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A note on biallelic expression of the *IGF2* gene in the liver and brain of adult pigs*

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ABSTRACT

The *IGF2* gene is imprinted in most mammalian tissues. Considering the potential large impact of the *IGF2* gene on breeding industry, further studies on *IGF2* expression in various tissues and at different stages of animal's development are required. Samples of blood, muscle, kidney, liver and brain were taken from 5.5-month-old pigs. Animals heterozygous for *SWC9* marker were selected. RNA was isolated from the muscle, kidney, liver and brain and RT-PCR was performed. The results of the study showed that there is a relaxation of imprinting of the *IGF2* gene in the liver and brain of adult pigs.

KEY WORDS: pigs, *IGF2* gene, imprinting, *SWC9* marker, RT-PCR, expression

INTRODUCTION

The *IGF2* gene is imprinted in most mammalian tissues. However, biallelic expression was detected in various tissues in humans (Kalscheuer et al., 1993), mice (De Chiara et al., 1991), rats (Pedone et al., 1994), cattle (Dindot et al., 2003) and sheep (Mc Laren and Montgomery, 1999) A partial relaxation of *IGF2* imprinting was also observed in muscles of 4-month-old pigs (Van Leare et al., 2003) The imprinting status of *IGF2* in pigs was first established by Nezer et al. (1999). They examined expression patterns in the muscle and liver of 10-week-old porcine foetuses and showed that *IGF2* is imprinted in these tissues. In recent times, a causative mutation in intron 3 of the *IGF2* gene, affecting muscle growth and heart weight, has been identified in pigs (Van Leare et al., 2003). It was also

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shown that this mutation affects the expression of *IGF2* antisense transcript, which has been identified recently (Braunschweig et al., 2004).

Considering the potential large impact of the *IGF2* gene on breeding industry, further studies on *IGF2* expression in various tissues and at different stages of animal's development are required. In this study we show that there is a relaxation of imprinting of the *IGF2* gene in the liver and brain of adult (5.5-month-old) pigs.

MATERIAL AND METHODS

Samples of blood, muscle, kidney, liver and brain were collected from fifteen 5.5-month-old pigs postmortem. All animals were healthy and their organs did not have any pathological changes. Samples of blood were used for genotyping of SWC9 and eight individuals heterozygous for this locus were selected. *SWC9* microsatellite was previously used in imprinting studies of the *IGF2* gene in pigs (Nezer et al., 1999; Van Leare et al., 2003). RNA was isolated from the muscle, kidney, liver and brain, using SV Total Isolation System (Promega). RT-PCR was performed using One Step RT-PCR System (Promega) with random hexamer primers and fluorescent-labelled specific primers for SWC9 microsatellite. RT-PCR conditions were as follows: 48°C 45 min, 94°C 2 min, 94°C 13 min (94°C 30 s, 58°C 30 s, 72°C 1 min) × 35, 72°C 1 h. Simultaneously negative control without reverse transcriptase was performed. The products were subjected to electrophoresis in a 4% polyacrylamide gel in an ABI PRISM 377 sequencer. Results were analysed using Genotyper v 2.0 software.

RESULTS

In all examined samples we observed monoallelic expression in the muscle and kidney, whereas in the liver and brain biallelic expression was detected (Figure 1).

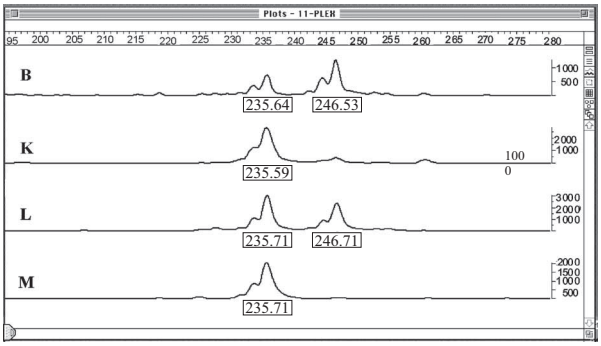

Figure 1. Expression of porcine *IGF2* gene in adult animal's tissues. Monoallelic expression in muscle (M) and kidney (K), and biallelic expression in liver (L) and brain (B)

The RT-PCR controls without reverse transcriptase were all negative. Our results suggest that there is a loss of imprinting in the adult liver and brain of pigs.

DISCUSSION

Similar results - biallelic expression in the adult liver - were obtained in sheep (Mc Laren and Montgomery, 1999) and humans (Kalscheuer et al., 1993). Loss of imprinting in the liver of both these species is supposed to be associated with the presence of transcript from P-1 promotor (Ohlsson et al., 1994; McLaren and Montgomery, 1999). *IGF2* gene in pigs is highly homologous to human *IGF2* (Amarger et al., 2002). It was also shown that transcript from P-1 promotor of porcine *IGF2* is used mainly in the liver (Amarger et al., 2002; Braunschweig et al., 2004). Therefore, the association between usage of P-1 promotor and relaxation of imprinting is likely to be common in all these mammalian species.

A partial relaxation of imprinting in the *IGF2* gene was also observed in skeletal muscle of four-month-old pigs. Before birth, *IGF2* was expressed exclusively from the paternal allel, whereas at four months of age, weak expression from the maternal allel was observed (Van Leare et al., 2003). In our studies we did not noticed expression from the maternal allel in muscles. However, we examined older – 5,5-month-old pigs. It is probable, that during development repression and partial derepression of the maternal allel occur in porcine skeletal muscles.

Loss of imprinting in brain tissues has been previously reported in the mouse (De Chiara et. al., 1991), rat (Pedone et al., 1994), sheep (McLaren and Montgomery, 1999) and humans (Ohlsson et al., 1994). Expression of the *IGF2* gene often varies in different parts of the brain. In mice, biallelic expression was observed in leptomeninges and choroids plexus (De Chiara et al., 1991), whereas in humans the loss of imprinting occurred in pons, but not in globus palladus (Pham et al., 1998). In our studies we collected samples from peripheral layers of the brain, without distinction of anatomical parts. We observed biallelic expression in all examined samples.

Recently, *IGF2* antisense transcript (IGF2 AS) has been found in foetal and adult porcine tissues (Braunschweig et al., 2004). It was shown that IGF2 AS are expressed exclusively from paternal allele in foetal and adult muscles and liver. It is hypothesised that there is an antagonistic relationship between sense and antisense loci (Ogawa and Lee, 2002). Many sense and antisense transcript pairs show reciprocal imprinting, although this is not a general rule since antisense *Igf2* transcripts as well as sense *Igf2* transcripts are paternally expressed in mice (Moore et al., 1997). It would be very useful to examine simultaneously the imprinting status of sense and antisense transcripts in one adult pig, as it could give new

insights into understanding the role of antisense transcripts in the regulation of gene expression and imprinting.

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