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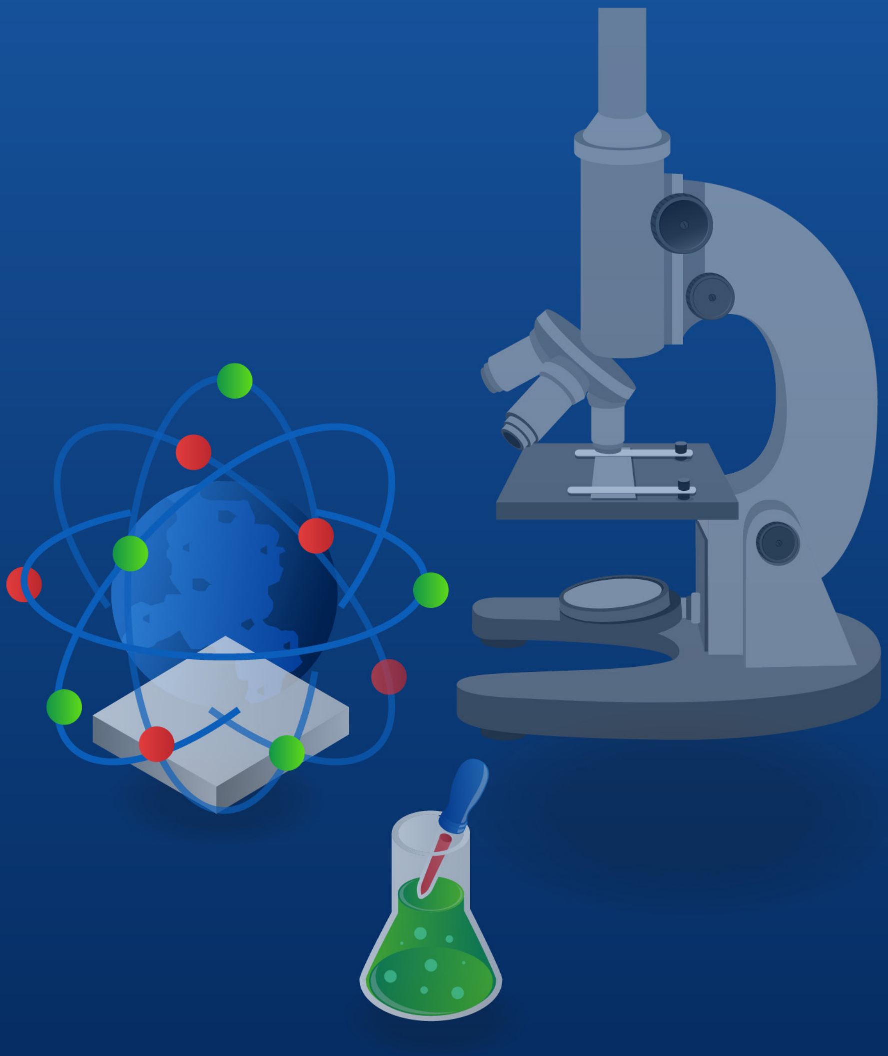

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Association of Neuropeptide Y, Dopamine Receptor D2, Bone Morphogenetic Protein Receptor 1B and Melanocortin 1C Receptor Genes with Body Weight in Indigenous Chicken Ecotypes

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ABSTRACT

The present study aimed to determine the association of single nucleotide polymorphisms (SNPs) within *DRD2, BMPR1B, NPY* and *Mel1C* genes with body weight (BW) in indigenous chicken (IC) ecotypes. A total of 234 chickens from six ecotypes of Ethiopia; Afar (n = 16), Amhara (n = 105), Gumuz (n = 13), SNNPR (n = 18), Tigray (n = 43), and Oromia (n = 17) were sampled, their blood drawn from wing vein, and genomic DNA (gDNA) extracted. Contigs of the gDNA were sequenced on a Hamilton MicroLab STAR system and Genologics Clarity LIMS X Edition. Four candidate genes, *DRD2, NPY, BMPR1B*, and *Mel1C*, were extracted based on GRCg6a chicken reference genome assembly and used to conduct downstream genomic and association analyses. Body weight (kg) was measured on all sampled chickens. BW ranged from 0.6kg to 2.60kg, with significant differences between sexes ($p \leq 0.01$) and among ecotypes ($p \leq 0.01$) and ecotype-by-sex interaction ($p \leq 0.5$) but no significance ($p \leq 0.05$) with age. Average BW was 1.38±0.32kg and 1.20±0.29kg for male and female chickens, respectively. BW was highest in SNNPR (1.39±0.07kg) and lowest in Oromia (1.06±0.04kg) ecotypes. Amhara, Afar, Gumuz and Tigray had average BW of 1.26±0.03kg, 1.13±0.01kg, 1.18±0.06kg and 1.36±0.05kg, respectively. There was no significant ($p \leq 0.05$) association between SNPs of *NPY, Mel1C, DRD2* and *BMPR1B* genes with BW. However, some SNPs (rs312679760 of *BMPR1B*, rs315981851 of *NPY* and rs314194962 of DRD2) with potential effects on BW showed suggestive associations. These findings demonstrate significant variation of BW across ecotypes and sexes, and no significant association of the genes with BW in the Ethiopian ecotypes, suggestive SNPs warrant further genome-wide research.

INTRODUCTION

Indigenous chickens (Gallus gallus domesticus) are the most populous poultry species globally due to their economic, social and cultural significance, making significant contributions to household food and income security (Muchadeyi *et al.,* 2007; Ekaluo *et al.,* 2025). In Ethiopia, ICs account for over 90% of the national chicken meat and egg production (Gheyas *et al.,* 2022). The primary reason for domesticating chickens in Africa was their socio-cultural significance; their role as a food emerged later (Clutton-Brock, 1992). High demand of meat and its preference for special dishes drives IC market prices to twice or thrice commercial products (Islam and Nishibori, 2009; Al-Jaryan *et al.,* 2021). Consequently, BW being a primary determinant of market prices, is the most important selection criterion used by farmers (Dana *et al.,* 2010c; Gebremariam *et al.,* 2017).

Indigenous chickens are traditionally kept in isolated, small unimproved flocks, which are left to scavenge with little or no supplemental feeding, better housing and veterinary care under a free-range scavenging system (Dana *et al.,* 2010d; Markos *et al.,* 2024). They are relatively poor producers of meat due to their slow growth rate and low mature weight (Ngeno *et al.,* 2013). As a result, they are gradually losing their importance in rural villages prompting widespread introduction of exotic genetic material through crossbreeding programs (Mathur *et al.,* 1989). However, introduction of exotic breeds often leads to the dilution and loss of valuable genetic variability in local chickens' ecotypes (FAO, 2007; Besbes, 2009; Yuan *et al.,* 2018).

The BW of chicken can be classified into large, medium and small (Wang *et al.,* 2023). Indigenous chickens are characterized by small body size varying by age, sex, breed and population (Halima *et al.,* 2009; Yang *et al.,* 2022). Increasing the BW using genetic means requires a prevailing genetic variation and effective selection methods to exploit it (Muchadeyi *et al.,* 2007). Taffa *et al.* (2022) and Ohagenyi *et al.* (2023) reported moderate to high heritability estimates, strong positive correlation with growth curve traits, negative to weak correlation with carcass and meat traits, indicating existing additive genetic potential which can be exploited through selective breeding and marker-assisted selection (Andini *et al.,* 2019; Khobondo, 2021; Kanlisi *et al.,* 2022).

Body weight is highly polygenic, and several QTLs and chromosomal regions identified (Yuan *et al.,* 2018; Kanlisi *et al.,* 2022). Candidate genes relating to adaptation, production, reproduction, and immunity and unique in their expressions in domestic chicken population

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depending on ecological, production environments under which chickens are kept have been identified (Lawal *et al.,* 2018; Ekaluo *et al.,* 2025). Genes such as growth hormone (Mehdi and Reza, 2012; Kazemi *et al.,* 2018), ACTA-1 (Andini *et al.,* 2019), IGFBP-2 (Insulin-like growth factor binding protein-2) (Zhao *et al.,* 2015), GNPDA2 (glucosamine-6-phosphate deaminase 2) gene (Ouyang *et al.,* 2016), ODC and PRDM16 (Cahyadi *et al.,* 2013) among others have been reported to influence BW in IC. Studies have associated Neuropeptide Y (NPY), Dopamine Receptor D2 (DRD2), Bone Morphogenetic Protein Receptor 1B (BMPR1B) and Melanocortin 1C Receptor (Mel1C) genes with egg production traits and broodiness (Rifki *et al.,* 2021). Their effects in regulation of growth, maturation, eating behaviour, feed intake and energy balance are well recognized (Fatemi *et al.,* 2012; Awad and El-Tabany, 2015; Lek *et al.,* 2018; Ahiwe *et al.,* 2018; Calislar *et al.,* 2018). Few studies have attempted to associate these genes with BW in indigenous chicken populations (Promket *et al.,* 2023). Therefore, we hypothesized that SNPs within BMPR1B, DRD2, NPY, and Mel1C genes are associated with BW in IC Ethiopian ecotypes. The aim of this study was to establish effects of ecotype, sex, age on BW and association of SNPs within the BMPR1B, DRD2, NPY, and Mel1C genes with BW. This will improve future breeding programs utilizing Market Assisted Selection (MAS).

MATERIALS AND METHODS

Study Animals

The chicken used in this study were sampled from 24 villages across six regional states of Ethiopia with different agro-climatic, ecological and geographical conditions as described by Gheyas *et al.* (2022). Each regional state was considered a separate ecotype. To capture heterogeneity within populations, 8 to 10 chickens were sampled randomly from each village, making a total of 234 chickens; Afar (n=16), Amhara (n=105), Gumuz (n=13), SNNPR (Southern Nations, Nationalities and Peoples' Region) (n=18), Tigray (n=43), and Oromia (n=17). The samples consisted of 146 female and 88 male birds and varied in age (4–30 months; average 10.3 months).

Phenotyping

The weight of the birds (in kilograms) was recorded once by using a digital scale with the sensitivity of 1g.

Genotyping

Genomic DNA Extraction

Blood samples (50–250µl) were drawn from the large brachial vein between biceps and triceps muscles with syringes and needles and deposited in a microcentrifuge tube containing ethylenediaminetetraacetic acid (EDTA). DNA extraction was done at the BecA-ILRI Hub facility, Nairobi, Kenya using the Qiagen DNeasy blood and tissue kit protocol. DNA concentration was evaluated by spectrophotometry and its integrity (quality and quantity) determined by agarose gel electrophoresis. The gDNA was then adjusted to a final volume and concentration of 100 µl and 50 ng/µl, respectively, and sent to Edinburgh Genomics, UK for whole genome sequencing (WGS). At Edinburgh Genomics, gDNA samples were re-assessed for integrity. The AATI ProSize 2.0 software was used to quantify and a give quality score, samples with a score >7 passed quality control were normalized to fall within the acceptable range for library preparation.

Sequence Library Preparation

Next generation sequencing libraries were prepared using Illumina SeqLab specific TruSeq Nano High Throughput Library preparation kits. The pre-normalized gDNA samples were clipped to a 450 bp mean insert size and ligated with blunt ended, A-tailed, size selected TruSeq adapters and enriched using eight cycles of PCR amplification. The libraries were evaluated for mean peak size and quantity, normalized to 5nM and actual concentration. The libraries were denatured, and pooled in groups of eight for clustering and sequencing. The clustered flow cells were sequenced in paired-end mode with read length of 150bps.

Data Processing, Mapping and Variant Calling

Demultiplexing was performed using bcl2fastq (v2.17.1.14), allowing a single mismatch when assigning reads to barcodes. Adapters (Read1: AGATCGGAAGAGCACACGTCTGAACTCCAGTC, Read2: AGATCGGAAGA GCGTCGTGTAGGGA AAGAGTGT) were trimmed during this process. Sequenced data quality was checked using the FASTQC package (v0.11.5) and reports aggregated in by the MultiQC package into single report for ease of review. No quality-based trimming was performed on the sequence reads prior to mapping and sequencing.

Sequence reads were mapped on chicken reference genome (GCA_000002315.5_ GRCg6a) using the BWA-mem (v0.7.15) algorithm. Picard v2.9.2 was used to undertake coordinate sorting using the SortSam function and duplicate reads marking using MarkDuplicates function.

GTAK v3.8-0 was used to undertake base quality score recalibration and SNP calling using best practice protocol for "Germline short variant discovery pipeline" using the HaplotypeCaller function on individual samples, then joint genotyping using GenotypeGVCFs function. Variant quality score recalibration (VQSR) approach was used to undertake variant filtration using about one million validated SNPs as training and true set, and over 20M known chicken SNPs from the Ensembl database as known variants. A tranche sensitivity threshold of 99% was applied for filtering variants.

Single nucleotide polymorphisms with a missing genotype rate of more than 20% across the samples were filtered out using VCFtools (option – max-missing 0.2) and resultant SNPs filtered using Plink v1.9 software (Purcell *et al.,* 2007); minimum SNP quality of 20, 5% missing SNP genotypes, Hardy–Weinberg equilibrium (p

<10-5), call rate >95%, heterozygosity >0.4, and minor allele frequency >0.05. Autosomal SNPs were pruned using indep-pairwise parameters described by Wang *et al.* (2009).

Extraction of Candidate Genes

After variant calling, the resulting variant call format (VCF) file was used to extract polymorphisms located within the four candidate genes (DRD2, BMPR1B, NPY, and Mel1C) using Vcftools v0.1.16 (Danecek *et al.,* 2011) as shown in table 1 below. Genomic coordinates of the candidate genes were obtained from the reference chicken genome; Gallus gallus GRCg6a using Ensembl gene databases. Using these coordinates, subsets of variants located within or near each candidate gene were extracted with the VCFtools commands --chr and --from-bp / --to-bp, specifying chromosome numbers and base-pair positions corresponding to the start and end of each gene as shown in table 1 below. The resulting gene-specific VCF files containing biallelic SNPs falling within the defined genomic regions were retained for downstream analyses.

Table 1: Number of SNPs extracted from chromosomes and position locations of DRD2, NPY, BMPR-1B and Mel1C candidate genes

Gene	Chromosome	Base pair positions	SNPs
DRD2	24	Chr24: 5740646 - 5763539	373
NPY	2	Chr2: 31464139 - 31472048	163
BMPR-1B	4	Chr4: 58350823 - 58589309	3741
Mel1C	4	Chr4: 17605471 – 17631574	447

Statistical Analysis

Analysis of Phenotypic Variation of BW

Descriptive statistical analyses were performed to summarize BW measurements of IC across the ecotypes and sexes. The data was first cleaned and checked for normality and completeness using shapiro normality test, and outliers were removed before analysis. The descriptive results were computed and presented as mean ± standard error (SE), and minimum and maximum of BW for each ecotype and graphically illustrated to visualize variations in BW among ecotypes and between sexes.

Analysis of variance (ANOVA) was used to test for significant differences among means of the ecotypes and between sexes. Pairwise comparisons between levels of ecotype, sex, and their interaction were performed using Duncan's Multiple Range Test in the agricolae package. Differences were considered statistically significant at $p \leq 0.05$.

Effects of Ecotype, Sex and Age on BW

The effect of ecotype and sex on BW was analyzed using linear model in R version 4.4.1 (R Core Team, 2025). A linear model was fitted using the lm function, with BW as the response variable, and ecotype, sex, their interaction (ecotype × sex), and age (as a covariate) as explanatory variables. The model was specified as:

$Y_{ijk} = \mu + \beta_i + \alpha_j + (\beta x \alpha)_{ij} + K_k + e_{ijk}$

Where;

Y_{ijk} = the observed body weight of kth individual chicken
μ = overall mean
β_i = fixed effect of ith ecotype
α_j = fixed effect of jth sex
$(\beta x \alpha)_{ij}$ = fixed combined effect of ith ecotype and jth sex
K_k =age of the birds fitted as a covariate
e_{ijk} = random error

Association Analysis

Genome wide association was performed using mixed linear model implemented in rrBLUP (v4.6.3) package in R (Endelman *et al.,* 2011), incorporating a genomic kingship matrix (VanRaden 2008), missing genotype data was imputed using expectation-maximization method (Hastie *et al.,* 2009) ensuring marker representation across the ecotypes while maintaining underlying LD patterns.

$Y = Xb + Zu + e$

where; Y is the vector of BW observations; X is the design matrix for fixed effects (i.e., PCs), b, vectors containing fixed effects coefficients (sex, ecotypes, and SNPs); u, is a vector of random SNP effects $\mu \sim N(0, \sigma_u^2)$, a vector of random residuals; Z is design matrices for random genetic effects to the observations, e is vector of residuals $\varepsilon \sim N(0, \sigma_u^2 I)$.

To account for population structure, PCA was performed using singular value decomposition (SVD) method using prcomp function in R. (Patterson *et al.,* 2006) and top 5 PCs explaining the largest proportion of the variance in the data were included as covariates in the model.

The genomic relationship matrix capturing additive genetic relatedness among individuals was calculated using method proposed by VanRaden *et al.* (2008). The genotype matrix was first centred by subtracting thrice the allele frequency at each SNP and the kinship matrix computed as normalized product of centred genotype and its transpose. The matrix was included as random effect in the model to account for familial structure and relatedness reducing confounding effect of the association testing.

The mixed linear model used in rrBLUP incorporated both fixed effects (i.e. PCs) and random effects (i.e., kinship matrix) to reduce false positives while retaining power to detect true associations. Significant thresholds were determined using Bonferroni correction at $p \leq 0.05$.

RESULTS AND DISCUSSION

Phenotypic Variation of BW

This study evaluated phenotypic variation, effect of ecotype, age (months), sex, ecotype and sex interaction and the association of SNPs with BW. The results of descriptive statistics for BW are presented in Figure 1 and Table 3. The mean BW across the ecotypes was 1.27±0.2kgs. The mean BW for the sexes was 1.21±0.03kgs and 1.36±0.03kgs for females and males, respectively. SNNPR (1.40 ± 0.07kg) and Tigray (1.39 ± 0.05kg) chickens had higher BW than those from Oromia (1.09 ± 0.07kg) and Afar (1.16 ± 0.06kg), Amhara (1.26±0.03kg) and Gumuz (1.17±0.06kg) ecotypes were midway.

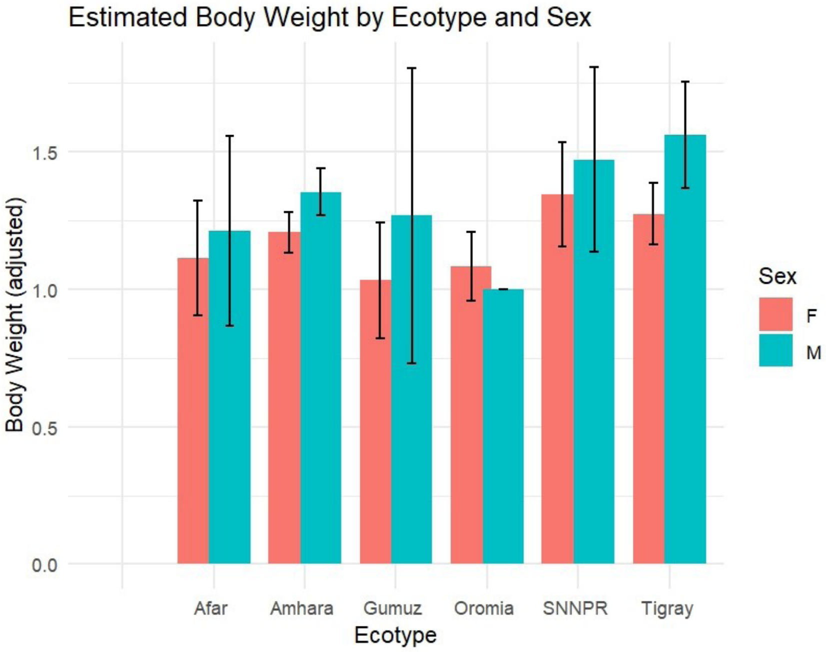

Figure 1: Average BW of Ethiopian IC

Among females, SNNPR (average1.35kg) and Tigray (average 1.26kg) had the heaviest bodies by weight while Gumuz (average 1.04kgs) is the lightest. Among males, Tigray (average 1.56kgs) and SNNPR (average 1.47kgs) were heaviest while Oromia (average 1.01kgs) was the lightest. The BW in the present study compares well with that reported by Dana *et al.* (2010b) and Birhanu *et al.* (2025) in Awi zone, Amhara Region of Ethiopia. The mean BW was lower than one reported in Rwanda IC (Mbakaya *et al.,* 2022), Ethiopia IC (Gebremariam and Girma, 2019) and IC of Belagaum division of Karnataka (Gowda *et al.,* 2020). However, the results are higher than 1.2-1.5kg found by Mahdabi *et al.* (2021). The differences between BW reported in the present study and other studies by Gowda *et al.* (2020), Mahdabi *et al.* (2021) and Mbakaya *et al.* (2022), could be because of differences in populations, management practices and selection histories of the populations.

Effect of Ecotype and Sex on BW of Ethiopian IC Ecotypes

Analysis of variance (table 2) indicated that the effect of ecotype and sex on BW was statistically significant (p ≤0.001). Ecotype-by-sex interaction had significant effects at p≤0.05, whereas age was fitted as a covariate, its effect was not significant (p= 0.543), indicating that age did not significantly influence BW in this populations. Statistical significance of ecotype and ecotype-by-sex interaction on BW agrees with Mebratie *et al.* (2019) and El-Henfnawy *et al.* (2022) but dissimilar to Shahin

Table 2: Summary of analysis of variance (ANOVA)

Variables	df	SS	MS	F value	p value and significance
Ecotype	5	2.2003	0.44007	5.1887	0.0001688 ***
Sex	1	1.2709	1.27091	14.9851	0.0001469 ***
Age	1	0.0314	0.03138	0.3700	0.5437125
Ecotype x Sex interaction	5	0.4208	0.8416	0.9923	0.04235810**
Residuals	199	16.8776	0.08481		

*Note: Significance: '***' p≤0.001; '**' p≤05; '*' p≤0.1, SS=Sum of Squares, MS= Mean of squares*

Table 3: Mean ± SE (kg) and DMRTs of BW among the ecotypes

Ecotype	Mean ± SE (Kg)	Min	Max	DMRT group
Afar	1.16±0.06	0.6	1.7	bc
Amhara	1.26±0.03	0.7	2.0	ab
Gumuz	1.17±0.07	0.6	1.9	bc
Oromia	1.09±0.07	1.0	1.5	c
SNNPR	1.40±0.07	1.0	2.0	a
Tigray	1.39±0.05	0.8	2.6	a
Overall	1.27±0.02	0.6	2.6	

and Elazeem (2005), who argue that ecotype and sex act independent of each other. Whereas age was not significant, contrary to Tenza *et al.* (2025) who found age had significant effects, while breed did not have significant effects on BW of village chicken in South Africa.

Duncan's Multiple Range Test (DMRT, α = 0.05), is shown in table 3 below, SNNPR (1.40±0.07kg) and Tigray (1.39±0.05kg) had the highest BW (group 'a') and did not differ significantly from each other. Amhara (1.26±0.03kg, group 'ab') had intermediate BW, not significantly different from either the heavier (SNNPR and Tigray) or lighter (Gumuz and Afar) ecotypes.

SE=Standard Error; Min=Minimum; Max=Maximum

Gumuz (1.17±0.07kg) and Afar (1.16±0.06kg) were intermediate (group 'bc'), while Oromia (1.09±0.07kg, group 'c') had the lowest BW. These results indicate a clear stratification of BW by ecotype, consistent with the significant ANOVA findings. DMTR (α = 0.05) showed that males (1.36±0.3kg, group 'a') differed significantly with female (1.20 ±0.3 kg, group 'b').

Variation between ecotypes could be attributed to superior genetics of SNNPR and Tigray than Amhara, Gumuz, Afar and Oromia as argued by Tenza *et al.* (2025). Variation between the sexes showed sexual dimorphism, likely occurring due to differential expression of the GH gene between male and female chickens (Baeza *et al.,* 2001; Parsch and Ellegren, 2013). Non-genetic factors such as inaccuracies in weighing scales, individual measurement differences and seasonal variations in feed availability, management or the negative effects of confined management could also explain variation in BW (Dana, 2010b; Jahan *et al.,* 2017; Birhanu *et al.,* 2025).

Association of SNPs with BW

Association of SNPs on BMPR1B, NPY, DRD2 and Mel1C genes with BW are shown in Figures 2,3,4 and

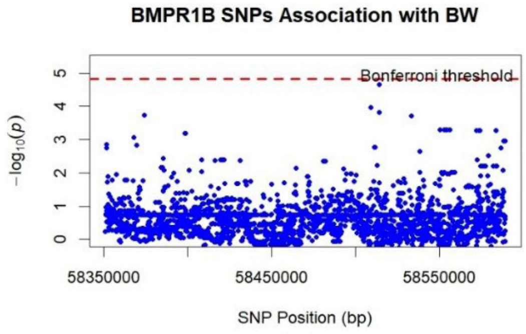

Figure 2: Association of BMPR1B SNPs with BW

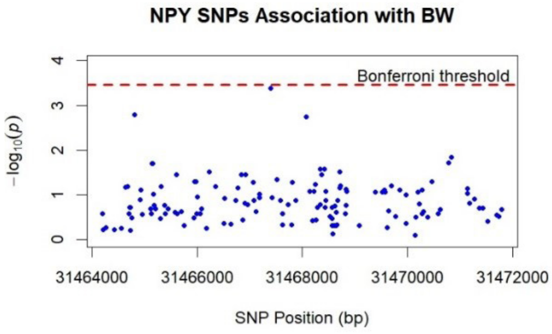

Figure 3: Association of NPY SNPs with

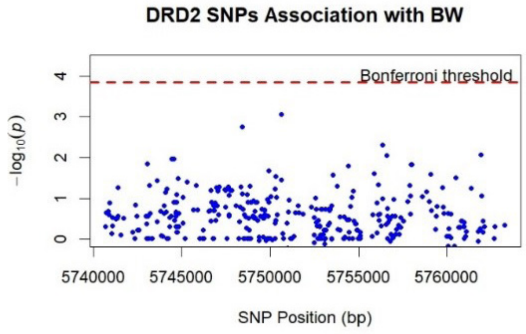

Figure 4: Association of DRD2 SNPs with BW

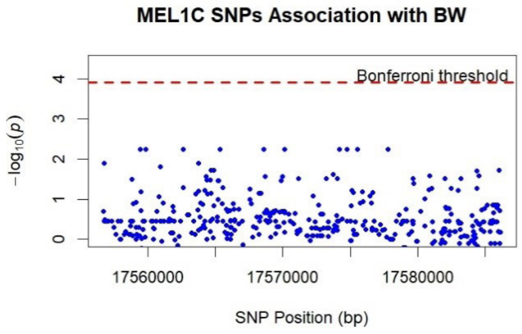

Figure 5: Association of Mel1C SNPs with BW

5, respectively. No SNPs within BMPR1B, NPY, DRD2 and Mel1C genes reached significant association with BW after Bonferroni correction, indicating that either their effect sizes are too small to detect with the current sample or the genes do not have association with BW in this population.

Similar results (i.e. no significance) were reported by Padwar and Thakur, (2021) and Abdi *et al.* (2014) in relation to 240 bp amplicon of the NPY gene in Jabalpur colour and Kadaknath chicken, and West Azerbaijan native poultry, respectively. Similarly, Niknafs *et al.* (2014) did not find association between the A287G SNP of BMPR-1B gene with growth traits. In relation to Mel1C gene, Li and Li (2019) did not find its association with BW when assessing time-dependent genome-wide SNP-SNP interaction analysis of BW. Although many studies have reported SNPs in these genes or SNPs in regions associated with these genes influencing growth traits. For instance, NPY by Fatemi *et al.* (2012) and Rahman *et al.* (2014), DRD2 by Rahman *et al.* (2014) and Mel1C and BMPR1B by Kudinov *et al.* (2019 and Habimana *et al.* (2021). Given likely relevance of these SNPs, further investigation may be required using genome-wide data.

The present study used few SNPs extracted from NPY (n=163), DRD2 (n=373), BMPR1B (n=3,705) and Mel1C (n=446) genes. These few SNPs could not have sufficient statistical power to detect associations after stringent filtering of SNPs before association analysis. Additionally, perhaps not all variants, both rare and common, in high LD with the SNP panel variants were captured during extraction (Iheshiulor *et al.,* 2016; Sengupta *et al.,* 2023). Many GWAS associations are mediated by non coding (regulatory) variants rather than coding ones (Alsheikh *et al.,* 2022). SNPs downstream and upstream of coding regions and regulatory sites have trait association potential, exerting their effects through modulating the transcriptional activities of genes; hence a large the sample size of SNP panel data is required to detect associations (Koufariotis *et al.,* 2014; Wu *et al.,* 2017).

However, several markers showed suggestive associations, potentially, playing key roles in regulating BW variation among IC ecotypes in rural villages. The rs314194962 in the DRD2 gene is associated with dopamine signaling, which influences feed intake, energy balance, and growth behavior. This variant may alter appetite regulation, leading to differences in growth performance among ecotypes. The rs315981851 in the NPY gene affects neuropeptide Y expression, a critical regulator of hunger and metabolism, thereby influencing BW through modulation of feed efficiency and energy homeostasis. The rs312679760 in the BMPR1B gene is linked to bone morphogenetic protein signaling, which governs skeletal growth and muscle development. Collectively, these SNPs may interact with environmental factors, in gene-by-environment (GxE) interaction to influence growth potential of IC under rural management systems (Zhao *et al.,* 2025).

CONCLUSION

The results of this study revealed that ecotype and sex and their interaction had significant effects on BW whereas age was not statistically significant. The results from the current study indicate that SNPs on BMPR1B, NPY, DRD2, and Mel1C genes are not associated with BW traits and are not potential markers for use in molecular marker-assisted selection programs. The present study used a relatively small sample size, which could have limited the statistical power needed to achieve significant associations. Whole genome sequence data as opposed to SNP panel data may be needed to detect the association of NPY, DRD2, BMPR1B, Mel1C genes with BW of Ethiopian IC.

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