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Polymorphism of *AA-NAT* gene and its relationship with litter size of Jining Grey goat of China*

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The polymorphisms of arylalkylamine-N-acetyltransferase (AA-NAT) gene in high-prolificacy Jining Grey goat, medium-prolificacy Boer goat and low-prolificacy Liaoning Cashmere, Inner Mongolia Cashmere and Angora goats were detected to analyse their relationships with litter size. Primers (P1-P5) were designed to detect the polymorphisms by PCR-SSCP and PCR-RFLP. For P2, *AA, AB* and *BB* genotypes were detected. Sequencing revealed one silent mutation (T132C) of *AA-NAT* gene in *BB* in comparison to *AA.* For P3, *CC* and *CD* genotypes were detected and sequencing revealed one mutation (C265T) of *AA-NAT* gene in *CD* in comparison to *CC*, and this mutation resulted in an amino acid change of Arg→Cys (R89C). The Jining Grey does with genotype *CD* delivered by 0.56 kids ($P<0.05$) more than those with *CC* genotype. For P5, *EE*, *EF* and *FF* genotypes were detected and sequencing revealed one mutation (C586T) of *AA-NAT* gene in *FF* in comparison

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to *EE*. This mutation caused an amino acid change of Arg→Trp (R196W). For both P2 and P5, the differences in litter size among three genotypes were not significant in Jining Grey goats (P>0.05). These results preliminarily indicate that allele D at the C265T *locus* of *AA-NAT* gene is a potential marker in genetic improvement of litter size in goats.

KEY WORDS: arylalkylamine-N-acetyltransferase (*AA-NAT*) gene / goat / polymorphism / prolificacy

Arylalkylamine-N-acetyltransferase (AA-NAT) belongs to the AA-NAT family, which is part of the large Gcn5-related acetyltransferase (G-NAT) superfamily [Dyda *et al*. 2000]. Melatonin (MLT) is synthesized with sequential enzymatic reaction from serotonin by the action of AA-NAT and hydroxyindole-O-methyltransferase (HIOMT), in which AA-NAT produces N-acetylserotonin (NAS) from serotonin and HIOMT catalyzes NAS into N-Acetyl-5-methoxytryptamine (MLT) - Ganguly *et al*. [2002], Park *et al*. [2010]. Earlier studies indicated that MLT rhythm was an important factor in both long- or short-day breeds to adjust physiological testicular and ovarian changes in different seasons [Barrett *et al*. 2003, Lincoln 2006, Dupre *et al*. 2008]. MLT can induce estrous cycles and is associated with an improvement of the ovulation rate [Zuniga *et al*. 2002] and litter size [Scott *et al*. 2009], enhances luteal function [Abecia *et al*. 2002], greater embryo viability [Forcada *et al*. 2006, Abecia *et al*. 2008], ovarian response to the ram effect [Abecia *et al*. 2006], percentage of progressive motile spermatozoa and number of spermatozoa attached per oocyte [Casao *et al*. 2010]. Because AA-NAT is a rate-limiting enzyme in MLT synthesis pathway involved in circadian oscillations of MLT levels [Zheng *et al*. 2001, Soria *et al*. 2010], AA-NAT is important for animal reproduction. However, to date, the association between polymorphism of *AA-NAT* and reproduction in animals remains unknown.

Human *AA-NAT* gene maps to chromosome 17q25 and has four exons separated by three introns, all of which are present within a 2.5 kb genomic region [Steven *et al*. 1996]. Among the four exons, exon 1 is untranslated, while the other three code for a 207-amino acid protein and are 238, 155 and 453 bp long, respectively [Steven *et al*. 1996]. The cDNA sequences of *AA-NAT* gene differ slightly among human, rat, mouse, cattle and sheep, and the homology ranges from 77% to 96.1%.

Most local goat breeds of China are of low prolificacy, but Jining Grey goat in Shandong Province has significant characteristics of sexual precocity, year-round estrus and high prolificacy. The mean litter sizes (born alive) of Jining Grey, Boer, Angora, Liaoning Cashmere and Inner Mongolia Cashmere goats have been reported to reach 2.94 [Tu 1989], 2.10 [Malan 2000], 1.31 [Roberts and Reeves 1988], 1.12 [Tu 1989] and 1.04 [Tu 1989], respectively.

The objectives of the present study were to detect the single nucleotide polymorphism (SNP) of *AA-NAT* in goats of five breeds with different prolificacy (Jining Grey(JG) – high, Boer (BO) – medium, and low – Angora goat(AN), Liaoning Cashmere(LC) and Inner Mongolia Cashmere(IM) goats) by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) and polymerase chain reaction-restriction

fragment length polymorphism (PCR-RFLP), and to investigate the association between polymorphism in *AA-NAT* gene and prolificacy in JG goat.

Material and methods

Animals and genomic DNA isolation

Samples of jugular vein blood (10 ml from a doe) on ACD anticoagulant were withdrawn from the following groups (breeds) of animals in China: 294 JG does in Shandong Province, 46 IM does in Inner Mongolia Autonomous Region, 54 LC does in Liaoning Province, 60 BO does and 41 AN does from Shanxi Province.

Genomic DNA was extracted from whole blood using phenol-chloroform method [Sambrook *et al*. 2002], then dissolved in TE buffer (10 mmol/l Tris-HCl [pH 8.0], 1 mmol/l EDTA [pH 8.0]) and kept at -20°C.

The 294 JG goat does were selected at random, being the progeny of five sires (n=52, 56, 60, 61, 65). Their blood samples were collected for genotype analysis and their litter sizes were recorded. Because the five bucks were sold, their blood was not collected for genotyping. No selection for litter size or other fertility traits was performed in the flocks over previous years. Kiddings were grouped into four 3-month seasons: March to May (season 1, spring, n=75), June to August (season 2, summer, n=69), September to November (season 3, autumn, n=84) and December to February (season 4, winter, n=66).

Primers and PCR amplification

Five pairs of primers (P1 to P5) were designed to amplify the *AA-NAT* gene and detect polymorphisms. Primers P1 and P5 were cited from Yu [2007], of which P1 was used to amplify the DNA sequence from partial exon 2 to partial exon 4**.** PCR was carried out in 25 µl volume containing approximately 0.5 µl of 10 µmol/l each primer, 2.5 µl of 10×PCR buffer (50 mmol/l KCl, 10 mmol/l Tris-HCl (pH 8.0), 0.1% Triton X-100), 1.5 µl of 25 mmol/l $MgCl_2$, 2.5 µl of 2.5 mmol/l each dNTP, 2.0 µl of 50 ng/µl caprine genomic DNA, 0.5 µl of 2.5 U/µl *Taq* DNA polymerase (PROMEGA, Madison, WI, USA) made up with H_2O. Amplification conditions were as follows: initial denaturation at 94°C for 5 min; followed by 34 cycles of denaturation at 94°C for 30 s, annealing at 59°C for 30 s, extension at 72°C for 1 min with a final extension at 72°C for 7 min on Mastercycler® 5333 (EPPENDORF, AG, Hamburg, Germany). Five randomly selected DNA samples from JG and LC goats were used to amplify by primer P1. The PCR products were cloned and sequenced, then aligned to identify potential polymorphic sites.

Primers P2, P3 and P4 were designed according to the amplified sequences of primer P1. PCR of primers P2, P3, P4 and P5 was carried out in 25 µl volume the same as primer P1 except for the annealing temperature (shown in Table 1) and extension times (at 72°C for 30 s).

Table 1. The primer sequence, amplified region, product size and annealing temperature of five pairs of primers analysed for goat *AA-NAT* gene

Primer	Primer sequence (5'→3')	Amplified region	Product size (bp)	Annealing temperature (°C)
P1	F:AGCGTCCACTGCCTGAAAC R:GGGATGGAAGCCAAACCTC	Through partial exon 2 to partial exon 4	1142	59
P2	F:ATGTCCACGCCGAGCATCCACT R:CCTCTCGCTCAATCTCAAACACG	Exon 2	163	59
P3	F:CCTGACACAGCCTTCATCTCT R:CCATCCTCACCTGAGTAAGT	Exon 3 and flanking	175	59
P4	F:TTTTGGGGTGCACAGAGGCGAC R:GGAAGCCGAACCTCTGGTAG	Partial exon 4 and flanking	252	61
P5	F:GGTTTGGCTTCCATCCCG R:ACCGTTTCCCTTCAGACCAAG	Partial exon 4 and 3' untranslation region	219	60

The primer sequence, product size, amplified region and annealing temperature are listed in Table 1. The primers were synthesized by Shanghai INVITROGEN Biotechnology Co. Ltd. (Shanghai, China).

SSCP analysis

PCR products of primers P2, P4 and P5 were used for SSCP analysis. A volume of 3 µl PCR product was transferred into the Eppendorf tube, mixed with 7 µl gel loading solution containing 98% formamide, 0.025% bromophenol blue, 0.025% xylene cyanol, 20 mmol/l EDTA (pH 8.0) and 10% glycerol. The mixture was centrifuged and denatured at 98°C for 10 min, then chilled on ice for 7 min and loaded on 12% neutral polyacrylamide gels (acrylamide:bisacrylamide=29:1). Electrophoresis was performed in 1×Tris borate (pH 8.3)-EDTA buffer at 9-15 V/cm for 14-16 hours at 4°C. The gels were stained with silver nitrate to identify SSCP, then photographed and analysed using an AlphaImager™ 2200 and 1220 Documentation and Analysis Systems (ALPHA INNOTECH Corporation, San Leandro, CA, USA).

RFLP analysis

Based on the results of sequence of P1, one mutation was revealed in exon 3 and restriction enzyme *Hae*III might be used to detect it. So the PCR products of P3 were digested by *Hae*III (NEB, Beijing, China) with 10 µl volume containing 5 µl of PCR product, 0.5 µl of 10 U/µl restriction enzyme *Hae*III, 1 µl 10× reaction buffer, 3.5 µl H_2O, incubated at 37°C for 5 h. The mixtures were detected by 3.0% agarose gels and genotyped using an AlphaImager™ 2200 and 1220 Documentation and Analysis Systems (ALPHA INNOTECH Corporation, San Leandro, CA, USA).

Cloning and sequencing

The PCR products of primer P1 and different genotypes analysed by SSCP were separated on 0.8% agarose gels, purified using Geneclean II kit (PROMEGA, Madison,

WI, USA) and inserted into the pGEM-T Easy vector (PROMEGA) according to the manufacturer's instructions. The recombinant plasmid was transformed into *E. coli* DH5α competent cell. Positive clones were identified by restriction enzyme digestion. Three positive clones of each genotype were sequenced in both directions for each individual using an ABI3730 automatic sequencer (PERKIN ELMER Applied Biosystems, Foster City, CA, USA) by Shanghai INVITROGEN Biotechnology Co. Ltd. (Shanghai, China).

For primers P2 and P5, the homozygotes detected by SSCP were also sequenced.

Statistical analysis

Chi-square test for allele and genotype frequencies was used to test whether the population remained in the Hardy-Weinberg equilibrium.

The following fixed-effects model was employed for analysis of litter size in JG goat does and least squares mean was used for multiple comparison in litter size among different genotypes.

$$y_{ijklm} = \mu + S_i + KS_j + P_k + G_l + e_{ijklm,}$$

where:

y_{ijklm} – the phenotypic value of litter size;

μ – the population mean;

S_i – the fixed effect of the i^{th} sire (i = 1, 2, 3, 4, 5);

KS_j – the fixed effect of the j^{th} kidding season (j = 1, 2, 3, 4);

P_k – the fixed effect of the k^{th} parity (k = 1, 2, 3);

G_l – the fixed effect of the l^{th} genotype (l = 1, 2, 3 or l = 1, 2);

e_{ijklm} – the random residual effect of each observation.

Analysis was performed using the general linear model procedure of SAS (Ver 8.1) (SAS Institute Inc., Cary, NC, USA). Mean separation procedures were conducted using the least significant difference test.

All procedures involving animals were approved by the animal care and use committees at the institutions where the investigations were conducted and authorized by the Chinese Ministry of Agriculture.

Results and discussion

PCR amplification of goat *AA-NAT* gene

Genomic DNA of goats of five breeds was successfully amplified using P1-P5 primers for *AA-NAT* gene. The PCR products were separated on 2.0% agarose gels (Fig. 1). The results showed that amplification fragment sizes were consistent with the target ones and had good specificity, which could directly be analysed by sequencing or SSCP and RFLP.

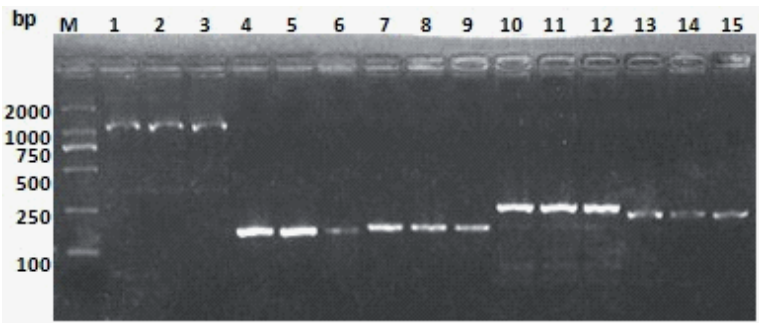

Fig. 1. PCR products of five pairs of primers. M: DL 2000 DNA Marker (TaKaRa, Dalian); Lanes 1 to 3: primer P1; Lanes 4 to 6: primer P2; Lanes 7 to 9: primer P3; Lanes 10 to 12: primer P4; Lanes 13 to 15: primer P5.

SSCP analysis of primers P2, P4 and P5 and sequencing of different genotypes

No polymorphism was identified in PCR products amplified by primer P4 (Fig. 2). The PCR products amplified by primers P2 and P5 displayed polymorphisms. For primer P2, three genotypes (*AA*, *AB* and *BB*) were detected (Fig. 3). Sequencing of homozygous amplicons revealed one silent mutation (T132C) in the CDS region in genotype *BB* compared to genotype *AA* according to mRNA sequence of ovine *AA-NAT* gene (GenBank No. NM_001009461) (Fig. 4). For primer P5, three genotypes (*EE*, *EF* and *FF*) were detected (Fig. 5). Sequencing of homozygous amplicons revealed

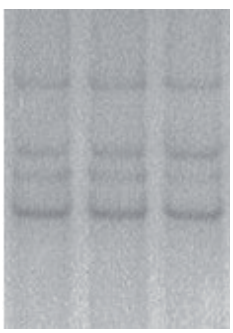

Fig. 2. SSCP analysis of PCR products of primer P4.

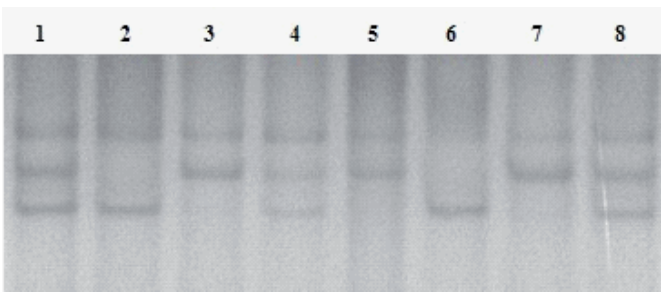

Fig. 3. SSCP analysis of PCR products of primer P2. Lanes 3, 5 and 7: *AA* genotype; Lanes 2 and 6: *BB* genotype; Lanes 1, 4 and 8: *AB* genotype.

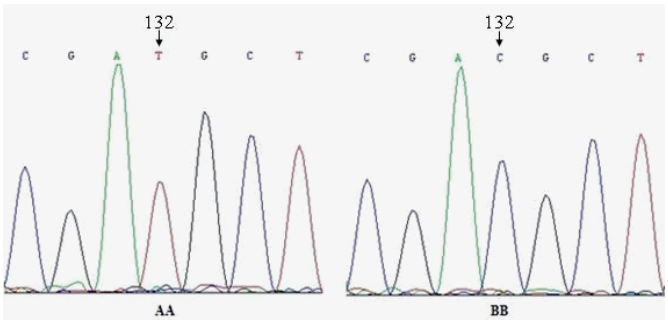

Fig. 4. Nucleotide mutation between genotypes *AA* and *BB* in caprine *AA-NAT* gene.

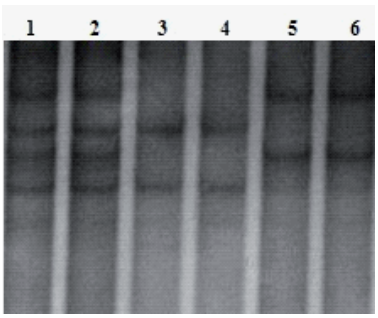

Fig 5. SSCP analysis of PCR products of primer P5. Lanes 3 and 4: *EE* genotype; Lanes 5 and 6: *FF* genotype; Lanes 1 and 2: *EF* genotype.

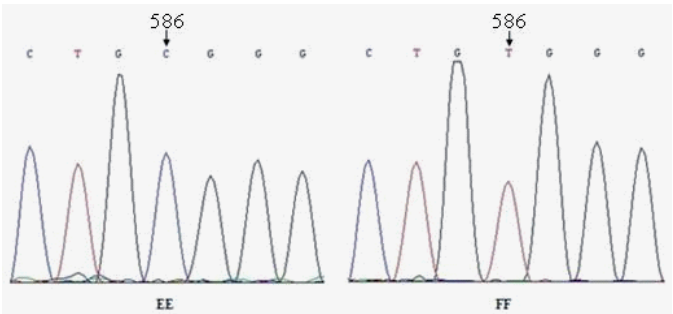

Fig 6. Nucleotide mutation between genotypes *EE* and *FF* in caprine *AA-NAT* gene.

one mutation (C586T) in the CDS region of caprine *AA-NAT* gene in genotype *FF* compared to genotype *EE* (Fig. 6) and this mutation caused an amino acid change of Arg→Trp (R196W).

RFLP analysis of primer P3 and sequencing of different genotypes

Sequencing of amplicon obtained with primer P1 showed one SNP in exon 3 (C265T). Thus, the PCR products of P3 were digested by restriction enzyme *Hae*III.

Two genotypes *(CC, CD*) were detected (Fig. 7). Because of existing more than one *Hae*III restriction sites in the fragment, the bands of genotype *CC* and *CD* should be 110/54/11 bp and 121/110/54/11 bp, respectively. It means that two bands, 121 bp and 54 bp, would make the electrophoretic pattern of *DD* genotype because of loss of restriction site for *Hae*III enzyme at 265 nt. Altogether, for primer P3, sequencing and restriction enzyme analysis revealed one mutation (C265T) in the CDS region of caprine *AA-NAT* gene as compared to *CC* according to NM_001009461 (Fig. 8), and this mutation resulted in an amino acid change of Arg→Cys (R89C).

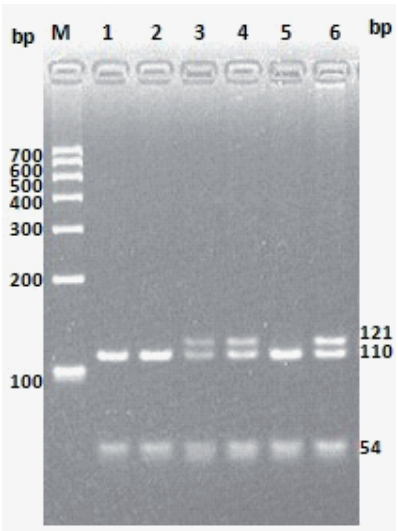

Fig. 7. RFLP analysis of PCR products of primer P3. M: DNA Marker I (Biomed, Beijing); Lanes 1, 2 and 5: *CC* genotype; Lanes 3, 4 and 6: *CD* genotype.

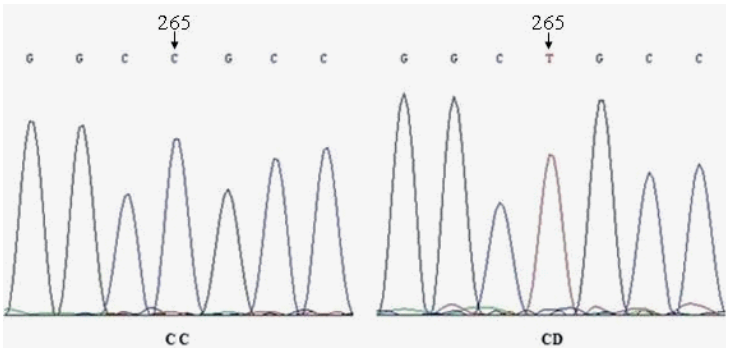

Fig. 8. Nucleotide mutation between genotypes *CC* and *CD* in caprine *AA-NAT* gene.

Allele and genotype frequencies of AA-NAT gene in five goat breeds

Allele and genotype frequencies of *AA-NAT* gene in five goat breeds are presented in Table 2. For primers P2, P3 and P5, Chi-square test showed that the JG goat population did not remain in Hardy-Weinberg equilibrium ($P<0.01$), in which genotype frequencies had been distorted by mutation, migration or other reasons.

As demonstrated in Table 2, for the T132C *locus*, *AA* showed the highest frequency in BO goats, whereas in AN goats a higher frequency was noticed for *BB* genotype; for the C265T *locus*, the *DD* genotype was not found and the highest frequency of *CD* was found in BO; for the C586T *locus*, LC was the only monomorphic breed with just *EE* genotype.

Table 2. Allele and genotype frequencies of *AA-NAT* gene in goats of five breeds

Locus	Breed		Jining Grey goat (JG)	Boer goat (BO)	Angora goat (AN)	Inner Mongolia Cashmere goat (IM)	Liaoning Cashmere goat(LC)
T132C	N of does		282	57	41	46	54
	Genotype frequency	*AA*	0.33(94)	0.82(47)	0.00(0)	0.26(12)	0.28(15)
		AB	0.59(166)	0.16(9)	0.10(4)	0.57(26)	0.48(26)
		BB	0.08(22)	0.02(1)	0.90(37)	0.17(8)	0.24(13)
	Allele frequency	*A*	0.63	0.90	0.05	0.54	0.52
		B	0.37	0.10	0.95	0.46	0.48
C265T	N of does		294	60	40	46	52
	Genotype frequency	*CC*	0.35(104)	0.02(1)	0.52(21)	0.65(30)	0.63(33)
		CD	0.65(190)	0.98(59)	0.48(19)	0.35(16)	0.37(19)
	Allele frequency	*C*	0.68	0.51	0.76	0.83	0.82
		D	0.32	0.49	0.24	0.17	0.18
C586T	N of does		278	60	38	46	53
	Genotype frequency	*EE*	0.79(220)	0.97(58)	0.76(29)	0.86(40)	1.00(53)
		EF	0.14(40)	0.00(0)	0.13(5)	0.07(3)	0.00(0)
		FF	0.07(18)	0.03(2)	0.11(4)	0.07(3)	0.00(0)
	Allele frequency	*E*	0.86	0.97	0.83	0.90	1.00
		F	0.14	0.03	0.17	0.10	0.00

The capital letters in the brackets are symbols of breed names.
The numbers in the brackets are the individuals that belong to the respective genotypes.

Influence of fixed effects on litter size in JG goats

Litter size in JG goats was significantly influenced by sire, kidding season and parity (all $P<0.05$). The least squares means and standard errors for litter size of different *AA-NAT* genotypes in JG goats are given in Table 3.

Concerning primers P2 and P5, no difference ($P>0.05$) was found in litter size among different genotypes in JG goats. Regarding primer P3, the JG goats of genotype *CD* have delivered by 0.56 ($P<0.05$) kids more than those of genotype *CC*.

AA-NAT gene displayed wide polymorphisms. Hitherto, 130 SNPs were published in NCBI databases including *Bos taurus* (10), *Gallus gallus* (17), *Mus musculus* (72), *Canis familiaris* (4), *Pan troglodytes* (2), *Homo sapiens* (21), *Rattus norvegicus* (2)

Table 3. Least squares means and standard errors for litter size of different genotypes of *AA-NAT* gene in Jining Grey goats

Locus	Genotype	Number of does	Litter size
T132C	*AA*	94	2.35^{a}±0.16
	AB	166	2.11^{a}±0.13
	BB	22	2.00^{a}±0.18
C265T	*CC*	104	1.82^{b}±0.17
	CD	190	2.38^{a}±0.14
C586T	*EE*	220	2.15^{a}±0.13
	EF	40	2.27^{a}±0.18
	FF	18	2.35^{a}±0.21

[a]Within the *locus* means bearing different superscripts are significantly different at P<0.05.

and *Danio rerio* (2). Sekine *et al*. [2001] found initially four SNPs in Japanese people by direct sequencing: two mutations (G-542T, C-263G) in the 5' flanking region, one in intron 3 (T39A), and one silent mutation in exon 4 (C150T). Except for C-263G mutation, Hohjoh *et al*. [2003] detected another three mutations in exon 4 of *AA-NAT* gene in Japanese individuals (n=200) including G619A, C702T and C756T, of which the former one caused an alanine to threonine change at position 129 (Ala129Thr). Ciarleglio *et al*. [2008] identified a C/G mutation (rs4238989) in human subjects from various global populations.

Yu [2007] detected four mutations in ovine *AA-NAT* gene consisting of an A→G mutation in exon 3 and three mutations in 3′ flanking region (C→G at 120 bp, C→G at 131 bp and A→G at 151 bp). In the present study, three mutations (C132T, C265T and C586T) were identified in exon 2, 3 and 4 of caprine breeds, respectively. Moreover, the latter mutation led to a change of positively charged amino acid Arg to nonpolar amino acid Trp.

AA-NAT is a critical enzyme involved in MLT biosynthesis. MLT plays a key role in regulation of the reproductive system of seasonal estrous animals. In ewes, MLT can induce estrous cycle, increase the ovulation rate [Zuniga *et al*. 2002] and litter size [Scott *et al*. 2009], enhance luteal function [Abecia *et al*. 2002], improve embryo viability [Forcada *et al*. 2006; Abecia *et al*. 2008] and enhance ovarian response to the ram effect [Abecia *et al*. 2006]. In rams, MLT can increase percentage of progressive motile spermatozoa and number of spermatozoa attaching oocytes [Casao *et al*. 2010]. Therefore, as the rate-limiting enzyme in MLT biosynthesis, AA-NAT is also critical for animal reproductive system.

In retinas, AA-NAT regulated by clocks and light had been reported to influence reproductive function in mammals through driving the daily rhythm in MLT production. The clock could generate a rhythm of *AA-NAT* gene transcription by interactions between circadian clock proteins (BMAL:CLOCK/MOP4) and an E-box enhancer element in its promoter, which leads to a circadian rhythm of AA-NAT mRNA and protein. Moreover, cAMP-dependent phosphorylation of transcription factors could enhance the E-box-driven increase of *AA-NAT* mRNA [Iuvone *et al*. 2005].

Yu [2007] found that allele A at the *Sma*I-NAT1 *locus* was associated with nonseasonal estrus in sheep, while allele B at the NAT4 *locus* had an association with seasonal estrus in sheep breeds. However, the associations between polymorphism of *AA-NAT* and litter size of animals were unknown.

In conclusion, the present study detected three SNPs of *AA-NAT* gene in five goat breeds and revealed that allele D at the C265T *locus* of *AA-NAT* gene maybe a potential marker for improving litter size in goats. This novel mutation provides further evidence that *AA-NAT* gene plays a key role in reproduction. Because of the lack of functional data, the conclusion is not solid and further studies are necessary for function validation of polymorphisms in question.

REFERENCES

1. ABECIA J.A., FORCADA F., CASAO A., VALARES J.A., ZUNIGA O., PALACIN I., 2008 – Effect of exogenous melatonin on the ovary, the embryo and the establishment of pregnancy in sheep. *Reproduction in Domestic Animals* 2, 399-404.
2. ABECIA J.A., FORCADA F., ZUNIGA O., 2002 – The effect of melatonin on the secretion of progesterone in sheep and on the development of ovine embryos in vitro. *Veterinary Research Communications* 26, 151-158.
3. ABECIA J.A., PALACIN I., FORCADA F., VALARES J.A., 2006 – The effect of melatonin treatment on the ovarian response of ewes to the ram effect. *Domestic Animal Endocrinology* 31, 52-62.
4. BARRETT P., SCHUSTER C., MERCER J., MORGAN J.P., 2003 – Sensitization: a mechanism for melatonin action in the pars tuberalis. *Journal of Neuroendocrinology* 15, 415-421.
5. CASAO A., VEGA S., PALACIN I., PEREZ-PE R., LAVINA A., QUINTN F.J., SEVILLA E., ABECIA J.A., CEBRIAN-PEREZ J.A., FORCADA F., MUINO-BLANCO T., 2010 – Effects of melatonin implants during non-breeding season on sperm motility and reproductive parameters in Rasa Aragonesa rams. *Reproduction in Domestic Animals* 45, 425-432.
6. CIARLEGLIO C.M., RYCKMAN K.K., SERVICK S.V., HIDA A., ROBBINS S., WELLS N., HICKS J., LARSON S.A., WIEDERMANN J.P., CARVER K., HAMILTON N., KIDD K.K., KIDD J.R., SMITH J.R., FRIEDLAENDER J., MCMAHON D.G., WILLIAMS S.M., SUMMAR M.L., JOHNSON C.H., 2008 – Genetic differences in human circadian clock genes among worldwide populations. *Journal of Biological Rhythms* 23(4), 330-340.
7. DUPRE S.M., BURT D.W., TALBOT R., DOWNING A., MOUZAKI D., WADDINGTON D., MALPAUX B., DAVIS J.R., LINCOLN G.A., LOUDON A.S., 2008 – Identification of melatonin regulated genes in the ovine pars tuberalis, a target site for seasonal hormonal control. *Endocrinology* 149, 5527-5539.
8. DYDA F., KLEIN D.C., HICKMAN A.B., 2000 – GCN5-related N-acetyltransferases: a structural overview. *Annual Review of Biophysics and Biomolecular Structure* 29, 81-103.
9. FORCADA F., ABECIA J.A., CEBRIAN-PEREZ J.A., MUINO T., VALARES J.A., PALACIN I., CASAO A., 2006 – The effect of melatonin implants during the seasonal anestrous on embryo production after superovulation in aged high-prolificacy Rasa Aragonesa ewes. *Theriogenology* 65, 356-365.
10. GANGULY S., COON S.L., KLEIN D.C., 2002 – Control of melatonin synthesis in the mammalian pineal gland: the critical role of serotonin acetylation. *Cell and Tissue Research* 309(1), 127-137.
11. HOHJOH H., TAKASU M., SHISHIKURA K., TAKAHASHI Y., HONDA Y., TOKUNAGA K., 2003 – Significant association of the arylalkylamine N-acetyltransferase (AA-NAT) gene with delayed sleep phase syndrome. *Neurogenetics* 4(3),151-153.

12. IUVONE P.M., TOSINI G., POZDEYEV N., HAQUE R., KLEIN D.C., CHAURASIA S.S., 2005 – Circadian clocks, clock networks, arylalkylamine N-acetyltransferase, and melatonin in the retina. *Progress in Retinal and Eye Research* 24(4), 433-456.
13. LINCOLN G.A., 2006-Decoding the nightly melatonin signal through circadian clockwork. *Molecular and Cellular Endocrinology* 252, 69-73.
14. MALAN S.W., 2000 – The improved Boer goat. *Small Ruminant Research* 36(2), 165-170.
15. PARK O.K., YOO K.Y., LEE C.H., CHOI J.H., HWANG I.K., PARK J.H., KWON Y.G., KIM Y.M., WON M.H., 2010 – Arylalkylamine N-acetyltransferase (AANAT) is expressed in astrocytes and melatonin treatment maintains AANAT in the gerbil hippocampus induced by transient cerebral ischemia. *Journal of the Neurological Sciences* 294(1-2), 7-17.
16. ROBERTS A.J., REEVES J.J., 1988 – Kidding rates of Angora goats passively immunized against estrogens. *Journal of Animal Science* 66(10), 2443-2447.
17. SAMBROOK J., RUSSELL D.W., 2002 – Molecular Cloning: a Laboratory Manual. Translation from English by Huang Pei Tang. Edition 3, Science Press, Beijing, China.
18. SCOTT P.R., SARGISON N.D., MACRAE A.I., GOUGH M.R., 2009 – Melatonin treatment prior to the normal breeding season increases fetal number in United Kingdom sheep flocks. *The Veterinary Journal* 182, 198-202.
19. SEKINE A., SAITO S., IIDA A., MITSUNOBU Y., HIGUCHI S., HARIGAE S., NAKAMURA Y., 2001 – Identification of single-nucleotide polymorphisms (SNPs) of human N-acetyltransferase genes NAT1, NAT2, AANAT, ARD1, and L1CAM in the Japanese population. *Journal of Human Genetics* 46(4), 314-319.
20. SORIA V., MARTÍNEZ-AMORÓS E., ESCARAMÍS G., VALERO J., CRESPO J.M., GUTIÉRREZ-ZOTES A., BAYÉS M., MARTORELL L., VILELLA E., ESTIVILL X., MENCHÓN J.M., GRATACŇS M., URRETAVIZCAYA M., 2010 – Resequencing and association analysis of arylalkylamine N-acetyltransferase (AANAT) gene and its contribution to major depression susceptibility. *Journal of Pineal Research* 49(1), 35-44.
21. STEVEN L.C., KRZYSZTOF M., MARIANNE B., PATRICK H.R., DAVID C.K., IGNACIO R.R., 1996 – The human SerotoninN- Acetyltransferase (EC 2.3.1.87) gene (AANAT): structure, chromosomal localization, and tissue expression. *Genomics* 34, 76–84.
22. TU Y.R., 1989 – The Sheep and Goat Breeds in China. Shanghai Science and Technology Press, Shanghai, pp. 88–90, 98–101, 112-114.
23. YU C.J., 2007 – Studies on genetic polymorphism of AA-NAT and HIOMT genes in nonseasonal and seasonal oestrus sheep. Yangling, Shaanxi province, China. Dissertation, Northwest A & F University.
24. ZHENG W., SCHEIBNER K.A., HO A.K., COLE P.A., 2001 – Mechanistic studies on the alkyltransferase activity of serotonin N-acetyltransferase. *Chemistry & Biology* 8(4), 379-389.
25. ZUNIGA O., FORCADA F., ABECIA J.A., 2002 – The effect of melatonin implants on the response to the male effect and on the subsequent cyclicity of Rasa Aragonesa ewes implanted in April. *Animal Reproduction Science* 72, 165-174.