DETECTING PRESENCE OF C/T POLYMORPHISM AT POSITION 34 SECOND INTRON OF THE MYOSTATIN GENE IN RABBITS

BADANIE OBECNOŚCI POLIMORFIZMU C/T W POZYCJI 34 DRUGIEGO INTRONU GENU MIOSTATYNY KRÓLIKÓW

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ABSTRACT

Myostatin gene is a negative regulator of skeletal muscles growth. It is responsible for normal development of skeletal muscles. The objective of the research was to detect variation of C/T at position 34 of the second intron of the MNST gene in rabbits. The research included 114 rabbits: 54 of them Polish Rabbits, and 60 of them White Flemish Giants, examined by means of the PCR-RFLP method using AluI restriction enzyme. We found allele C with a frequency of 0.6184 of the examined rabbit population, and allele T with a frequency of 0.3816 of the examined rabbits.

Key words: MNST, polymorphism, rabbit

STRESZCZENIE

Gen miostatyny jest ujemnym regulatorem wzrostu mięśni szkieletowych. Gen MNST odpowiedzialny jest za prawidłowy rozwój mięśni szkieletowych. Celem badań była detekcja zmienności C/T w pozycji 34 drugiego intronu genu MNST królików. Badaniu poddano 114 królików: 54 osobniki rasy hermelin i 60 osobników rasy belgijski olbrzym biały metodą PCR-RFLP przy użyciu enzymu restrykcyjnego AluI. Wykryto allel C stanowiący 0,6184 przebadanej populacji królików oraz allel T stanowiacy 0,3816 przebadanych osobników.

Słowa kluczowe: MNST, polimorfizm, królik

STRESZCZENIE SZCZEGÓŁOWE

Badania nad genem miostatyny, który jest odpowiedzialny za prawidłowy rozwój mięśni szkieletowych [5], przeprowadzono na 114 królikach rasy belgijski olbrzym biały oraz hermelin. Materiał biologiczny do badań stanowiła zarówno krew obwodowa, jak i sierść zwierząt. W celu wykrycia polimorfizmu jednego nukleotydu C/T w pozycji 34 w drugim intronie genu miostatyny użyto metody PCR-RFLP przy zastosowaniu enzymu restrykcyjnego AluI. W wyniku przeprowadzenia detekcji SNP w pozycji 34 drugiego intronu genu miostatyny królików wykryto allel C oraz allel T u osobników rasy: hermelin oraz belgijski olbrzym biały. Allel C stanowił 0,6184, natomiast allel T 0,3816 przebadanej populacji królików. Frekwencja allela C była wyższa od frekwencji allela T w przypadku obu badanych ras. Wykazano występowanie zarówno genotypów w układzie homozygotycznych C/C i T/T, jak i heterozygotycznych C/T. Genotyp C/C wykryto u 31 osobników przebadanej populacji królików, 21 u rasy hermelin i 10 rasy belgijski olbrzym biały. Genotyp T/T wykryto u 4 królików, 1 u rasy hermelin i 3 rasy belgijski olbrzym biały. Genotyp C/T wykryto u 79 osobników, 32 u rasy hermelin i 47 rasy belgijski olbrzym biały. SNP w króliczym genie miostatyny w pozycji 34 drugiego intronu jest wykrywalna przy zastosowaniu metody PCR-RFLP. W badaniach własnych frekwencja allela C stanowiła 0,6184 badanej populacji królików oraz 0,6852 rasy hermelin i 0,5583 rasy belgijski olbrzym biały. Allel T stanowił 0,3816 przebadanych osobników oraz 0,3148 rasy hermelin i 0,4417 rasy belgijski olbrzym biały. Badania należy rozszerzyć o kolejne rasy królików.

INTRODUCTION

Myostatin (MNST) gene, also known as GDF8, belongs to TGF-β (transforming growth factor-beta) family. Myostatin gene expression is observed in skeletal muscles of adults [5]. The GDF8 gene was mapped in cattle on the long arm of chromosome 2. The Blue Flemish breed and asturiasa are carriers of two mutated alleles which determine occurrence of muscle hypertrophy. Animals being carriers of mutated myostatin allele are characterized by increased mass of skeletal muscles, caused by overgrowth of muscle fibre, and decreased amount of fat and connective tissue [1]. Research conducted on mice, to which antibodies being GDF8 inhibitors were administered pharmacologically, proved that myostatin is a negative regulator of growth and development of mature skeletal muscles. Increase of skeletal muscle mass does not lead to alteration of organ sizes or histological changes [8]. It has been proved that carcass of a mouse being carrier of mutated myostatin gene weighs twice as much as a normal mouse carcass [4].

The rabbit myostatin gene underwent sequencing. The gene is built of 3 exons and 2 interons [3]. Only a single polymorphism of the rabbit myostatin gene was found: in the second intron, at position 34, C/T [3, 6].

The research on myostatin gene in rabbit was conducted in Italy [3] and Slovakia [6]. SNP may be detected by means of genotyping with the use of the PCR-RFLP method. The objective of this research was to detect C/T polymorphism at position 34 in the second intron of the myostatin gene in the Polish Rabbit and White Flemish Giant populations.

MATERIAL AND METHODS

The research was based on 114-strong rabbit population. Two breeds were examined: Polish Rabbit, and White Flemish Giant. Biological material was constituted by the animals' peripheral blood and fur. Genomic DNA isolation from blood stains was carried out in accordance with the methods devised by Słomski [7] combined with authors' own modifications: blood drops were placed on absorbent paper, from which 5mm round shapes were cut out, to be put inside an Eppendorf test tube. As the next step 20 µl of 200mM NaOH was added, and the mixture was incubated at the temperature of 75 °C for 5 minutes. Then, 180 µl of 40mM Tris-HCl pH 7.5 was added. Genomic DNA isolation from fur was conducted in accordance with the methods devised by Drissing [2] combined with the authors' own modifications: twenty 5mm long hairs with bulbs were placed in an Eppendorf test tube. Fifty µl of 200mM NaOH was added, and the mixture was then incubated for 20 minutes at the temperature of 95 °C. The tubes were vortexed for 30 seconds, and then 50 µl of 200nM HCl + 100mM Tris_ HCl pH 8.5 was added. PCR reactions were conducted in 25 µl. The reaction mixture was composed of 1 U of Dream Taq (Fermatas) polymerase, 2.5 mM of each dNTP, 1 mM of $MgCl_2$, 10 pmol of both the Forward 5`TAAC TGAAAAGAACCCTCTAGTAGC3` and Reverse 5`TC GGTAGTTGTTTCCCACTTT3`primers, 50 ng of DNA as well as BSA. The reaction thermal profile consisted of initial denaturation at 95 °C for 180 seconds, and then 40 cycles including 95 °C for 30 seconds, 60 °C for 30 seconds, 72 °C for 30 seconds, and the final synthesis at 72 °C for 10 minutes. The amplified stretch was 80 pz long. In order to detect myostatin gene mutation in the 2nd intron, at position 34, C/T, AluI enzyme was used (Fermentas). The amplified stretch underwent enzymatic hydrolysis with 3U restriction enzyme in 5µl restriction

mixture, at the temperature of 37 °C, throughout the night, in accordance with methods devised by Fontanesi et al [3]combined with the authors' own modifications. The enzymatic digestion product was separated in 3.5% of agarose gel with addition of ethidium bromide in 0.5 x concentrated TBE buffer (10xTBE: 0.89 Tris, 0.89M boric acid, 0.02M EDTA, pH 8.0) at the voltage of 120V for 30 minutes. When C/C homozygote occurred, 80 pz long stretch was obtained; when T/T homozygote occurred, two stretches of 56 pz and 24 pz were obtained; when C/T heterozygote occurred, three stretches of 80 pz, 56 pz, and 24 pz were obtained. After that, C/C, T/T, and C/T genotype frequencies as well as C and T allele frequencies were calculated for the entire group covered by the examination, depending on the breed of a given rabbit.

RESULTS AND DISCUSSION

In order to obtain high quality genomic DNA, two different isolation methods were applied, depending on what biological material was used for examination. The method based on DNA isolation from blood stains proved effective when trace amounts of biological material were at our disposal. The concentration of isolated DNA was very low. DNA isolated from rabbit fur enabled us to obtain high quality PCR product, which then underwent enzymatic hydrolysis with the use of AluI restriction enzyme.

Following SNP detection at position 34 of the second intron of the myostatin gene in rabbit, C and T alleles were found in animals of the Polish Rabbit and White Flemish Giant breeds. C allele constituted 0.6184, and T allele 0.3816 of the examined rabbit population. Allele C frequency was higher than allele T frequency in both examined breeds. Presence of genotypes was found to occur in homozygotic C/C and T/T groups, as well as heterozygotic C/T group. The C/C genotype was found in 31 animals of the examined rabbit population, 21 in the Polish Rabbit and 10 in the White Flemish Giant. The T/T genotype was found in 4 rabbits, 1 in the Polish Rabbit and 3 in the White Flemish Giant. The C/T genotype was found in 79 rabbits, 32 in the Polish Rabbit, and 47 in the White Flemish Giant. Genotype and allele frequencies in the examined rabbit population are presented in Table 1.

The genetic tests on the polymorphism of the second intron of the myostatin gene in rabbit conducted in Italy and Slovakia, as well as the authors' own research, proved presence of C/C and T/T homozygotic animals, as well as C/T heterozygotic animals [3, 6]. In the Italian research, the C allele constituted 0.51, and the T allele 0.49 of the examined rabbit population [3]. The results of the Slovakian research were the opposite, there was a higher percentage of the T allele, which constituted 0.67, and the C alle constituted only 0.33 of the examined animals [6]. In the authors' own research it was found that the C allele frequency in the examined rabbit population was 0.62, and the T allele 0.38.

The Italian research was based on 47 rabbits. The myostatin gene was sequenced in 4 rabbits of the following breeds: Belgian Hare, Burgundy Fawn, Checkered Giant, and Giant Grey. Based on the conducted examinations it was found that mutation occurs in the second intron at position 34 of the myostatin gene. The PCR-RLFP method was used to detect SNP in Checkered Giant, Giant Grey, Dwarf, Burgundy Fawn, Giant White, Lop, Belgian Hare, and New Zealand White breeds. Animals with genotypes C/C, T/T, C/T were found. The C allele constituted 0.51 of the examined population, and the T allele 0.49 [3].

The Slovakian research included 127 broilers of lines

Table 1. Genotype and allele frequency in the examined rabbit population.
Tabela1. Frekwencja genotypów oraz alleli badanej populacji królików

MSTN	examined population badana populacja		White Flemish Giant belgijski olbrzym bialy		Polish Rabbit hermelin	
genotypes genotyp	number liczba	frequency frekwencja	number liczba	frequency frekwencja	number liczba	frequency frekwencja
C/C	31	0.272	10	0.167	21	0.389
C/T	79	0.6929	47	0.783	32	0.5925
T/T	4	0.0351	3	0.05	1	0.0185
total suma	114	1.00	60	1.00	54	1.00
allele allel	number liczba	frequency frekwencja	number liczba	frequency frekwencja	number liczba	frequency frekwencja
C	141	0.6184	67	0.5583	74	0.6852
T	87	0.3816	53	0.4417	34	0.3148
total suma	228	1.00	120	1.00	108	1.00

M91 and P91. Polymorphism of the myostatin gene in rabbit at position 34 of the second intron was found in the homozygotic C/C group in 13 animals, which equalled 0.1 of the examined population. The T/T genotype was found in 56 animals, which equalled 0.44, and the heterozygotic C/T group was found in 58 animals, which equalled 0.46 of the examined rabbit population. The C allele constituted 0.33, and the T allele 0.67 of the examined animals [6].

CONCLUSION

The SNP at position 34 second intron of the myostatin gene in rabbits. In own examinations the allele C and allel T was found in the population of Polish Rabbit and White Flemish Giant. Myostatin gene is responsible for meat quality. It is advisable to conduct similar studies covering other rabbit breeds. Basing on the research of the relationships between polymorphism myostatin gene with different genes influencing features farm it is possible to make the farm plan out to the purpose of getting populations of rabbit standing out with better functional indicators.

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REFERENCES

[1] Charon K.M., Świtonski M., Genetyka zwierząt, Wydawnictwo Naukowe PWN. (2006) 195-196, 294-295.

[2] Drissing J., Rudbeck L., Marcher H., A five minute procedure for extraction of genome DNA from whole blood, semen and forensic stain for PCR, In: Advances in Forensic Haemogenetics (Carracedo, A.,ed.). Springer-Verlag, New York. (1996) pp. 269-271.

[3] Fontanesi L., Tazzoli M., Scotti E., Russo V., Analysis of candidate genes for meat production traits in domestic rabbit breeds, 9th World Rabbit Congress Verona – Italy. (2008) June 10-13.

[4] Lee S.J., McPherron A.C., Regulation of muscle mass by myostatin, Ann. Rev. Cell Dev. Biol. (2004) 20: 61-86.

[5] McPherron AC, Lawler AM, Lee SJ, Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member, Nature. (1997) 387(6628):83-90.

[6] Rafayova A., Lieskovska Z., Trakovicka A., Kovacik A., Detection of MSTN polymorphism In Rabbit, Zootehnie si Biotehnologii. (2009) 42 (2): 637-641.

[7] Słomski R., Analiza DNA teoria I praktyka. (2008) 49-50.

[8] Whittemore L-A., Song K., Li X., Aghajanian J., Davies M., Girgenrath S., Hill J.J., Jalenak, Kelley P., Knight A., Maylor R, O_Hara D., Pearson A., Quazi A., Ryerson S.,Tan X-Y, Tomkinson K.N., Veldman G.M., Widom A., Wright J.F., Wudyka S., Zhao L., Wolfman N.M., Inhibition of myostatin in adult mice increases skeletal muscle mass and strength, Biochem. Biophys. Res. Commun. (2003) 300: 965–971.