**RESEARCH ARTICLE**

# Single nucleotide polymorphism of the Growth Hormone Receptor (GHR) encoding gene in *Oryctolagus cuniculus*

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**ABSTRACT**

Considered the rabbit Growth Hormone Receptor (GHR) gene as a candidate gene for growth efficiency, understanding the genetic variation in this locus is of particular relevance. The aim of the present research was to investigate populations from the rabbits (*Oryctolagus cuniculus*) and to identify single nucleotide polymorphism (SNP) with respect to the GHR gene through PCR-RFLP assay. Genotype profiles were established in a total of 100 rabbits from two populations (New Zealand White, NZW, n=51 and Californian, n=49), reared at The Institute of Animal Science, Kostinbrod. As expected, a 479bp amplicon of the polymorphic site (exon 3) of GHR gene was amplified using PCR and digested with endonuclease enzymes HinfI. The restriction pattern obtained in agarose gel electrophoresis was constituted by three bands (210bp, 162bp and 107 bp) for allele C and by two bands for allele G (317bp and 162 bp). The obtained restriction fragments revealed three genotypes: CC, CG and GG, observed in 7.8%, 53% and 39.2% of the NZW rabbit population and in 28.6%, 51% and 20.4% of the Californian rabbit population, respectively, without departure from the Hardy-Weinberg equilibrium (P>0.20) in the investigated groups. The allele frequencies determined a prevalence of the G allele (0.657) over the C allele (0. 343) in NZW rabbit population, while in the Californian rabbits, the frequency of the C allele (0.541) was higher than allele G (0.459). Observed heterozygosity was higher than expected, resulting in a negative inbreeding coefficient (Fis= -0.174 for NZW rabbits and Fis= -0.027 for Californian rabbits), indicating a sufficient number of heterozygous forms in both studied groups of rabbits. The obtained results from the present investigation confirmed the presence of the SNP in rabbit GHR gene. Therefore, the genetic variability established in this polymorphic locus could be applied in further association studies with growth traits in domestic rabbits.

**Key words:** *Oryctolagus cuniculus,* Growth Hormone Receptor (GHR) gene, single nucleotide polymorphism (SNP), PCR-RFLP

## Introduction

The European rabbit (*Oryctolagus cuniculus*) is one of the domesticated animal species with the broadest ranges of economic and scientific applications and fields of investigation (Bertolini et al., 2014). On the other hand, domestic rabbits have been recently considered as a good alternative source of animal protein for the increasing human population in the developing countries (Lukefahr & Cheeke, 1991; El-Sabrout & Aggag, 2017). In this connection, revelation the single nucleotide polymorphism in the Growth Hormone Receptor encoding (GHR) gene is of particular relevance, taking into consideration that this locus is a candidate gene for growth efficiency in rabbits due to its biological roles.

As a member of the cytokine receptor superfamily, GHR is constituted by three functional domains – an extracellular (ligand-binding) domain, one transmembrane domain and a signal-transducing, cytoplasmic domain (VanderKuur et al., 1994; Postel-Vinay & Finidori, 1995; Rosenboom, 2000). The binding of Growth Hormone (GH) to GHR causes receptor-dimerization and initiates signaling cascades via the cytoplasmic domain that leads to cell growth and differentiation, increased blood glucose levels, increased free fatty acid levels, decreased fat mass, increased lean body mass, as well as increased linear growth and organ size (Frank, 2001).

The rabbit GHR genes have been already sequenced and Leung et al. (1987) established ten exons encoding 638 amino acids. In this gene, SNP polymorphisms and their association with body weight and growth performance traits in different domestic rabbit breeds and strains were successfully determined. The genetic variability in the rabbit GHR gene have been reported by Deng et al. (2008), who sequenced a part of exon 10 and identified two polymorphisms (g.63537066 C>T and g.63537228 A>G) that were used in an association study with carcass traits and feed efficiency. Also, by Zhang et al. (2012), who sequenced three exons of this gene and identified a missense polymorphism that was associated with carcass traits and body weight in three rabbit breeds. All coding exons and portions of flanking and intronic regions of the GHR gene have been re-sequenced in a panel of several rabbits by Fontanesi et al. (2016) in order to identify polymorphisms and performed an association study between a SNP (g.63453192C>G or c.106C>G if based on the predicted cDNA), located in exon 3 and finishing weight in a commercial meat rabbit line, genotyped by PCR-RFLP. The single nucleotide polymorphism examined by authors is a missense mutation that changes the amino acid at position 36 of the GHR protein (p.L36V). The 1st exon of the growth hormone receptor gene with expected amplicon size of 263 bp had been carried out by Sahwan et al. (2014) for revealing GHR gene polymorphism in three rabbit breeds reared under Egyptian conditions and to investigate the association between growth performance and detecting of SNP in this locus.

The present investigation was carried out for identification C/G nucleotide polymorphism in exon 3 of the GHR gene in New Zealand White and Californian rabbit populations reared in Bulgaria and to establish the genetic structure, through PCR-RFLP assay.

**Figure 1.** *New Zealand White rabbit reared in The Institute of Animal Science, Kostinbrod.*

**Figure 2.** *Californian rabbit reared in The Institute of Animal Science, Kostinbrod.*

## Materials and Methods

### *Sample source and DNA isolation*

The present investigation was carried out with a total of 100 rabbits from 2 populations, reared in The Institute of Animal Science, Kostinbrod: New Zealand White (22♀ and 29♂; Figure 1) and Californian (25♀ and 24♂; Figure 2).

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 genetic assay. Genomic DNA was extracted from the whole rabbit blood using Illustra Blood Genomic Prep DNA Purification Kit (GE Healthcare, UK). The quality and quantity (about 30-70 ng) of the obtained DNA was determined using NanoVue Plus Spectrophotometer (GE Healthcare) and through agarose gel electrophoresis (Figure 3).

### *PCR amplification and genotyping*

The genotypes of the studied rabbits with respect to the exon 3 of the GHR gene were established through PCR-RLFP analysis. PCR amplifications were carried out in total volume of 20 µl, containing 100 ng DNA template, 2×Red Taq DNA Polymerase Master mix (VWR, Belgium) and 10 pM of each primer with sequence described by Fontanesi et al. (2016). PCR reactions were performed in Gradient thermocycler (VWR) under the following conditions: an initial denaturation at 94ºC/5 min, followed by 30 cycles at

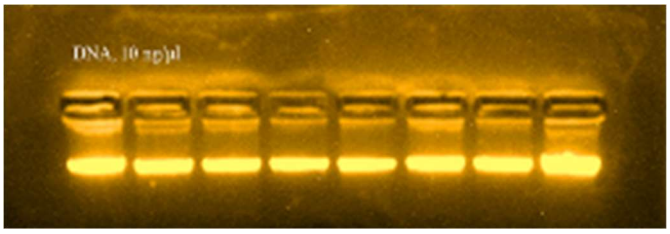

**Figure 3.** *Changes in seed coat colours in the $M_2$ generation and the control variant (upper left corner).*

94ºC/30 sec, primer annealing at 56.4ºC/45 sec, extension at 72ºC/1 min and final extension at 72ºC/7 min. 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 HinfI (Bioneer). The obtained PCR products and restriction fragments were separated on 2.5% agarose gel and visualized using Electrophoresis Gel Imaging Analysis System (Bio-Imaging Systems, Israel).

***Statistical analysis***

The data were processed using PopGene 32, v.1.31 software (Yeh & Yong, 1999; Labate, 2000). In both studied rabbit populations, the following parameters were calculated: allele and genotype frequencies, observed (Ho) and Nei-expected (He) heterozygosity, coefficient of inbreeding (Fis) and chi-square ($\chi^2$) for testing deviation from Hardy-Weinberg Equilibrium (HWE).

## Results and Discussion

As expected, a 479bp amplicon of the target polymorphic site (exon 3) of the GHR gene was successfully amplified in both rabbit populations, through gradient PCR (Figure 4).

The restriction pattern with endonuclease enzyme HinfI, obtained in 2.5 % agarose gel electrophoresis was constituted by three bands (210bp, 162bp and 107 bp) for allele C and by two bands for allele G (317bp and 162 bp). The obtained restriction fragments revealed three genotypes: two homozygous CC and GG and one heterozygous CG, observed in 7.8%, 53% and 39.2% of the New Zealand White rabbit population and in 28.6%, 51% and 20.4% of the Californian rabbit population, respectively (Figure 5).

The presented on Figure 6 allele frequencies determined a prevalence of the G allele (0.657) over the C allele (0.343) in NZW rabbit population. On the contrary, in the Californian rabbits, the frequency of the C allele (0.541) was higher than allele G (0.459).

Our results for the NZW breed agreed with data reported by Zhang et al. (2012) for higher prevalence of allele G (frequency 0.676) over allele C (frequency 0.323) in Tianfu rabbit population, also in Iraq rabbits (0.610 for allele G and 0.384 for allele C).

The genotype frequencies of the GHR gene in each of rabbit populations are presented in Figure 7.

The heterozygous CT genotype was detected in 27 NZW rabbits, with frequency 0.529, while the homozygous CC genotype was found out only in 4 rabbits (0.078). In this population, homozygous GG genotype was presented in 20 rabbits, with frequency 0.392.

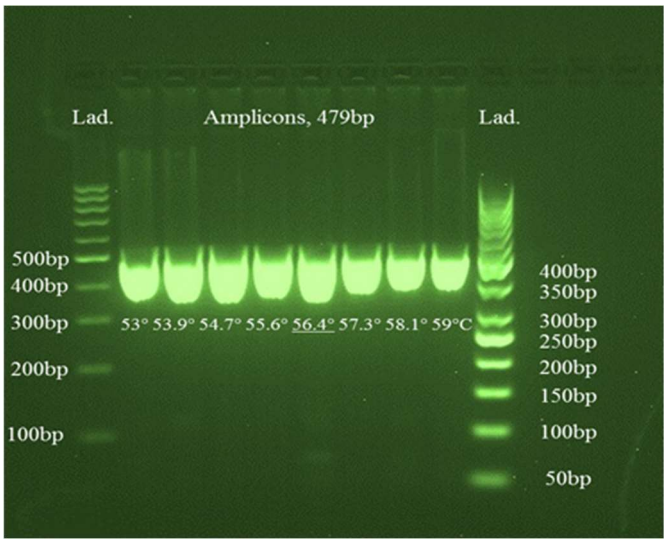


**Figure 4.** *PCR gradient-obtained amplicons of exon 3 at rabbit GHR gene on 2.5% agarose gel.*

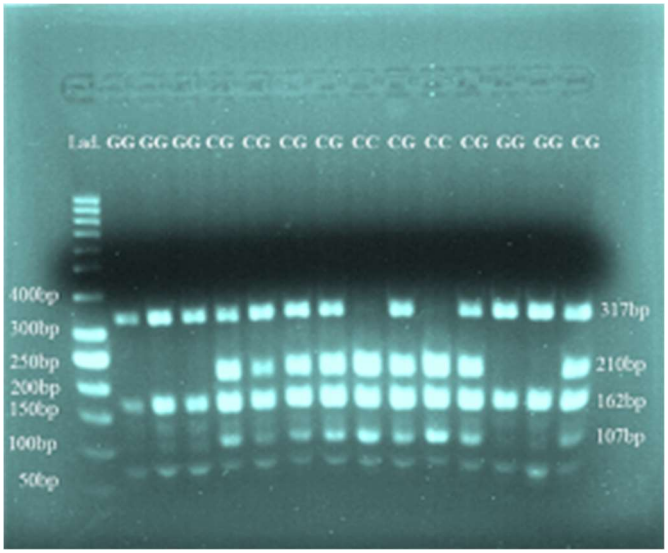


**Figure 5.** *HinfI - restriction fragments represented different GHR genotypes of rabbit populations.*

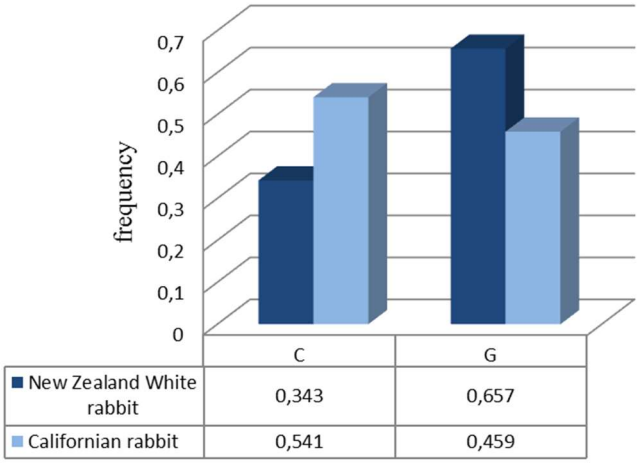


**Figure 6.** *Allele frequencies of the polymorphic site in GHR gene in the studied rabbit populations.*

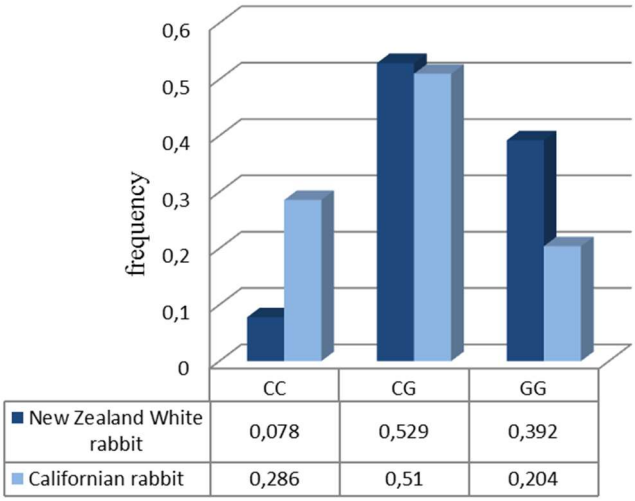


**Figure 7.** *Genotype frequencies of the polymorphic site in GHR gene in the studied rabbit populations.*

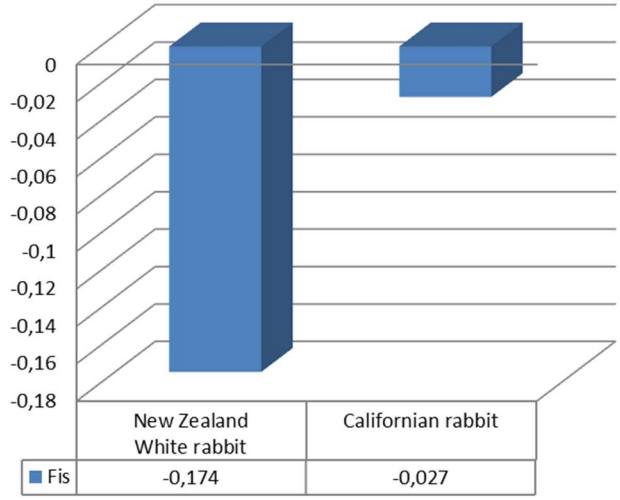


**Figure 8.** *Coefficient of inbreeding (Fis) for the polymorphic locus in GHR gene in the studied rabbit populations.*

In the Californian rabbit population, the frequency of the heterozygous CT genotype was 0.510 (25 rabbits), whereas the homozygous CC and TT genotypes had frequencies of 0.286 (14 rabbits) and 0.204 (10 rabbits), respectively.

In a study by Zhang et al. (2012) comprising 111 rabbits from Champagne breed, the frequency of homozygous genotype GG was higher (0.657) compared to another homozygous CC genotype (0.018). This implied a prevalence of the G allele over allele C (0.819 vs 0.180) in this group of rabbits.

The values of theoretically expected heterozygosity in the studied rabbit populations with respect to GHR gene are presented on Figure 7.

The values of theoretically expected heterozygosity ranging from 0.451 for NZW rabbit population to 0.497 for Californian rabbits were lower than observed ones (0.529 and 0.510, respectively, see Figure 7). Therefore, this tendency resulting in a negative inbreeding coefficient (Fis= -0.174 for NZW rabbits and Fis= -0.027 for Californian rabbits), indicating a sufficient number of heterozygous forms in both studied groups of rabbits. The values of the inbreeding coefficient as a measure of heterozygosity deficiency or excess in a given population are presented in Figure 8.

The chi-square test for Hardy-Weinberg equilibrium (Table 1) showed a value of $\chi^2$ with level of probability P>0.2 and at degree of freedom df=1, confirming the validity of the HWE for both rabbit populations in the present research.

**Table 1.** *Nei-expected heterozygosity, chi-square test of HWE ($\chi^2$) and P-value for the polymorphic locus in GHR gene in the investigated rabbit populations.*

| Rabbit populations | n | Nei* | $\chi^2$ | P-value |
|---|---|---|---|---|
| New Zealand White | 51 | 0.451 | 3.707 | 0.230 |
| Californian | 49 | 0.497 | 4.437 | 0.906 |

*Expected heterozygosity calculated as per Nei (1973).

## Conclusions

The obtained experimental results based on PCR-RFLP analysis confirmed the presence of C/G SNP at exon 3 of the GHR gene in studied rabbit populations. The distribution of allele frequencies with respect to GHR gene suggested that the observed genetic polymorphism could be a useful marker in future research on association analysis for growth performance traits in rabbits. Therefore, additional investigations are planned to estimate the favourable GHR genotypes that would allow accurate rabbit selection.

## Acknowledgement

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