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QTL and Association Analysis of Mx1 Gene with Economic Traits in Commercial Pigs

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Abstract: Mx proteins, as interferon-induced GTPases had been shown to have antiviral activity in different species. Recently, it has been recognized that an 11 bp-deletion in coding region of porcine Mx1 gene can lead to a special Mx1 protein isoform, which is lacking antiviral activity to influenza *in vitro*. This Mx1 11 bp-deletion polymorphism is a potential gene marker in pig breeding for disease resistance in future but there is no report on its relationship with economic traits in pig. It's interesting to know, whether it associates with the economic QTLs or traits in commercial pigs, as well as its distribution in different breeds. In this pilot study, the pig Mx1 gene was found within three QTL regions which were related with fiber percentage, average daily gain and average backfat thickness. Seven economic traits of pure Landrace and Yorkshire were used to analyze the association with the Mx1 11 bp-deletion polymorphism. These traits include body length, body height, lean meat percentage, correction backfat thickness, average daily gain from birth to 100 kg, average daily gain from 30-100 kg and age to 100 kg. The results showed no statistical significant associations between the Mx1 11 bp-deletion allele and the seven porcine economic traits in the tested pure Landrace and Yorkshire population. We also detected the allele frequencies of the Mx1 11 bp-deletion in seven different porcine breeds and estimate its population homogeneity between breeds. However, more research is still necessary before this gene marker is utilized in porcine breeding.

Key words: Pig, Mx1, QTL, 11bp-deletion, disease resistance, economic trait, association

INTRODUCTION

Mx proteins, as interferon-induced GTPases, were well known to have antiviral activity in different species, especially to influenza virus. They exists in mammalian (Haller *et al*., 2007), bird (Sarmento *et al*., 2008) and fish (Das *et al*., 2008) cells in response to virus infection and other stimulations, e.g., poly I:C, following a type I Interferons (IFNs) dependent pathway. The Mx1 orthologous gene in pig was firstly cloned by Muller *et al*. (1992) as an anti-virus gene implicated with selective resistance to influenza virus and was proved to provide antiviral resistance to Vesicular Stomatitis Virus (VSV) (Asano *et al*., 2002) and influenza virus (Palm *et al*., 2007) *in vitro*. The Mx1 gene is polymorphic in pig (Morozumi *et al*., 2001) as well as in mouse (Garber *et al*., 1993) and chicken (Ko *et al*., 2002; Livant *et al*., 2007). Studies in mouse (Garber *et al*., 1993) and chicken (Ko *et al*., 2002) showed that allelic isoforms of Mx1 protein have differential antiviral activity. An 11 bp deletion of GGCGCCGGCTC in coding region of porcine Mx1 gene was found to result in frame shift to yield 8 amino acid substitutions and a 23 amino acid extension of the carboxyl terminal region of Mx1 protein (Morozumi *et al*., 2001). Recently, two independent studies had shown that, compared with wild type, the Mx1 protein isoform of 11 bp-deletion allele is lacking anti-influenza activity *in vitro* (Nakajima *et al*., 2007; Palm *et al*., 2007). Therefore, this 11 bp-deletion polymorphism of porcine Mx1 gene is a potential gene marker in porcine breeding program for disease resistance, especially for anti-influenza. Mx1 gene may link with and effect on economic traits in chicken (Livant *et al*., 2007). As a potential disease resistance gene marker in pig genetic selection, it's interesting and promising to know whether the Mx1 11 bp-deletion polymorphism associates with the economic QTLs or traits in commercial pigs, as well as its distribution in different breeds. But in pig, there is no report on this to date. In this study we detected the allele frequencies of the 11 bp deletion in seven different

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porcine breeds, illustrated the QTLs containing Mx1 gene region in pig and analyzed its association with seven economic traits in pure breed pig population consisting of Landrace and Yorkshire.

MATERIALS AND METHODS

Animal samples: Ear samples for DNA extraction were collected from 308 pure breed pigs consisting of Landrace (140) and Yorkshire(168) in breeding farm of Wen's Group in Guangdong Province, China. Seven economic traits were recorded in these two breeds, including body length, body height, lean meat percentage, correction backfat thickness, average daily gain from birth to 100 kg, average daily gain from 30-100 kg age to 100 kg.

DNA preparation: DNA samples from Chinese local pig breeds (ErHuaLian, DaHuaBai, TongCheng, Yushanhei) and Duroc were conserved in our lab. For each pig used for association analysis, genomic DNA form ear tissue was extracted by the conventional phenol/chloroform method and dissolved in TE buffer.

Primer design, PCR conditions, sequencing and PAGE genotyping: The porcine Mx1 gene mRNA sequence (GenBank accession No. DQ095779.1) obtained from the NCBI website was used to design primers. The primer sequences are: PF1-CCAGCGACAAGAGGAAGT and PR1-TAGTCTGTTAGGGAAGGAGGT. The PCR reactions were performed in 10 μL reactions containing 1×PCR buffer, 0.3 μM of each primer, 75 μM of each dNTP, 20 mM $MgCl_2$, 0.5U Taq DNA polymerase (Takara Biotechnology) and 25 ng of porcine genomic DNA. Amplification protocol was 5 min at 94°C followed by 35 cycles of 40 sec at 94°C, 30 sec at 55°C, 25 sec at 72°C and a final extension of 5 min at 72°C. PCR fragments were separated in 8% Polyacrylamide Gel Electrophoresis (PAGE) to detect the length polymorphisms.

Genotyping and allele frequencies: The 11 bp-deletion polymorphism was detected in 308 pure breed pigs consisting of Landrace (140) and Yorkshire (168) in breeding farm of Wen's Group in Guangdong Province, China.

QTL analysis: Quantitative trait locus analysis was performed by searching the QTLs linking the position of porcine Mx1 gene and its linked markers in PigQTLdb (Hu *et al*., 2005; Hu and Reecy, 2007; Rothschild *et al*., 2007).

Statistics analysis: Homogeneity tests between breeds were conducted by Chi-square and Fisher's exact test using the FREQ procedure in SAS 8.01 (SAS Institute Inc. Cary, NC, USA). Analyses of variance were performed to estimate the effects of the genotypes on all the economic traits using the GLM procedure in SAS 8.01. For each trait, the least-square means between genotypes were computed and compared using lsdmeans statement. All values are presented as mean±SE of the mean. The following models were used to estimate the effects of the genotypes on the economic traits.

$$Y_{ijkm} = \mu + G_i + S_j + F_k + B_m(F_k) + \varepsilon_{ijkm}$$

Where:

- Y = The response vector for the economic traits above
- μ = The overall mean of each traits
- G_i = The genotype effect (i = 3: AA AD DD)
- S_j = The sex effect (j = 2: male, female)
- F_k = The farm effect (k = 2: QinYuan, ShuiTai)
- $B_m(F_k)$ = The effect of breed nested within the farm (m = 2: Landrace, Yorkshire)
- ε_{ijkm} = The random residual

A $p<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

PCR-PAGE genotyping and allele frequencies: The PCR fragments containing the Mx1 gene the 11 bp-deletion allele polymorphism were amplified and separated by 2% agarose gel (Fig. 1). The PCR products were confirmed by sequencing. To detect the length difference between 11 bp-deletion and non-deletion alleles in porcine Mx1 gene, the PCR products from different animal samples

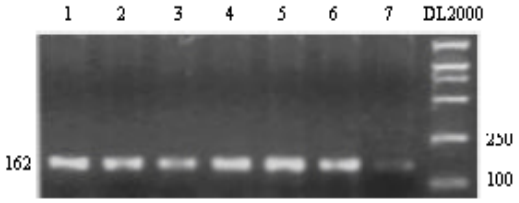

Fig. 1: The PCR fragment of the Mx1 gene containing the 11 bp deletion allele polymorphisms

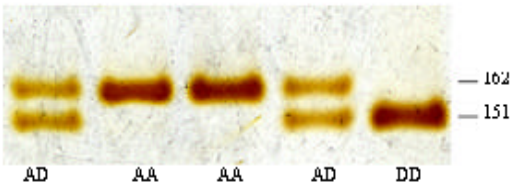

Fig. 2: The 11 bp-deletion allele polymorphisms of the Mx1 gene, 'D' represents the allele with 11 bp deletion in Mx1 gene. 'A' represents the normal allele of Mx1 gene. The D allele is 11bp shorter than A in length due to the deletion mutation

were separated by 8% polyacrylamide gel electrophoresis (PAGE, Fig. 2). The result of the 8% PAGE showed that the two PCR fragments were 162 bp (AA, the alleles without deletion) and 151 bp (DD, the alleles with 11 bp deletion), respectively. Here, 'D' represents the allele with 11 bp-deletion in Mx1 gene; 'A' represents the normal allele of Mx1 gene.

By using this method, the Mx1 gene 11bp-deletion allele distribution in seven different pig breeds were detected and shown in Table 1. According to the Table 1, there is no Mx1 11 bp deletion homozygote in Yourshire, Dahuabai and Tongchen. The 11 bp-deletion allele frequency of the 11 bp-deletion was lowest (0.021) in Yorkshire pig. This was also supported another two studies (Palm *et al*., 2007).

Homogeneity tests between breeds were conducted by Chi-square and Fisher's exact test and shown in Table 2. It shows that for porcine Mx1 11 bp-deleltion polymorphism, the population homogeneity of Yorkshire pig is extremely different with others breed tested. Because 11 bp-deletion allele is unfavorable for antivirus, selection pressure may influence the Mx1 locus especially in Yourshire pig.

QTL analysis of porcine Mx1 region: The pig Mx1 was localized on SSC13 in pig (Rettenberger *et al*., 1996). But its detailed localization was not previously reported. Recently, the pig Mx1 and Mx2 were found closely linked with SW769 and S0291 (Morozumi *et al*., 2009). According to these cues and the comparative genome evidence with human (Fig. 3), we estimate that porcine Mx1 were on ssc13p48-49. Using closely linked microsatellites SW769 and S0291, QTLs matched with pig Mx1 were found in PigQTLdb. These QTLs mostly affect pig growth and meat quality (Fig. 3). The QTLs in corresponding region include percentage type IIa fibers (FIB2AP) (Estelle *et al*., 2008), Average Daily Gain (ADG) and average Backfat Thickness (BFT) (Kim *et al*., 2006). These suggest that pig Mx1 may associate with or effect pig economic traits, especially for the growth and meat quality traits.

Table 1: Frequency table of the Mx1 11bp-deletion alleles and genotype in different pig breeds

		Genotype			Allele frequency	
Pig breed	Number	AA	AD	DD	A	D
ErHuaLian	20	0	17	3	0.425	0.575
DaHuaBai	19	9	10	0	0.737	0.263
TongCheng	20	5	15	0	0.625	0.375
Yushanhei	17	2	12	3	0.471	0.529
Duroc	20	12	6	2	0.750	0.250
Landrace	140	55	73	12	0.653	0.347
Yorkshire	168	161	7	0	0.979	0.021

Association analysis: For association analysis, all the 308 pure breed pigs consisting of Landrace (140) and Yorkshire (168) with economic traits were genotyped by PCR-PAGE as describe above. The numbers of genotypes AA, AD and DD were 216, 80 and 12, respectively. The allele frequency of the 11 bp-deletion was 0.169 in this population. The allele frequency of the 11 bp-deletion in Yorkshire (0.021) was less than Landrace within the population (0.347).

Seven economic traits recorded were used to analyze the association with the Mx1 11 bp-deletion polymorphism in this population. The seven economic traits include Body Length (BL, cm), Body Height (BH, cm), Lean Meat Percentage (LMP, %), Correction Backfat Thickness (CBT, cm), Average Daily Gain from Birth to 100 kg (ADGBT100, g), average daily gain from 30-100 kg (ADG30T100, g) and age to 100 kg (AT100, day).

Briefly, no statistically significant associations were found between the Mx1 11 bp-deletion polymorphism and the seven porcine economic traits in the tested pure Landrace and Yorkshire population (Table 3). In the chicken, significant favorable effect of one Mx1 polymorphisms on body weight at 40 days in one line were detected, this polymorphism was also associated with higher vaccinal antibody titers to infectious bursal disease virus (Livant *et al*., 2007).

We think that similar results can occur in pig too. So, it's interesting and promising to continue this research in other large size population. But it's better to choose Landrace but not Yorkshire, for the Mx1 11 bp-deletion allele frequency was very low in Yorkshire pig.

Table 2: Homogeneity of the porcine Mx1 11bp-deletion alleles between different pig breeds

Pig breed	ErHuaLian	DaHuaBai	TongCheng	Yushanhei	Duroc	Landrace	Yorkshire
ErHuaLian	1	0.001	0.0172	0.2676	0.0002	0.0025	<0.0001
DaHuaBai	2.64E-04	1	0.1455	0.023	0.1822	0.3866	<0.0001
TongCheng	0.0115	0.1908	1	0.1105	0.0127	0.1148	<0.0001
Yushanhei	0.4145	0.017	0.1646	1	0.0103	0.0665	<0.0001
Duroc	2.89E-05	0.2104	0.1646	0.0081	1	0.1676	<0.0001
Landrace	3.31E-04	0.48	0.0144	0.0439	0.1338	1	<0.0001
Yorkshire	1.10E-21	1.14E-07	3.90E-13	2.59E-15	7.85E-06	3.04E-29	1

[a]Upper triangular matrix indicates the p-value of χ^2-test between two breeds. [b]Lower triangular matrix indicates the p-value of Fisher's exact test between two breeds

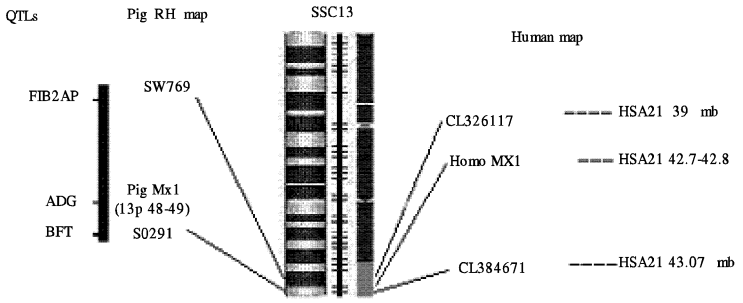

Fig. 3: QTLs covering the porcine Mx1 region, porcine Mx1 was mapped to ssc13, shown in the pig karyotypes map with its putative detailed position. At the further left, the QTLs in corresponding region include percentage type IIa Fibers (FIB2AP), Average Daily Gain (ADG) and average Backfat Thickness (BFT). At the right, human Mx1, mapped to Hsa21, also agrees with the comparative maps of human chromosomes 21 (Hsa21) and pig chromosomes 13 (Ssc13)

Table 3: Association analysis of 11 bp-deletion polymorphism with 7 economic traits

	Genotype (LS Mean±SE)			
Traits[a]	AA (216)	AD (80)	DD (12)	p-value[b]
BL	116.89±0.31	117.97±0.48	118.74±1.11	0.0940
BH	60.33±0.19	59.91±0.29	60.67±0.67	0.3277
LMP	63.55±0.16	63.17±0.25	62.80±0.59	0.3095
CBT	14.29±0.17	14.36±0.26	14.24±0.61	0.9635
ADG-BT100	716.74±3.94	721.31±6.03	717.11±13.82	0.8074
ADG-30T100	893.01±6.41	899.26±9.78	890.17±22.41	0.8311
AT100	149.86±0.68	149.01±1.04	149.12±2.39	0.7914

[a]BL: Body Length (cm); BH: Body Height (cm); LMP: Lean Meat (%); CBT: Correction Backfat Thickness (cm); ADGBT100: Average Daily Gain from Birth to 100 kg (g); ADG30T100: Average Daily Gain from 30-100 kg (g); AT100: Age to 100 kg (day). [b]p-value for test the effect of genotype. *$p<0.05$

CONCLUSION

In this study, we found the pig Mx1 gene was localized in three QTL regions which related with fiber percentage, average daily gain and average backfat thickness. But no statistical significant associations were found between the Mx1 11 bp-deletion allele and the seven porcine economic traits in the tested pure Landrace and Yorkshire population. The tested traits include Body Length (BL, cm), Body Height (BH, cm), Lean Meat Percentage (LMP, %), Correction Backfat Thickness (CBT, cm), Average Daily Gain from Birth to 100 kg (ADGBT100, g), Average Daily Gain from 30-100 kg (ADG30T100, g) and age to 100 kg (AT100, day). Yorkshire pig has the smallest unfavorable allele (D) in tested breeds. The allele frequencies of different breeds described here is also useful for further study or breeding selection. However, more research is still needed before the Mx1 11 bp-deletion gene marker is utilized in porcine breeding program. Continuing this research in other population with large size or different breed will be interesting and promising for illustrating the relationship between the Mx1 marker and the porcine economic traits in future. But it's better to choose Landrace but not Yorkshire, for the Mx1 11 bp-deletion allele frequency was very low in Yorkshire pig.

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