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Porcine IGF1 synonymous mutation alter gene expression and protein binding affinity with IGF1R

Yunyun Cheng [a,1], Songcai Liu [a,b,1], Gang Wang [a], Wenzhen Wei [a], Shan Huang [a], Rui Yang [a], Hongwei Geng [a], Haoyang Li [a], Jie Song [a], Lidan Sun [c], Hao Yu [a,*], Linlin Hao [a,*]

[a] College of Animal Science, Jilin University, 5333 Xi'an Road, Changchun, Jilin 130062, China
[b] Five-Star Animal Health Pharmaceutical Factory of Jilin Province, 5333 Xi'an Road, Changchun, Jilin 130062, China
[c] Beijing Tong He Sheng Tai Institute of Comparative Medicine, Beijing, China

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ABSTRACT

Context: Insulin like growth factor 1 (IGF1) is privotal in the regulation of animal growth and is a single-chain globular protein composed of B, C, A, and D regions, of which the C region is involved in maintaining high affinity binding to the IGF1 receptor (IGF1R).
Purpose: In this study, significant expression differences between large pigs and miniature pigs were detected and only one synonymous SNP (c.258G>A) in the C region of the coding sequence of *IGF1* gene was screened. The aim of this manuscript was to clear the function of the SNP and clarify the mechanism of its influnce.
Methods: The expression vectors contained A allele and G allele were constructed, and the expression assays of the two groups were determined by qRT-PCR and western blotting, then the stability assays of the mRNA and protein were carried out under the inhibitation of actinomycin D and cycloheximide, respectively. At last, the binding affinity of IGF1-G and IGF1-A with IGF1R were indicated by co-immunoprecipitation and double immunofluorescence labeling methods, the conformation difference was detected by differential immunodetection.
Results: The IGF1-G expressed higher than IGF1-A in both transcription and translation levels, and the mRNA and protein stabilities of IGF1-G were lower than IGF1-A ($P < 0.05$). Furthermore, the relative binding affinity of GG-genotype IGF1 with IGF1R was significantly higher than that of the AA-genotype IGF1 ($P < 0.05$), and there was a difference in the conformation of the IGF1 with two genotypes.
Conclusion: Our findings indicated the synonymous mutation altered the IGF1 gene expression and confirmed the synonymous mutation affected the IGF1 folding and the interactions with the IGF1R preliminarily.

1. Introduction

Insulin-like growth factor 1 (IGF1), as one of the most important factors in the IGF family, has many growth-promoting and metabolic activities [1]. Researchers found numerous organ weights were larger in mice overexpressing IGF1 and indicated IGF1 acts from birth and through puberty and leads to proportional size increases in a variety of tissues [2]. IGF1 regulates the proliferation and differentiation of various cell types and is capable of exerting insulin-like metabolic effects by binding to IGF receptors (IGF1R and IGF2R) [3,4], of which IGF1 binds with the highest affinity to the IGF1R [5,6]. IGF1 is produced by most tissues or organs in the body, especially the liver [7].

IGF1 is a 70 amino acid residues single-chain globular protein composed of B, C, A, and D regions from the N-terminus to the C-terminus [8] and researchers indicated that the C region of IGF1 is involved in maintaining high affinity binding to the IGF1R [9]. In addition, the *IGF1* gene is fairly conserved in animal evolution, and it is reported that the amino acid sequences of human, pig, and bovine are identical [10,11]. Based on the effect of IGF1 on the animal growth and the fact that miniature pigs have been widely used as experimental animals in recent years, the Large White pigs (LW, Large pigs) and Bama Xiang pigs (BM, Miniature pigs) were selected for this study because the two breeds have the opposite growth rate and body size, of which the BM pigs, as an excellent Chinese miniature pig breed, are produced in Guangxi Province of China and they are highly inbred, genetically stable and with mini-body size (adult mean body weight is 40 kg) [12], while the LW pigs also known as Yorkshire, have the traits of high growth rate and large bodysize, which adult weight can reach 250 kg and are treated as the most typical representative of large pigs [13,14]. Moreover, only one missense mutation and three synonymous mutations in the coding region of *IGF1* gene were retrieved within the pigs according to the Ensembl genome browser.

* Corresponding authors.
E-mail addresses: yu_hao@jlu.edu.cn (H. Yu), haolinlin@jlu.edu.cn (L. Hao).
[1] Yunyun Cheng and Songcai Liu contributed equally to this study.

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Synonymous mutations were once thought to be functionally neutral, but evidence now indicates it is shaped by evolutionary selection and affects other aspects of protein biogenesis beyond specifying the amino acid sequence of the protein [15]. In addition, a large number of studies have shown that synonymous mutations can affect protein biogenesis and protein folding [16] because the codon usage evolved to govern the rhythm of translation and to facilitate co-translational folding [17,18]. In the present study, significant difference was detected in gene expression level between LW pigs and BM pigs (Fig. 1) and there was only one synonymous mutation in the coding region of the *IGF1* gene within the two pig breeds, which was located in the C region of the *IGF1* gene (Fig. 2A). Therefore, the aim of this study was to confirm whether the synonymous mutation affects the gene expression and protein binding affinity with its receptor, and further clarify the molecular mechanism, expecting to provide new data for the IGF1 role in the formation mechanism of miniature pigs.

2. Materials and methods

2.1. Samples

The liver, muscle and cartilage tissues were collected from LW pigs ($n = 80$) and BM pigs ($n = 36$) that were one week after birth. All tissues were obtained within 30 min after slaughter and immediately frozen in liquid nitrogen until used. Eight LW pigs and eight BM pigs were selected randomly for the tissue expression assays. The total protein of the tissues were extracted under the introductions of the Tissue or Cell Total Protein Extraction Kit (KeyGEN, China), the total RNA was extracted according to the Eastep® Super Total RNA Extraction Kit

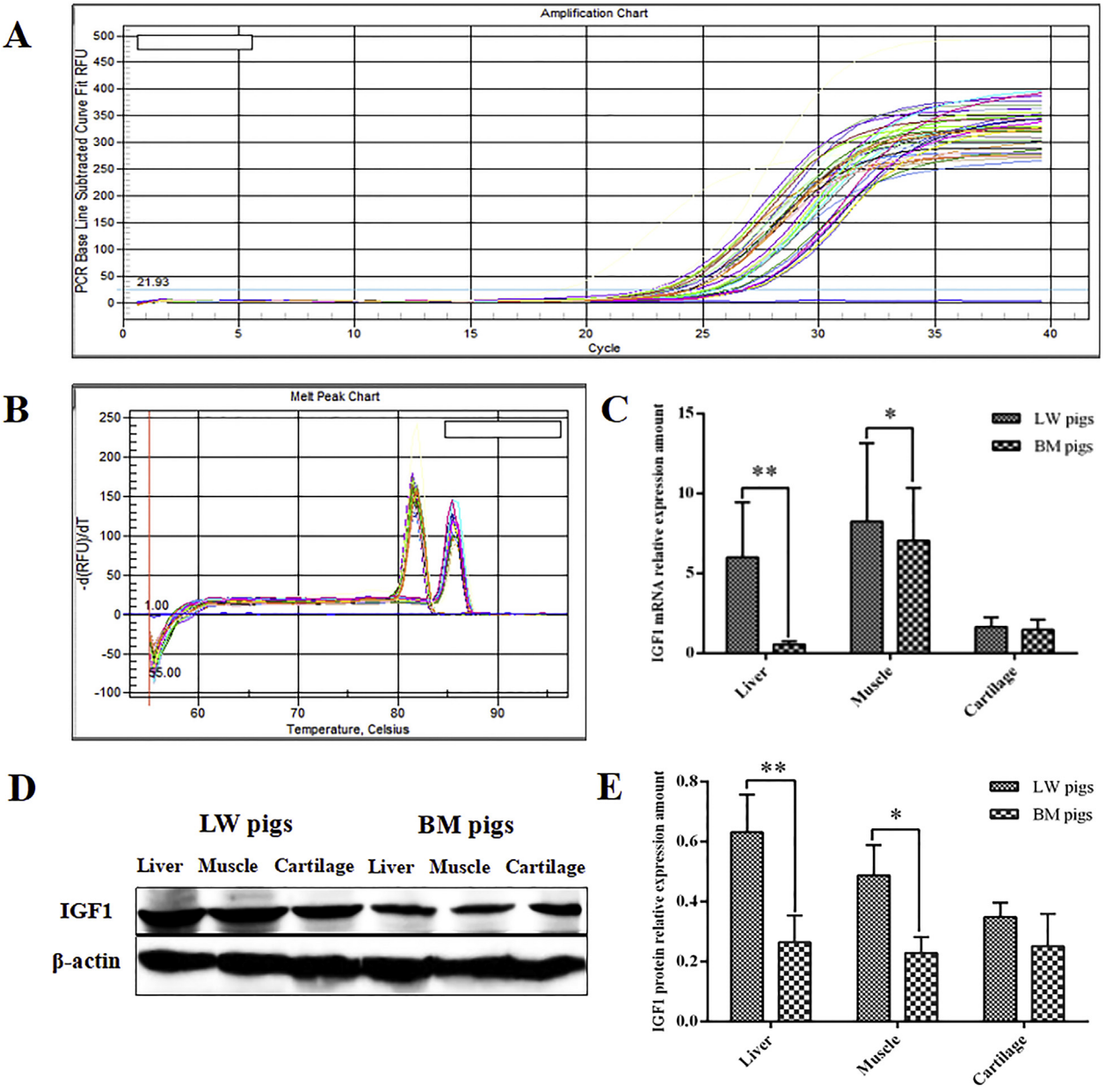

Fig. 1. Expression profiles of the *IGF1* gene in the liver, muscle and cartilage of LW pigs and BM pigs (A) Amplification curves of *IGF1* gene and *β-actin* gene. (B) Melt curves of the amplification of *IGF1* gene and *β-actin* gene. (C) mRNA expression profiles of *IGF1* gene. (D and E) Protein expression profiles of *IGF1* gene.