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Association Between Growth Hormone Receptor Gene Polymorphism and Body Weight in Growing Rabbits

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Abstract | The current study was performed to investigate the association of the SNP c.106C>G which is located in exon 3 of growth hormone receptor (GHR) gene with body weight in rabbits. Baladi Red (local breed) and New Zealand White rabbits (exotic breed) were used, body weights at 6, 8, 10 and 12 weeks of age of 10 individuals/sex/breed were considered as the phenotypic parameters. The individuals were genotyped by PCR-RFLP method using *Hinf1* as a restriction enzyme. The results showed that the body weight of NZW rabbits was significantly higher than the local breed over all ages, however the sex effect was mostly insignificant. The gene frequency results showed that the frequency of CC genotype was 0.10 overall individuals of the two breeds. The frequencies of the two alleles were found to be 0.225 (allele C) and 0.775 (allele G), and the frequency of allele C was slightly higher in NWZ than local Baladi rabbits. Highly significant ($p \leq 0.002$) associations were found between CC genotype and body weights at 6, 10 and 12 weeks of age. The results suggested the possibility of using polymorphism of exon 3 of the GHR gene as a candidate gene for body weight in both local and exotic rabbit breeds in Egypt.

Keywords | Association study, Body weight, GHR gene, Local rabbits, SNP

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INTRODUCTION

Rabbits (*Oryctolagus cuniculus*) are considered a good source of animal protein. Although consumption of rabbit meat is not prevalent in some countries, it is acceptable in Egypt. The per capita consumption of rabbits in Egypt is about 0.7 Kg/year compared with 3.6 and 3.5Kg/year in France and Spain, respectively (FAO, 2017). The rabbit production in Egypt depends mainly on the exotic breeds, due to the poor productivity of local ones. Exotic rabbit breeds in Egypt are suffering from the Egyptian hot climate in the summer season. However, the local and synthetic rabbit lines are well adapted for the Egyptian environmental conditions. Accordingly, improving the productivity of local breeds is mandatory for sustainable development.

Molecular genetics offered new approaches for the genetic improvement of animals. Such approaches accelerate the improvement steps and increase the genetic gain. One of these approaches is a candidate gene strategy (Fontanesi et al., 2008), where genes with known functions are screened for polymorphism and linked to traits of interest, and employed in genetic selection programs (Dekkers, 2004; Van Oers et al. 2005).

Growth hormone is very important for growth in human and animals as well, when growth hormone binds to growth hormone receptor (GHR), it causes receptor-dimerization which promotes cell growth and increases blood glucose and fatty acids levels (Frank, 2001; Zhang et al. 1999). GHR affects growth hormone either directly by the activation of tyrosinase kinase or indirectly by the induction of insulin-like growth factor 1 (Brooks and Waters, 2010). The GHR gene has been sequenced in rabbits, it is located on the 11th chromosome (ENSOCUG00000008496.2) of

the rabbit genome, it contains 10 exons and annotated to 17 domains with transcript length of 4024 bp which translated to a protein length of 638 amino acids (Leung et al. 1987).

SNP can be detected by several ways. Ota et al. (2007) proposed a new method for SNP detection and analyzing DNA sequences using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, where the PCR products are incubated with restriction endonuclease to detect point mutations based on the fragmentation of the PCR products. The association of single nucleotide polymorphisms (SNPs) with different economical traits in rabbits was successfully explored (Peiró et al., 2008; Kotsyubenko et al., 2017; Wu et al., 2015). Deng et al. (2008) analyzed the polymorphism of the GHR gene by PCR-SSCP and identified two mutations (C705T and C810T) in five rabbit populations. Fontanesi et al. (2016) studied the polymorphism of GHR gene by sequencing the exons and non-coding region in rabbits and identified 10 SNPs, including one missense mutation (g.63453192C>G or c.106C>G).

This research was done to analyze the relationship between the single nucleotide polymorphism (c.106C>G), which is located at the third exon of GHR gene, and the body weight at different ages of one local and one exotic rabbit breeds in Egypt.

MATERIALS AND METHODS

Ethical Approval

This work was done within the framework of the project entitled "Genomic selection as a tool to develop a new rabbit line adapted for local environmental conditions". All the project activities were approved from the institutional animal care and use committee at Cairo University (CU-IACUC) with the approval number of CU/I/F/49/17.

Animals

A total of forty growing rabbits were used in the current study. Twenty post-weaning (30 days old) individuals (10 males and 10 females) from each of Baladi Red as a local breed and New Zealand White rabbits (NWZ) as an exotic commercial line were analyzed. Animals were bred during the winter season of 2018, and were housed in a semi-closed system with individual cages, and supplied with a commercial pelleted diet with free access to water (nipple system). Post-weaning body weights were recorded biweekly from 6 to 12 weeks of age and considered as the phenotypic data for the association analysis.

Blood Samples

Three milliliters of blood samples were collected from the marginal vein of the ear of each individual to tubes containing ethylene-diaminetetracetic acid (EDTA) and stored at -20°C until the DNA extraction

DNA Extraction and Amplification

DNA was extracted using a genomic DNA extraction kit (BioFlux®, China) according to the manufacturer's instructions with minor modifications by using 0.75X of all quantities. The purity and quantity of the extracted DNA were measured using Spectrophotometry (PG instruments, UK), the DNA was then diluted (60 ng/µl) and stored at 4°C until use. PCR-RFLP technique was chosen to detect the polymorphism of GHR gene mutation c.106G>C (Zhang et al., 2012), accordingly the primers (designed by Fontanesi et al., 2016) were used to amplify exon 3 (479 bp) of the GHR gene (F: AGGTGAAGCGTGCTCTCATT, R: TTTGGCCTAGCTTAGCCTTT). The total PCR reaction volume was 20 µl, the mixture was composed 10 µL 2X Taq PCR Master Mix (Vivantis, Malaysia), 1 µl of DNA template, 1.5 µL (10 pmol) of each primer and 6 µL of ddH_2O. The PCR program started with an initial denaturation step (95°C for 10 minutes), followed by 35 PCR cycles of denaturation (94°C for 30 seconds), annealing (56°C for 30 seconds) and elongation (72°C for 60 seconds), the final extension step was set to 72°C for 10 minutes. The genotyping was done according to Fontanesi et al. (2016), where the PCR product was digested with the restriction enzyme *HinfI*. The digestion process was done by incubation for 15 hours at 37°C in a 10 µL reaction mixture includes 5 µL of PCR product, 4 U/µL of the restriction endonuclease and 1X buffer. The resulted fragments were then electrophoresed in 2% agarose gel, stained with ethidium bromide and visualized using a UV transilluminator.

Statistical Analysis

Phenotypic data were analyzed using SAS procedures (SAS, 2000). The association analysis between the SNP (c.106C>G) and body weights was also performed using General Linear Model (GLM) procedure of SAS, using the following model:

$$Y_{ijklm} = \mu + B_i + S_j + G_k + e_{ijkl}$$

where, Y is the dependent variable under study, µ is the overall mean, B, S and G indicate the fixed effects of breed, sex and genotype, respectively. The effect of litter size was not included in the model because each rabbit was selected randomly from different does litter. Significant differences were shown by Duncan's multiple range test (Duncan, 1955).

Table 1: Body weight (LSM±SE, g) with age of the different breed and sex of the genotyped rabbits

	NWZ		Baladi Red	
	Males	Females	Males	Females
BW6	853.2[a]± 53	786.8.8[a]±53	600.0[b]± 53	496.4[b]±53
BW8	1210.0[a]±45	1097.0[a]±45	869.8[b]±45	799.6[b]±45
BW10	1767.0[a]±68	1492.8[b]±68	1459.66[b]±68	1146.4[c]±68
BW12	2288.6[a]±119	2047.0[ab]±119	1698.6[bc]±119	1505.2[c]±119

[a, b, c], means with different superscripts within a row indicate significant differences (P ≤ 0.05).

Table 2: Genotypic and allelic frequencies of the genotyped animals

	n	Genotype frequency			Allele frequency	
		CC	CG	GG	C	G
All individuals	40	0.10	0.25	0.65	0.225	0.775
Baladi	20	0.10	0.10	0.80	0.150	0.850
NWZ	20	0.10	0.40	0.50	0.300	0.700
Male Baladi	10	0.20	0.20	0.60	0.300	0.700
Female Baladi	10	0.00	0.00	1.00	0.000	1.000
Male NWZ	10	0.20	0.40	0.40	0.400	0.600
Female NWZ	10	0.00	0.40	0.60	0.200	0.800

Table 3: Association analysis of GHR genotypes with body weight (g) at different ages in rabbits (values are LSM ± SE)

Trait	Breed	Genotype			P value
		CC	GC	GG	
BW6	All individuals	933.8[a]±65.9	657.4[b]±43.9	575.7[b]±49.8	0.0015
	NWZ	945.0[a]±23.3	779.3[b]±20.2	749.3[b]±23.3	0.0011
	Baladi Red	900.0[a]±50.2	560.0[b]±22.5	445.5[b]±25.1	0.0003
BW8	All individuals	1154.0[a]±93.5	1216.7[a]±71.8	966.0[a]±98.7	0.192
	NWZ	958.9[a]±62.3	1127.8[a]±62.2	823.8[a]±44.1	0.6013
	Baladi Red	948.4[a]±70.6	1125.7[a]±71.8	815.5[a]±49.3	0.4177
BW10	All individuals	1836.0[a]±97.6	1397.1[b]±65.1	1344.4[b]±73.8	0.0021
	NWZ	1868.0[a]±83.3	1552.5[b]±72.1	1495.0[b]±83.3	0.0306
	Baladi Red	1740.0[a]±167.9	1272.8[b]±75.1	1231.0[b]±83.9	0.0453
BW12	All individuals	2351.3[a]±148.6	1906.3[b]±99.0	1590.7[b]±112.3	0.002
	NWZ	2514.3[a]±116.9	2131.3[b]±101.3	1870.0[b]±116.9	0.0171
	Baladi Red	1862.0[a]±131.3	1726.4[a]±58.7	1381.3[b]±65.6	0.0092

[a, b, c], means with different superscripts within a row indicate significant differences (P ≤ 0.05).

RESULTS AND DISCUSSION

Body Weight

Average body weights of the genotyped rabbits are summarized in Table (1). The effect of breed was significant at 6 and 8 weeks of age, NWZ rabbits had significantly (p≤0.05) higher body weight than Baladi Red rabbits. However, the effect of sex was significant only at 10 weeks of ages for the two breeds.

Allele Frequency and Polymorphism

Since the study was targeting the polymorphism of the SNP c.106C>G of exon 3 of GHR gene, the restriction endonuclease *Hinf1* was used for targeting the GANTC restriction site, which exists once in the allele G and twice in the allele C. Accordingly, the incubation of the PCR fragment (479 bp) with *Hinf1* generates three fragments (210, 162 and 107 bp) for allele C and only two fragments for allele G (317 and 162 bp). Then the genotypes of animals can be distinguished directly from the generated banding pattern for each individual, whereas the number of bands appeared is two (317 and 162 bp) for CC genotype, three (210, 162 and 107 bp) for GG genotype, and four (317, 210, 162 and 107 bp) bands for the heterozy-

gous CG. The genotypic frequencies of the genotyped animals are presented in Table (2), the genotype CC was not detected in the females of both Baladi and NWZ rabbits which was unexpected, the heterozygous genotype also was not detected in the females of Baladi rabbits. In general, the frequency of CC genotype was found to as low as 0.10 overall individuals of the two breeds. On the contrary, the frequency of GG genotype was 0.65 overall individuals while the heterozygous was 0.25 only. The frequency of GG genotype was higher in Baladi (0.80) than in NWZ (0.50) rabbits. The same frequency (0.20) of CC genotype was found in the males of the two breeds. Gencheva et al. (2017) genotyped 51 NWZ rabbits and reported a similar heterozygous genotype (CG) frequency of 0.529, while the GG genotype was less frequent (0.392) than the current study. They also reported a very low frequency of (0.078) for CC genotype. The frequencies of the two alleles were found to be 0.225 and 0.775 for C and G alleles, respectively. These results were in accordance with the results of Zhang et al. (2012) as the existence of the C allele was low (0.323) compared to G allele (0.677) in a Chinese rabbit population. Migdal et al. (2019) used PCR-RFLP for genotyping the same mutation, CC genotype frequencies were ranged between 0.03 and 0.47. The low frequencies of CC genotypes (≤0.10) with a moderate frequency of the C allele (0.15 – 0.30) may suggest that the presence of a kind of natural selection against the C allele.

Genotype Effect and Association with Body Weight

The associations between genotype and body weight over age are presented in Table (3). The effect of sex was not considered for association analysis not only because the insignificance of sex effect in most ages, but also because the CC genotype was not detected in the females. When all the 40 rabbits were analyzed together, the difference between CC genotype and the other two genotypes was highly significant ($p \leq 0.002$) at 6, 10 and 12 weeks of age, these high levels of significance indicate that the differences between individuals were attributed to the genotype effect. Nevertheless, insignificant differences were found between CG and GG genotypes. Similar results were obtained when each breed was analyzed separately. There were no significant differences between the genotypes GG and GC except for Baladi Red rabbit at 12 weeks of age. It is obvious that the presence of allele C in the heterozygous genotype did not affect the difference between GC and GG genotypes, although the considerable increases in body weights when allele C was presented in the homozygous form. Similar findings were reported by Zhang et al. (2012) where the highest values for 84-day weight and slaughter weight were associated with CC genotypes. Deng et al. (2008) found a significant ($p \leq 0.05$) association of two GHR mutations (G.63537066C>T and G.63537228A>G) located in exon 10 of the GHR gene with live weight, vis-ceraste weight, and slaughter percentage in 5 rabbit populations. Moreover, Fontanesi et al. (2016) identified one a GHR gene mutation (g.63453192C>G or c.106C>G) which was associated ($P < 0.05$) with 70-days body weight in rabbits. The same mutation (c.106C>G) was associated with carcass traits (Zhang et al., 2012; Migdal et al., 2019) and meat weight (Migdal et al., 2019). Another mutation was reported located in the first exon of GHR gene and was correlated with growth performance in NWZ, V-line and Alexandria rabbits (Sahwan et al., 2014). On the contrary, El-Sabrout and Aggag (2017) detected a missense mutation in the GHR gene in Alexandria and V-line rabbits which was not associated with any economical trait. The results indicated that CC genotypes were less frequent but had superior body weights competed to other genotypes. The c.106C>G mutation of exon 3 of the GHR gene constitute a good candidate gene for increasing selection efficiency for body weight in rabbits.

CONCLUSION

The individual variation in GHR gene plays an important role in controlling growth and differentiation. The obtained results showed that GHR c.106C>G polymorphism is associated with body weight at 6, 10 and 12 weeks of age. These results indicated the possibility of using polymorphism of the third exon GHR gene as a candidate gene for growth in both local and exotic rabbit breeds in Egypt, and it should be employed in a marker assisted selection program to improve the productivity of local rabbits. Moreover, the polymorphism of the other nine exons of the GHR gene needs to be deeply investigated further in the future.

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