
ASSOCIATION OF GROWTH HORMONE RECEPTOR (*GHR*) GENE POLYMORPHISMS WITH BODY WEIGHT OF RABBITS: A SYSTEMATIC REVIEW AND META-ANALYSIS

Bashiru, H. A.* and Oseni, S.O.

Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria

*Corresponding Author's Email: hbashiru@oauife.edu.ng /+2347037292065

ABSTRACT

The growth hormone receptor (*GHR*) gene plays a key role in regulating growth and metabolism. However, the relationship between polymorphic variants of this gene and the body weight of rabbits under different genetic models has not been established. Thus, the objective of this study was to evaluate relationship between *GHR* gene polymorphisms and body weight of rabbits. Relevant literatures were systematically reviewed using the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) guideline. 248 studies were identified and screened against pre-determined inclusion criteria and data were extracted from 17 studies. Each study was subjected to Hardy-Weinberg Equilibrium (HWE) test and six genetic models consisting of co-dominant, dominant, recessive and over-dominant models were fitted using OpenMeta[®] Analyst Software. The Cohen's *d* method was used to calculate standardized mean differences (SMDs) to estimate individual study effect size as well as the overall effect size. Heterogeneity measures were inferred from the *Q* statistic (based on Chi-square test) and its associated *p*-value, Tau-squared (τ^2), H-squared (H^2) and I-squared (I^2) values. Publication bias was assessed by Egger's regression asymmetry test and sensitivity analysis was conducted to detect if the overall effect size was largely due to a single study effect size. There was significant association ($P < 0.05$) of *GHR* gene with body weight of rabbits under one of the co-dominant models (CG vs GG). However, this association was not significant ($P > 0.05$) under all other genetic models. Further, the results indicated that, cumulatively for all studies included in the meta-analysis of association of *GHR* gene with body weight of rabbits, GG genotype had higher body weight than CG genotype. However, there was no difference ($P > 0.05$) between CC and CG and CC and GG genotypes. These findings implied that the impact of *GHR* gene polymorphisms on body weight in rabbits may be influenced by specific genetic models.

Keywords: *GHR* gene, meta-analysis, systematic review, rabbit, polymorphisms

INTRODUCTION

The growth hormone receptor (*GHR*) is important for vital life processes such as growth and metabolism regulation. According to Ramadan *et al.* (2020), the growth hormone, growth hormone receptor and insulin-like growth factor are jointly involved in the coordination of growth regulation in rabbits. The activation of the growth hormone receptor occurs upon the binding of growth hormone to its receptor, initiating the activation and expression of insulin-like growth factors and other genes (Herrington and Carter-Su, 2001). This process, as described by Dehkhoda *et al.* (2018) results in the stimulation of amino acid uptake and protein synthesis. Single nucleotide polymorphisms (SNPs) in the *GHR* have been reported to be associated with body weight of various breeds of rabbits (Deng *et al.*, 2008; Zhang *et al.*, 2012; Fontanesi *et al.*, 2016; Gencheva *et al.*, 2022). In addition, three genotypic variants (CC, CG and GG) have been identified in rabbits. However, there have been contrasting reports on the relationship between these variants. An elucidation of such relationship could be crucial for marker-assisted selection in rabbit genetic improvement programmes. Therefore, the objective of this study was to evaluate the relationship between *GHR* gene and body weight and establish the most appropriate genetic model(s) for the relationship.

MATERIALS AND METHODS

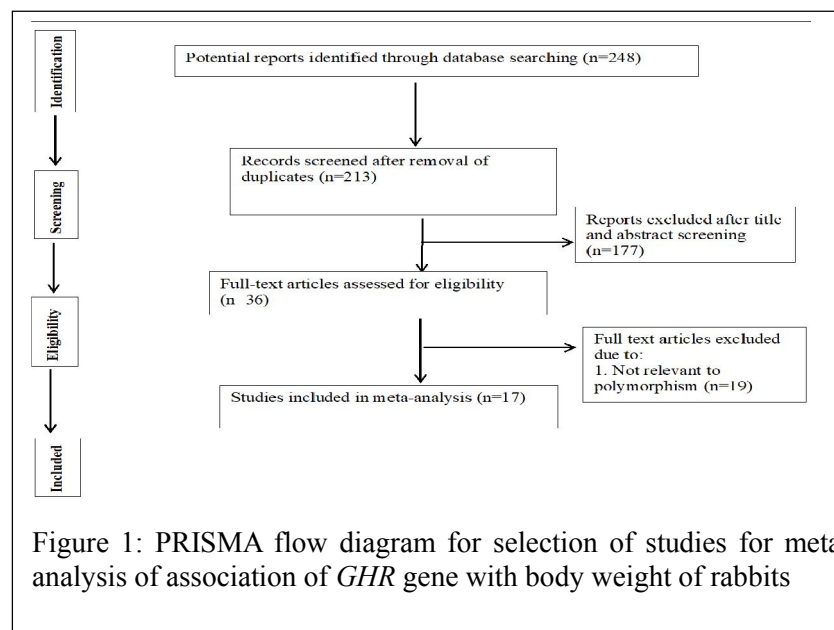
Database and search strategy

A comprehensive database search was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline (Moher *et al.*, 2009). The PRISMA flow diagram is presented in Figure 1. Screening of the title and abstract of each article identified through database search was done and relevant articles were selected for in-depth and full text assessment using

the pre-determined inclusion and exclusion (eligibility) criteria. A total of 248 studies were retrieved from databases. Out of these, only 17 studies fulfilled the inclusion criteria and were included in the final analysis.

Data analysis

Each study was subjected to Hardy-Weinberg Equilibrium (HWE) test using the gene calculator software freely available online: <https://gene-calc.pl/hardy-weinberg-page>.



OpenMeta[®] Analyst Software (Wallace *et al.*, 2012) was used for the meta-analysis. Six genetic models under the assumption of co-dominance (3 genetic models: CC vs CG; CC vs GG and CG vs GG), recessive (1 genetic model; CC vs CG+GG), dominance (1 genetic model: CC +CG vs GG) and over-dominance (1 genetic model: CG vs CC+GG) were fitted to evaluate the association between *GHR* gene

polymorphism and body weight of rabbit. The Cohen's *d* method was used to calculate standardized mean differences (SMDs) to estimate individual study effect size as well as the overall effect size. Heterogeneity measures were inferred from the Q statistic value, Tau-squared (T^2), H-squared (H^2) and I-squared (I^2) values. The random effects model was used because I^2 values were greater than 20% for all models (Ryan-Moore *et al.*, 2020). In addition, Forest plots of heterogeneity test were plotted to visualize individual study's effect size, standard error, upper and lower limit of estimates as well as the overall effect size. Publication bias was assessed by Egger's regression asymmetry test.

RESULTS AND DISCUSSION

Heterogeneity tests

Table 1 presents the heterogeneity tests of six genetic models for the meta-analysis. The P values for the Chi-square test were significant ($P < 0.01$) for the co-dominant models (CC vs CG and CG vs GG), the recessive model and the over-dominant model. The I^2 values were high ($I^2 > 59\%$, ranged from 59.4% to 78.4%) and thus, all genetic models fitted were considered heterogeneous which indicated that effect size reported by different studies included in the meta-analysis of the association between *GHR* gene polymorphism and body weight of rabbits are due to systematic differences and not solely due to sampling error (Ryan-Moore *et al.*, 2020).

Estimation of overall effect size

The overall effect size estimates of the six genetic models for the meta-analysis of the association between *GHR* gene polymorphism and body weight of rabbits are presented in Table 2. The overall effect size under different genetic models evaluated ranged from -0.288 (co-dominant -CG vs GG) to 0.203 (co-dominant -CC vs CG). There was significant association ($P < 0.05$) of *GHR* gene polymorphism with body weight of rabbits under the co-dominant (CG vs GG) model (SMD = -0.288 ± 0.1229 ; 95% CI = -0.327 ± 0.1362 , 95% CI = -0.529 to -0.47; $p = 0.019$). However, *GHR* polymorphisms showed no significant association ($P > 0.05$) with body weight of rabbit under the co-dominant models (CC vs GG and CC vs CG), recessive, dominant

Table 1: Heterogeneity test of six genetic models for the meta-analysis of the association between *GHR* gene and body weight of rabbits (n=17)

Category	Genetic model	T ²	H ²	I ²	Chi-square (Q statistic)	P value
Co-dominant	CC vs CG	0.171	2.983	66.5	47.412	<0.001
Co-dominant	CC vs GG	0.202	2.462	59.4	18.073	0.320
Co-dominant	CG vs GG	0.138	2.690	62.8	27.096	0.040
Recessive	CC vs CG+GG	0.217	3.926	74.5	41.739	<0.001
Dominant	CC+CG vs GG	0.189	3.675	72.8	26.164	0.052
Over-dominant	CG vs CC+GG	0.187	4.637	78.4	57.850	<0.001

T²=Tau-squared; H²=H-squared; I²=I-squared

and over-dominant models. The negative significant overall effect size ($P < 0.05$) under the co-dominant genetic model (CG vs GG) indicated that, cumulatively for all studies included in the meta-analysis, GG genotype had higher body weight than CG genotype. There are contrasting reports on these associations in literature. Deng *et al.* (2008) reported no significant difference between the body weight of CG and GG genotype but the two were significantly higher than CC genotype of Harbin white rabbits in China. Fontanesi *et al.* (2016) reported that 10 different breeds of GG genotype had higher body weight ($P < 0.05$) than rabbits of CC and CG genotype. However, Gencheva *et al.* (2022) reported that Californian rabbits of CC genotype had higher significant body weight at 35, 70 and 90 days than CG and GG genotypes. Helal (2019) reported no difference ($P > 0.05$) in the body weight of CC, CG and GG genotypes of New Zealand and Baladi Red rabbits in Egypt at 8 weeks of age. However, the author reported that CC genotype had higher body weight than CG and GG genotype at 6, 10 and 12 weeks of age. Migdal *et al.* (2019) reported no significant difference ($P > 0.05$) in body weight between CC, CG and GG genotype of crossbred New Zealand x Belgian Giant Grey rabbit at day-old, 5 weeks and 12 weeks of age. However, significant higher body weight ($P < 0.05$) was reported for GG genotype of Belgian Giant Grey rabbit at 5 weeks of age while no difference was reported at day-old and 12 weeks of age. Similarly, significant higher body weight ($P < 0.05$) was reported for CG genotype of Termond White rabbit at day-old while no difference was reported at 5 and 12 weeks of age. Zhang *et al.* (2012) also reported that CC genotype of Tianfu, Iraq and Champagne rabbit had higher body weight ($P < 0.05$) than CG and GG genotypes of these breeds of rabbits.

Table 2: Overall effect size estimates of the six genetic models for the meta-analysis of the association between *GHR* gene and body weight of rabbits (n=17)

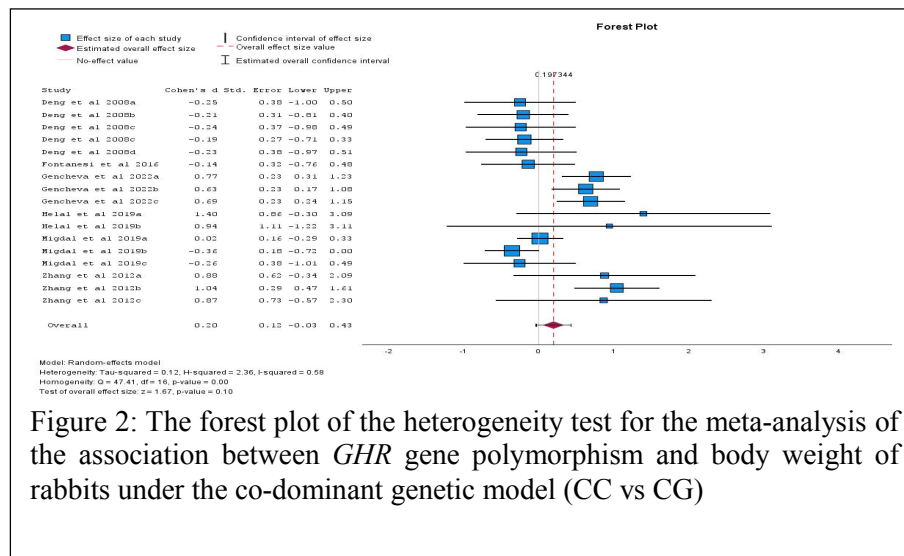
Category	Genetic model	Effect size (SMD ±SE)	95% CI	95% PI	P value
Co-dominant	CC vs CG	0.203±0.1331	-0.057 to 0.464	-0.723 to 1.130	0.126
Co-dominant	CC vs GG	0.044±0.1508	-0.252 to 0.339	-0.966 to 1.054	0.772
Co-dominant	CG vs GG	-0.288±0.1229	-0.529 to -0.47	-1.122 to 0.545	0.019
Recessive	CC vs CG+GG	0.126±0.1405	-0.149 to 0.401	-0.911 to 1.164	0.369
Dominant	CC+CG vs GG	-0.170±0.1326	-0.430 to 0.090	-1.139 to 0.799	0.200
Over-dominant	CG vs CC+GG	-0.209±0.1244	-0.453 to 0.034	-1.168 to 0.749	0.092

SMD- Standardized Mean difference; SE-Standard error; CI- Confidence interval; PI -Prediction interval

Forest plot of the genetic models for the association between growth-regulatory complex genes and growth performance traits of rabbit

Figure 2 shows the forest plot of the heterogeneity test for the meta-analysis of the association between *GHR* gene polymorphism and body weight of rabbits under the co-dominant genetic model (CC vs CG). The overall effect size was not significant ($P > 0.05$) suggesting no association between *GHR* gene polymorphism and body weight of rabbits under this genetic model. The non-significance

of the overall effect size could be interpreted that effect size from most of the studies included in the meta-analysis were not different from zero under this model.



Egger's regression test for the assessment of publication bias

Table 3 present the Egger's regression test for six genetic models evaluated. The Egger's regression tests were significant ($P < 0.05$) under the co-dominant (CC vs CG) model [$b = 0.806 \pm 0.2249$; 95% CI= -0.663 to 2.274; $P = 0.0261$], co-dominant (CG vs GG) model [$b = -$

0.617 ± 0.1536 ; 95% CI= -0.945 to -0.290; $P = 0.001$], recessive model [$b = -0.195 \pm 0.1314$; 95% CI= -0.688 to 0.298; $P = 0.0413$] and over-dominant model [$b = -0.715 \pm 0.2523$; 95% CI= -1.253 to -0.177; $P = 0.013$]. Furthermore, the tests predicted two theoretical missing studies for the analysis under these genetic models and thus suggest the existence of publication bias. Conversely, the Egger's regression-based tests were not significant ($P > 0.05$) under the co-dominant (CC vs GG) model [$b = -0.313 \pm 0.2904$; 95% CI= -0.932 to 0.306; $P = 0.299$] and dominant model [$b = 1.825 \pm 1.8408$; 95% CI= 0.033 to 4.617; $P = 0.461$].

Table 3: Egger's regression test for six genetic models for the assessment of publication bias for the random effects meta-analysis of association between *GHR* gene and body weight of rabbits

Category	Genetic model	Regression coefficient $\pm$ SE	95% CI	P value	Number of missing studies
Co-dominant	CC vs CG	0.806 $\pm$ 0.2249	-0.663 to 2.274	0.0261	2
Co-dominant	CC vs GG	-0.313 $\pm$ 0.2904	-0.932 to 0.306	0.299	0
Co-dominant	CG vs GG	-0.617 $\pm$ 0.1536	-0.945 to -0.290	0.001	2
Recessive	CC vs CG+GG	-0.195 $\pm$ 0.1314	-0.688 to 0.298	0.0413	2
Dominant	CC+CG vs GG	1.825 $\pm$ 1.8408	0.033 to 4.617	0.461	0
Over-dominant	CG vs CC+GG	-0.715 $\pm$ 0.2523	-1.253 to -0.177	0.013	2

SE- Standard error; CI- Confidence interval

CONCLUSION

The findings from this study implied that the impact of *GHR* gene polymorphisms on body weight in rabbits may be influenced by specific genetic models, with the co-dominant model (CG vs GG) showing a significant association. The observed higher body weight in rabbits with the GG genotype suggests a potential role of this genetic variant in promoting growth. However, further research is needed to explore the underlying mechanisms and confirm these associations.

ACKNOWLEDGEMENT

This study was funded under the TETFund IBR Code 11-801-BSG, awarded to the second author. This is acknowledged with gratitude.

REFERENCES

- Dehkhoda, F., Lee, C. M. M., Medina, J. and Brooks, A. J. (2018). The growth hormone receptor: mechanism of receptor activation, cell signaling, and physiological aspects. *Frontiers in Endocrinology*, 9. <https://doi.org/10.3389/fendo.2018.00035>
- Deng, X.S., Wan, J., Chen, S.Y., Wang, Y., Lai, S.J., Jiang, M.S., Xu, M. and Chuan, Y. (2008). The correlations between polymorphism of growth hormone receptor gene and butcher traits in rabbit. *Hereditas*, 30 (11): 1427-1432. <https://doi.org/10.3724/SP.J.1005.2008.01427>
- Fontanesi, L., Sparacino, G., Utzeri, V.J., Scotti, E., Fornasini, D., Dall'Olio, S. and Frabetti A. (2016). Identification of polymorphisms in the rabbit growth hormone receptor (GHR) gene and association with finishing weight in a commercial meat rabbit line. *Animal Biotechnology*, 27:77-83 <http://dx.doi.org/10.1080/10495398.2015.1101697>
- Gencheva, D. G., Velikov, K. P., and Veleva, P. M. (2022). Association analysis of nucleotide polymorphisms in growth hormone (GH) and its receptor (GHR) with body weight in Californian rabbits. *World Rabbit Science*, 30: 95-102 <https://doi.org/10.4995/wrs.2022.13127>
- Helal, M. M. (2019). Association between growth hormone receptor gene polymorphism and body weight in growing rabbits. *Adv. Anim. Vet. Sci.* 7(11):994-998. <http://dx.doi.org/10.17582/journal.aavs/2019/7.11.994.998>
- Herrington, J. and Carter-Su, C. (2001). Signaling pathways activated by the growth hormone receptor. *Trends in Endocrinology and Metabolomics*, 12: 252-257
- Migdal, L., Pałka, S., Kmiecik, M. and Derewicka, O. (2019). Association of polymorphisms in the GH and GHR genes with growth and carcass traits in rabbits (*Oryctolagus cuniculus*). *Czech Journal of Animal Science*, 64, 2019 (6): 255–264 <https://doi.org/10.17221/27/2019-CJAS>
- Moher, D., Liberati, A., Tetzlaff, J. and Altman, D. G. (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med.* 6:e1000097. <https://doi.org/10.1371/journal.pmed.1000097>
- Ramadan, S. I., Manaa, E. A., El-Attrouny, M. E., and El Nagar, A. G. (2020). Association of growth hormone (GH), insulin-like growth factor 2 (IGF-2) and progesterone receptor (PGR) genes with some productive traits in Gabali rabbits. *World Rabbit Science*, 28(3), 135–144. <https://doi.org/10.4995/WRS.2020.12610>
- Ryan-Moore, E., Mavrommatis, Y. and Waldron, M. (2020). Systematic Review and Meta-Analysis of Candidate Gene association studies with fracture risk in physically active participants. *Frontiers in Genetics*, 16:11:551. <https://doi.org/10.3389/fgene.2020.00551>
- Wallace, B. C., Dahabreh, I. J., Trikalinos, T. A., Lau, J., Trow, P., and Schmid, C. H. (2012). Closing the Gap between Methodologists and End-Users: R as a Computational Back-End. *Journal of Statistical Software*, 49(5), 1–15. <https://doi.org/10.18637/jss.v049.i05>
- Zhang, W.X., Zhang, G.W., Peng, J. and Lai, S.J. (2012). The polymorphism of GHR gene associated with the growth and carcass traits in three rabbit breeds. In: *Proceedings 10th World Rabbit Congress*, 2012 September, Sharm El-Sheikh, Egypt, 75-78.