Relationship between genetic variants of *POU1F1*, *PROP1*, *IGFBP3* genes and milk performance in Guanzhong dairy goats

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ABSTRACT

The aim of this study was to determine and verify if the potential genetic variations in the *POU1F1*, *PROP1* and *IGFBP3* genes could be related to dairy goat milk performance. This study detected the polymorphisms of *POU1F1-Pst*I, *POU1F1-Dde*I, *POU1F1-Alu*I, *PROP1-Hin*6I and *IGFBP3-Xsp*I loci, and evaluated their associations with milk performance in Guanzhong dairy goats. The present results confirmed the previous genotypes and found three unreported genotypes in Guanzhong dairy goats (e.g. genotype D_2D_2 at *POU1F1-Dde*I locus, genotype TC at *POU1F1-Alu*I locus, genotype X_2X_2 at *IGFBP3-Xsp*I locus, respectively). The genotypic frequencies of all analyzed loci were at Hardy-Weinberg equilibrium ($P > 0.05$) except *POU1F1-Pst*I locus ($P < 0.01$). The PIC values of *POU1F1-Pst*I locus and *IGFBP3-Xsp*I locus were 0.328 and 0.329, respectively, suggesting that these two loci possessed median polymorphism. Association analysis demonstrated no significant relationship between the *POU1F1-Pst*I, *POU1F1-Dde*I, *PROP1-Hin*6I and *IGFBP3-Xsp*I loci and milk performance in Guanzhong dairy goats ($P > 0.05$). Interestingly, in the *POU1F1-Alu*I locus, this SNP was significantly associated with a total of six milk traits ($P < 0.05$). Moreover, the individuals with genotype TT possessed superior morning milk fat content, afternoon milk fat content and average milk fat when compared with those of genotype TC ($P = 0.026$, $P = 0.029$, $P = 0.036$, respectively); Consistently, genotype TT had higher morning milk acidity, afternoon milk acidity and average milk acidity than genotype TC ($P = 0.028$, $P = 0.027$, $P = 0.038$, respectively). These findings suggested that the *POU1F1-Alu*I locus had significantly effect on milk performance, and genotype TT could become an effective DNA marker for senior milk fat content or acidity, which could benefit to dairy goat selection and breeding through marker-assisted selection (MAS).

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1. Introduction

Goat milk production is a dynamic and growing industry that is fundamental to the wellbeing of billions of people worldwide (Silanikove et al., 2010). The recent literatures manifested that in relation to some parameters goat milk has more advantages than the milk of other species, and has specific benefits in human nutrition and food security (Silanikove et al., 2010; Devendra and Liang, 2012; Pacinovski et al., 2015), For example, goat milk fat drops are smaller and more evenly distributed in the milk, allowing faster and easier digestion in human organisms. Moreover, many researches characterize goat milk as a drink for convalescent but also as basic detoxifier of human organism (Pacinovski et al., 2015), so goat milk has increasingly become popular. As we know, dairy goats are mainly reared in the northern China, and large export of dairy and its related products give great chance for rural and western families to improve the economic situation (Dubeuf et al., 2004).

Therefore, the further improvement and increase of the quantity and quality in goat dairy will better contribute to the Chinese society, particularly in economy, nutrition, tradition and religion (Lan et al., 2007a). Milk production traits, e.g. milk fat/protein content (%), lactose content (%), total solids content (TS) (%), milk solids-not-fat (SNF) content (%), milk acidity, milk density and milk FPD (°C) are essential in dairy goats. However, improving milk production traits by traditional selective breeding methods has proved to be difficult due to their low heritability (An et al., 2015). So, a lot of breeders mainly focus on DNA markers for potential milk traits candidate genes selection through marker-assisted selection (MAS). Unfortunately, little information about the candidate genes affecting goat milk performance is available, and thereby exploring suitable DNA markers from candidate genes that correlate with milk production traits has become a major desire in breeding and genetics of dairy goats (Lan et al., 2013b).

According to some researches, we know that goat milk traits are correlated with growth hormone (GH), prolactin (PRL) etc. (Dettori et al., 2013; Zhao et al., 2013).And the expression of these hormones is regulated by many genes (Mukherjee and Porter, 2012; Baş et al., 2015), the most common gene of them is *POU1F1*. Moreover, as a prophet of *POU1F1*, *PROP1* also affects the expression of *GH*, *PRL* and *POU1F1* (Carvalho et al., 2006; Davis et al., 2010). Interestingly, some researchers found GH and PRL regulate *IGFBP-3* gene expression (De et al., 1995; Breves et al., 2014), which affects growth and reproduction by influencing insulin-like growth factors (IGFs) system (Bale and Conover, 1992; Hastie et al., 2004; Lan et al., 2007b; Salazar-Ortiz et al., 2014). And in view of our previous reports, *POU1F1*, *PROP1* and *IGFBP3* genes were all found polymorphisms at some loci (Lan et al., 2007a,b,c,d; Lan et al., 2009a,b,c; Pan et al., 2013). So, to make our research more deeply and to detect the candidate genes that associated with milk production traits, we picked these three genes to be our research subjects.

POU1F1 (also named PIT-1 or GHF-1), representing for pituitary specific transcription factor-1, is a positive regulator for GH, PRL and thyroidstimulating hormone β (TSHβ) in mammals (Cohen et al., 1997; Baş et al., 2015). Hence, *POU1F1* mutations result in different expression of *GH*, *PRL*, *TSH* and *POU1F1* gene itself (Li et al., 1990; Cohen et al., 1997; Baş et al., 2015). These genes participate in a variety of signaling pathways that are important for many developmental and physiological processes including pituitary and mammary gland development and growth, milk protein expression and milk production and secretion (Li et al., 1990; Svennersten-Sjaunja and Olsson, 2005; Akers, 2006; Jia et al., 2011; Molee et al., 2015). Mutations of *POU1F1* were shown to be associated with mice Snell dwarf (dw) and Jackson dwarf (dw-J), human dwarfish (Li et al., 1990; Pfäffle et al., 1992; Reynaud et al., 2004), swine economic traits (Stanceková et al., 1999), bovine growth and development (Renaville et al., 1997a; Zhao et al., 2004; Selvaggi et al., 2011), cattle milk production traits (Renaville et al., 1997b; Yan et al., 2011), sheep economic traits and production performance (Bastos et al., 2006; Sadeghi et al., 2014; Jalil-Sarghale et al., 2014), chicken body weight and egg numbers (Yan et al., 2013), rabbit meat quality traits (Wang et al., 2015). In goat, our previous report firstly showed four genetic variations within *POU1F1* exon 6 in China goat breeds (Lan et al., 2007c). A silent mutation(S241S, *POU1F1-Dde*I) was significantly associated with milk yield, litter size, and body weight (Lan et al., 2007a); A synonymous SNP (S279S, *POU1F1-Alu*I) was closely related to milk yield and birth weight (Lan et al., 2007c); The G > A mutation at position 92 of the 3′ untranslated region (UTR) was genotyped by PCR-RFLP (Lan et al., 2009c); T > C transition (*POU1F1-Pst*I) at position 110 of the 3′ UTR was strongly correlated with goat cashmere yield (Lan et al., 2009c). Recently, Daga et al. (2013) has reported nine exonic and seven non-coding regions SNPs within the Sarda goat *POU1F1* gene. They

also found the statistically significant associations between several polymorphisms (e.g. *POU1F1-Dde*I and *POU1F1-Mly*I loci) and milk traits (Daga et al., 2013). However, to date, no relationship between the *Dde*I, *Alu*I and *Pst*I polymorphisms within the *POU1F1* gene and goat milk performance (e.g. milk fat content (%), milk protein content (%) and milk acidity etc.) has been revealed in Chinese Guanzhong dairy goats.

As a paired class homeodomain transcription factor, *PROP1* (prophet of POU1F1) is specifically expressed in the pituitary gland, and plays a direct or indirect crucial role in the ontogenesis of pituitary gonadotropes, somatotropes, lactotropes and caudomedial thyrotropes (Lan et al., 2009b; Baş et al., 2015). Numerous studies have identified that *PROP1* mutations were not only responsible for deficiencies in GH, PRL and TSHβ, but also significantly affected the expression level of the *POU1F1* gene (Carvalho et al., 2006; Davis et al., 2010). SNPs of human *PROP1* gene have been reported to associate with combined pituitary hormone deficiency (CPHD) (Wu et al., 1998; Carvalho et al., 2006; Baş et al., 2015). In cattle, mutations of *PROP1* gene were demonstrated significantly affect growth, male fertility and milk production traits (Lan et al., 2013a; Pan et al., 2013). Lan et al. (2009b) found there was no significant association between SNP of *PROP1-Hin*6I locus and production traits as well as average milk yield in goat (Lan et al., 2009b). So far, it has been unknown whether there was significant association between the *Hin*6I polymorphism and goat milk performance or not in Guanzhong dairy goats.

Insulin-like growth factor binding protein-3 (IGFBP3) gene is a structural gene responsible for the multiple influence of insulin-like growth factors (IGFs) system (Lan et al., 2007b; Sharma et al., 2013).The IGFs signaling system plays an important role in development, growth and reproduction as well as aging (Bale and Conover, 1992; Hastie et al., 2004; Salazar-Ortiz et al., 2014). Polymorphisms have been described in bovine *IGFBP3* gene associated with growth, fat content and production traits (Maciulla et al., 1997; Choudhary et al., 2007; Xi et al., 2012). SNPs of buffalo *IGFBP3* gene also have been reported (Kumar et al., 2004; Padma et al., 2004). Similar mutations have not been found in a fragment of sheep *IGFBP3* gene (Kumar et al., 2006; Ali et al., 2009). Recently, *IGFBP3* A-202C polymorphism has been shown may be associated with Korean prostate cancer and Chinese breast cancer (Park et al., 2010; Ma et al., 2015). In goat, four mutations of *IGFBP3* were found in the exon 2 and intron 2 (Lan et al., 2007d). Polymorphisms of *IGFBP3* gene in the exon 2 were detected associated with litter size (Lan et al., 2007d). Furthermore, the *Hae*III and *Xsp*I polymorphisms were revealed not significantly associated with the milk yield (Lan et al., 2007b). However, to date, the further relationship between the *IGFBP3* gene potential polymorphism and other milk performance has not been reported yet.

Guanzhong dairy goat is the predominant dairy goat breed in China. Some literatures data showed there were around 12 million dairy goats reared in China in 2012(Tian et al., 2014), and Guanzhong dairy goats account for around 10% of them. In addition, the annual average milk yield of Guanzhong dairy goats is generally 500–600 kg (Han et al., 2010; Tian et al., 2014). Many researches have shown that polymorphisms of *POU1F1*, *PROP1* and *IGFBP3* genes in several positions were/were not associated with some economic traits and production performance in Guanzhong dairy goats (Lan et al., 2007a,b,c,d; Lan et al., 2009a,b,c). However, whether the genetic variants of goat *POU1F1*, *PROP1* and *IGFBP3* genes have a significant relationship with milk performance in Guanzhong dairy goats is unknown. Therefore, it is an interesting and important work to explore the genetic variants of *POU1F1*, *PROP1*, *IGFBP3* genes and their association with milk performance in Guanzhong dairy goats, which could provide useful genetic markers for dairy goat MAS.