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Full Length Research Paper

Origin and differentiation of a special fragment from *Capra hircus agouti* gene

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A 136 bp fragment previously considered to only exist in *Capra hircus agouti* gene was analyzed. The results indicated that it existed in the *agouti* gene of *C. hircus* and *Ovis aries* and in other genes of *O. aries* and *Bos taurus* with forward and reverse sequences. The reverse sequences from *O. aries* had higher genetic diversity than forward ones. The differentiation between *B. taurus* and *C. hircus* was more obvious than that between *B. taurus* and *O. aries*. AC150540 (BACR5) from *B. taurus* and EE751186 (OEEF2) from *O. aries* could be considered as the farmost ancestral sequences.

Key words: Special fragment, 136 bp, *Agouti* gene, origin, differentiation.

INTRODUCTION

Agouti gene plays an important role in the synthesis and distribution of coat color in domestic animals. Its encoding product, agouti-signaling protein (ASIP), acts as an antagonist of MC1R by nullifying the action of alpha-melanocyte-stimulating hormone (alpha-MSH). Alpha-MSH and MC1R combine to make black pigment (eumelanin). If MC1R lost its function, yellow pigment (pheomelanin) will be produced (Barsh, 1996). Recently, most attention has been focused on the study of sequencing, identification of single nucleotide polymorphisms (SNPs) and association analysis with color variation in a number of animals as well as human beings (Voisey and Van Daal, 2002; Voisey et al., 2006; Stefan et al., 2001; Vage et al., 1997; Eizirik et al., 2003). Altogether, ten color patterns have been observed and were postulated to be caused by ten alleles at the *agouti* locus, with the allele for white or tan color been dominant and the nine others codominant (Adalsteinsson et al., 1994). Previously, we sequenced intron 2 (AY291442), exon 4 (DQ058664) and got 5719 bp (EF587236) including 17 SNPs of goat *agouti* gene (Li et al., 2010). Meanwhile, we found that allele T of g.423G>T in DQ058664 might be responsible for the black phenotype or be linked with the causative site in goats (Tang et al., 2008), and T128 del in EF587236 might have an effect on pigment synthesis of goat (Tang et al., 2009). Interesti-ngly, it was found that a special fragment, 136 bp fragment (CTAAACTGGAGAAGGAAATGGCAACCCACTCCAGTGTT CTTGGAGAATCCCAGGGACGGAGGAGCCTGGTGGGCT ACCGTCTATGGGGTTGCACAGAGTCGGACATGACTAAA GCGACTTAGCAGCAGCAGCAAA) existed in goat agouti gene but not in the agouti gene of cow (X99691), pig (AJ251837) and horse (AF288358). We also proved that the 136 bp fragment did not exist in the *agouti* gene of cow, sheep and pig after testing experiments (Li et al., 2010). Bioinformatics analysis of this special fragment revealed many homologous regions of the sequences in cow with high identity (90.00 to 93.89%) in some genes, and with forward and reverse orientation in the same gene, inferring that the sequence might be a retro-transposon in those genes (Li et al., 2010). In this study, genetic diversity of this special fragment was analyzed and network tree including its ancestral fragment was reconstructed in order to investigate its origin and differentiation among different species.

MATERIALS AND METHODS

The BLAST program (human genomic plus transcript (Human G+T), mouse genomic plus transcript (Mouse G+T) and nucleotide collection (nr/nt) databases) was used to search homologous

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regions of the special fragment in the GenBank of NCBI. A total number of 80 fragments with identity of over 90% belonging to 3 species (*Capra hircus*, *Ovis aries* and *Bos taurus*) were obtained from GenBank (Table 1). All the subject fragments plus the query fragment were aligned using Clustal W program in BioEdit (version 7.0.5). DnaSP (version 5.0) software was used to analyze the genetic diversity of the fragments, including number of haplotypes (h), haplotype diversity (Hd), average number of nucleotide differences (K), nucleotide diversity (p), polymorphic site (S), singleton variable sites (SP), parsimony informative sites (PIP) and genetic distance representing genetic differentiation (Gst) for forward and reverse fragments of each species. The phylogenetic tree among directional fragments of each species based on the Gst was constructed using unweighted pair group method with arithmetic mean (UPGMA) method in Mega 4.0 software. Network (version 4.5.1.6) was used to reconstruct phylogenetic network of all subject fragments.

RESULTS AND DISCUSSION

Results of BLAST

A total of 80 fragments with identity of over 90% belonging to 3 species (*C. hircus*, *O. aries* and *B. taurus*) was obtained from GenBank after BLAST program (version 7.0.5) was carried out using the special fragment from goat *agouti* gene as query sequence (Table 1). There were 2 forward fragments in *C. hircus*, 6 forward and 12 reverse fragments in *O. aries* and 37 forward and 23 reverse fragments in *B. taurus*. The query sequence of the 136 bp fragment from goat *agouti* gene was not found in other animals. All of the forward and reverse fragments were renamed according to their origins and directional characteristics (Table 1).

Interestingly, it was found that the special 136 bp query fragment of goat *agouti* gene not only existed in *agouti* gene of *C. hircus* and *O. aries*, but also existed in other genes of *C. hircus*, *O. aries* and *B. taurus*. For example, the subjected sequence (AF506740) in *C. hircus* was *cyclin T1* related with transcription of HIV-1(Xavier et al., 2002); only OEUF5 (EU420022) and OEUF6 (EU420023) in *O. aries* were from *agouti*, none of the fragments in *B. taurus* were from *agouti* gene. In our previous study, we did not detect the special 136 bp fragment in the *agouti* gene of *O. aries* and *B. taurus* (Li et al., 2010), and the existence of OEUF5 and OEUF6 in sheep *agouti* gene illustrated that the special 136 bp fragment also existed in *O. aries agouti* gene, but not in the same region. The non-existence of the special 136 bp fragment in *B. taurus agouti* gene was in accordance with our previous study (Li et al., 2010), but it existed in other genes and clones of *B. taurus*, such as T cell receptor gamma cluster 1 (TCRG1) gene (AY644517), T cell receptor gamma cluster *2* (TCRG2) gene (AY644518), and X-inactivation center region in the *Jpx* and *Xist* gene (AJ421481), etc.

In *O. aries* and *B. Taurus,* it was found that the special 136 bp fragment existed in other genes and existed in different chromosomes, even in the same gene with forward and reverse sequences such as X-inactivation center region in the *Jpx* and *Xist* gene (AJ421481), *TCRG1* and *TCRG2* in *B. taurus* (Table 1). So it could be inferred that the special fragment might be a retrotransposon in these genes (Li et al., 2010).

Meanwhile, we also noticed that the special 136 bp query fragment not only existed in the non-coding region of some genes, such as ATP-binding cassette superfamily G member 2 transporter (ABCG2) gene (AJ871176), polycystic kidney disease 2 (PKD2) gene (AJ871176), amelogenin (AF083090), epidermal growth factor precursor (EGF) gene (AY192564) and signal transducer and activator of transcription 3 (STAT3) gene (AJ620661) of *B. taurus*, but also existed in the coding region of some genes or clones, such as the cDNA from early embryo (DN842617), liver (DV787781), fat (DV778412), brain (CO892005), spleen (BM364118) and loin (DV794276) of *B. taurus*. It could be inferred that the special 136 bp fragment might be transposed or recombined during the long period of evolution within and among species, and might be related to the expression of genes.

Genetic diversity of the special 136 bp fragment

There were 110 sites excluding sites with gaps after the 80 fragments (Table 1) which were treated with BioEdit and DnaSP, in which there were 43 invariable sites and 67 variable sites including 32 singleton variable sites (SP) and 35 parsimony informative sites (PIP). Among the 32 singleton variable sites, there were 28 sites with 2 variants, 3 sites with 3 variants and 1 site with 4 variants. There were 18 sites with 2 variants, 11 sites with 3 variants and 6 sites with 4 variants in the 35 parsimony informative sites. A total of 76 haplotypes (h) with high haplotype diversity (Hd) (0.952 and 1.000) were sorted from 80 fragments, illustrating the plentiful genetic diversity of these special 136 bp fragments. The nucleotide diversity (p) and the average number of nucleotide differences (K) for all sequences were 0.0578 and 6.3600, respectively. In *B. taurus*, the subject forward and reverse sequences had similar average number of nucleotide differences (6.697 and 6.830) and the nucleotide diversity (0.059 and 0.054), although, the forward sequences had higher number of fragments (N), polymorphic sites (S), mutations (Eta), SP, PIP and h than the reverse sequence. In contrast, the subject reverse sequence from *O. aries* had higher genetic diversity than the forward sequence (N: 13 and 7; S: 45 and 7; Eta: 56 and 24; SP: 24 and 9; PIP: 21 and 12; h: 13 and 6; Hd: 1.000 and 0.952; K: 13.321 and 9.619; P: 0.099 and 0.071). Meanwhile, we also noticed that the

Table1. BLAST results of the 136bp fragment in *Capra hircus agouti* gene

Species	Accession No.	Genes or clones	Location of the chrosome	Subject range (bp)	Strength (bp)	Identity (%)	Type	Renamed fragment
Capra hircus	AF506740	*CCNT*1		990-1129	140	93	F	SAFF1
	EF587236	*Agouti*		2286-2421	136	100	F	SEFF2
	EU420022	*ASIP*		1740-1879	140	95	F	OEUF5
	EU420023	Nonfunctional *ASIP*		1746-1885	140	96	F	OEUF6
	EE751186	*OAPP*		240-379	140	95	F	OEEF2
				42873-43004	121	90	F	OACF1
				18522-18392	130	90	R	OACR5
	AC148039	Clone CH243-463D18		58849-58710	140	91	R	OACR6
				99416-99275	140	91	R	OACR7
				130777-130644	133	92	R	OACR8
Ovis aries				125935-126064	130	90	F	OEUF3
				9772-9642	131	91	R	OEUR9
	EU185098	Clone INRA-164HB		14146-14017	130	90	R	OEUR10
				84799-84669	131	90	R	OEUR11
				140157-140018	140	96	R	OEUR12
	EU185099	Clone INRA-218G7		9542-9681	140	95	F	OEUF4
				23759-23630	130	90	R	OEUR4
				62545-62416	140	91	R	OACR1
	AC147927	Clone CH243-129I22		109844-109717	128	92	R	OACR2
				130215-130083	133	92	R	OACR3
				73854-73989	136	92	F	BAJF7
				111986-112120	135	92	F	BAJF8
				113471-113606	136	91	F	BAJF9
	AJ421481	X-inactivation center region in the *Jpx* and *Xist* genes	X	24196-24063	131	90	R	BAJR7
				107007-106964	134	90	R	BAJR8
				113218-113086	133	93	R	BAJR9
				115010-114877	134	90	R	BAJR10
				202353-202221	133	93	R	BAJR11
Bos taurus				226580-226719	140	92	F	BAYF14
				227210-227350	140	92	F	BAYF15
	AY644517	T cell receptor gamma cluster 1(*TCRG*1)	4	127434-127306	129	90	R	BAYR12
				57645-57516	130	91	R	BAYR13
				163960-163821	140	91	R	BAYR14
				19185-19315	131	90	F	BACF32
	AC150644	BAC CH-240-223I2	4	185146-185276	131	93	F	BACF33
				117643-117767	125	91	F	BACF34
				11911-11781	131	91	R	BACR1

Table 1 contd.

AJ871176	ABGG2, PKD2 and SPP1	6	130671-130543	139	93	R	BAJR3
			132132-131992	140	90	R	BAJR4
			24206-24333	128	90	F	BAJF6
AC150540	BAC CH240-385 H19	4	136497-136367	131	93	R	BACR5
			139540-139408	133	90	R	BACR6
			142308-142438	131	92	F	BACF35
			63657-63787	131	90	F	BACF36
			117658-117788	131	90	F	BACF37
AC107065	Clone rp42-513 g13	8	92482-92349	134	93	R	BACR15
			27618-27488	131	91	R	BACR16
			157570-157410	131	90	R	BACR17
			8243-8116	128	90	R	BACR18
			142954-142824	131	90	R	BACR19
			153019-152889	131	90	R	BACR20
			20751-20881	131	91	F	BACF22
AC149694	BAC CH240-423 c21	5	170067-169935	133	90	R	BACR21
			63956-63826	131	90	R	BACR22
			124325-124195	131	90	R	BACR23
			9261-9391	131	93	F	BACF25
			135160-135290	131	91	F	BACF26
			140740-140870	131	90	F	BACF27
			178358-178487	130	90	F	BACF28
			119217-119342	126	91	F	BACF29
			77870-78010	140	90	F	BACF30
			185779-185909	131	90	F	BACF31
AY644518	*TCRG2*	4	164674-164804	131	91	F	BAYF10
			14960-15090	131	91	F	BAYF11
			62848-62978	131	93	F	BAYF12
			37950-38089	140	91	F	BAYF13
AC236839	Y Chr CH240-203 F12		116366-116496	131	93	F	BACF23
			168166-168294	129	90	F	BACF24
EU915048	*IL*-5		2608-2747	140	92	F	BEUF1
AJ620661	*stat*3	19	639-778	140	93	F	BAJF2
AY192564	*EGF*	6	3836-3975	140	90	F	BAYF3
AF083090	Y-ch *amelogenin*		608-747	140	91	F	BAFF4
BC126641	cDNA 152009	X	1969-2108	140	91	F	BBCF5
EH209254	cDNA 86222675	3	486-625	140	92	F	BEHF16
DV787781	Liver cDNA	3	772-911	140	90	F	BDVF17

Table 1 contd

DV778412	CF-24-HW fat cDNA	26	221-360	140	90	F	BDVF18
CO892005	Brain cDNA	3	390-529	131	92	F	BCOF19
BM364118	Spleen cDNA	7	319-458	140	91	F	BBMF20
DV794276	Loin cDNA		111-241	131	91	F	BBDF21
DN842617	Early embryo cDNA	13	94-233	130	94	R	BDNR2

A total of 80 fragments with identity of over 90% belonging to 3 species (*C. hircus, O. aries* and *B. taurus*) were obtained from GenBank in NCBI with the BLAST program (human genomic plus transcript (human G + T), mouse genomic plus transcript (Mouse G+T) and nucleotide collection (nr/nt) databases). The symbol of F and R represents the abbreviation of forward (5'→3') and reverse (3'→5') direction, respectively.

subject reverse sequence from *O. aries* had the largest value of K (13.321) and p (0.099), although, it had lower value of N, S, Eta, SP, PIP and h than the forward and reverse fragments from *B. taurus*. So, it could be inferred that the special 136 bp query fragment might evolve differently between *O. aries* and *B. taurus*.

Origin and differentiation of the special 136 bp fragment

Phylogenetic tree of these special 136 bp fragments from different species

The genetic distance (Gst) of the forward and reverse subject fragments from different species was analyzed by DNAsp (version 5.0), and their phylogenetic tree based on the Gst is shown in Figure 1. The smallest genetic distance (0.002) was in *B. taurus* F. The largest genetic distance (0.097) existed between *B. taurus* F and *C. hircus* F. The relative smaller genetic distance (0.009) was also detected between *O. aries* F and *O. aries* R. Meanwhile, the genetic distance (0.003 and 0.033) between *O. aries* and *B. taurus* was smaller than that (0.087 and 0.097) between *C. hircus* and *B. taurus*, and that (0.052 and 0.069) between *C. hircus* and *O. aries*. It was obvious that there was a smaller genetic differentiation for this special 136 bp fragment within species, and larger genetic differentiation among species, even if they existed in forward and reverse style. The genetic differentiation of the special 136 bp fragment between *B. taurus* and *O. aries* was smaller than that between *B. taurus* and *C. hircus*.

Phylogenetic network of these special 136 bp fragments

The phylogenetic network of all subject fragments with median vectors based on their variable sites is shown in Figure 2. The phylogenetic network was constructed first using all subject fragments, from which 9 torso fragments were gotten: BDVF18 (DV778412), BACF33 (AC150644,), OEEF2 (EE751186), BAYF12 (AY644518), BAJR9 (AJ421481), BACF25 (AC236839), OEUR12 (EU185098), OEUF6 (EU420023) and BACR5 (AC150540) as shown in Figure 2A with blue color. Then, 2 novel torso fragments, BACR5 (AC150540) and OEEF2 (EE751186) shown in Figure 2B with green color, were gotten after 11 fragments including 9 torso fragments and two goat fragments (SAFF1 and SAFF2) which were used to reconstruct phylogenetic network. Among the 80 subject sequences, the 9 torso fragments could be considered as the latest original sequences, in which 6 (BDVF18, BACF33, BAYF12, BAJR9, BACF25 and BACR5) were from *B. taurus* and 3 (OEEF2, OEUR12, and OEUF6) were from *O. aries*. The 2 novel torso fragments, BACR5 from *B. taurus* and OEEF2 from *O. aries*, could be considered as the far most ancestral sequences.

It was also obvious from Figure 2B that the SEFF2 of *C. hircus* originated from the OEUF6 (one of the 9 latest original sequences) of *O. aries* without median vector, and the SAFF1 of *C. hircus* originated from the OEEF2 of *O. aries* with two median vectors (mv2 and mv5). It was consistent with the genetic distance (0.052 or 0.069) between *C. hircus* F and *O. aries* F (or R) which was smaller than that (0.097 or 0.087) between *C. hircus* F and *B. taurus* F (or R), and also consistent with their relationship (Figure 1).

Conclusion

It could be concluded that the special 136 bp fragment existed in the *agouti* gene of *C. hircus* and *O. aries* and did not appear in the *agouti* gene of *B. taurus*, but existed in coding or non-coding regions with forward and reverse sequences in other genes of *O. aries* and *B. taurus*. This special fragment had high genetic diversity in the involved genome. The number of the forward sequences was higher than that of the reverse ones but with similar genetic diversity in *B. taurus*, while the reverse sequences from *O. aries* had higher genetic diversity

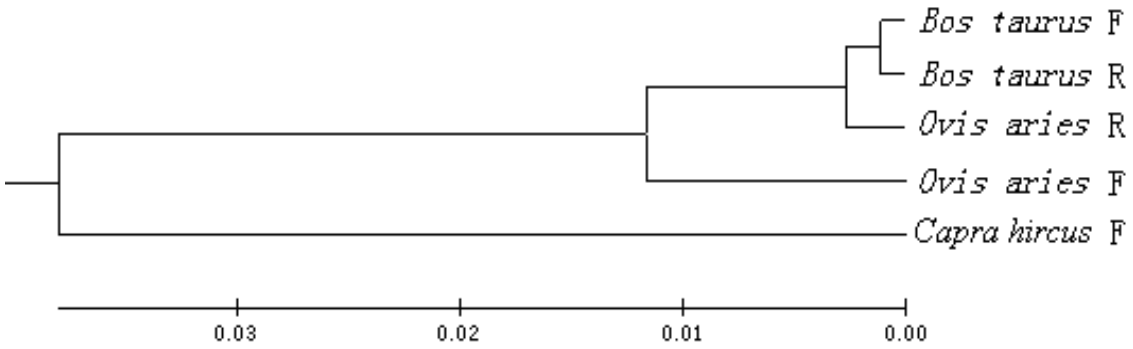

Figure 1. The phylogenetic tree among directional fragments of each species based on the Gst was constructed using unweighted pair group method with arithmetic mean (UPGMA) method in Mega 4.0 software.

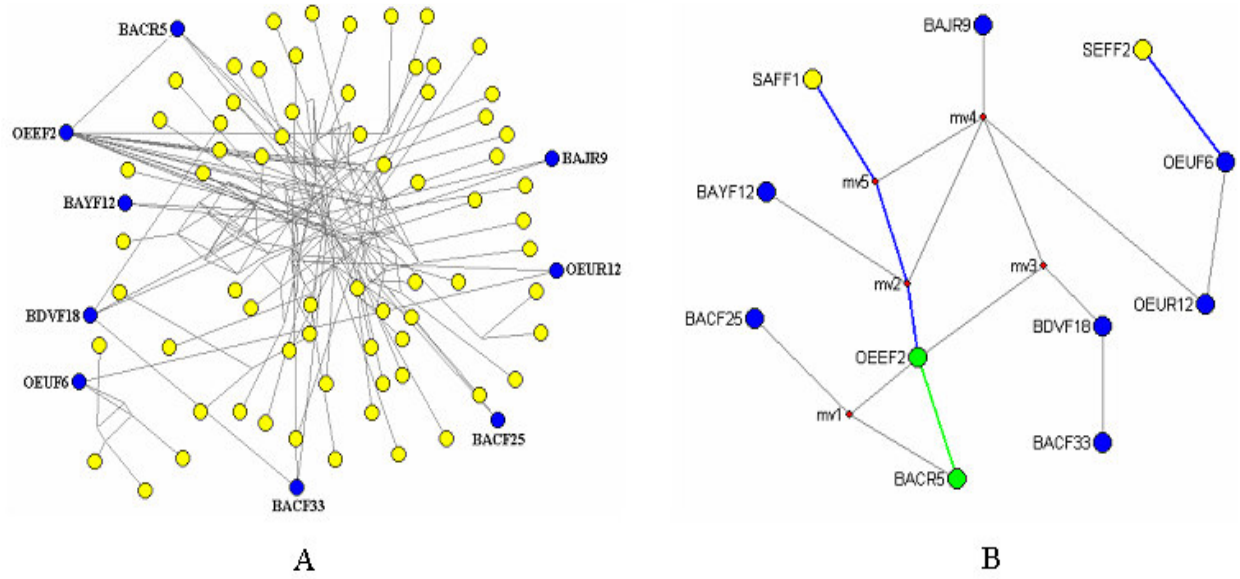

Figure 2. The phylogenetic network of all subject fragments was gained with Network (version 4.5.1.6) based on their variable sites. The yellow and red solid circles represent the subject fragments and median vectors, respectively. The blue and green solid circles represent the torso sequences.

than the forward ones. The BACR5 (AC150540) from *B. taurus* and OEEF2 (EE751186) from *O. aries* could be considered as the farmost ancestral sequences. The differentiation between *B. taurus* and *C. hircus* was more obvious than between *B. taurus* and *O. aries*, but not within species.

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