MEDWIN PUBLISHERS
Committed to create value for Researchers

Open Access Journal of Agricultural Research
ISSN: 2474-8846

# Rabbit Growth Hormone and Myostatin Gene Polymorphisms

**Hristova D[1]*, Tanchev S[1], Velikov K[2], Gonchev P[1] and Georgieva S[1]**

[1]Department of Genetics, Breeding and Reproduction, Faculty of Agriculture, Trakia University, Bulgaria

[2]Department of Special branches, Institute of Animal Science –Kostinbrod, Bulgaria

**Research Article**

Volume 2 Issue 3

**Received Date**: February 16, 2017

**Published Date:** March 03, 2017

***Corresponding author:** Hristova D, Department of Genetics, Breeding and Reproduction, Trakia University, Bulgaria, E-mail: dhristova@uni-sz.bg

## Abstract

The aim of the present research was to investigate two rabbit populations from the New Zealand White breed and to identify single nucleotide polymorphisms with respect to the genes encoding growth hormone (GH) and myostatin (MSTN) by PCR-RFLP assay. Genotype profiles were established in a total of 50 rabbits from two populations: one reared at The Institute of Animal Science, Kostinbrod (NZW-KB, n= 26) and another (NZW-SZ, n=24), reared at the Experimental farm of the Faculty of Agriculture, Trakia University, Stara Zagora. As expected, a 231bp fragment of the polymorphic site (part of the 5'-flanking region, 5'-untranslated region and exon 1) of GH gene and a80 bp fragment of the intron 2of MSTN gene were amplified using PCR and digested with endonuclease enzymes Bsh1236 and Alu I, respectively. The obtained restriction fragments revealed three genotypes: CC, CT and TT for the GH gene, observed in 27%, 62%,and 11% of the NZW-KB rabbit population and in 42%, 50%,and 8% of the NZW-SZ population, respectively, without departure from the Hardy-Weinberg equilibrium (P>0.10) in these groups. The allele frequencies determined a prevalence of the C allele (0.577 and 0.667) over the T allele (0.423 and 0.333) in both populations. This tendency was preserved with regard to MSTN gene, where the frequency of the C allele (0.692 and 0.813) was higher than that of the T allele (0.308 and 0.187). The homozygous genotype TT was absent in the investigated rabbit populations. The observed heterozygosity value (0.615) in NZW-KB rabbit population was higher compared to the expected one (0.426) resulting in deviation from Hardy-Weinberg Equilibrium (P<0.1), in contrast to the NZW-SZ population. The results from the present investigation confirmed the presence of the polymorphisms in GH and MSTN genes. Therefore, the genetic variability established in these polymorphic loci could be applied in further association studies with growth and meat production traits in rabbits.

**Keywords:** *Oryctolaguscuniculus*; Growth hormone gene (GH); Myostatin gene (MSTN); Single nucleotide polymorphism (SNP); PCR-RFLP

## Introduction

DNA polymorphism associated with economic traits can be identified by investigating candidate genes that are directly or indirectly related to the physiological mechanisms influencing important traits for selection. Mutations in the growth hormone and Myostatin genes associated with production traits have been already successfully described and applied in several livestock species, including *Oryctolaguscuniculus*. For this reason, GH and MSTN could be interesting candidate genes in the rabbit genome. Growth hormone (somatotropin; GH) is a protein hormone isolated from the pituitary, which regulates somatic growth in most vertebrates, and has effects on various metabolic activities. Like other mammalian GH genes for which sequences are available, the rabbit growth hormone gene consists of five exons split by four introns [1]. Southern blotting analysis revealed that unlike other species, this gene is present as a single copy gene without GH-like genes in the rabbit genome [2]. Fontanesi et al. (2012) [3] have re-sequenced a fragment of 1337bp of the growth hormone gene in rabbits (EMBL accession numbers: HE646284 and HE646285) from different breeds and identified two single nucleotide polymorphisms (SNPs) in the 5'-flanking region: c.-78C>T andc.-33A>G.

Myostatin (MSTN), also known as GDF8, is a member of the transforming growth factor (TGF)-β-superfamily that actively represses skeletal muscle growth [4]. The rabbit MSTN gene sequence has been recently assembled after the initiative of the Broad Institute that shotgun sequenced the rabbit genome (Ensembl Gene ID: ENSOCUG00000012663, http://www.ensembl.org/Oryctolagus_cuniculus/index.html). It comprises three coding exons and two introns, as observed in other species. Fontanesi et al. (2008) [2] sequenced exon 1, 2, 3 and intron 1, 2of rabbit MSTN gene and found only one SNP in intron 2, which is C-T transition in position 34. Also, Kurkute et al. (2011) [5] have resequenced myostatin coding regions in three different rabbit breeds (White giant, Soviet chinchilla and Desi) in order to perform a comparative study. Resequencing and comparison of three MSTN exonic regions of these rabbit breeds showed only one variation (A>G) in exon 1 in the Soviet chinchilla and one variation (G>A) in exon 3 in the White giant breed, respectively.

The genetic polymorphisms in loci encoding growth hormone and myostatin have so far been described in several livestock species, but only few studies have investigated their variability in the rabbit genome. Based on PCR-RFLP method, SNP polymorphisms of the GH and MSTN genes in New Zealand White and their lines and cross-bred populations have been reported by Rafayovà et al. (2009), Markowska et al. (2010), Bindu et al. (2012), Fontanesi et al. (2012), Amalianingsih et al. (2014), Hussein et al. (2015), etc [6-10,3] Also, the GH and MSTN the gene polymorphisms and their association with different performance traits were successfully determined in *Oryctolaguscuniculus* [11-15]. The present study was focused on identification of the polymorphisms at GH and MSTN genes in rabbits from the New Zealand White breed, reared in Bulgaria and on establishment of the genetic structure of their populations, by means of PCR-RFLP analysis.

## Material and Methods

### Animals and DNA Isolation

The present investigation was carried out with a total of 50 New Zealand White rabbits from 2 populations: NZW-KB reared in the Institute of Animal Science, Kostinbrod (11♀ and 15♂) and NZW-SZ reared at the Experimental farm at the Faculty of Agriculture, Trakia University, Stara Zagora (9♀ and 15♂). Blood samples (3 ml) were obtained from the auricular vein of rabbits in sterile EDTA tubes, mixed thoroughly and stored at -20°C until the assay. Genomic DNA was extracted from the whole rabbit blood using Illustra Blood Genomic Prep DNA Purification Kit (GE Healthcare, UK). The quality of the obtained DNA (about 30-90 ng) was determined using NanoVue Plus Spectrophotometer (GE Healthcare).

### PCR amplification and genotyping

PCR amplifications were carried out in total volume of 20 µl, containing 80 ng DNA template, 2×Red Taq DNA Polymerase Master mix (VWR, Belgium) and 20 pM of each primer with sequence described by Fontanesi et al. (2008, 2011) [2,16]. PCR reactions were performed in GeneAmp thermocycler (Applied Biosistems, USA) under the following conditions: an initial denaturation at 94ºC for 5 min, followed by 30 cycles at 94ºC for 30 sec, primer annealing at 60ºC for 45 sec for GH gene and 59ºC for 45 sec for MSTN gene, respectively; extension at 72ºC for 1 min and final extension at 72ºC for 10 min. The genotypes of the analyzed individuals at the GH and MSTN genes were established through RLFP analysis. The digestion reactions were carried out in 25 µl final volume, containing 10 µl PCR product, incubated at 37ºC/15h using 10 U/µl enzyme Bsh1236I (Bioneer) for GH gene and at 37ºC/10min. using 10U/µl enzyme AluI for MSTN gene, respectively. The obtained PCR products and

restriction fragments were separated on 2 % agarose gel and visualized using Electrophoresis Gel Imaging Analysis System (Bio-Imaging Systems, Israel).

## Statistical Analysis

The data were processed using PopGene32, v.1.31 software [17,18]. In both studied NZW rabbit populations, the following parameters were calculated: allele and genotype frequencies, observed heterozygosity, Nei-expected heterozygosity and chi-square ($\chi^2$) for testing deviation from Hardy-Weinberg Equilibrium (HWE).

## Results and Discussion

### Growth Hormone (GH) Gene

The PCR products of the polymorphic region from rabbit growth hormone (GH) gene (part of the 5'-flanking region, 5'-untranslated region and exon 1) were amplified successfully and a single 231 bp band, was obtained in both studied populations (Figure 1).

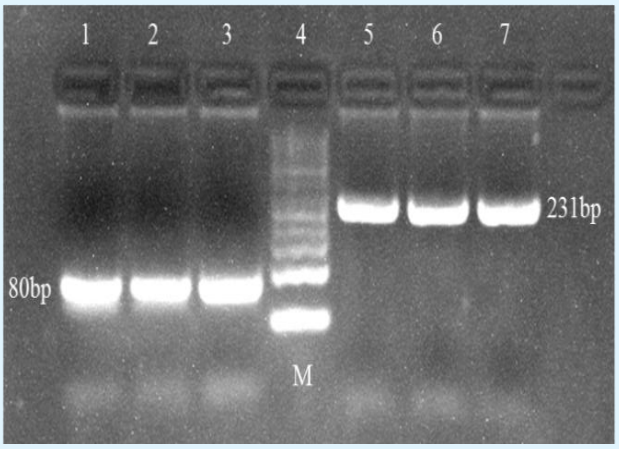


Figure 1: Agarose gel (1%) image of the amplified PCR products of the polymorphic sites in rabbit GH and MSTN genes. Lane 1-3 – PCR products of the MSTN gene; Lane 4 (M) – Molecular weight marker, 50bp; Lane 5-7 –PCR products of the GH gene

Through the RFLP approach with digestion with the restriction enzyme Bsh1236 I at a determined specific site at 5'…CG↓CG…3' identification of the alleles at rabbit GH gene was possible. As a result, three different genotypes were identified in the observed locus in the studied rabbit populations – two homozygous (CC and TT) and one heterozygous (CT).

The homozygous CC genotype, represented with two fragments 169 and 62 bp of size, was detected in 7 NZW-KB and in 10 NZW-SZ rabbits. The homozygous TT genotype was identified in few rabbits –3 from the NZW-KB and in 2 from the NZW-SZ population, respectively. The heterozygous genotype CT (three fragments – 231, 169 and 62 bp) was found in 16 inbred NZW-KB rabbits and in 12 NZW-SZ rabbits, respectively. On the basis of PCR-RFLP analysis, genotype structure of the examined rabbit populations was established and allele frequencies were calculated. The distribution of the alleles and genotypes frequencies of the GH gene in rabbit populations are summarized in Table 1.

From the below table, NZW-KB rabbit population the frequency of the heterozygous genotype CT was 0.615, while those of the homozygous genotypes CC and TT were 0.270 and 0.115, respectively. This suggested a prevalence of the C allele – 0.577 over the T allele – 0.423 in this rabbit group. Similar results were obtained in NZW-SZ, where the genotype frequencies were 0.500 for the heterozygous genotype. In this group, the frequency of homozygous genotype CC – 0.417 was higher than that of the homozygous TT – 0.083. The observed preponderance of allele C compared to allele T was also reported by Amalianingsih *et al.* (2014) [9] in New Zealand White and in Californian rabbit breeds (0.625 vs 0.375). Our results disagreed with data reported by Fontanesi et al. (2012) [3] for higher prevalence of allele T (frequency 0.594) over allele C (frequency 0.406) in Checkered Giant rabbits. In a recent study of Hussein et al., (2015) [10], comprising 202 rabbits from the APRI line, observed frequencies were 0.540 for allele T and 0.460 for allele C, respectively. The chi-square test for Hardy-Weinberg equilibrium (Table 1) at degree of freedom df=1 showed a value of $\chi^2$ with level of probability P>0.1, confirming the validity of the HWE for the both NZW-KB and NZW-SZ rabbit populations.

| Rabbit population | n | Allele frequency | | Genotype frequency | | | Nei* | $\chi^2$ | P** |
|---|---|---|---|---|---|---|---|---|---|
| | | C | T | CC | CT | TT | | | |
| NZW-KB | 26 | 0.577 | 0.423 | 0.27 | 0.615 | 0.115 | 0.615 | 1.514 | 0.219 |
| | | | | -7 | -16 | -3 | | | |
| NZW-SZ | 24 | 0.667 | 0.333 | 0.417 | 0.5 | 0.083 | 0.454 | 0.261 | 0.609 |
| | | | | -10 | -12 | -2 | | | |

Table 1: Allele and genotype frequencies, expected heterozygosity, chi-square test for HWE ($\chi^2$) and P-value for the GH gene in the examined rabbit populations.

*Expected heterozygosity calculated as per Nei (1973)
**P-value (P) and degree of freedom (df) = 1

## Myostatin (MSTN) Gene

Based on PCR-RFLP analysis a 80bp fragment from the polymorphic region of the rabbits myostatin (MSTN) gene – intron 2 was amplified, as seen on figure 1. Digestion with restriction enzyme Alu Icut the sequence of nucleotides at a specific site at 5'… AG↓CT… 3'. As a result two different genotypes in the observed locus were identified in the both researched rabbit populations – CC and CT. The homozygous genotype CC (one undigested fragment with length 80 bp) was detected in 10 NZW-KB rabbits and in 15 NZW-SZ rabbits of. The heterozygous genotype CT (three fragments with length 80, 56 and 24 bp) was detected in 16 NZW-KB and 9 NZW-SZ rabbits, respectively. The homozygous genotype TT (two fragments with length 56 bp and 24 bp) was not identified in the investigated rabbit populations.

The frequencies of detected MSTN genotypes and alleles in both rabbit populations are presented in Table 2.

| Rabbit population | n | Allele frequency | | Genotype frequency | | | Nei* | ($\chi^2$) | P** |
|---|---|---|---|---|---|---|---|---|---|
| | | C | T | CC | CT | TT | | | |
| NZW-KB | 26 | 0.692 | 0.308 | 0.385 | 0.615 | 0 | 0.426 | 4.762 | 0.029 |
| | | | | -10 | -16 | 0 | | | |
| NZW-SZ | 24 | 0.813 | 0.187 | 0.625 | 0.375 | 0 | 0.305 | 1.117 | 0.29 |
| | | | | -15 | -9 | 0 | | | |

Table 2: Allele and genotype frequencies, expected heterozygosity and chi-square test for HWE ($\chi^2$) and P-value for the MSTN gene in the studied rabbit populations.

*Expected heterozygosity calculated as per Nei (1973)
**P-value (P) and degree of freedom (df) = 1

Following SNP detection at position 34 of the second intron of the rabbit's myostatin gene alleles C and T were found in examined populations. The frequency of allele C (0.692 in NZW-KB and 0.813 for NZW-SZ) was higher than that of allele T (0.308 in NZW-KB and 0.187 for NZW-SZ), which is in agreement with the results reported by Fontanesi et al., 2008 [2] for two rabbit populations – Badana with frequencies 0.618 for allele C and 0.381 for allele T and for Polish Hermelin with frequencies 0.685 for allele C and 0.348 for allele T. Opposite results for the distribution of the allele frequencies in this locus were obtained by Rafayová et al., 2009 [6] demonstrating a high value of the allele T (0.669) vs another one (0.331) in 127 broiler rabbits.

In the present study, the CC genotype was found in 10 rabbits of the NZW-KB population with frequency0.385 and in 15 NZW-SZ rabbits of with frequency 0.625. The homozygous TT genotype was not found in both rabbit populations. The CT genotype was detected in 16 NZW-KB rabbit sand 9 NZW-SZ rabbits with frequencies of 0.615and 0.375, respectively. The results in a Polish research by Markowska et al. (2010) [7] were contrary to ours for the NZW-SZ rabbit group as the authors reported a prevalence of heterozygous CT (0.783, 0.692 and 0.592) over the homozygous CCgenotype (0.167, 0.272 and 0.389) in three rabbit breeds – White Flemish Giant, Badana and Hermelin. The authors also reported very low frequencies of the homozygous genotype TT (0.050, 0.035

and 0.018, respectively) in these examined rabbit populations. The chi-square values in the rabbit populations corresponding to MSTN gene (Table 2) showed deviation from the Hardy-Weinberg equilibrium in NZW-KB rabbit population with level of probability $P<0.05$ and degree of freedom df=1. This could be explained by the fact that the obtained values of the observed heterozygosity (0.615) were significantly higher than the theoretically expected (0.426) in this rabbit group. The obtained P-value (0.290) confirmed the validity of HWE in assessed NZW-SZ rabbit population.

## Conclusions

The obtained experimental results, based on PCR-RFLP analysis confirmed the presence of SNP in the polymorphic regions in GH and MSTN genes in studied rabbit populations. Nevertheless, the identified polymorphisms in GH and MSTN genes did not affect the coding sequence because they were localized in an intronic region without any presumed functional role. Considering their allele frequencies distribution, these SNPs could be useful DNA markers for future association studies with growth performance and meat production traits in rabbits. Additional investigations are planned to estimate the possible effects of these markers on different production traits before using them in inbreeding programme (selection) to improve rabbit production traits.

## Acknowledgments

This research was funded by scientific project number 14.G.15 and realized in the DNA laboratory of the Faculty of Agriculture in Trakia University, Stara Zagora. We thank people from the Institute of Animal Science in Kostinbrod who helped to collect rabbit samples.

## References

1. Wallis C, Wallis M (1995) Cloning and characterisation of the rabbit growth hormone-encoding gene. Gene 163(2): 253-256.

2. Fontanesi L, Tazzoli M, Scotti E, Russo V (2008) Analysis of candidate genes for meat production traits in domestic rabbit breeds. Verona, Italy: 79-84.

3. FontanesiL, Dall'Olio S, Spaccapaniccia E, Scotti E, Fornasini D, et al. (2012) A single nucleotide polymorphism in the rabbit growth hormone (GH1) gene is associated with market weight in a commercial rabbit population. Livest Sci 147(1-3): 84-88.

4. Lee SJ (2004) Regulation of muscle mass by myostatin. Ann Rev Cell Dev Biol 20: 61-86.

5. Kurkute AS, Tripathi AK, Nadeem S, Jawale CV, Ramani1 UV, (2011) Molecular cloning and characterization of rabbit myostatin Gene. IIOAB 2(5): 1-7.

6. Rafayová A, Lieskovská Z, Trakovická A, Kováčik A (2009) Detection of MSTN polymorphism in rabbit. Zootechniesi Biotehnologii 42(2): 637-641.

7. Markowska A, Rafayova A, Trakowicka A (2010) Detecting presence of C/T polymorphism at position 34 second intron of the myostatin gene in rabbits. Journal of Central European Agriculture 11: 449-452.

8. Bindu KA, Arun R, Siby A, Raghunandanan KV (2012) Association of myostatin gene (MSTN) polymorphism with economic traits in rabbits. In Fibre production in South American camelids and other fibre animals (eds Pérez- Cabal MA, Gutiérrez JP, Cervantes I, Alcalde MJ) pp. 131- 133. Wageningen Academic Publishers, Wageningen, The Netherlands.

9. Amalianingsih T, Brahmantiyo B, Jakaria K (2014) The Variability of Growth hormone gene associated with ultra soundimaging of *Longissimus dorsi* muscle and perirenal fatin rabbits. Media Peternakan 1-7.

10. Hussein B, Abdel-Kafy EM, Abdel-Ghany SM, Gamal AY, Badawi YM (2015) Single nucleotide polymorphisms in growth hormone gene are associated with some performance traits in rabbit. Int J Biol Allied Sci 4: 490-504.

11. Qiao XB (2010) Polymorphisms of Gh, GHR and MSTN and Their Relationships with Meat Production Traits in Domestic Rabbits. Thesis of PhD, Shandong Agricultural University, China.

12. Peng J, Zhang G, Zhang W, Liu Y, Yang Y, et al. (2013) Rapid Genotyping of MSTN Gene Polymorphism Using High-resolution Melting for Association Study in Rabbits. Asian-Australian J Anim Sci 26(1): 30-35.

13. Sternstein I, Reissmann M, Maj D, Bieniek J, Brockmann GA (2014) A new single nucleotide polymorphism in the rabbit (*Oryctolaguscuniculus*) myostatin (MSTN) gene is associated with carcass composition traits. Animal genetics 45(4): 596-599.

14. Qiao XB, Xu KY, Li B, Luan X, Xia T, et al. (2014) Rabbit MSTN gene polymorphisms and genetic effect analysis. Genet Mol Res 13(2): 2590-2597.

15. Abdel-Kafy EM, Darwish SF, Elkhishin D (2016) Correlating single nucleotide polymorphisms in the myostatin gene with performance traits in rabbit. World Rabbit Sci 24(3): 213-221.

16. Fontanesi L, Scotti E, Frabetti A, Fornasini D, Picconi A,et al. (2011) Identification of polymorphisms in the rabbit (*Oryctolaguscuniculus*) myostatin (MSTN) gene and association analysis with finishing weight in a commercial rabbit population. Anim Genet 42(3): 339.

17. Yeh F, Yong R (1999) POPGENE version 1.31 (02.04.2011) Microsoftbased Freeware for Population Genetic Analysis. University of Alberta, Edmonton, Canada.

18. Labate J (2000) Software for population genetic analyses of molecular marker data. Crop Sci 40(6): 1521-1528.