c]	Flanking marker		*p* value					
				Upper	Lower	All[d]		DL[d]		DL × DE / DE × DL[d]	
						chr[a]	exp[b]	chr[a]	exp[b]	chr[a]	exp[b]
1	0.69	SW1301	141	S0155	–	0.11	0.48	0.21	0.75	0.18	0.69
2	0.61	SW1564	55	SW240	SW834	0.15	0.96	0.4	0.99	0.05*	0.62
3	1.16	S00164	61	SW72	SW2570	0.02*	0.36	0.56	0.99	0.05*	0.63
4	1.06	S0214	120	S0214	SW857	0.02*	0.33	0,.7	0.69	0.09	0.78
5	1.45	SW967	146	IGF1	–	0.07	0.75	0.28	0.99	0.01*	0.19
6	1.16	S0035	7	–	S0087	0.02*	0.16	0.18	0.87	0.07	0.52
6	1.64	S0059	93	S0220	S0003	≤0.01**	0.02*	0.03*	0.27	0.05*	0.41
7	0.77	S0101	135	S0115	–	0.1	0.75	0.17	0.92	0.13	0.84
8	0.9	S0086	62	SW2611	S0144	0.06	0.8	0.06	0.79	0.02*	0.79
9	0.36	SW54	67	SW911	S0109	0.33	0.99	0.19	0.97	–	–
10	0.64	SW830	0	–	S0070	0.14	0.92	0.07	0.73	0.29	0.99
11	1.95	SW703	76	S0009	–	≤0.01**	0.24	0.04*	0.83	0.05	0.90
12	0.74	SW874	65	GH	SW605	0.21	0.99	–	–	0.17	0.99
13	0.73	SW344	35	S0219	PIT1	0.2	0.97	0.42	0.99	0.04*	0.44
14	0.51	SW857	7	–	S0007	0.21	0.99	0.12	0.99	0.43	0.99
15	0.77	SW936	89	SW1111	SW1119	0.08	0.99	0.15	0.99	0.24	0.99
16	0.5	S0111	0	–	S0026	0.20	0.99	0.31	0.99	0.28	0.99
17	0.7	SW840	49	SW335	GHRH	0.12	0.85	0.19	0.96	0.13	0.89
18	0.17	SW1023	32	–	LEP	0.37	0.99	0.29	0.99	0.42	0.99

* Significant at 5% level; ** significant at 1% level

[a] Chromosome-wide, [b] experiment-wide, [c] position of marker closest to QTL on public linkage map (USDA-MARCv2)

[d] All = merged analysis of German Landrace and F_1 (DL*DE/DE*DL); DL = German Landrace; DE = Large White

pig, respectively, showing an increase of liability for the inverted teat defect (Table 3).

Linkage and association analyses of SSC4 revealed significant QTL with a peak at marker S0097 in animals of the experimental population (Table 1). A significant NPL value could be detected in animals of commercial families in the region between markers S0214 and S0097, i.e., in close proximity to the QTL found in the DUMI (Table 2). Family-based association tests revealed significant association and linkage of alleles of marker S0097 in both populations.

On SSC5 a highly significant QTL was detected in the region of the microsatellite marker SW1482 (Table 1). The analysis of the crossbreed animals in the commercial group revealed a QTL on SSC5 with a NPL value significant at a 5% chromosome-wide significance level more distal (Table 2).

The highest NPL value of 9.25 was detected on SSC6 with a peak at marker S0220 (Fig. 1). In the dam lines the highest NPL value was detected at marker S0059, distal of S0220. This QTL was the only locus with experiment-wide significance in the commercial animals. There was good agreement between the NPL plots found in the analysis of all commercial animals and analysis separated by breeds. A NPL value of 1.16 implementing a chromosome-wide 5% significance level was detected in the commercial animals with a peak at marker S0035 telomeric of SSC6. At this position a QTL reaching a 5% experiment-wide significance level was also obtained in the DUMI population. Alleles of marker S0220 were significantly associated with the defect in both populations, whereas alleles of markers S0059 and S0035 showed association only in the commercial and experimental populations, respectively. Results of linkage and association analyses in both populations indicate that there are at least two QTL segregating on SSC6 that affect the inverted teat defect.

The distal region of SSC7 showed significant NPL values for the heritable inverted teat defect, as revealed by full-sib analysis, between markers S0115 and S0101 at the experiment-wide 5% significance level only in the experimental population.

SSC8 carried a QTL at the proximal end in the DUMI population. The region around marker S0086, which is more distal, showed a significant NPL value in the analysis of crossbreed animals of the commercial population (Table 2).

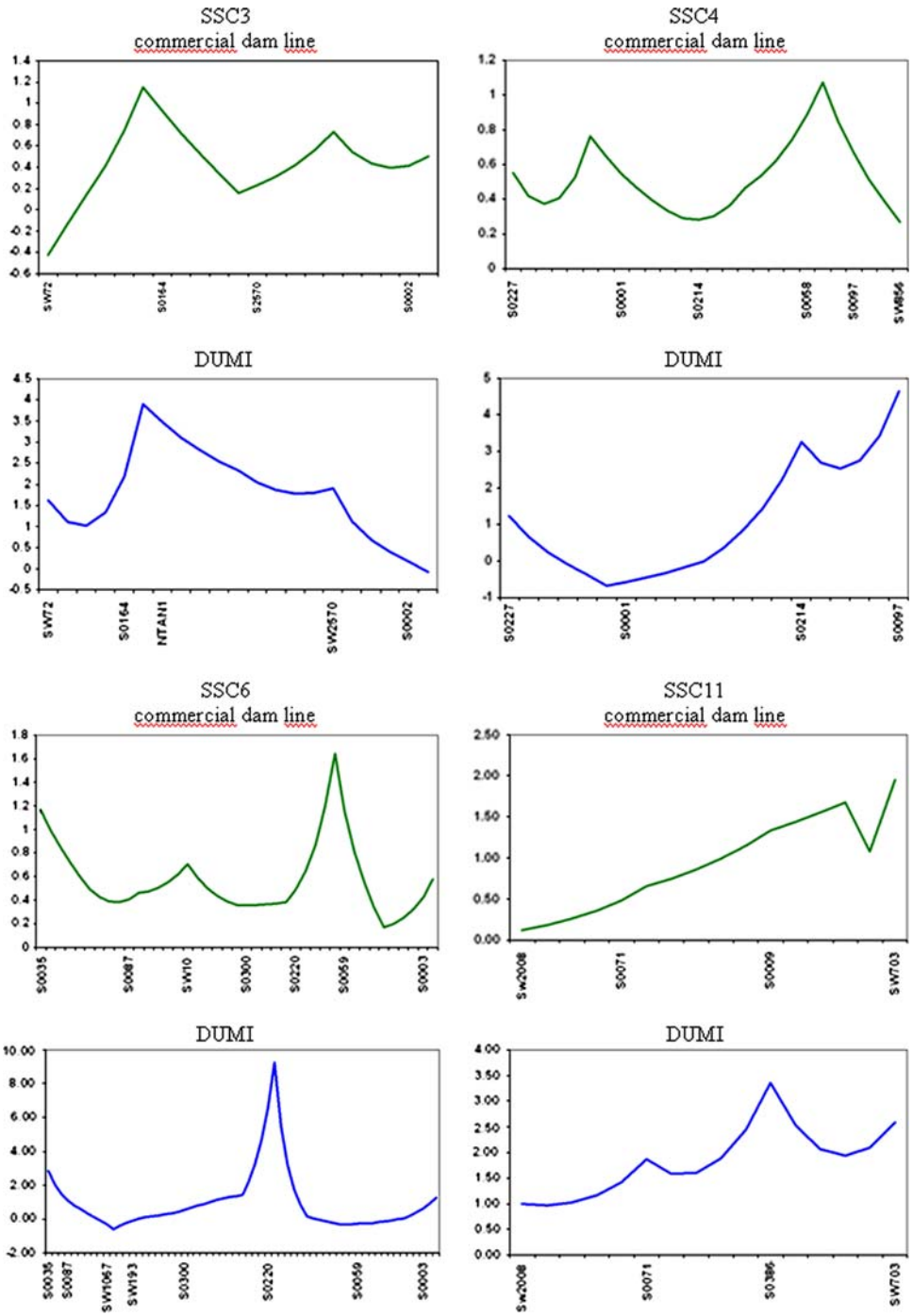

Fig. 1 Profiles of multipoint NPL scores for SSC3, 4, 6, and 11, respectively, obtained from QTL analysis in the experimental population DUMI and commercial dam lines. Positions of markers genotyped are given on the *x* axis; numbers on the *y* axis indicate NPL values

On SSC11 a QTL reaching the 1% experiment-wide significance level in the DUMI population was found surrounding marker S0386 (Table 1). In the commercial animals, a NPL value reaching the 1% chromosome-wide significance level was detected in close proximity at SW703. The peak was also detected in the families that included only animals of the German Landrace breed (Table 2). Both regions could be confirmed by association analysis that revealed alleles of marker S0386 being associated with the defect status in the experimental population and alleles of marker SW703 being associated with the defect in both populations (Table 3).

A significant QTL could be detected on SSC12 between the markers SW605 and L147 in animals of the DUMI population. The NPL score of 6.7 was the second highest in this analysis (Table 1). There was no evidence found for a QTL nor an associated allele in the commercial animals.

SSC14 contained a significant QTL next to the marker S0007, but no significant QTL was detected in the

Table 3 Overview of results, including markers at positions of QTL detected in either the DUMI population or the commercial dam lines or both

SSC	Marker	QTL (Genehunter) NPL value		Association study (FBAT)					
				Significant allele[e]		*Z* value[d]		*P* value	
		DUMI	comm	DUMI	comm	DUMI	comm	DUMI	comm
1	SW1301	4.43[a]	0.69	161[1]	.	7.197	.	≤0.001	.
				163[3]	.	−5.604	.	≤0.001	.
				165[2]	.	−3.757	.	≤0.001	.
				171[2]	171	−1.897	−2.425	0.058	0.015
2	SW2443	4.56[a]	0.43	203[3]	.	2.455	.	0.014	.
				207[1]	207	−2.568	1.866	0.010	0.062
3	S0164	3.9[a]	1.16[c]	214[3]	ns	−2.496	ns	0.013	ns
				218[2]		2.958		0.003	
				266[3]		−2.395		0.017	
				286[1]		2.475		0.013	
				302[1]		−2.297		0.022	
4	S0097	5.2[a]	0.67	205[3]	148	7.19	3.888	≤0.001	≤0.001
				212[1]	170	−1.972	−3.458	0.049	≤0.001
				234[2]	172	−7.296	−2.513	≤0.001	0.012
	S0058	–	1.06[c]	–	ns	–	ns	–	ns
5	SW1482	3.83[a]	–	108	ns	−2.465	ns	0.014	ns
				110		2.21		0.027	
6	S0035	2.82[b]	1.16[c]	179	ns	−3.007	ns	0.003	ns
	S0059	0.83	1.64[c]	ns	144	ns	−1.896	ns	0.058
					146		2.419		0.016
					154		−2.121		0.034
	S0220	9.25[a]	0.1	146[3]	.	4.248	.	≤0.001	.
					148	.	−2.530	.	0.011
				154[2]	.	−4.9	.	≤0.001	.
8	SW2410	3.41[a]	–	ns	ns	ns	ns	ns	ns
11									
	S0386	3.35[a]	–	154	ns	−2.037	ns	0.042	ns
				158		−3.889		≤0.001	
	SW703	2.58	1.95[c]	127	.	3.741	.	≤0.001	.
				133	.	−1.941	.	0.052	.
				.	135	.	2.236	.	0.025
				.	137	.	−2.076	.	0.038
12	S0143	6.7[a]	–	155	ns	7.673	ns	≤0.001	ns
				157		−7.013		≤0.001	
14	S0007	3.78[a]	0.24	158[3]	.	5.755	.	≤0.001	.
				174[2]	.	−5.149	.	≤0.001	.
				.	177	.	−2.041	.	0.041
				.	187	.	2.064	.	0.039
16	S0061	3.14[a]	–	166	ns	−2.405	ns	0.016	ns
				170		1.899		0.058	
18	SWR414	3.23[a]	0.13	123	.	2.181	.	0.029	.
				147	.	−1.882	.	0.06	.
				.	153	.	−2.566	.	0.01

ns = not significant

[a] QTL according to the definition of Lander and Kruglyak (1995), i.e., NPL > 3, [b] QTL reaching 5% genome-wide significance, [c] QTL according to the chromosome-wide *p* value of ≤0.05 in all animals of commercial population, [d] Positive *Z* scores indicate that alleles can be found more often in affected animals, [e] Names of alleles derived from their length in bp, [1] Origin breed Miniature pig, [2] Origin breed Duroc, [3] Allele inherited from both breeds

commercial animals (Table 1). Significant association of the marker S0007 was found in both populations (Table 3).

On SSC16 and SSC18 suggestive QTL were detected in DUMI population; in addition, significant associated alleles of markers S0061 and SWR414 were also found. In the commercial population, significant association of alleles of markers SWR414 on SSC18 was found, however, none were found on SSC16 (Table 3).

Even though the NPL values calculated on SSC9, 10 and 13 were significant and highly significant at the chromosome-wide level, they could not be defined as QTL according the definition of Lander and Kruglyak (1995). Significant associated alleles were detected only on the respective markers on SSC9 and 10. On SSC17 no significant QTL was detected.

Discussion

A linkage study was performed for the heritable defect inverted teat in the pig in an experimental population that showed a high incidence of the defect. To evaluate the relevance of the results in commercial pig breeds and to confirm the results, a linkage study was done in an independent set of animals of the German Landrace breed and its cross with Large White; these breeds represent the most important dam lines in pig production in Germany. The study showed that the inverted teat defect is governed by genetic variation at several loci distributed throughout the genome. This is in agreement with more recent models of inheritance of the inverted teat defect that propose a polygenic background, while early works suggested autosomal recessive inheritance with reduced penetrance (Nordby 1934; Mayer and Pirchner 1995).

The emergence of inverted teats depends on insufficient mesenchymal proliferation at the teat ground during teat development (Günther 1984). As far as proliferation processes are concerned, the defect of inverted teats is related to the number-of–teats trait. Because this etiologic link of the traits, QTL for inverted teats and number of teats can be expected to be partly identical. The number of teats may also depend on local signaling between adjacent embryonic tissues that determine the somite patterning and cell fate decisions leading to the establishment of the mammary bud but is less important for the liability to express the inverted teat phenotype. There are a few reports on QTL for the number of teats in the pig that are summarized in a publicly accessible database, PigQTLDB (Hu and Reecy 2007; Hu et al. 2005). Most studies used an experimental population of crosses with the breed Meishan (Hirooka et al. 2001; Rodriguez et al. 2005; Rohrer et al. 1999; Yue et al. 2003). Meishan is known for its high number of teats, about 17.0 on average (Haley et al. 1995), compared with that of the Large White breed, with about 14 teats (Clayton et al. 1981; Haley et al. 1995).

On SSC1 QTL for the number of teats were detected between markers SJ029 and SWR485 and between markers SW803 and SW373 in resource populations from different groups (Beeckmann et al. 2003b; Cassady et al. 2001; Geldermann et al. 2003; Rohrer et al. 1999; Wada et al. 2000). In the latter region with upper marker S0155 and peak at marker SW1301, a QTL for the inverted teat defect was detected in the experimental population in this study. Comparing the results from different QTL studies, we can conclude that there might be a number of genes influencing teat development on SSC1. The relaxin gene (*RLN*) maps to SSC1 at position 1q28–q29, showing association with the inverted teat defect (Wimmers et al. 2002). *RLN* knockout mice were shown to exhibit impaired mammary development resulting in a phenotype similar to inverted teats that cannot be suckled by the offspring (Zhao et al. 1999). Another gene involved in teat development is the estrogen receptor (*ESR*) gene, in the region 1p24–p25 where a QTL for the number of teats was found (Wada et al. 2000). The *ESR* gene is already used as a marker for the litter size in different porcine populations (Short et al. 1997). Even though no QTL could be detected in commercial families, SSC1 contains candidate genes for the development of functional teats. By the results of association analysis using the marker SW1301 on SSC1, there is more evidence corroborating this assumption.

QTL for the number of teats were located in the proximal region of SSC2 (Hirooka et al. 2001; Lee et al. 2003a), close to the QTL for inverted teats reported here. Positional candidate genes are insulin-like growth factor 2 (*IGF2*) and follicle-stimulating hormone beta (*FSHB*). Insulin-like growth factor-2 is a growth factor that mediates prolactin induced in mammary gland development that shows dose-dependent mitogenic properties on primary human breast epithelial cells and stroma cells (Hovey et al. 2003; Strange et al. 2002, 2004). The follicle-stimulating hormone (FSH) is essential for normal reproductive function in males and females; it is more important for female than for male fertility (Tapanainen et al. 1997). In another study it was also found that the *FSH* gene plays a role in the morphologic development of the mammary gland within lactogenesis (McNeilly 1994).

For SSC3 one QTL for the number of teats is published at 84 cM with an *F* statistic of 15.78 (Rohrer 2000). In the resource population a QTL in the same region next to marker S0164 was detected. The transforming growth factor alpha (*TGFA*) gene and the follicle-stimulating hormone receptor (*FSHR*) gene map to 3q22–q23 and 3q21, respectively (Remy et al. 1995). FSHR mediates effects of FSH on mammary development. Growth-promoting TGFA and growth-inhibiting TGFB are

coexpressed in the bovine mammary gland. Higher mRNA contents of both factors during mammogenesis and involution may indicate autocrine or paracrine functions for these growth factors during proliferation and reorganization of the mammary tissue (Plath et al. 1997).

On SSC4 a QTL for the inverted teat defect was found in the commercial population where the thyroid-stimulating hormone beta (*TSHB*) gene at 4q21 is a functional and positional candidate gene. The metabolic hormone TSH is necessary for alveolar morphogenesis in proliferation and lactogenesis of the mammary gland. Thyroid hormone, as well as prolactin, is an important regulator of the functional development of the mammary gland. A lower level of TSH in the serum leads to a loss of the ductal growth and a lower or no proliferation of alveolar tissue (Bhattacharjee and Vonderhaar 1984; Vonderhaar and Greco 1979).

In studies by Lee et al. (2003b), a QTL for the number of teats was detected between markers SWR453 and SW2425 on SSC5. In our study a high NPL value for the heritable inverted teat defect was detected in the same region. The insulin-like growth factor-1 (*IGF1*) gene is suggested to be a candidate gene for teat development in pig; however, a microsatellite within *IGF1* showed no association with the inverted teat defect in this study. Another candidate gene, the parathyroid-hormone-like hormone (*PTHLH*) gene on SSC5 is found to be expressed in the stroma cell of the mammary gland. An overexpression of PTHLH in transgene mice leads to a mismatch of the gland and ductal growth in different stages (Dunbar et al. 2001; Wysolmerski et al. 1994, 1995). It was found that a C/T nonsynonymous single nucleotide polymorphism (S19L) at nucleotide position 375 of the porcine PTHLH cDNA was associated with the inverted teat defect (Chomdej 2005; Chomdej et al. 2004).

In this study two significant QTL in different regions on SSC6 could be detected. The NPL value had its peak in the DUMI and the commercial population at markers S0220 and S0059, respectively. Furthermore, in both populations a significant NPL value could be detected at marker S0035. A QTL for the number of teats was detected in a three-generation resource population between markers DG93 and SW1328 with a peak at 171 cM (Cassady et al. 2001). The result of linkage analysis in this study was partly verified by the association analysis in both populations.

Our linkage analysis in experimental and commercial families highlight the telomeric region of SSC6 close to marker S0035 and the central region close to markers S0059 and S0220 as regions containing QTL for the inverted teat defect segregating in both populations. The comparative map of pig and human indicate synteny of QTL at marker S0035 with the telomeric q-arm of HSA16. S0220 and S0059 direct to HSA1 (Meyers et al.2005). However, whereas the current status of the comparative map clearly shows correspondence of the genomic region of S0059 to HSA1, it still has to be proven that S0220 may also correspond to HSA19. Establishment of this relationship will finally contribute to clarification of whether the NPL plot peak at S0220 in the DUMI represents the same QTL as the NPL plot peak at S0059 obtained in the dam lines. There were several genes already mapped on SSC6 that are involved in the development of the mammary gland. The *TGFB* gene at 6q11–q21 (directing to HSA19) and the leptin receptor (*LEPR*) gene at 6q33–q35 (directing to HSA1) are suggested to be candidate genes for teat development. Leptin receptors have been also appeared in mammary epithelial cells and it has been suggested that leptin is involved in the control of the proliferation of both breast cells (Dundar et al. 2005). Other candidate genes, such as the wingless-type MMTV integration site family, member 4 (*WNT4*) gene, which plays a part in the early development of the mammary gland, could be found in the comparative region between markers S0220 and SW59. The LIM domain only 4 (*LMO4*) gene was mapped at HSA1p22.3; a higher expression of this gene could be shown to suppress the differentiation of epithelial cells in mammary gland (Visvader et al. 2001). The impact of the protein tyrosine phosphatase, receptor-type, F (*PTPTF* or *LAR*) gene was shown in knockout mice, in which female mice could not deliver milk. LAR-mediated signaling may have an impact on development and function of the mammary gland (Schaapveld et al. 1997). Another gene involved in mammary gland development that mapped onto HSA1 is the fatty acid-binding protein 3 (*FABP3* or *MDGI*) gene, also called mammary-derived growth inhibitor. It was the first gene identified as a growth inhibitor in the lactating bovine mammary gland (Bohmer et al. 1987). In summary, several positional and functional candidate genes could be identified; further studies need to be done to identify the exact position of the QTL on SSC6 and compare it to the human genome map to detect the regions with the highest concordance in these two species.

Several QTL for the number of teats have been found on SSC7 (Cassady et al. 2001; Wada et al. 2000). A QTL for the inverted teat defect on 7q25 close to marker S0101 was detected only in the experimental population. The prolactin (*PRL*) gene on 7p11–p12 is a functional candidate gene for teat characteristics. PRL is involved in different physiologic processes and is a key factor for the development of mammary gland and lactation (Bole-Feysot et al. 1998). The activity of PRL over its receptor in lactation is essential for the regulation of the metabolism of the adipose tissue (Ling et al. 2003).

Different QTL for the number of teats were detected on SSC8, suggesting that there may be different genes involved in mammary gland development (Beeckmann et al. 2003a; Cassady et al. 2001; Hirooka et al. 2001;

King et al. 2003). On SSC8 a QTL with a peak at SW268 was suggested (King et al. 2003). The QTL between SW1070 and S0144 includes the marker S0086, where in our study a chromosome-wide significant NPL value was detected in the commercial crossbreed population (Beeckmann et al. 2003a). A QTL for the number of nipples in pigs between markers SY23 and SW905 was detected, and a QTL with a peak at marker SW2410 was detected in the DUMI population (Cassady et al. 2001). The relaxin receptor leucin-rich G-protein-coupled receptor 7 (LGR7) was mapped on HSA4 in the comparative region of SSC8. LGR7 is likely to be involved in the development of the mammary gland and is a candidate for the inverted teat defect, because its ligand showed an association with the defect (Chomdej 2005; Wimmers et al. 2002). Radiation hybrid (RH) mapping of LGR7 showed that this gene is located next to SW368.

QTL for the number of nipples detected on SSC10 include regions between markers SW1708 and SW2067, between markers SW1991 and SW1626, between markers SWR1849 and SW2000, and between markers SW1041 and SW951 (Dragos-Wendrich et al. 2003; Hirooka et al. 2001; Rodriguez et al. 2005; Rohrer 2000). It can be summarized from these studies that in the region between markers SWR1849 and SW2067, QTL for the number of nipples were found. In DUMI population the peak of the experiment-wide significant NPL value was detected at marker SW2067 (Table 1). There are no candidate genes proposed in these studies. The orthologous human genomic region on chromosome 10 harbors calmodulin-like 3 protein (CALML3), a protein specifically expressed in mammary epithelial cells that is regulated during differentiation and might be involved in Ca-dependent pathways during mammary development (Yaswen et al. 1992).

The QTL with the highest peak at the marker S0386 on SSC11 could be confirmed in the commercial families. A QTL for the number of teats was found with an *F* value of 3.25 (Cassady et al. 2001). The breast cancer susceptibility (*BRCA2*) gene, which is involved in teat development, was detected on SSC11 (Bignell et al. 1997; Musilova et al. 2000).

QTL for the number of teats were detected in several experimental populations in different regions of SSC12 (Hirooka et al. 2001; Rodriguez et al. 2005; Yue et al. 2003). The detected region in our own study promotes the growth hormone as a positional candidate gene for the teat defect.

Our detected significant and suggestive QTL in the experimental population on SSC14, 16, and 18 could not be confirmed in the commercial population. Interestingly, alleles of most significant markers S0007 on SSC14 were shown to be significantly and highly significantly associated with the defect status in both populations.

Only one publication suggests a QTL region on SSCX in pig (Cepica et al. 2003). The androgen receptor (*AR*) gene was mapped to the X chromosome. This gene shows an association with the number of teats but no association with the inverted teat defect could be detected (Trakooljul 2004). In the present study no linkage mapping on SSCX was conducted.

The mothering ability of a sow plays an important role in the economic efficiency of pig production. Sows cannot raise more piglets than they have functional teats. The teat characteristics are used as selection criteria in pig breeding, with typically a minimum of 12 to 14 functional teats and no defect teats requested.

To detect the genetic cause of heritable defects, different approaches can be used. In our study the application of linkage analysis, which is *a priori* hypothesis-free, was chosen. The QTL analysis was performed in different populations: an experimental population with high defect incidence to increase the power of this approach, and commercial populations to confirm results and to evaluate their relevance for commercial breeding. QTL regions in overlapping marker intervals or close proximity in both populations were found on SSC3, SSC4, SSC6, and SSC11. Further association analysis of each marker separately provided further evidence of regions being involved in the phenotype of the inverted teat defect. For some regions with significant NPL values in both populations or significant QTL in the DUMI population, significant association and linkage of different alleles to the defect could be shown by the family-based association test. However, the genetic origin of the inverted teat defect has not been detected yet. Future studies are necessary to detect genes that are involved in the development of the inverted teat defect. Combining these results with current attempts to identify genes expressed during mammary gland development will facilitate the detection of the causal genes.

Acknowledgments This work was supported by funds provided by the Development Association for Biotechnology Research (FBF) and the Research Federal Ministry of Education and Research (BMBF).

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