PROCEEDINGS OF THE 11th WORLD RABBIT CONGRESS
Qingdao (China) - June 15-18, 2016
ISSN 2308-1910

Session
Breeding and Genetics

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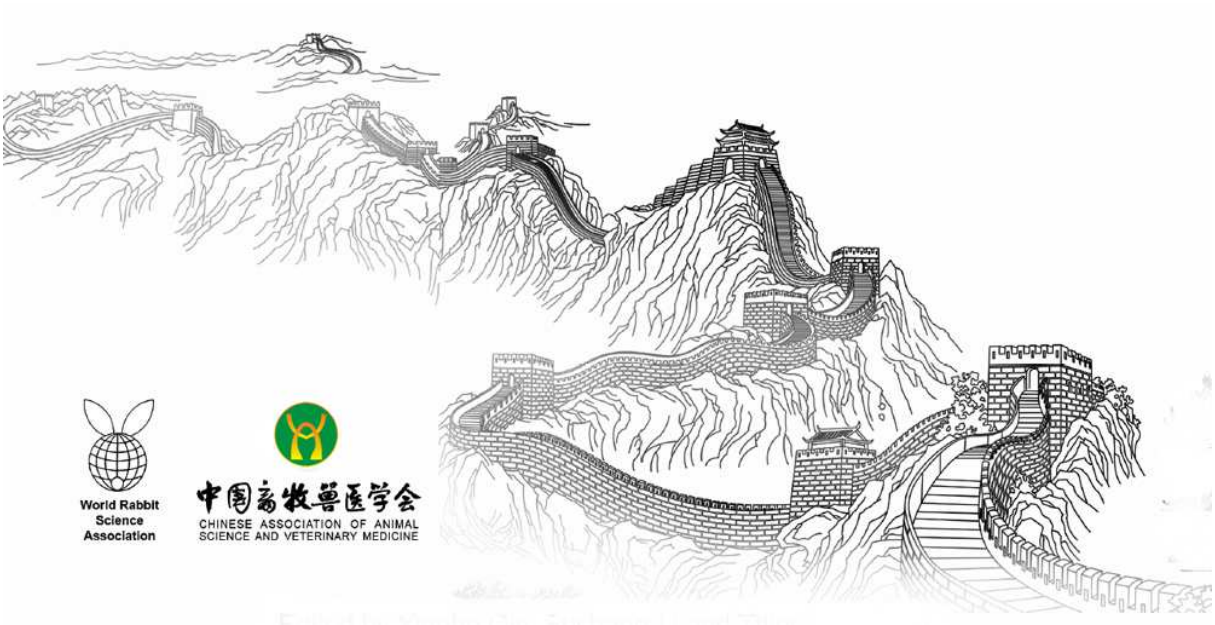
Full text of the communication

How to cite this paper :
Wang L., Jia X., Chen S., Wang J., Lai S., 2016 - Polymorphisms of *PIK3CA* and *AKT3* genes and their association with growth traits of rabbits. *Proceedings 11th World Rabbit Congress - June 15-18, 2016 - Qingdao - China, 133-135.*



POLYMORPHISMS OF *PIK3CA* AND *AKT3* GENES AND THEIR ASSOCIATION WITH GROWTH TRAITS OF RABBITS

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ABSTRACT

The PI3K-AKT signaling pathway has an important role in cell growth and survival in a variety of tissues. In this study we investigated the polymorphisms of *PIK3CA* and *AKT3* genes and their association with growth traits of rabbits (N=711). Six SNPs of *PIK3CA* and two SNPs of *AKT3* are present in the genomic sequences. The polymorphism c.1213A>C of *AKT3* was only found in the Kangda rabbit, and the AC and AA genotypes were associated significantly with higher body weight at every growth stage (P<0.05) compared to the CC genotype. For the *PIK3CA*:c.3068A>G polymorphism, the homozygous individuals for the G allele had the lowest weight at all growth stages in Fujian and Kangda rabbits. Our results indicated that the polymorphisms of both *PIK3CA* and *AKT3* genes are associated significantly with production traits in rabbits, which can be taken advantage of in breeding programs with marker-assisted selection.

Key words: *PIK3CA*, *AKT3*, SNP, Growth performance, Rabbit

INTRODUCTION

Studies in the literature demonstrated inhibition of the PI3K pathway resulted in cell apoptosis (Davis et al. 2014; Mccubrey et al. 2015); however, there are few reports about the influence of the PI3K-AKT signaling pathway on the growth of rabbits. There was a need, therefore, to investigate the association between growth traits and polymorphisms of the *PIK3CA* and *AKT3* rabbit genes.

MATERIALS AND METHODS

Animals and growth traits

Rabbits used in this study were healthy local varieties collected from farms in Qingdao, Chongqing and Fujian China. We collected a total of 711 rabbits (248 Qingdao Kangda rabbits, 225 Chongqing Ira and 238 Fujian black). The bodyweight (BW) of each rabbit was measured at 35, 42, 56 and 70 days of age (BW35, BW42, BW56, and BW70 respectively).

Mutation screening of *PIK3CA* and *AKT3* genes

Seven PCR primer pairs were designed to amplify the available rabbit *PIK3CA* (NC_013682.1) and *AKT3* (NC_013684.1) sequences. PCR and direct sequencing technology were used for mutation screening.

Genotyping

After amplifying fragments of 682 bp for the *PIK3CA* gene and 700 bp for the *AKT3* gene, they were digested with the FastDigest restriction enzyme *Pst*I and *Taq*I (Fermentas, Vilnius, Lithuania), respectively.

RESULTS

Small nuclear polymorphisms (SNPs) in rabbit *PIK3CA* and *AKT3* genes

One synonymous mutation was identified in the exon20 of *PIK3CA* (c.3068A>G) and in the exon10 of *AKT3* (c.1213A>C). The other six mutations were detected in the intron region and 5'UTR of both genes (g.12178A>G, g.12388C>T, g.12605T>C, g.12711C>G and c.3377G>C in *PIK3CA*; g.258389T>C in *AKT3*).

Genetic diversity of the two polymorphisms

For *PIK3CA*c.3068A>G, AA, AG and GG had similar frequencies of allele and genotype in Kangda and Fujian black rabbits (0.56, 0.38 and 0.05 in Kangda rabbits versus 0.53, 0.44 and 0.03 in Fujian black rabbits). AA was the predominant genotype, whereas only AG and GG genotypes were detected in Ira with the predominant genotype of GG. For *AKT3*c.1213A>C, AA, AC and CC genotypes were detected in Kangda rabbits; the frequencies of them were 0.75, 0.21 and 0.04, respectively. However, only the AA genotype was detected in Ira and Fujian black rabbits

The *PIK3CA*c.3068A>G locus has great degree of diversity in Ira rabbits, which indicated there was a relatively large selection potential of *PIK3CA*c.3068A>G in Ira rabbits.

Association of *PIK3CA* and *AKT3* genes with growth traits

Associations of *PIK3CA*c.3068A>G and *AKT3*c.1213A>C polymorphisms with growth traits in rabbits are given in Table 1.

Table 1. Association of *PIK3CA*c.3068A>G and *AKT3*c.1213A>C polymorphisms with growth traits

SNP	breed	trait	genotype		
			AA	AG	GG
PIK3CA	Kangda	BW35	915.53±128.61^{ab}	932.70±117.24^{a}	819.50±112.62^{b}
c.3068A>G		BW56	1687.80±140.96^{ab}	1723.23±139.17^{a}	1571.67±131.79^{b}
		BW70	2159.76±196.74^{ab}	2213.50±191.04^{a}	2023.50±154.88^{b}
	Ira	BW35		934.00±15.67^{b}	954.58±52.69^{a}
		ADG		35.10±1.92^{A}	33.10±3.84^{B}
	Fujian black	BW35	916.44±114.84^{a}	929.43±89.71^{a}	845.70±63.96^{b}
		BW56	1694.90±115.76^{a}	1710.84±103.58^{a}	1621.30±66.51^{b}
		BW70	2173.08±159.19^{ab}	2191.96±144.86^{a}	2076.70±58.08^{b}
			AA	AC	CC
AKT3	Kangda	BW35	925.65±114.20^{a}	913.61±75.26^{ab}	818.80±90.62^{b}
c.1213A>C		BW56	1717.58±125.79^{A}	1685.72±73.29^{AB}	1574.60±94.64^{B}
		BW70	2209.20±183.88^{a}	2157.61±114.89^{ab}	2030.40±117.63^{b}

Only significant association between traits and SNPs are given. Superscripts lacking a common lowercase differ significantly ($P<0.05$). Superscripts lacking a common uppercase are highly significantly different great ($P<0.01$). BW35, bodyweight at 35 days of age. BW56, bodyweight at 56 days of age. BW70, bodyweight at 70 days of age. ADG, average daily gain of bodyweight.

DISCUSSION

We documented synonymous glutamine and arginine mutations in the PIK domain of PI3K and the S-TKc domain of AKT, respectively. The *PIK3CA*c.3068A>G polymorphism is located in the PIK domain that is the accessory domain of the PI3K family. The downstream products of the PI3K (Hiles et al. 1992) function as secondary messengers in many cell signaling pathways. The catalytic domain of PI3K has the bilobal structure typical of other ATP-dependent kinases (Djordjevic et al. 2002; Huang et al. 2009). The S-TKc domain could participate positively in a multitude of cellular processes, including division, proliferation and apoptosis.

There are few reports of associations between the SNPs of the PI3K/AKT pathway and growth traits in other species of livestock. The PI3K/AKT pathway, however, is associated with intermediate growth and survival signaling pathways, via the RTKs, mTOR, MAPK, and insulin-like growth factor pathways (Luks et al. 2015; Zawel 2010). Liu (Liu et al. 2014) constructed two strips of Akt1 short hairpin RNA

eukaryon expression vector in rabbits, and these two strips could effectively inhibit the proliferation of smooth muscle cells. An *AKT2* mutation caused severe insulin-independent hypoglycemia, mild asymmetric overgrowth, and progressive obesity, and an *AKT3* mutation was associated with brain overgrowth (Carpten et al. 2007). Somatic activating mutations in *PIK3CA* have been identified in a spectrum of mosaic overgrowth disorders ranging from isolated digit enlargement to more extensive overgrowth of the body, brain or vasculature (Guo et al. 2007; Maclellan et al. 2014). An activating mutation of *PIK3CA* could lead to pathological tissue growth(Board et al. 2010).

In this study, homozygous individuals for the G allele of *PIK3CA*c.3068A>G were associated with the lowest body weight in all growth stages of Fujian black and Kangda rabbits. The polymorphism of *AKT3*c.1213A>C was found only in Kangda rabbits, and the AC and AA genotypes were associated significantly with greater bodyweight at every growth stage compared to CC.

Mutation frequencies were different among different rabbit species. For example, the mutation *AKT3*c.1213A>C was not detected in Ira or Fujian black rabbits. AA individuals of *AKT3*c.1213A>C were not found in Ira rabbits which might be the result of comprehensive selective breeding or a relatively small amount of samples.

CONCLUSIONS

Polymorphisms of both *PIK3CA* and *AKT3* genes were significantly associated with production traits in rabbits. However, such association was different among breeds.

ACKNOWLEDGEMENTS

This study was supported by the Earmarked Fund for China Agriculture Research System (CARS-44-A-2). We thank Gen-Yan Li and Cong Tu for helping data collection.

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