Identification of porcine *hernia inguinalis/scrotalis* using single nucleotide polymorphism in *INSL3* and *BAX* genes

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Abstract

Scrotal hernia is a congenital defect of great concern to pig producers that leads to economic loss and poor animal welfare. Several candidate genes have been proposed to be causative for the disorder. This study focused on the analysis of single nucleotide polymorphisms in the genes encoding the Leydig insulin-like hormone *(INSL3)* and the BCL2-associated X protein (*BAX*). *INSL3* has recently been mapped to *SSC2q12-q13* and *BAX* to *SSC6q21*. In total, 250 bp in *INSL3* (promotor region) and 416 bp in BAX (Intron1) were comparatively sequenced using affected and un-affected commercial pigs as well as autochthonous Thai pigs. PCR-RFLP was used to screen SNPG-224A (*INSL3*) and C119T (*BAX*). A total of 212 commercial pigs (179 unaffected (u) and 33 herniated (h) pigs) were used for *INSL3* genotyping. Allele frequency estimations revealed no significant differences between the two phenotypes at this loci (G_u = 0.97; A_u = 0.03; G_h = 0.91; A_h= 0.09) indicating that this mutation cannot be used to identify the disease. Interestingly, the allele frequency for G in Thai native pigs (n=7) was 0.07. It appears that the breed differences exist in the *INSL3* gene. Screening of *BAX* was done in 151 commercial pigs (125 unaffected and 26 herniated pigs) showing significant differences in allele frequencies between unaffected and herniated pigs (C:T = 0.62:0.38 and 0.83:0.17) ($p < 0.01$). Allele C in Thai native pigs (n=7) was 1.00. Currently, further mutations in the regulatory and coding regions of *BAX* are identified to assess their possible role in this congenital disorder.

Keywords: *INSL3*, *BAX*, Porcine hernia inguinalis

Introduction

The Foerderverein Biotechnologieforschung der Deutschen Schweineproduktion (FBF) reported a frequency of herniated pigs with about 2% in Germany. In Thailand, hernia inguinalis/scrotalis is an economical problem for Thai pig breeders. The frequencies of herniated animals ranged from 1% (industrial pig farms) to 5% in small-sized farms (Gatphayak *et al*., 2005). A genome scan with DNA-markers and affected siblings revealed an association on chromosome 3,6,7,12

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and 15 with the hernia phenotype (Borneman-Kolatzki, 2004). Moreover, Grindflek *et al.* (2006) reported the QTLs regions of inguinal hernia in Chromosome 1,2,5,6,15,17 and SSCX. Several candidate genes in this QTLs regions have been proposed to be causative for the disorder such as *COL9A1, ESR1, INSL3, CGRP, MIS, BAX, HOXB9* and *HOXB5.* This study focused on the analysis of single nucleotide polymorphisms in the genes encoding the Leydig insulin-like hormone (*INSL3*; G-224A) (Knorr *et al*., 2004) and the BCL2-associated X protein (*BAX*; C119T) (Laenoi *et al*., 2006) in commercial pigs in Thailand.

Materials and methods

In total, 250 bp in *INSL3* (promotor region) and 416 bp in BAX (Intron1) were comparatively sequenced using affected and un-affected commercial pigs as well as autochthonous Thai pigs. PCR-RFLP was used to screen SNPG-224A (*INSL3*) and C119T (*BAX*). A total of 212 commercial pigs (179 unaffected (u) and 33 herniated (h) pigs) were used for *INSL3* genotyping. Screening of *BAX* was done in 151 commercial pigs (125 unaffected and 26 herniated pigs). Thai native pig (n=7) were used for genotyping in both genes. Primer sequences used for amplification DNA fragments for SNPs screen shown in table1.

Table 1. Primer used in this study.

Primer Name	Primer Sequence (5'→ 3')	PCR product	Tm (°C)
BAX SNP-2 for	TCA GTT CAT CTA GCA GGG AC	416 bp	61.1
BAX SNP-2 rev	CCATGT TAC TGT CCA GTT CAT C		
INSL3 -for	GTC TAC TCT TGT ATA GAT GA	250 bp	49.7
INSL3 -rev	AGA GCA TTC CCA AAG GAC		

The RFLP reactions were performed according to the manufacturer (NEB, USA) with the respective restriction enzymes (*HpyCH4 IV* and *Ear*I) for overnight at 37 °C in a volume of 30 µl. The digested products were subsequently separated on 1% agarose gel containing ethidium bromide.

Result and discussion

SNPG-224A (*INSL3*), 250 bp PCR fragment was amplified by INSL3 primer combination with *HpyCH4 IV* digestion. Allele G has recognition site for *HpyCH4 IV* and showed 2 bands after digestion (fragments 153 and 97 bp), where as allele A has no recognition site for *HpyCH4 IV* and showed an undigested PCR product (Figure 1). SNPC-119T *(BAX)*, a 416 bp PCR fragment was amplified by *BAX*-SNP2 primer combination with *EarI* digestion. Two alleles could be distinguished. Allele T has no recognition site for *EarI* and shows an undigested PCR product, where as allele C has recognition site for *EarI* and shows the fragments 120 and 296 bp (Figure 2).

Allele frequency estimations of *INSL3* genotyping revealed no significant differences between the two phenotypes at this loci (G_u = 0.97; A_u = 0.03; G_h = 0.91; A_h= 0.09) indicating that this mutation cannot be used to identify the disease. Interestingly, the allele frequency for G in Thai native pigs (n=7) was 0.07. It appears that the breed differences exist in the *INSL3* gene. Screening of the SNP in *BAX* gene showing significant differences in allele frequencies between unaffected and herniated pigs (C:T = 0.62:0.38 and 0.83:0.17) ($p < 0.01$). Allele C in Thai native pigs (n=7) was 1.00.

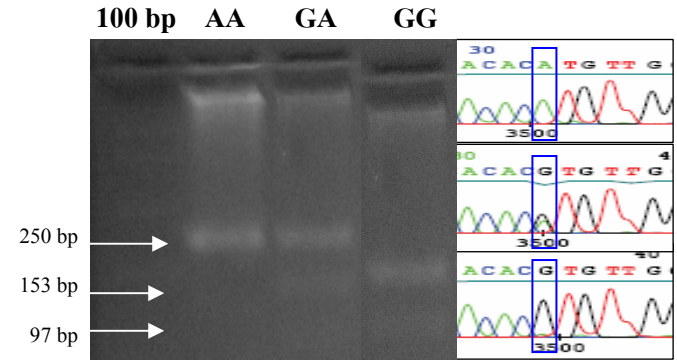

Figure 1. Restriction pattern and sequencing chromatograms of the SNP G-224A of the *INSL3* gene.

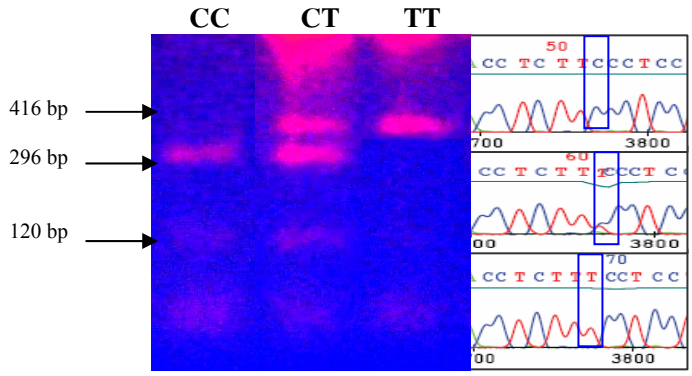

Figure 2. Restriction pattern and sequencing chromatograms of the SNP C-119T of the *BAX* gene.

Although the SNP in BAX gene is locate in the intronic region, it is possible that it might affect the splice process and that are alterations of alternative splicing lead to disease. In human, *BAX* gene mutation in the promoter (G 125A) was associated with lower *BAX* mRNA and protein level in chronic lymphocytic leukemia patients (Moshynska *et al*., 2005). The study of the biological role of the BAX protein is necessary to conduct a potential function of the characterized SNP. Further mutations in the regulatory and coding regions of *BAX* are identified to assess their possible role in this congenital disorder.

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