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Full Length Research Paper

Determination of taste receptor type 1 member 1 (TAS1R1) gene polymorphism and association with some body measurements in goats

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Umami taste plays a key role in intake of amino acids. It is believed that the taste receptor type 1 member 1 (TAS1R1) and taste receptor type 1 member 3 (TAS1R3) receptors are to function in combination as a heteromeric umami taste receptor in humans and other mammals. Most of the previous studies laid particular emphasis on the taste sensitivity to umami substances or the relation of umami taste sensitivity to variations in candidate umami receptor genes in rodents or in humans. Taste thresholds were associated with body weight. In this article, the objective was to investigate variations in goat TAS1R1 gene and their associations with growth traits in 317 goats by PCR-SSCP and DNA sequencing methods. The results showed two novel single nucleotide polymorphisms (SNPs): HM449123:g. [T3974C, C4037T]. In detail, two different alleles, A and B, were identified and three genotypes were observed, AA, AB, and BB with the frequency distribution of allele B from 0.59 to 0.67 in analyzed populations. The genetic diversity analysis revealed that all PIC values were between 0.34 and 0.37, implying that this locus within TAS1R1 gene possessed moderate genetic diversity in goat. Furthermore, nucleotide sequence analysis showed that HM449123:g. [T3974C, C4037T] resulted in two synonymous mutations. But association analysis demonstrated significant differences between different genotypes and production traits (such as body length, chest circumference and cannon circumference) of Haimen goats. We hope that it can provide valuable information for molecular marker-assisted selection.

Key words: Goat, PCR-SSCP, polymorphism, production traits, TAS1R1 gene

INTRODUCTION

It has been reported that taste thresholds were associated with body weight. L-glutamate intensity was very weak but significantly correlated with body mass index in women (Donaldson et al., 2009). L-Glutamate signaling via taste and gut L-glutamate receptors could influence multiple physiologic functions, such as thermoregulation and energy homeostasis (Kondoh et al., 2009).

The 3 genes of the T1Rs family (Zhao et al., 2003; Kim et al., 2006; Ren et al., 2009), taste receptor type 1 member 1 (TAS1R1), taste receptor type 1 member 2 (TAS1R2), and taste receptor type 1 member 3 (TAS1R3), combine to generate at least two heteromeric receptors: TAS1R1 and TAS1R3 form an L-amino acid sensor, which in rodents recognizes most amino acids (Nelson et al., 2002), and TAS1R2 and TAS1R3 associate to function as a broadly tuned sweet receptor. On the condition of only TAS1R3, it can also identify sweetening (Damak et al., 2003). Like all G protein-coupled receptors (GPCRs), these receptors have a seven helix TM domain

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(7 TM), which contains the active site for typical ligands. Variations in these genes have been characterized (Kim et al., 2006; Fushan et al., 2009; Shigemura et al., 2009b), but neural and behavioral responses to umami is not eliminated. In one study (Chaudhari et al., 2009; Sakthivel et al., 2009; Shigemura et al., 2009b), knockout of either TAS1R1 or TAS1R3, the responses to oral glutamate is not completely eliminated. However, in another study, knockout of TAS1R3 and TAS1R1 potentiation of glutamate, taste responses effected little on umami taste (Shigemura et al., 2009b). It was reported that the TAS1R1 to TAS1R3 receptor was likely to be the receptor mediating L-glutamate detection in human fungiform papillae and that naturally occurring variants of this receptor might underlie inter-individual variability of sensitivity to L-glutamate in humans (Raliou et al., 2009b).

We assume that goat TAS1R1 play similar role on L-glutamate sense, the functional variations of TAS1R1 gene may affect thermoregulation and energy homeostasis of goat. In this study, the coding and flanking region of TAS1R1 gene was screened in three goat populations for SSCP polymorphism and SNPs, and the association of SSCP genotypes with caprine body weight and growth traits were analyzed.

MATERIALS AND METHODS

Sampling and DNA extraction

Boer goat (BE, n = 81), Chinese Xuhuai white goat (XH, n = 89) and Chinese Haimen goat (HM, n = 117) were collected from Jiangsu province of China. All these goat breeds represent the main breeds of this region. Blood samples were obtained from these goat populations and genomic DNA was extracted from the blood samples according to standard procedures. Adult growth traits and body sizes (body height, body length, chest circumference, cannon circumference) in sampled animals were measured for association analysis.

DNA pool construction

To screen TAS1R1 gene for polymorphisms and to evaluate the association with caprine growth traits, we construct DNA pools. The DNA samples of every animal in three goat breeds were selected to the pools. The selected DNA was diluted to be a standard concentration. DNA pool was constructed by combining 50 ng of DNA from each individual. Transfer individual aliquots of DNA into a single tube, and mix gently. Quantitate it before further dilution to a working concentration of 5 ng/µl.

PCR amplification

Based on the bovine TAS1R1 gene (GenBank accession number: NC_007314), the following 10 pairs of polymerase chain reaction (PCR) primers were designed using the Primer V 5.0 software to amplify the coding regions. E_1 was used to amplify the exon I, including a fraction of intron I, other 9 pairs primers were used to amplify other 5 exons. The 10 pair's primers can amplify all the coding sequences. PCR solution contained 50 ng DNA template, 0.20 mM dNTP, 2.5 mM $MgCl_2$, and 0.5 U Taq DNA polymerase (MBI). The PCR was performed using the following program: 94°C for 5 min followed by 35 cycles of 94°C for 35 s, annealing for 45 s, and 72°C for 1 min and a final extension at 72°C for 10 min (Table 1).

Single stranded conformation polymorphism (SSCP)

PCR products were analyzed for single-strand conformation polymorphisms (SSCP). Aliquots of 5 µl of above PCR products were mixed with 5 µl of the denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene–cyanole and 0.025% bromophenol blue), heated for 10 min at 98°C and chilled on ice. Denatured DNA was subjected to PAGE (80 × 73 × 0.75 mm) analysis which was run with 0.5 × TBE buffer (89 mM tris–borate, 2 mM EDTA, pH 8.3) for 2 h at room temperature under a constant voltage (180 V). The gel was stained with silver nitrate and visualized with 2% NaOH solution (containing 0.1% formaldehyde).

DNA sequencing analysis

The PCR products which represented different PCR–SSCP genotypes, including both homozygous and heterozygous genotypes were purified with the GenElute PCR DNA purification kit (Sigma-Aldrich Corporation, USA) and sequenced using the ABI 377 sequencer from both directions (Applied Biosystems, USA). The Blast algorithm of NCBI was used to search the NCBI GenBank (http://www.ncbi.nlm.nih.gov/) database for homologous sequences.

Statistical analysis

Gene heterozygosity, gene homozygosity, effective allele numbers, were calculated for each breed using the PopGen software (version 3.2) and the polymorphism information content (PIC) was calculated by Botstein's methods. Data of the breeds on the traits were analyzed by analysis of variance (ANOVA) indicated. Differences for genotypic and haplotype frequencies at the goat TAS1R1 loci among/between the breeds were analyzed using χ^2-test which was performed by SPSS software (version 17.0). The effects associated with season of birth (spring vs. fall), age of dam and sire were not included into the linear model, as the preliminary statistical analyses indicated that these effects did not significantly influence on variability of traits in the population. Therefore, the effects of genotype on the traits were analyzed by the least-squares method as applied in the general linear model (GLM) procedure of SPSS according to the following statistical model:

$$Y_{ijk} = \mu + Age_j + Breed_i + Marker_k + e_{ijk}$$

Y_{ijk} is the observation of the trait, μ is the least square mean, $Breed_i$ is the effect of breed, Age_j is the effect of age, $Marker_k$ is the effect of marker genotype and e_{ijk} is the residual effect. The correction for multiple testing was performed by Bonferroni method.

RESULTS

SSCP was screened in the ten fragments of the caprine TAS1R1 gene. The number of bands and their positions in the gel clearly showed the occurrence of DNA sequence variations. The PCR products of different SSCP variants were sequenced and 2 SNPs were found at exon 3. In

Table 1. Primers used for polymerase chain reaction (PCR) amplification.

Loci	Position (bp) ref. NC_007314	Primer sequence	Annealing temperature (℃)
E_1	-98~155	F: 5'GGGAAGCATCTGGGCAACT3' R: 5'TCTGGAAGGGCACTGTGGA3'	60.5
E_2	1618~2088	F:5'TCCAGGACCCATGTTGAGG3' R:5'AGGGGAGATGAGTGAACTGTTAG3'	62.5
E_3	3746~4143	F:5'AGGCTGATGCCACTGAACTT3' R:5'GCACCACGGATTCAAAGAA 3'	62
E_4	4122~4465	F:5'GTGTTCTTTGAATCCGTGGTG3' R:5'ACAGCGTAGACAGCCTGGTAT3'	62.5
E_5	4431~4654	F:5'ACAGCGTAGACAGCCTGGTAT3' R:5'CCTCTAGCGGTCACCCTTT3'	58.5
E_6	4773~5210	F:5'TCACTCAGGAGGCTGGTTAGG3' R:5'CGTCCCTGTTACCTGGTTGTC3'	62.5
E_7	5441~5754	F:5'GTCCCTTGACATGAGCATC3' R:5'TCCTCCAAACTGTCCCTAA3'	56.5
E_8	6902~7292	F:5'GCTGTCCTCACCTGGCTCTC3' R:5'CAGGAAGATGGCAAAACCGA3'	66
E_9	7278~7577	F:5'TTTGCCATCTTCCTGTCCTGC 3' R:5'GCTGATCGAGAGGAGGCCATT3'	63
E_{10}	7558~7905	F:5'ATGGCCTCCTCTCGATCAG3' R:5'GGCTTGACCACGCCCGAT3'	63

Note: Exons 1, 2, 3, 4, 5 and 6 are located at base pairs 1~191, 1746~2052, 3777~4538, 4986~5198, 5495~5615, 6949~7880, respectively.

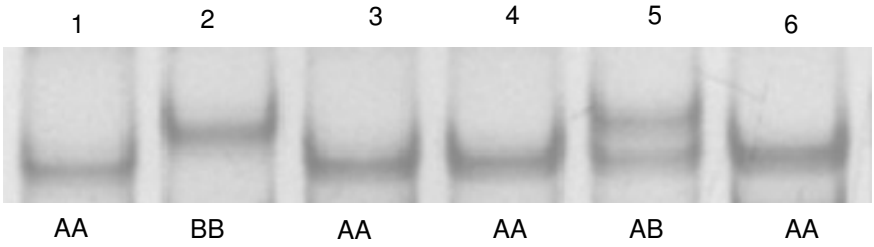

Figure 1. The electrophoresis patterns of PCR-SSCP for E_3 locus in TAS1R1 gene.

detail, genetic polymorphism of TAS1R1 E3 locus in the caprine TAS1R1 gene (Figure 1) shows the SSCP pattern of the E_3 locus of the gene. Two different alleles, A and B, were identified and three genotypes were observed: the slower migrating band was designated as A and the faster one as B, while the heterozygous individual (AB) presented two distinct bands. In order to better understand the detailed genetic variation within the caprine TAS1R1 gene, the polymorphic DNA amplification fragments comprising exon3 were sequenced. Sequence determination of the entire length of the fragment revealed two novel polymorphic sites at position 3974 and 4037, respectively (Figure 2), compared with reference sequence (GenBank accession no.: NC_007314). Both base substitutions were located in the third exon. The mutation at position 3974 was a T-to-C transversion, while the mutation at 4037 was a C-to-T transversion. Regrettably, SSCP analysis detected half (A: T3974 … T4037 and B: C3974 … C4037) of the four possible combinations from the two mutations. The two other possible combinations (T3974…C4037 and C3974 … T4037) either do not produce unique band migration or do not exist in the sample population investigated. Interestingly, we found there was a subtle linkage relationship between the two novel mutations: when the position 3974 was a T, the position 4037 must be a T, too; when C was located in the position 3974, C in the position 4037, definitely. Therefore, the two mutations in Exon3 give rise to merely two haplotypes (A: T3974 … T4037 and B: C3974 … C4037) in this study, just like one mutation

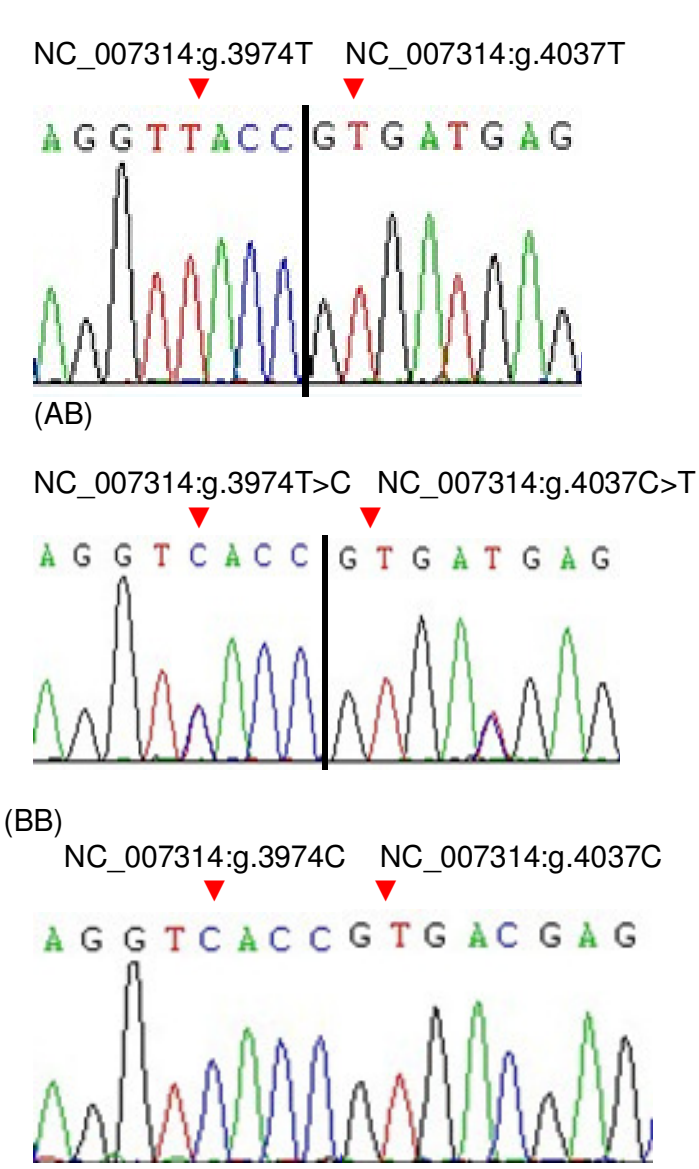

Figure 2. The sequencing and sequence comparison results of caprine *TAS1R1* gene and SNPs are indicated by red arrows.

does, which means the frequency of allele is equal to that of haplotype at E3 locus. Genotypic and allelic frequencies of TAS1R1 gene in capine were shown in Table 2. The frequencies of genotype AA in the above three breeds were 0.22, 0.20, 0.20, respectively, with genotype AA the lowest. Accordingly, any frequency of haplotype A in the population investigated was lower than that of haplotype B, with allele B dominating. The genotype distribution of TAS1R1 gene at E3 locus were found to be significantly deviating from the Hardy–Weinberg equilibrium ($p < 0.05$) in Xuhuai white goat ($p = 0.03$) and Haimen goat ($p = 0.00$), that resulted in excess of homozygotes. The genotype distribution is at Hardy-Weinberg equilibrium in Boer goat ($p = 0.06$).

On the basis of Nei's and Botstein's methods, the population genetic indexes, including gene heterozygosity (He), effective allele numbers (Ne), and polymorphism information content (PIC) were calculated (Table 2). Because the mean Ne approximated to 2 in every population investigated, it indicated that the two alleles contributed almost equally on the whole to the polymorphisms of E_3 locus. According to the classification of PIC (low polymorphism if PIC value < 0.25, median polymorphism if 0.25 < PIC value < 0.5, and high polymorphism if PIC value > 0.5), E_3 locus of TAS1R1 gene in the three population analyzed was at median

Table 2. Genotype and allele frequencies , PIC, He, and Ne at E3 loci of caprine TAS1R1 gene.

Breed	Genotype frequency		Allele frequency					
	Paa	Pab	Pbb	P_A	P_B	He	Ne	PIC
XH (89)	0.22	0.37	0.41	0.41	0.59	0.37	1.94	0.37
HM (117)	0.20	0.26	0.54	0.33	0.67	0.27	1.79	0.34
BE (81)	0.20	0.37	0.43	0.38	0.62	0.37	1.89	0.36

Note: XH = Xuhuai goat; BE = Boer goat; HM = Haimen goat; He = Gene heterozygosity; Ne = Effective allele; PIC = Polymorphic information.

polymorphic level (PIC_{xh} = 0.37, PIC_{be} = 0.36, PIC_{hm} = 0.34, respectively). This reflected that there was a moderate genetic diversity within the gene in analyzed populations, which implied that TAS1R1 gene has some potential for breeding selection.

DISCUSSION

Previous research has focused on the discussion in the relationship between TAS1Rs genes mutations and changes in taste thresholds. Like all GPCRs, these receptors have a seven helix TM domain (7 TM), which contains the active site for typical ligands. T1Rs combine to generate at least two heteromeric receptors: TAS1R1 and TAS1R3 form an L-amino acid sensor, which in rodents recognizes most amino acids (Nelson et al., 2002). Previous studies suggested that the extracellular domain (ECD) of TAS1R1 contains binding sites of umami stimuli (Xu et al., 2004). Indeed, there are strong arguments in favor of the involvement of several receptors for umami taste (Harland et al., 1989; Lugaz et al., 2002; Damak et al., 2003). Sequencing of the exons encompassing the coding region of the corresponding genes, detected 9 cSNPs (Raliou et al., 2009a) including 3 non-synonymous SNPs (nsSNPs) in the TAS1R1 gene all of which are located in the ECD: C329T (A110V), G1114A (A372T), G1520A (R507Q). Raliou et al. (2009a) and Shigemura et al. (2009a) discover five non synonymous mutations (in E1 G11A: Cys4Tyr, in E2 C329T: Ala110Val and in E3 G1039A: Glu347 Lys, C1067G: Ser356 Cys G1114A: Ala372 Thr). Two of these SNPs (A110V and A372T) were analyzed because of their relatively high allele frequency ($p > 0.01$). The significant difference of the prevalence of nsSNPs has been shown in tasters compared with non tasters and hypo tasters. Three SNPs with an amino acid substitution in mouse TAS1R1 (M347T, K443N, and K626E) between C57BL/6J and 129P3/J (Shigemura et al., 2009a). These results suggest that the differences in umami sensitivity between inbred strains may be related to SNPs with these amino acid mutations in TAS1R1 but not to amino acid mutations in TAS1R3.

As it has been reported that taste thresholds were associated with body weight (Donaldson et al., 2009; Kondoh et al., 2009). Therefore, this paper will elaborate the relationship between SNPs of TAS1R1 and growth traits. To investigate the effects of these mutations (HM449123), we analyzed the relationship between TAS1R1 genotypes and the effects on variation in body height, body length, cannon circumference, chest circumference in three goats breeds (Table 3). From Table 3, we can see that Boer possesses significantly larger body sizes (body height, body length, chest circumference and cannon circumference) than Xuhuai and Haimen goat breeds. In Boer population, significant association of polymorphisms loci with body height, body length and cannon circumference was identified. Multiple-comparison results showed that genotype AB individuals had 9.04% body height and 22.05% body length more than genotype AA individuals, while genotype BB population was also significantly shorter than genotype AB individuals ($p < 0.01$). However, in Haimen population, significant statistical difference was found in chest circumference cannon circumference. Genotype AB individuals was obviously thicker than Genotype AA (in ChC and in CaC $p < 0.01$) and Genotype BB analysis revealed that Genotype AB, as heterosis, has been predominant in the population investigated.

By alignment to the sequences of the bovine TAS1R1 gene, that in caprine has two novel SNPs and these novel SNPs do not result in any substantial change in the quantity or electrostatic charges of amino acid: p. V(GTT)232V(GTC) and D(GAT)254(GAC). Synonymous mutations like above, can disrupt splicing efficiency, reduce tRNA speed or interfere with mRNA binding. Hence, synonymous mutations are not inconsequential but vital (Nackley et al., 2006; Kimchi-Sarfaty et al., 2007). The real importance for breeding is the economical values of the different traits after all.

Haimen goat and Xuhuai white goat are the local species while Boer goat, native to South Africa, was introduced into China. These three breeds are raised for meat production at main land. By studying the genes related with their growth traits and analysis of the association between genetic variation and growth traits, we hope that it can provide valuable information for molecular marker-assisted selection.

In summary, two novel SNPs, HM449123:g. [T3974C, C4037T] were detected. The genotype of the two SNPs

Table 3. Association of genotypes at the *E3* loci with growth traits in 317 goats.

	BH (cm)	BL (cm)	ChC (cm)	CaC (cm)
XH Genetype(number)				
AA(20)	65.90±0.62 ^{AB}	75.50±1.33	81.50±1.60	8.95± 0.15^{a}
AB(33)	66.48±0.7 2^{A}	74.70±1.30	80.15±1.53	8.50±0.11^{b}
BB(36)	64.06±0.71 ^{B}	72.50±1.24	78.33±1.25	8.63±0.16^{ab}
HM Genetype (number)				
AA(23)	54.13±2.26	82.70±3.73	62.78±2.46 ^{A}	7.33±0.31 ^{A}
AB(31)	59.65±2.17	83.45±3.80	75.58±2.24 ^{AB}	8.85±0.26 ^{AB}
BB(63)	55.49±1.11	84.60±2.51	63.24±1.33 ^{B}	7.44±0.13 ^{B}
BE Genetype(number)				
AA(16)	62.69±1.01^{A}	68.94±1.89^{A}	80.69±1.63	9.66±0.31^{ab}
AB(30)	68.63±1.64^{B}	85.88±1.95^{AB}	85.42±2.68	10.83±0.36^{a}
BB(35)	65.91±1.07 ^{AB}	75.60±0.57^{A}	80.89±1.80	10.66±0.24^{b}

Note: LSM in a row with no common superscripts differ significantly, low-case character represents the significance at level of $p < 0.05$, capital character represents the significance at level of $p < 0.01$. BH = body height, BL = body length, ChC = chest circumference, CaC = cannon circumference.

was associated with the growth traits of goat. Further work need to be done to investigate if the SNPs could be applied in molecular marker-assisted selection of meat type goat.

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