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ASSOCIATION OF THE PORCINE TRANSFORMING GROWTH FACTOR BETA TYPE I RECEPTOR *(TGFBR1)* GENE WITH GROWTH AND CARCASS TRAITS

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Background: *Growth and carcass traits are of great economic importance in livestock production. A large number of quantitative trait loci (QTL) have been identified for growth and carcass traits on porcine chromosome one (SSC1). A key positional candidate for this chromosomal region is* **TGFBR1** *(transforming growth factor beta type I receptor). This gene plays a key role in inherited disorders at cardiovascular, craniofacial, neurocognitive, and skeletal development in mammals.*

Results: *In this study, 27 polymorphic SNPs in the porcine* **TGFBR1** *gene were identified on the University of Illinois Yorkshire × Meishan resource population. Three SNPs (SNP3, SNP43, SNP64) representing major polymorphic patterns of the 27 SNPs in F1 and F0 individuals of the Illinois population were selected for analyses of QTL association and genetic diversity. An association analysis for growth and carcass traits was completed using these three representative SNPs in the Illinois population with 298 F2 individuals and a large commercial population of 1008 animals. The results indicate that the* **TGFBR1** *gene polymorphism (SNP64) is significantly associated ($p < 0.05$) with growth rates including average daily gains between birth and 56 kg ($p = 0.049$), between 5.5 and 56 kg ($p = 0.024$), between 35 and 56 kg ($p = 0.021$). Significant associations ($p < 0.05$) were also identified between* **TGFBR1** *gene polymorphisms (SNP3|SNP43) and carcass traits including loin-eye-area ($p = 0.022$) in the Illinois population, and back-fat thickness ($p = 0.0009$), lean percentage ($p = 0.0023$) and muscle color ($p = 0.021$) in the commercial population. These three SNPs were also used to genotype a diverse panel of 130 animals*

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representing 11 pig breeds. Alleles SNP3_T and SNP43_G were fixed in Pietrain and Sinclair pig breeds. SNP64_G allele was uniquely identified in Chinese Meishan pigs. Strong evidence of association ($p < 0.01$) between both SNP3 and SNP64 alleles and reproductive traits including gestation length and number of corpora lutea were also observed in the Illinois population.

Conclusion: This study gives the first evidence of association between the porcine **TGFBR1** *gene and traits of economic importance and provides support for using* **TGFBR1** *markers for pig breeding and selection programs. The genetic diversities in different pig breeds would be helpful to understand the genetic background and migration of the porcine* **TGFBR1** *gene.*

BACKGROUND

To date, over 6300 pig quantitative trait loci (QTLs) representing 593 different traits have been identified across the whole genome, including 1391 (21.93%) QTLs detected on porcine chromosome one (SSC1) (1). The porcine transforming growth factor beta type I receptor *(TGFBR1)* gene has been mapped between microsatellite markers *SW803* (94.3 cM) and *SW705* (122.6 cM) on SSC 1q (2). TGF-β receptors are centrally involved in the TGF-β mediated cell growth and differentiation (3). Mutations in the *TGFBR1* and *TGFBR2* genes have been associated with inherited disorders in cardiovascular, craniofacial, neurocognitive, and skeletal development in humans (4). The porcine *TGFBR1* gene has been physically associated with approximately 218 putative QTLs (94.3–122.6 cM) related to production including growth rate and vertebra number (5–14). This locus has also been associated with meat quality including drip loss, fat composition, fatness, muscle color, conformation, and carcass composition (15–34). Importantly, *TGFBR1* has been associated with QTLs for reproduction traits, including teat number and age at puberty (35–39). Finally, both behavior (40), and immune parameters (41) have been associated with the SSC1 region containing *TGFBR1*. Considering its physiological function in growth and development, the large number of ligands for this receptor, and its position in the QTL-rich region, polymorphisms of the porcine *TGFBR1* gene could contribute several important QTLs (42).

TGF-β, a potent inhibitor of normal epithelial cell growth belongs to a large superfamily of structurally related multifunctional proteins that includes activins, inhibins, bone morphogenetic proteins, myostatin, and Müllerian inhibiting substance (3, 43, 44). TGF-β signaling is mediated by two specific cellular serine/threonine kinase receptors (*TGFBR1* and *TGFBR2*). In particular, during skeletal development, TGF-βs and receptors have unique functions and act sequentially to modulate chondrocyte and osteoblast differentiation (45). *TGFBR1* mutations are associated with Loeys-Dietz aortic aneurism syndrome (4), and various human cancers including kidney, bladder, head-neck, invasive breast cancers, and cervical and ovarian carcinomas (46–54).

The porcine *TGFBR1* gene has been well characterized and a variety of polymorphisms within this gene are known (42, 55). The porcine *TGFBR1* gene contains 9 exons spanning a transcription unit of 62 kb. A total of 85 gene polymorphisms (77 SNPs and 8 indels), including 3 SNPs in exons, 4 SNPs in 5′- and 3′- flanking regions, and 78 nucleotide variations in introns were detected in the porcine *TGFBR1* gene

utilizing a panel of DNA from eight diversified pig breeds. In the present study, to analyze the association of the porcine *TGFBR1* gene with traits of economic importance, the polymorphic regions of the porcine *TGFBR1* gene utilizing all the 18 F1 and 6 founder pigs of the University of Illinois Yorkshire × Meishan reference family were re-sequenced (56). This sequence information was then used to initiate an association of polymorphisms within the porcine *TGFBR1* gene and growth, carcass and reproductive traits in this resource population as well as a large commercial population with 1008 progeny from 138 families.

RESULTS

Polymorphism Detection

By direct DNA sequencing, 27 polymorphisms were identified in *TGFBR1* gene in the Illinois resource population (Table 1). Among these 27 SNPs, six were transversion SNPs (purine ↔ pyrimidine) and 21 were transition SNPs (purine ↔ purine, or pyrimidine ↔ pyrimidine). As expected, there was an excess of transition over transversion substitutions in the *TGFBR1* gene observed in the Illinois resource population. Although there are twice as many possible transversions as transitions, there is a universal bias in favor of transitions over transversions, possibly as a result of the molecular mechanisms by which each is generated. In addition, transitions are less likely to result in amino acid substitutions, thus they are more likely to persist because the protein structure is not altered.

One non-synonymous SNP (SNP3, T^{84}-C^{84}), leads to an amino acid exchange Pro^{8}-Ser^{8}. One SNP was detected in the regulatory 5' flanking region (SNP2, G^{-415}-C^{-415}), and the remaining 25 SNPs were located in introns throughout the *TGFBR1* gene. The results showed that the SNP3 and SNP43 shared the same distribution pattern of genotypes in the F1 individuals, and parents of the commercial population also have the same distribution pattern in the F2 individuals and the progeny of the commercial population. The frequency of minor allele SNP3_C/SNP43_A was 0.33 in 298 F2 animals of Illinois resource population, and was 0.71 in the 1008 progeny of the commercial population. The minor allele frequency of SNP64_G was 0.24 in the 298 F2 individuals of the Illinois resource population.

Association of *TGFBR1* Polymorphisms and Growth Traits

The association of the *TGFBR1* polymorphism with growth and carcass traits were analyzed using a model with sire and dam as covariates. Sire was treated as a random effect and age as fixed effect. Significant effects ($p < 0.05$) of SNP64 of the *TGFBR1* gene were observed on growth traits in the Illinois resource population (Table 2). The SNP64G allele was identified as the favorable allele for growth rates in the Illinois resource population. Fifteen of the F1 sows carried the SNP64_AA genotype, and the 3 F1 boars carried SNP64_AGgenotype, and thus no animal with SNP64_GG genotype was detected in the F2 individuals. The SNP64_AG genotype imparted a larger final weight (3.3 kilograms, $p = 0.029$), and a faster daily growth rate of 0.07 kg/day ($p = 0.021$) from grower to finisher compared to the SNP64_AA genotype (Table 2).

Table 1 SNP genotyping of porcine *TGFBR1* gene in F0 and F1 of the University of Illinois Meishan × Yorkshire Swine Family

NO ID	Loc	1 28le	2 F8	3 J48	4 7ST	5 23H	6 H20	7 7B	8 28B	9 31B	10 13B	11 16B	12 14B	13 24B	14 6B	15 12B	16 30B	17 27B	18 15B	19 23B	20 17B	21 11B	22 29B	23 26B	24 25B
S02 (G/C)	**-415**	GG	CG	CG	GG	GG	CC	GG	CG	CG	GG	GG	GG	CG	GG	GG	CG	CG	GG	CG	GG	CG	CG	CG	CG
S03 (T/C)	**84**	TT	CT	CT	TT	TT	CC	TT	CT	CT	TT	TT	TT	CT	TT	TT	CT	CT	TT	CT	TT	CT	CT	CT	CT
S10 (T/C)	**5483**	TT	CT	CT	TT	TT	CC	TT	CT	CT	TT	TT	TT	CT	TT	TT	CT	CT	TT	CT	TT	CT	CT	CT	CT
S11 (A/G)	**5492**	AA	AG	AG	AA	AA	GG	AA	AG	AG	AA	AA	AA	AG	AA	AA	AG	AG	AA	AG	AA	AG	AG	AG	AG
S12 (G/T)	**10012**	GG	GT	GT	GG	GG	TT	GG	GT	GT	GG	GG	GG	GT	GG	GG	GT	GT	GG	GT	GG	GT	GT	GT	GT
S13 (T/C)	**10437**	TT	CT	CT	TT	TT	CC	TT	CT	CT	TT	TT	TT	CT	TT	TT	CT	CT	TT	CT	TT	CT	CT	CT	CT
S35 (C/T)	**47814**	TT	CT	CT	TT	TT	CC	TT	CT	CT	TT	TT	TT	CT	TT	TT	CT	CT	TT	CT	TT	CT	CT	CT	CT
S42 (G/A)	**50844**	GG	AG	AG	GG	GG	AA	GG	AG	AG	GG	GG	GG	AG	GG	GG	AG	AG	GG	AG	GG	AG	AG	AG	AG
S43 (G/A)	**51499**	GG	AG	AG	GG	GG	AA	GG	AG	AG	GG	GG	GG	AG	GG	GG	AG	AG	GG	AG	GG	AG	AG	AG	AG
S44 (T/C)	**51592**	TT	CT	CT	TT	TT	CC	TT	CT	CT	TT	TT	TT	CT	TT	TT	CT	CT	TT	CT	TT	CT	CT	CT	CT
S51 (T/C)	**52463**	TT	CT	CT	TT	TT	CC	TT	CT	CT	TT	TT	TT	CT	TT	TT	CT	CT	TT	CT	TT	CT	CT	CT	CT
S53 (C/T)	**53206**	CC	CT	CT	CC	CC	TT	CC	CT	CT	CC	CC	CC	CT	CC	CC	CT	CT	CC	CT	CC	CT	CT	CT	CT
S55 (T/C)	**53498**	CC	CT	CC	CC	CC	CT	CC	CC	CC	CC	CC	CC	CT	CC	CC	CC	CT	CC	CT	CC	CT	CC	CC	CT
S63 (G/C)	**55736**	GG	CG	CG	GG	GG	CC	GG	CG	CG	GG	GG	GG	CG	GG	GG	CG	CG	GG	CG	GG	CG	CG	CG	CG
S64 (G/A)	**56211**	AA	AG	AA	AA	AA	AG	AA	AA	AA	AA	AA	AA	AG	AA	AA	AA	AA	AA	AG	AA	AG	AA	AA	AG
S66 (A/G)	**56485**	AA	AG	AG	AA	AA	GG	AA	AG	AG	AA	AA	AA	AG	AA	AA	AG	AG	AA	AG	AA	AG	AG	AG	AG
S67 (C/T)	**56546**	CC	CT	CT	CC	CC	TT	CC	CT	CT	CC	CC	CC	CT	CC	CC	CT	CT	CC	CT	CC	CT	CT	CT	CT
S70 (G/A)	**57277**	GG	AG	AG	GG	GG	AA	GG	AG	AG	GG	GG	GG	AG	GG	GG	AG	AG	GG	AG	GG	AG	AG	AG	AG
S71 (T/C)	**57282**	TT	CT	CT	TT	TT	CC	TT	CT	CT	TT	TT	TT	CT	TT	TT	CT	CT	TT	CT	TT	CT	CT	CT	CT
S72 (C/T)	**57288**	CC	CT	CT	CC	CC	TT	CC	CT	CT	CC	CC	CC	CT	CC	CC	CT	CT	CC	CT	CC	CT	CT	CT	CT
S73 (T/C)	**57290**	TT	CT	CT	TT	TT	CC	TT	CT	CT	TT	TT	TT	CT	TT	TT	CT	CT	TT	CT	TT	CT	CT	CT	CT
S74 (C/A)	**57407**	CC	CC	AC	CC	CC	CC	CC	AC	AC	CC	CC	CC	CC	CC	CC	AC	AC	CC	CC	CC	CC	AC	AC	CC
S76 (G/T)	**57653**	GG	GT	GT	GG	GG	TT	GG	GT	GT	GG	GG	GG	GT	GG	GG	GT	GT	GG	GT	GG	GT	GT	GT	GT
S77 (A/G)	**58866**	AA	AG	AG	AA	AA	GG	AA	AG	AG	AA	AA	AA	AG	AA	AA	AG	AG	AA	AG	AA	AG	AG	AG	AG
S78 (T/C)	**59229**	TT	CT	CT	TT	TT	CC	TT	CT	CT	TT	TT	TT	CT	TT	TT	CT	CT	TT	CT	TT	CT	CT	CT	CT
S79 (G/A)	**59340**	GG	AG	AG	GG	GG	AA	GG	AG	AG	GG	GG	GG	AG	GG	GG	AG	AG	GG	AG	GG	AG	AG	AG	AG
S85 (T/A)	**62351**	TT	AT	AT	TT	TT	AA	TT	AT	AT	TT	TT	TT	AT	TT	TT	AT	AT	TT	AT	TT	AT	AT	AT	AT

Founders (♀ = Yorkshire, ♂ = Meishan): 28le (♂), F8 (♀), J48 (♀), 7ST (♂), 23H (♂), H20 (♀).
F1 individuals: 7B (♂), 28B (♂), 31B (♂), 13B (♂), 16B (♂), 14B (♂), 24B (♀), 6B (♂), 12B (♂), 30B (♂), 27B (♂), 15B (♂), 23B (♀), 17B (♂), 11B (♂), 29B (♂), 26B (♂), 25B (♀).
Loc: SNP location (+1 corresponds to the transcription initiation point of the longest porcine *TGFBR1* cDNA).
S03 (T/C), S43 (G/A), S64 (G/A) were representative SNPs selected for analyses of association and genetic diversity in diverse pig panel.

Table 2 Least squares means and P-values of porcine TGFBR1-SNP64 in the University of Illinois Meishan × Yorkshire Swine Family

SNP64	Yvar	Cvar	Zvar	p-value	SNP64_AA	SNP64_AG	AA-AG
Reproduction	GL	None	family	0.0008	44.44	46.37	−1.93
SE					0.40	0.41	0.56
p-value							0.0008
Reproduction	GL	None	SD (sire)	0.0009	44.29	46.14	−1.86
SE					0.38	0.40	0.54
p-value							0.0009
Reproduction	CL	None	family	0.0838	14.26	15.41	−1.15
SE					0.51	0.53	0.66
p-value							0.0838
Growth*	GF_ADG	None	SD (sire)	0.0213	0.63	0.70	−0.07
SE					0.02	0.02	0.03
p-value							0.0213
Growth*	GF_ADG	None	family	0.0333	0.65	0.72	−0.06
SE					0.02	0.02	0.03
p-value							0.0333
Growth*	WF_ADG	None	SD (sire)	0.0236	0.52	0.55	−0.04
SE					0.02	0.01	0.02
p-value							0.0236
Growth*	WF_ADG	None	family	0.0258	0.53	0.56	−0.04
SE					0.01	0.01	0.02
p-value							0.0258
Growth*	F_weight	None	SD (sire)	0.0291	52.64	55.95	−3.32
SE					1.72	1.53	1.51
p-value							0.0291
Growth*	F_weight	None	family	0.0317	53.83	57.15	−3.32
SE					1.37	1.10	1.53
p-value							0.0317
Growth*	BF_ADG	None	family	0.0497	0.44	0.47	−0.03
SE					0.02	0.02	0.02
p-value							0.0497
Growth*	WG_ADG	None	family	0.0832	0.46	0.49	−0.03
SE					0.01	0.01	0.01
p-value							0.0832
Growth*	G_weight	None	family	0.0874	33.93	35.62	−1.69
SE					0.97	0.81	0.98
p-value							0.0874

Measurement: growth or carcass or reproduction; Yvar: traits; Cvar: covariate term; Zvar: random effect terms; SE: standard error; GL: gestation length (days); CL: number of corpora lutea; GF_ADG: average daily growth rate from grower to finisher (kg/day); WF_ADG: average daily growth rate from weaning to finisher (kg/day); BF_ADG: average daily growth rate from birth to finisher (kg/day); WG_ADG: average daily growth rate from weaning to grower (kg/day); F_weight: final weight (kg); G_weight: grower weight (kg); SNP64_AA: Least squares means for SNP genotype AA; SNP64_AG: Least squares means for SNP genotype AG; AA-AG: Difference between SNP genotypes AA and AG; *When both SNPs were included in statistical model.

Neither SNP3 nor SNP43 was significantly associated with measured growth traits in the Illinois or commercial populations. Suggestive associations ($0.05 < p < 0.10$) were observed between SNP3/SNP43 and growth rates in the Illinois resource population. Animals carrying the SNP3_TT genotype have an average daily gain (ADG) from grower to finisher nearly 0.1 kg/day greater than those pigs with

SNP3_CC ($p = 0.064$). Similarly, the SNP3_TT genotype had a daily growth rate of 0.05 kg/day higher than SNP3_CC pigs ($p = 0.086$). Interestingly, pigs with a single SNP3_T allele had an intermediate values for all three growth traits compared.

Association of *TGFBR1* Polymorphisms and Carcass Traits

Results of association analysis between *TGFBR1* gene polymorphism and carcass traits in Illinois resource population and a commercial population revealed significant effects of *TGFBR1* gene on carcass traits in both populations. The SNP3_TT animals had a larger loin-eye-area by 2.13 cm^2 ($p = 0.022$) than animals carrying the SNP3_CC genotype in the Illinois resource population (Table 3). The SNP3_TT animals have thicker back-fat by 1.39 mm ($p = 0.0009$), smaller lean percentage ($p = 0.0023$), larger Japanese-colour of 0.30 ($p = 0.021$) than those with SNP3_CC in the commercial population (Table 4). Suggestive association was also observed between SNP3 and leaf fat ($p = 0.075$), marbling score ($p = 0.087$) in the Illinois population. No significant or suggestive association was detected between the SNP64 and carcass traits in the Illinois resource population.

Association of *TGFBR1* Polymorphisms and Reproductive Traits

TGFBR1 gene polymorphisms were tested for association with four reproductive traits and four litter size traits in the Illinois resource population. The sows carrying genotype SNP3_CC revealed a longer gestation of 1.98 days than the ones having the genotype SNP3_TT ($p = 0.0099$). Sows carrying genotype SNP3_CT have 1.84 and 1.64 more corpora lutea compared with females carrying genotype SNP3_CC and SNP3_TT, respectively (Table 3). It was also revealed that females having the genotype SNP64_AG have a longer gestation of 1.93 days ($p = 0.0008$) than those with genotype SNP64_AA (Table 2). No significant association was detected between these SNPs and the litter size traits.

Allele Frequencies in Diverse Pig Panel

Allele frequencies for three SNPs were analyzed using a total of 130 animals representing 11 genetically divergent pig breeds (Table 5). The SNP3_T and SNP43_G alleles were swept in the Pietrain and Sinclair pig breeds, and the SNP64_G allele was only detected in the Chinese originated Meishan pig breed with an allele frequency of 0.27. The minor allele frequency of the SNP3_T/SNP43_G in the three miniature pig breeds variedfrom 0.17 in Ossabaw, to 0.75 in Yuctan and 1 in Sinclair. The three miniature pig breeds are most likely to have different genetic background on this gene since the high variations of the allele frequencies among them. The average minor allele frequency of the SNP3_C/SNP43_A of the porcine *TGFBR1* gene in the 7 western pig breeds (Duroc, Hampshire, Hanford, Landrace, Large White, Pietrain, and Yorkshire) was 0.39, and 0.36 on average in the 3 miniature pig breeds. In contrast, the frequency was 0.92 in the Chinese originated Meishan pigs.

Table 3 Least squares means and P-values of porcine TGFBR1-SNP3 in the University of Illinois Meishan × Yorkshire swine family

SNP3	Yvar	Cvar	Zvar	p-value	SNP3_CC	SNP3_CT	SNP3_TT	CC-CT	CC-TT	CT-TT
Reproduction	GL	None	family	0.0099	46.39	46.09	44.41	0.30	1.98	1.68
SE					0.77	0.45	0.42	0.89	0.88	0.60
p-value								0.7368	0.0268	0.0067
Reproduction	GL	None	SD (sire)	0.0104	46.20	45.88	44.26	0.31	1.93	1.62
SE					0.74	0.44	0.41	0.86	0.85	0.59
p-value								0.7151	0.0248	0.0077
Reproduction	CL	None	family	0.0453	13.99	15.83	14.19	−1.84	−0.20	1.64
SE					0.97	0.58	0.55	1.09	1.08	0.70
p-value								0.0959	0.8536	0.0212
Reproduction	CL	None	SD (sire)	0.0608	14.26	15.92	14.36	−1.66	−0.10	1.56
SE					1.06	0.79	0.78	1.06	1.06	0.70
p-value								0.1210	0.9240	0.0275
Carcass*	LEA	None	SD (sire)	0.0215	22.71	22.19	24.84	0.52	−2.13	−2.58
SE					1.29	0.90	0.97	1.03	1.48	0.97
p-value								0.6263	0.1550	0.0074
Carcass*	LEA	None	family	0.0242	22.65	21.94	24.52	0.65	−1.94	−2.58
SE					1.10	0.58	0.77	1.03	1.48	0.97
p-value								0.5226	0.2001	0.0095
Carcass*	Leaf_fat	SW	SD (sire)	0.0754	6.80	6.12	5.09	0.68	1.72	1.04
SE					0.58	0.30	0.38	0.55	0.77	0.51
p-value								0.2161	0.0290	0.0466
Carcass*	Leaf_fat	SW	family	0.0960	6.77	6.08	5.14	0.69	1.63	0.93
SE					0.55	0.27	0.36	0.53	0.75	0.51
p-value								0.1952	0.0337	0.0731
Carcass	MS	None	family	0.0873	3.58	3.31	3.19	0.27	0.39	0.12
SE					0.16	0.08	0.08	0.18	0.18	0.11
p-value								0.1255	0.0301	0.2824
Growth*	GF_ADG	None	SD (sire)	0.0639	0.62	0.67	0.72	−0.05	−0.10	−0.05
SE					0.03	0.02	0.02	0.03	0.04	0.03
p-value								0.0655	0.0201	0.1011

(*Continued*)

Table 3 Continued

SNP3	Yvar	Cvar	Zvar	p-value	SNP3_CC	SNP3_CT	SNP3_TT	CC-CT	CC-TT	CT-TT
Growth*	WF_ADG	None	SD (sire)	0.0857	0.51	0.54	0.56	−0.02	−0.05	−0.02
SE					0.02	0.01	0.02	0.01	0.02	0.02
p-value								0.0601	0.0322	0.1872

Measurement: growth or carcass or reproduction; Yvar: traits; Cvar: covariate term; Zvar: random effect terms; SE: standard error; GL: gestation length (days); CL: number of corpora lutea; LEA: loin-eye-area (cm^2); MS: marbling score; GF_ADG: average daily growth rate from grower to finisher (kg/day); WF_ADG: average daily growth rate from weaning to finisher (kg/day); SNP3_CC: Least squares means for SNP genotype CC; SNP3_CT: Least squares means for SNP genotype CT; SNP3_TT: Least squares means for SNP genotype TT; CC-TT: Difference between SNP genotypes CC and TT; CT-TT: Difference between SNP genotypes CT and CT; *When both SNPs were included in the statistical model.

Table 4 Least squares means and P-values of porcine TGFBR1-SNP3 in the commercial population

SNP3	Yvar	Zvar	Cvar	F-value	p-value	SNP3_CC	SNP3_CT	SNP3_TT	CC-CT	CC-TT	CT-TT
Carcass	BF_depth	SD (sire)	H_Weit	7.1696	0.0009	16.88	17.98	18.26	−1.11	−1.39	−0.28
SE						0.31	0.31	0.59	0.30	0.61	0.56
p-value									0.0003	0.0222	0.6158
Carcass	BF_depth	SD (sire)		5.7876	0.0033	16.92	17.97	18.17	−1.05	−1.25	−0.19
SE						0.33	0.33	0.62	0.32	0.64	0.59
p-value									0.0009	0.0505	0.7446
Carcass	Lean %	SD (sire)	H_Weit	6.1339	0.0023	55.50	54.80	54.53	0.70	0.97	0.27
SE						0.23	0.23	0.42	0.21	0.42	0.39
p-value									0.0009	0.0216	0.4896
Carcass	Lean %	SD (sire)		5.4609	0.0045	55.48	54.81	54.58	0.68	0.90	0.23
SE						0.23	0.23	0.42	0.21	0.43	0.39
p-value									0.0016	0.0347	0.5629
Carcass	JColor_1	SD (sire)		3.8827	0.0213	3.29	3.41	3.59	−0.12	−0.30	−0.18
SE						0.07	0.07	0.12	0.06	0.12	0.11
p-value									0.0437	0.0105	0.0965
Carcass	JColor_1	SD (sire)	H_Weit	3.6876	0.0258	3.29	3.41	3.59	−0.12	−0.30	−0.18
SE						0.07	0.07	0.12	0.06	0.12	0.11
p-value									0.0517	0.0122	0.0995
Carcass	L_color	SD (sire)	H_Weit	2.7425	0.0655	47.58	47.22	46.52	0.36	1.06	0.70
SE						0.24	0.24	0.46	0.24	0.47	0.44
p-value									0.1317	0.0258	0.1137
Carcass	L_color	SD (sire)		2.3401	0.0975	47.56	47.23	46.56	0.33	1.00	0.67
SE						0.25	0.25	0.47	0.24	0.48	0.44
p-value									0.1764	0.0377	0.1313

Measurement: growth or carcass; Yvar: traits; Cvar: covariate term; Zvar: random effect terms; SE: standard error; BF_depth: backfact depth (mm); Lean %: lean percentage; Jcolor1: Japanese color 1; L_color: L-color; H_weit: Hot weight; SNP3_CC: Least squares means for SNP genotype CC; SNP3_CT: Least squares means for SNP genotype CT; SNP3_TT: Least squares means for SNP genotype TT; CC-TT: Difference between SNP genotypes CC and TT; CT-TT: Difference between SNP genotypes CT and CT.

Table 5 Allele frequencies of porcine TGFBR1-SNPs in diverse pig panel

SNPs		SNP3		SNP43		SNP64	
Breeds	No.	T	C	G	A	G	A
Duroc	13	0.5385	0.4615	0.5385	0.4615	0	1
Hampshire	13	0.5769	0.4231	0.5769	0.4231	0	1
Hanford	12	0.5833	0.4167	0.5833	0.4167	0	1
Landrace	10	0.35	0.65	0.35	0.65	0	1
Large white	10	0.60	0.40	0.60	0.40	0	1
Meishan	13	0.08	0.92	0.08	0.92	0.27	0.73
Ossabaw	9	0.1667	0.8333	0.1667	0.8333	0	1
Pietrian	13	1	0	1	0	0	1
Sinclair	12	1	0	1	0	0	1
Yorkshire	13	0.6154	0.3846	0.6154	0.3846	0	1
Yucatan	12	0.75	0.25	0.75	0.25	0	1

DISCUSSION

The aim of the study was to conduct association analysis between the gene polymorphisms of a positional candidate gene *TGFBR1* and economically important traits in both experimental and commercial populations, and perform allele frequency analysis of the *TGFBR1* genomic variants in genetically divergent pig breeds to understand their genetic diversity for potential application in pig selection and breeding practice. The initial studies conducted a SNP screen of the founder and F1 animals from The Yorkshire × Meishan Illinois resource population. Having identified SNPs in the experimental population, three SNPs defining this genomic region were tested for association analysis. Significant results from both the reference and commercial populations revealed that the *TGFBR1* gene has significant effects on multiple phenotypic traits of carcass and reproduction and a suggestive effect on porcine growth rates.

Many association studies have been done in the past years, but most of them were performed in experimental populations (e.g., 57–59). In the present study, we examined the association using both experimental pedigrees as well as a large commercial population. As the phenotypic traits between these two populations were different, it might be difficult to conclude if the *TGFBR1* gene has the same level of effects on both populations or not. In general, association between *TGFBR1* gene polymorphisms and carcass traits were detected in both Illinois resource and commercial populations, that is, loin-eye-area ($p = 0.022$) in Illinois population, and backfat depth ($p = 0.0009$), lean percentage ($p = 0.0023$), and muscle color ($p = 0.021$) in the commercial population. Significant associations between the *TGFBR1* gene polymorphism (SNP64) and growth traits were observed in the Illinois resource population with ADGs between 35 kg (grower) and 56 kg (finisher) ($p = 0.021$), between 5.5 kg (weaning) and 56 kg ($p = 0.024$), and between birth and 56 kg ($p = 0.049$) (Table 2). However, no association analysis could be performed between TGFBR1_SNP64 and growth traits in the commercial population due to the monomorphism of TGFBR1_SNP64 in the commercial population. As the SNP64_G allele was only found in the Chinese Meishan pig breed from the diversified pig panel, it would not

be surprising that the sires and dams of the commercial population were not polymorphic for the TGFBR1_SNP64. These findings suggest that genetic effects could vary to some extent in different populations. We are fully aware of this fact and recommend caution on selecting genetic markers to utilize in animal breeding programs.

Strong evidence of an association of SNP64 in the porcine *TGFBR1* gene with gestation length ($p = 0.0008$), and significant association between SNP3 and number of copora lutea ($p = 0.045$) were found in the present study (Tables 2 and 3). However, since no phenotypic data of reproductive traits was available for the commercial population, validation of this effect could not be confirmed. It is not surprising that association was found between the *TGFBR1* gene polymorphisms and reproduction traits since some of the ligands of the TGF-beta receptor complex such as bone morphogenetic proteins (e.g., BMP15, BMPR1B) play important roles in ovulation in sheep (60–62) and pigs (63, 64). Moreover, the *TGFBR1* gene is located in a QTL region responsible for age of puberty (35, 37), and teat numbers (23, 24, 36, 38).

A genomic scan of the Illinois resource population resulted in a significant QTL effect defined by microsatellites *SW373* and *SW1301* containing the *TGFBR1* gene on SSC1 for post-weaning average daily gain between 5.5 and 56 kg of body weight ($p = 0.000007$), between birth and 56 kg body weight ($p = 0.000227$), between 35 and 56 kg body weight ($p = 0.00077$) (6). Using the Illinois population, QTLs for carcass composition and meat quality were detected on SSC1, SSC6, SSC7, SSC8, and SSC12 (65); whereas QTLs for reproductive traits were identified on SSC1, SSC4, SSC5, SSC6, SSC7, SSC8, SSC9, and SSC15 (66). Significant association identified between the *TGFBR1* gene polymorphism and growth rates with $0.01 < p < 0.05$ suggested that the *TGFBR1* gene could partly contribute to the growth QTL, but other genes also probably contribute to the growth QTL in the region. With respect to the effects on carcass traits and reproductive traits, the association analyses showed *TGFBR1* gene has significant effects ($p < 0.01$) on both carcass and reproductive traits, but this gene is not located in the QTL regions previously detected using this population. This could possibly be accounted for some other genes in the region surrounding the *TGFBR1* gene having opposite effect on these traits and subtracting the effects of this region. It would also possibly be due to the low resolution of markers used for QTL screen resulting in large QTL intervals with about 10–30 cM on the pig chromosomes that make some QTLs/genes with significant effects to be undetected.

Shimanuki et al. (55) showed that the frequency of SNP3_C allele was 0.39 in European pigs ($n = 9$) including Landrace, large white, and Duroc, and that of 1.00 in Asian pigs ($n = 6$) including Chinese Jinhua and Japanese wild pig. The present study showed that the allele frequency of SNP3_C in these three European pig breeds varies from 0.40 in Large Whites to 0.65 in Landrace with an average of 0.50 ($n = 33$), and was 0.92 in the Chinese Meishan breed. The allele frequency of SNP3_C was lower than that in the present study. This study extends and confirms the prior study and the small number of tested animals by Shimanuki et al. (55) likely account for the differences between the two studies.

Analysis of secondary structure prediction of *TGFBR1* was performed with the GOR IV secondary structure prediction method (67). Possible effects on the protein conformation (alpha helix, extended sheet, and random coil) caused by these two amino acid substitutions from the non-synonymous SNP3 were analyzed. The

predominant secondary structure surrounding Ser^8 is predicted to be random coil. An amino acid substitution to Pro^8, an alpha-helical structure-breaker further decreased the possibility of an alpha-helical structure and increasing the probability of a random coil structure. Thus, no changes were predicted in the secondary structure mediated by the replacement of Ser^8-Pro^8. However, it is unknown if this change alters the signal transduction capacity of the *TGFBR1*.

Surprisingly, SNP3_TT and SNP43_GGgenotypeswere fixed in both fast-growing Pietrain and slow-growing Sinclair miniature pig breeds. In contrast, the SNP3_T and SNP43_G alleles are nearly absent in the Chinese Meishan (SNP3_T/SNP43_G = 0.08), and have a low frequency of distribution in Ossabaw miniature breed (SNP3_T/SNP43_G = 0.17). The low frequency of the beneficial alleles for growth rate in Meishan and Ossabaw pig breeds are inconsistent with their slow growth compared to western pig breeds. The observations suggest that *TGFBR1* is not a common genetic determinant for slow growth in pigs. Further investigation of the porcine *TGFBR1* gene polymorphisms in wild ancestors of domestic pigs for defining their ancestral alleles and evolutionary history would be interesting. The uniqueness of SNP64_G in the Chinese Meishan pig suggests that this could possibly be due to the genetic background of its Asian origin and environments compared to the other European-originated pig breeds. In addition, it could possibly have arisen from intensive artificial selection on some agricultural important traits (e.g., female reproduction). Considering that the SNP64_G allele was unique to the Chinese originated Meishan pig, it would also be interesting to do an extensive survey of this allele in Chinese indigenous pig breeds to better illustrate the gene flow among them. Provided the significantly high fertility of the Meishan pig, it would not be surprising that the SNP64 has a significant effect on reproduction traits in the Illinois resource population which was constructed by Meishan and Yorkshire pigs.

CONCLUSIONS

TGFBR1 is considered to play a key role in the regulation of skeletal and tissue development through the TGF-beta pathway. The porcine *TGFBR1* gene has been mapped to QTL regions associated with growth and carcass traits in several independent studies. The association analysis presented here provides the first evidence of such an association in pigs. The results revealed that the *TGFBR1* gene polymorphisms (TGFBR1_SNP3/SNP43) have significant effects on carcass traits in both Illinois resource population and the commercial population. However, the genetic effects of TGFBR1_SNP64 on growth traits was only identified in the Illinois resource population, but could not be validated in the commercial population due to the locus monomorphism in the commercial population. The genetic diversities in different pig breeds would be helpful to understand the genetic background and evolution history of the *TGFBR1* gene. The results are useful for gene-assisted-selection in pig selection and breeding programs.

METHODS

Animals

Illinois resource population. The University of Illinois F2 pig resource family (56) was constructed by using Yorkshire × Meishan consisting of 10

grandparents, 18 F1 and 298 F2 to provide DNA samples and growth and carcass traits data from three generations of animals.

Growth data. Phenotypic data for nine ADG traits, birth weight, and weight at two weeks of age for 298 F2 animals were recorded. The ADG were calculated between body weights for the following standard phases in swine production: weaning weight (average weight of 5.5 kg), nursery (between 5.5 and 35 kg), grower (between 35 and 56 kg), and finishing (between 56 and 105 kg). Three ADG traits, birth to 105 kg, 35 to 105 kg, and 56 to 105 kg body weight, were collected only on male animals ($N = 116$).

Carcass data. Ten carcass traits of from 116 F2 barrows (i.e., castrated males) including live weight, slaughter weight, leaf fat, average fat thickness, loin eye area, color score, firmness score, marbling score, 10th rib fat thickness, and carcass length were collected.

Reproductive data. Four reproductive traitsincluding gestation length, number of corpora lutea, total fetuses, uterine length, and four litter size traits including total pig born, number of piglets born alive, number of stillborn piglets, and number of piglets weaned were collected from 122 females in the Illinois resource population.

Commercial population. The commercial population consists of 1008 progeny from 138 families produced from 8 boars and 86 dams. The 8 sires were selected from Pipestone Artificial Breeders (Pipestone, MN) and the 86 dams were selected from Buttercup, Pipestone Systems (Pipestone MN). These breeding animals were selectedas being heterozygous at three microsatellites (*SW373, S0112* and *SW1301*) that flank the average daily gain (ADG) QTL as defined by Paszek and colleagues (6).

Growth data. At birth all piglets were ear-tagged on both ears, weighed, and tailed. Piglets were weighed at several stages of growth: birth, weaning, end of nursery, middle of finisher, and end of finisher.

Carcass data. 8 carcass traits including hot weight, pH value (24 hrs), loin depth, lean percentage, backfat, L value, Japanese color 1, Japanese color 2, were collected on progeny at the "Swift & Company" packing plant (Worthington, MN, USA).

Divergent pig breed panels. A panel of 130 unrelated (no shared grandparents) pigs, representing 11 breeds, were genotyped to investigate the pattern and variability of *TGFBR1* SNP polymorphism distribution in domestic pig breeds. The panel consisted of Duroc ($n = 13$), Hampshire ($n = 13$), Hanford ($n = 12$), Landrace ($n = 10$), Large white ($n = 10$), Meishan ($n = 13$), Ossabaw ($n = 9$), Pietrain ($n = 13$), Sinclair ($n = 12$), Yorkshire ($n = 13$), Yucatan ($n = 12$). The panel comprises the main inter-boundary commercial breeds (Duroc, Landrace, Large white, Pietrain, Yorkshire), two American pig breeds (Hampshire, Hanford), one Asian breed (Meishan), and three miniature breeds (Ossabaw, Sinclair, Yucatan).

SNP detection and DNA-Sequencing. The SNPs in the porcine *TGFBR1* gene were detected by a direct sequencing approach throughout the *TGFBR1* coding and non-coding regions as well as the 5′ and 3′-flanking regions with DNAs from 6 F0

and 18 F1 individuals from the University of Illinois reference population. The PCR amplifications were performed in a final volume of 20 μL containing 20 ng genomic DNA, 10 pmol of each primer, 200 μM of each dNTP, 1 U *Taq* DNA polymerase, 1 × PCR buffer (2.5 mM $MgCl_2$) and 1 × Q-solution (Qiagen, Valencia, CA, USA).

To investigate the polymorphisms of the porcine *TGFBR1* gene at the Illinois resource population, 24 amplicons containing 27 SNPsrepresenting all polymorphic patterns in the discovery panel of 8 diversified pig breeds (Table 6) were bidirectionally re-sequenced with the ABI Prism BigDye Terminator Cycle Sequencing Kit (version 3.0). PCR products were sequenced with respective gene-specific primers and 20–50 ng DNA-template directly after purification using the Qiagen MinElute 96 UF PCR purification kit (Qiagen, Valencia, CA, USA) or ExoSAP-IT (USB Corporation, Cleveland, OH, USA). The sequencing reactions were performed in a final volume of 10-μL containing 3.2 pmol of primer, 0.25 μL Bigdye terminator premixture, and 1.875 μL of 5 × sequencing buffer. All sequencing reactions were analyzed on an ABI 3730 DNA capillary sequencer (Applied Biosystems, Foster, CA, USA). The reaction conditions contained initial denaturation at 96°C for 5 min, 35 cycles with 96°C for 10 s, 53°C for 5 s, 60°C for 4 min, and a final cycle with an extension at 65°C for 5 min. Sequence comparison for SNP discovery was done by Phrap and Gap4 integration (http://staden.sourceforge.net/phrap.html).

SNP selection for association study. To investigate the association between the polymorphisms of the porcine *TGFBR1* gene and related phenotypic traits, 27 SNPs representingthe polymorphic patterns in the 8 divergent pig breeds were genotyped in the F1 and F0 individuals of the University of Illinois Meishan × Yorkshire swine resource population. According to the distribution patterns of the 27 polymorphic SNPs of *TGFBR1* in the F1 individuals, three representative SNPs (SNP3, SNP43, SNP64) were selected for the association analysis using the F2 population with 298 individuals. SNP3 and SNP43 sharing the same distribution pattern among the F1 individuals of the Illinois resource population, and also in the parents of the commercial population, were chosen to test the distribution pattern of the genotypes in F2 individuals, and the progeny of the commercial population. The SNP64 is monomorphic in the parents of 8 boars and 86 dams of the commercial population and was, therefore, removed from further genotyping of the 1008 progeny and association analysis in the commercial population.

SNP genotyping by PCR-RFLP. For SNP genotyping, PCR-RFLP tests were developed for the two SNPs located in exon 1 (SNP3) and intron 6 (SNP64). RFLP reactions were performed in a total reaction volume of 20 μL containing 10 μL of PCR-sample, 1 U of the respective restriction enzyme, appropriate buffer, and BSA. Restriction results were subsequently electrophoretically separated on 3% agarose gels.

The SNP3 ($T^{84}C$) was screened after PCR of a 230-bp amplicon generated by primers SNP3_F1 and SNP3_R1 (Table 7) on a PTC-100 Peltier Thermal Cycler (MJ research, Waltham, MA, USA): initial denaturation at 95°C followed by 35 cycles of 95°C 45 sec, 58°C 45 sec, 72°C 1 min, and 72°C 10 min. The mutant nucleotide C creates a polymorphic recognition site for *BSP*1286 (allele T = 207 bp + 23 bp; allele = 118 bp + 89 bp + 23 bp) (Fig. 6a).

To genotype alleles in SNP64 ($G^{56211}A$), a PCR-*Hinf*I-RFLP assay was established. PCR was performed to amplify an 872-bp fragment using primer SNP64_F1

Table 6 Primers used for re-sequencing the porcine *TGFBR1* gene in the F0 and F1 animals of the University of Illinois Meishan × Yorkshire swine family

Primer name	Forward primer sequences (5′-3′) Reverse primer sequences (5′-3′)	Primer position*		Size (bp)	Tm
		beginning	end		
TBRS-5UF2/5UR2	TCTGGATGAATGTGGGGAAT	−515	−496	757	58
	GCGCCATGTTTGAGAAAGAG	222	241		
TBRS-I1F1/I1R1	CTCCTGGACTTGGAGAGCTG	498	517	973	58
	CCCTCTCCACACCTCAGTTT	1451	1470		
TBRS-I1F4/I1R4	GGATCTTTAGCCTGCTGTGC	5112	5131	736	60
	GAGCAAAATCCTGCCAACTC	5828	5847		
TBRS-I1F5/I1R5	TTTACCGTTCTTCCCACAGG	9834	9853	1377	60
	TTGATGTGCCAGGAGTATGC	11191	11210		
TBRS-I1F6/I1R6	GTGCTTCCTGCACACTTTGTC	16266	16286	807	58
	GGATATTTAACCCTATGGAGTATGG	18048	18072		
TBRS-E2F/E2R	AGGTCCATCCACGAATTTTG	29438	29457	671	58
	TGGGCTTTATCAGGATTTGC	30089	30108		
TBRS-I2F3/I2R3	GGTGCAGCCATAAAAAGGAA	31484	31503	992	58
	TGATATTCCATTGCCTTCTGG	32455	32475		
TBRS-I2F4/ I2R4	AAATACCAGAAGGCAATGGAA	32450	32470	804	58
	CGTCTTGTTTCAAGGCCAGT	33234	33253		
TBRS-I3F1/I3R1	TTCTCAGGCTAGGGGTTGAA	33990	34009	984	58
	CAAAGCACATGCAAGGAAAA	34954	34973		
TBRS-I3F5/ I3R5	TCACATTCTGACACGGCTTC	47056	47075	860	58
	ATTTTCCTCGCCAAACCTCT	47896	47915		
TBRS-I4F1/I4R1	TCCTGACCATTAGGGCATTC	48422	48441	811	58
	TGCATCTGGAACCTACACCA	49213	49232		
TBRS-I4F2/I4R2	ACCCAGGAACACCAACTGAG	48982	49001	954	58
	GGAGTAGGATGGAGGGGAAG	49916	49935		
TBRS-I4F4/I4R4	TGTGACTTATGGCTGGTGATG	50786	50806	858	58
	CACTAGCTTCATCTGCATGCTT	51622	51643		
TBRS-I4F6/I4R6	CCTAGGCTAGAAGCCCAACC	52234	52253	811	58
	CCAACCAAAGCTGAGTCCAT	53025	53044		
TBRS- E5F/E5R	CCCTTTCTCATTTCCCTTCC	52652	52671	594	58
	TTCCAAGTGGACATCAGATCC	53225	53245		
TBRS-E6F/E6R	TTGGATTACCCTTTATGCAACC	55339	55361	834	58
	TGTGATGGATGCTGGGAATA	56153	56172		
TBRS-I6F/I6R	GCTTGAGAGCAGTCTTGTATT	56136	56156	872	60
	TCATTCCATTACTGCCACACA	56987	57007		
TBRS-I6F1/I6R1	TCGTTCCCTGGAGTATGTCC	56640	56659	874	58
	CTTCAGGGGCCATGTACCTA	57494	57513		
TBRS-E7F/E7R	ATCCCTGGCTTTGTTCAGTG	57168	57187	722	58
	CAAGACAACAAGGGGTTGGT	57870	57889		
TBRS-E8F/E8R	AGGAGGTGAATGGTTGATGC	58451	58470	691	58
	ACTGAAGTGGTTGCCCAAAG	59122	59141		
TBRS-I8F1/I8R1	GCCGTGGAACATTTTAGTGG	58934	58953	847	58
	CTGGGTTCTTGTAGCCAAGG	59761	59780		
TBRS-I8F2/I8R2	GTTGCCACAGCTGCAGTTTA	59646	59665	933	58
	GAGATTCGGCAGTGAACACA	60559	60578		
TBRS-I8F3/I8R3	CACTTTCCTTCCCTGGCATA	60416	60435	918	58
	GAACTTCCTCCCCCAAACAT	61314	61333		
TBRS-3UF/3UR	TCTTTGGACCCAGGAAACAG	62035	62054	838	58
	GCAAACATGACCATGACGAC	62853	62872		

*+1 corresponds to the transcription initiation point of the longest porcine *TGFBR1* cDNA.

Table 7 Primers used for SNP genotyping of porcine *TGFBR1* gene

Primers	Primer Sequences (5′-3′)	Size (bp)	Tm (°C)
TBR1-SNP3F1	GAGGCGAAGCTTGTTGAGG		
TBR1-SNP3R1	GAGAAGGAGCGAGCCAGAG	230	58
TBR1-SNP64F1	GCTTGGGAGCAGACTTGTATT		
TBR1-SNP64R1	TCATTCCATTACTGCCACACA	872	58
TBR1-SNP43I1	CCTCAGGAAAATCTCCCATTCTTACA	200	TD*
TBR1-SNP43I2	ACAAGAAATAAATAGGAACATAGTCATAC	137	
TBR1-SNP43O1	TTAGTTAATTCCACCTCAGACAATCC		
TBR1-SNP43O2	ACCTTTTCTTTTCCTTAATACAGGTACA	282	

TD*, touchdown PCR.

and SNP64_R1 (Table 7). AlleleAis represented by a 341-bp fragment, and alleleGby by two fragments of 265 bp and 76 bp (Fig. 1b). Cleavage of PCR products using *Hinf*I was completed at 37°C. Restriction products were then separated on 3% agarose gel and visualized after ethidium bromide staining on an UV transilluminator.

SNP genotyping by tetra-primer ARMS-PCR. In order to genotype the animals for the SNP43 ($G^{51499}A$) located in intron 4, a simple and convenient SNP genotyping procedure tetra-primer ARMS (Amplification Refractory Mutation System)-PCR (68) was used. Primers (sequences and combinations given in Table 7) were designed using a web-based program (http://cedar.genetics.soton.ac.uk/public_html/primer1.html). The PCR reactions contained 10–20 ng of template DNA, 10 pmol of primers TBR1-S43I1 and TBR1-S43I2, 5 pmol of primers TBR1-S43O1 and TBR1-S43O2, 200 μM dNTP, 1 × PCR buffer and 1 unit QIAGEN *Taq*-Polymerase (QIAGEN, Valencia, CA, USA) in a total reaction volume of 25 μL. The PCR reaction was performed on a PTC-100 Peltier Thermal Cycler (MJ research, Waltham, MA, USA) using a touchdown-PCR profile: initial denaturation for 5 min at 95°C, followed by 35 cycles of 45 sec denaturation (95°C), 45 sec annealing, and 1 min extension (72°C), and a final extension at 72°C for 8 min at the end of the 35 cycles. The annealing temperature was 72°C for the first cycle, decreasing by 1°C per cycle until 58°C was reached, then continuing at 58°C in the annealing step of the remaining cycles. An aliquot of 10 μL of PCR products was mixed with 2 μL of loading buffer and analyzed by agarose gel (2.5%) electrophoresis as described previously (Fig. 1c).

Statistical Analysis

In this present study, two distinctive populations were examined for QTL association where the distribution of SNP alleles and phase association between the SNP alleles and QTL may differ between populations. Given that specific additive and dominance effects may vary in the two populations, an overall genetic effect that includes additive and dominance effects was tested for QTL association. An ANOVA model was used for the association analysis which allows thetesting of both additive and dominance effects and is applicable to situations when not all SNP genotypes are present or different but closely located SNP makers are utilized.

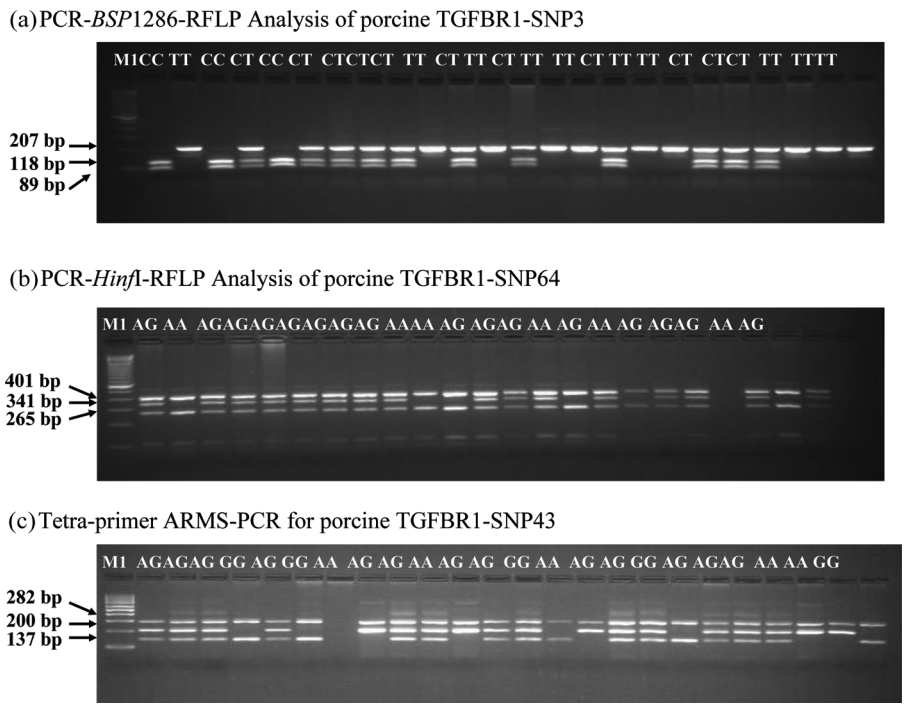

Figure 1 (a) Pattern of SNP3 after restriction with *Bsp* 1286. PCR amplification with primers SNP3_F1 and SNP3_R1 (Table 7) creates an amplicon of 230-bp. Allele C is represented by fragments of 207-bp and 23-bp which is not visible in the agarose gel picture, whereas allele T is represented by fragments of 118-bp, 89-bp, and 23-bp. M1 = Hyperladder IV (Bio-line, Taunton, MA, USA). (b) Pattern of SNP64 after restriction with *Hinf*I. PCR amplification with primers SNP64_F1 and SNP64_R1 (Table 7) creates an amplicon of 872-bp. Allele A is represented by fragments of 341-bp, whereas allele G is represented by fragments of 265-bp and 76-bp. M1 = Hyperladder IV (Bio-line, Taunton, MA, USA). (c) Detection of SNP43 by tetra-primer ARMS-PCR. Pattern of SNP43 after tetra-primer ARMS-PCR amplification with primers TBR1_S43I1, TBR1_S43I2, TBR1_S43O1, and TBR1_S43O2 (Table 7). Allele G is represented by fragment of 137-bp, whereas allele A is represented by fragment of 200-bp. The fragment of 282-bp is an internal control. M1 = Hyperladder IV (Bio-line, Taunton, MA, USA).

The overall statistical significance *P*-value indicates whether either or both effects are significant. If the overall test wasnot significant, then neither additive nor dominance effects were considered.

The general model used to detect associations between either growth, carcass, and reproduction traits and *TGFBR1* polymorphisms included the fixed effect of SNP and the random effects of sire and sow nested within sire. For the Illinois resource population, the model for carcass traits included the covariate of weight at slaughter. For the commercial population, all models included sex of the pig and the carcass traits included the covariate of hot weight at slaughter. An *F*-test was used to test for the association between SNP and phenotypes and a *t*-test was used to evaluate all pair-wise contrasts between SNP genotypes. Pair-wise contrasts were only considered at locations with *F*-values surpassing the significance threshold. All models were implemented using PROC MIXED (SAS, 2006) (69). Genetic correlations

between phenotypic traits of the Illinois resource population were unknown. Therefore, analyses for each trait were conducted independently.

LIST OF ABBREVIATIONS

ADG: average daily gain; ARMS-PCR: amplification refractory mutation system-PCR; BMP15: bone morphogenetic protein 15; BMPR1B: bone morphogenetic protein receptor-IB; BSA: bovine serum albumin; PCR: polymerase chain reaction; PCR-RFLP: PCR restriction fragment length polymorphism; QTL: quantitative trait locus; SNP: single nuclear polymorphism; SSC: *sus scrofa* chromosome; TGF-β: transforming growth factor beta; TGFBR1: transforming growth factor beta type 1 receptor; TGFBR2: transforming growth factor beta type 2 receptor.

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