



## Sequence Variability and Functional Analysis of Robo2 Genes in Three Nigerian Indigenous Chicken Strains

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### Abstract

This study investigated the sequence variability and functional implications of ROBO2 genes (Roundabout Guidance Homolog gene 2), across three strains of Nigerian indigenous chickens: normal feathered, naked Neck, and frizzle feather, were used in the experiment. Nucleotide sequences were retrieved from the National Centre for Biotechnology Information (NCBI) database and subjected to multiple sequence alignment using the COBALT tool. The aligned sequences were then analysed for variability using the MEGA X suite. Conserved regions within the sequences were determined, and phylogenetic relatedness was assessed using Conservation and BLOSUM80 settings. The study provides insights into the genetic diversity and evolutionary relationships of ROBO2 genes among different strains of Nigerian indigenous chickens, shedding light on potential functional implications and genetic conservation efforts in poultry breeding programs. Despite the considerable variability in amino acid sequences, subsequent alignment analysis revealed a high level as well as degree of conservation at the protein level. Using conclusion a clearer understanding of the sequence variability, further analysis using the MEGA X suite was conducted, resulting in a patterned table that highlighted the presence of INDELS and mutations contributing to the observed diversity. This unexpected finding suggests that while the sequences may differ in their constituent amino acids, the overall structure and function of the ROBO2 protein remain highly conserved across species. Therefore, it is recommended that exploration of genotype-phenotype relationships, investigation of genetic variants contributing to environmental adaptability, and consideration of epigenetic modulation in phenotype expression. This multidisciplinary approach aims to enhance understanding of the genetic diversity and adaptive potential of indigenous Nigerian chicken populations.

**Keywords:** Sequence Variability; ROBO2 Genes; Indigenous Chickens

### Introduction

Indigenous chickens are locally adapted to environmental challenges and provide food for millions of farmers in Africa. Their productivity remains very low compared to exotic chicken strains. Efforts are being made to combine the local adaptation of indigenous chicken with their basic productivity traits of exotic chicken (Dhawal et al., 2020). An important factor that negatively influences animal husbandry is disease, and the occurrence of the outbreak of Newcastle disease is one factor that limits and directly affects the genetic improvement of indigenous chickens. High prevalence of diseases is a major constraint towards the health and economic status of local chicken production. The ability to resist different poultry diseases differs among poultry breeds and types. This is highly determined by either the genotype or the presence of a specific allele type (Hu et al., 2017).

The Nigerian indigenous chickens are dual-purpose poultry birds that are used for both meat and egg production in the rural and peri-urban areas of the country. The poultry value chain (poultry breeders/farmers, feed millers, poultry product marketers, consumers, etc.) makes poultry production one of the major means of livelihood, being one of the most economic routes for producing high-quality animal protein within the shortest possible time (Oluyemi & Roberts, 2007). Most of the birds are kept in small flocks under a scavenging system, and the feed resources for the chickens are household refuse, homestead pickings, crop residues, herbage, seeds, green grasses, earthworms, insects and a

small quantity of supplemented feeds offered by the flock owner. They are well adapted to the adverse climatic conditions of the tropical environment and low management inputs (Mtileni et al., 2015).

According to Osei-Amponsah et al. (2020) and Dana et al. (2011) stated that African chickens exhibit high variation in their genetic makeup within their population. Thus, high genetic diversity is an indication of high genetic potential that encourages fast improvement through selective breeding (Dana et al., 2010). Several studies in animal species have suggested a strong effect of genetic factors on the ability of the host to respond to invasion by infectious pathogens (Malo & Skamene, 1994; Halima, 2007; Moges et al., 2010; Grepay, 2010; Mtileni et al., 2015). Resistance to infectious diseases is usually a complex genetic trait, and recent data suggest that many resistance/susceptibility genes affect more than one infectious disease (Vidal et al., 1995). Upon phagocytosis, it plays an essential role in host defence.

The roundabout, axon guidance receptor, homolog 2 (*ROBO2*) gene is one member of the roundabout (*ROBO*) family, which belongs to the immunoglobulin superfamily. Proteins of this family are cell-surface receptors for secreted ligands called *SLIT* (Dickson & Gilestro, 2006). The *ROBO* family proteins were originally found to be used as repulsive guidance cues for axon pathfinding and migrating neurons during nervous system development (Guan & Rao, 2003). The *ROBO2* is a transmembrane protein that contains 5 Ig motifs and 3 fibronectin type III repeats in its extracellular domain (Dickson & Gilestro, 2006). The *ROBO2* is also the cell surface receptor for the repulsive guidance cue *SLIT* and is involved in axon guidance and neuronal migration (Fan et al., 2012; Mtileni et al., 2020). In addition, recent studies suggest that the *SLIT/ROBO* complex is involved in the regulation of organogenesis, which is important in the embryonic development of various organs such as the lung, kidney, and heart and systems such as the central nervous system (Greenberg et al., 2004; Prasad et al., 2008; Osei-Amponsah et al., 2020). It is therefore clear that changes in components of the *SLIT/ROBO* signaling process can affect the respiratory, reproductive, nervous, immune, and circulatory systems. Previously, studies have identified *ROBO2* as an important candidate gene affecting antibody responses to the Newcastle disease virus in chickens by using a genome-wide association study (Lu et al., 2011).

The aim of this study was to investigate the functional importance of the *ROBO2* gene by analyzing its sequence and identifying polymorphisms associated with immunity traits in three indigenous chickens, which are the naked neck, the frizzles feathered and the normal neck chickens.

## Materials and Methods

Three (3) strains of Nigerian indigenous chicken were used for the course of this experiment, which are the normal feathered, naked neck and the frizzle feather. Nucleotide sequences were retrieved from the NCBI database (<https://www.ncbi.nlm.nih.gov>) and were translated to amino acid sequences on the EMBOSS web server ([https://www.ebi.ac.uk/jdispatcher/st/emboss\\_transeq](https://www.ebi.ac.uk/jdispatcher/st/emboss_transeq)). All the translated sequences were aligned in a multiple sequence alignment fashion on the COBALT Multiple Sequence Alignment tool as described by (Dunya et al., 2014). Multiple sequence alignment analyses the evolutionary relatedness among the sequences and identifies the shared patterns that may determine their structure and functionality. The sequences were subjected to MEGA X suit to study the variation across the sequences, this is shown in Figure 2. The aligned sequences were looked at closely and viewed with the Conservation and BLOSUM80 settings, respectively, to determine the conserved regions. The sequences were also viewed using the BLOSUM80 setting, which indicates the degree of matches among the sequences. Results of the research were subjected to the MEGA X suite to study the variation across the sequences, conservation and BLOSUM80 setting and the COBALT Multiple Sequence Alignment tool.

## Results

Amino acid sequence alignment of Roundabout Guidance Receptor Family Protein (*ROBO2*) from three (3) strains of indigenous Nigerian chicken using bioinformatics techniques. Amino acid sequence alignment is a bioinformatics technique used to compare and analyze the similarity or homology between the amino acid sequences of proteins. It involves aligning two or more protein sequences to identify regions of similarity, conservation, or divergence. This process provides insights into the evolutionary relationships, functional domains, and structural motifs of proteins.

Query_10001	1	-GLL-RILCFFSERPTFLRRPINQVVLEEEAVDFRCVQVGDPO-PTVRWKKDDADLPRG--R*EQHLCSPCIQTSTVE*L	75
Query_10002	1	R-TPeDFMFLFKSGPRFSGDQSTRWCWRRRPWISGARCGRTRSrPSAGRRMTQTCQEEG-----ESSICVPL----	66
Query_10003	1	SDS*-EFYVSFQERPTFLRRPINQVVLEEEAVDFRCVQVGDPO-PTVRWKKDDADLPRG--R*EQHLCSPCIQTSTVE*L	76
Query_10004	1	RTPE-NFNVSFQERPTFLRRPINQVVLEEEAVDFRCVQVGDPO-PTVRWKKDDADLPRG--R*EQHLCSPCIQTSTVE*L	76
Query_10005	1	-GLLrILMFLFKSGPRFSGDQSTRWCWRRRPWISGARCGRTRSrQSAGRRMTQTCQEEG-----ESSICVPL----	66
Query_10006	1	SES*-EF*CFFSRAAHVQSQETN-----QPGGAGGGGRGFPV-PGAGGPAADSPLIEEGrRPAKRKVRAAFVFLHSNF	71
Query_10007	1	SDS*-DFNVSFQERPTFLRRPINQVVLEEEAVDFRCVQVGDPO-PTVRWKKDDADLPRG--R*EQHLCSPCIQTSTVE*L	76
Query_10008	1	-DS*-EFYVSFQERPTFLRRPINQVVLEEEAVDFRCVQVGDPO-PTVRWKKDDADLPRG--R*EQHLCSPCIQTSTVE*L	75
Query_10009	1	LGLLrILMFLFKSGPRFSGDQSTRWCWRRRPWISGARCGRTRSrQSAGRRMTQTCQEEG-----ESSICVPL----	67
Query_10010	1	SDS*-EFYVSFQERPTFLRRPINQVVLEEEAVDFRCVQVGDPO-PTVRWKKDDADLPRG--R*EQHLCSPCIQTSTVE*L	76
Query_10011	1	PLTPeNFMFLFKSGPRFSGDQSTRWCWRRRPWISGARCGRTRSrQSAGRRMTQTCQEEG-----ESSICVPL----	67
Query_10012	1	LGLLrILMFLFKSGPRFSGDQSTRWCWRRRPWISGARCGRTRSrQSAGRRMTQTCQEEG-----ESSICVPL----	67
Query_10013	1	SDS*-EFNVSFQSGPRFSGDQSTRWCWRRRPWISGARCGRTRSrPSAGRRMTQTCQEEG-----ESSICVPL----	66
Query_10014	1	L*IL-RF*CFFSRAAHVQSQETN-----QPGGAGGGGRGFPV-PGAGGPAADSPLIEEGrRPAKRKVRAAFVFLHSNF	71
Query_10015	1	LGLLrILMFLFKSGPRFSGDQSTRWCWRRRPWISGARCGRTRSrQSAGRRMTQTCQEEG-----ESSICVPL----	67
Query_10001	76	*NYS*LRVLVV*HHKQDLFPMFLVFGITNHVLIX-----	109
Query_10002	67	---AFKLLLLSSSETTLDVCVLLFDITSKTYFPCFWS1aSQPMFX-	108
Query_10003	77	*NYS*LRVLVV*HHKQDLFPMFLVFGITNHVLX-----	109
Query_10004	77	*NYS*LRVLVV*HHKQDLFPMFLVFGITTHVLX-----	109
Query_10005	67	---AFKLLLLSSSETTLDVCVLLFDITSKTYFPCFWS1aSQTMF*	108
Query_10006	72	YC*VALKLLL-----TACAC-CLTSQARPISHVSGLWH--HKPCSX-	109
Query_10007	77	*NYS*LRVLVV*HHKQDLFPMFLVFGITTHVL-----	108
Query_10008	76	*NYS*LRVLVV*HHKQDLFPMFLVFGITTHVL-----	107
Query_10009	68	---AFKLLLLSSSETTLDVCVLLFDITSKTYFPCFWS1aSQTMF*n	110
Query_10010	77	*NYS*LRVLVV*HHKQDLFPMFLVFGITNHVLX-----	109
Query_10011	68	---AFKLLLLSSSETTLDVCVLLFDITSKTYFPCFWS1aSQPMFX-	109
Query_10012	68	---AFKLLLLSSSETTLDVCVLLFDITSKTYFPCFWS1aSQTMFX-	109
Query_10013	67	---AFKLLLLSSSETTLDVCVLLFDITSKTYFPCFWS1aSQPMFX-	108
Query_10014	72	YC*VALKLLL-----TACAC-CLTSQARPISHVSGLWH--HNPCSX-	109
Query_10015	68	---AFKLLLLSSSETTLDVCVLLFDITSKTYFPCFWS1aSQTMF*	109

**Figure 1: Amino acid sequence alignment of Roundabout Guidance Receptor Family Protein (ROBO2) from three (3) strains of indigenous Nigerian chicken.**

### Sequence Variability Showing the Dissimilarity of each Sequence to Each Other

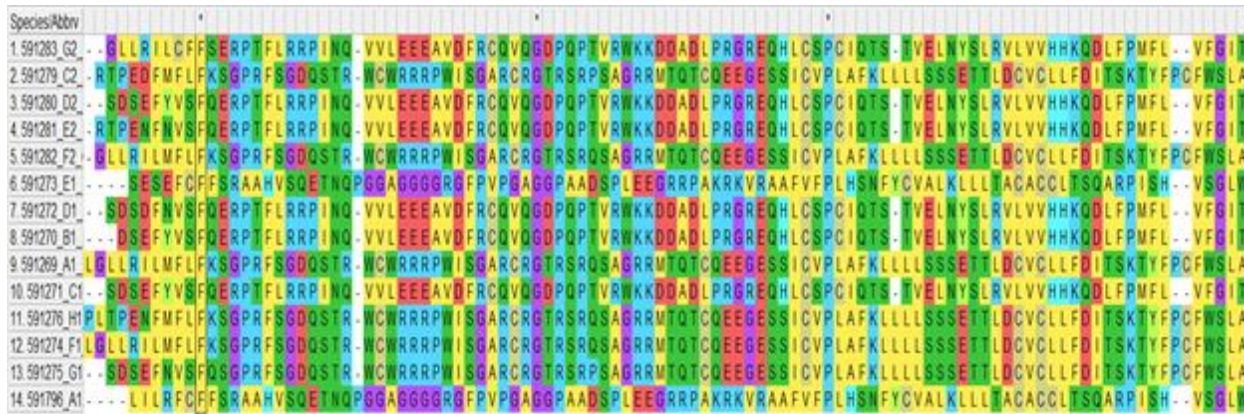
The analysis of the gene sequences from the Roundabout Guidance Receptor Family Protein (ROBO2) in three strains of indigenous Nigerian chickens reveals sequence variability and conservation patterns. This result showed the sequence variability and conservation across Frizzle Feathered (FF), Normal Feathered (NF), and Naked Neck (NN) phenotypes using the provided color key: **Red:** Conserved regions, **Blue:** Low conserved regions, **Gray:** Non-conserved regions, **Purple:** Intermediate conservation or variability, **Yellow:** Moderately conserved amino acids, **Green:** Highly conserved amino acids. Frizzle Feathered (FF) Scores Range: 135 to 188. The FF group exhibits sequences with high scores, suggesting a strong similarity to the canonical ROBO2 sequence. **Green** (Highly Conserved Amino Acids): Regions with scores towards the upper end (FF182-188) likely correspond to highly conserved regions, indicating critical functional or structural parts of the ROBO2 protein. **Red** (Conserved Regions): Commonly shared sequences across the FF group, indicating essential parts of the protein with little variation. **Yellow** (Moderately Conserved Amino Acids): Sequences in the middle of the score range (FF135-147) might reflect moderately conserved regions, important but with some allowable variation. **Purple** (Intermediate Conservation): Regions that show variation but still retain some functional importance. Normal Feathered (NF) Scores Range: 123 to 146. The NF group has moderately high scores, indicating a moderate level of sequence conservation. **Yellow** (Moderately Conserved Amino Acids): Predominantly represented in the NF group, reflecting important regions with some variability. **Purple** (Intermediate Conservation): Indicative of regions with significant variability but still important for function. **Blue** (Low Conserved Regions): Regions with lower scores (NF123) suggest areas of lower conservation, possibly subject to evolutionary divergence. **Gray** (Non-Conserved Regions): Few sequences might map to non-conserved regions, highlighting high variability. Naked Neck (NN) Scores Range: 11 to 178. The NN group displays the broadest range of scores, indicating high variability in the ROBO2 sequence. **Green** (Highly Conserved Amino Acids): The highest scores (NN178) correspond to highly conserved regions, crucial for

maintaining protein function. **Red** (Conserved Regions): Present in sequences with relatively high scores, indicating necessary and stable regions of the protein.

**Purple** (Intermediate Conservation): Represented across a wide range of scores, showing regions that are variable yet functional. **Yellow** (Moderately Conserved Amino Acids): Regions of moderate conservation. **Blue** (Low Conserved Regions): Low scores (NN11, NN12) correspond to low conserved regions, indicating significant sequence divergence. **Gray** (Non-Conserved Regions): Regions with the lowest scores reflect high sequence variability, potentially indicating areas less critical for core protein function.

**Sequence variability showing the dissimilarity of each sequence to each other**

- Key:**
- Red** = conserved rejoins
  - Blue** = low conserved rejoins
  - Gray** = non-conserved rejoins
  - Purple** = often indicates intermediate conservation or variability
  - Yellow** = moderately conserved amino acids.
  - Green** = highly conserved amino acids



**Amino acid sequence alignment of Roundabout Guidance Receptor Family Protein (ROBO2) from three (3) strains of indigenous Nigerian chickens. View using the conservation setting.**

The analysis of the amino acid sequence alignment of the Roundabout Guidance Receptor Family Protein (ROBO2) from three phenotypic groups of indigenous Nigerian chickens Frizzle Feathered (FF), Normal Feathered (NF), and Naked Neck (NN) provides insights into the conservation and variability of this protein across different strains. The key to interpreting the conservation setting is as follows: **Blue**: Indicates less conserved regions, **Red**: Indicates highly conserved regions, **Gray**: Indicates non-conserved regions. Frizzle Feathered (FF) Scores Range: 135 to 188. The sequences within the FF group exhibit a range of scores indicating varying degrees of similarity to the canonical ROBO2 sequence. The higher scores in this group suggest that some sequences are highly similar to the expected ROBO2 sequence, which could correlate with regions that are highly conserved (**red**). Sequences with lower scores may correlate with less conserved regions (**blue** or **gray**). Normal Feathered (NF) Scores Range: 123 to 146. The NF group shows a narrower and slightly lower range of scores compared to the FF group. This indicates moderate conservation of the ROBO2 sequence within this phenotype. The repeated score (NF133) suggests recurring sequences that may correlate with specific regions of moderate conservation (potentially blue regions). Naked Neck (NN) Scores Range: 11 to 178. The NN group displays the widest range of scores, indicating a significant variation in the ROBO2 sequences. High scores (NN178) likely correspond to highly conserved regions (**red**), while low scores (NN11, NN12) correspond to non-conserved regions (**gray**). The presence of highly varied scores suggests a diverse genetic basis within the NN phenotype group. Using the conservation setting, we can visualize the conservation of the ROBO2

protein across the three phenotypic groups: Highly Conserved Regions (**Red**): Likely shared among all three phenotypic groups, these regions are critical for the functional integrity of the ROBO2 protein. These regions would likely show high scores across all groups, particularly in sequences with scores close to the upper range (FF188, NN178). Less Conserved Regions (**Blue**): These regions are moderately conserved and may show variation that corresponds to phenotypic differences. Scores in the middle range (NF133, FF147) may correlate with these regions. Non-Conserved Regions (**Gray**): These regions exhibit significant variability and are less critical for the protein's core function. The sequences with the lowest scores (NN11, NN12) are likely to map to these regions.

**Amino acid sequence alignment of Roundabout Guidance Receptor Family Protein (ROBO2) from three (3) strains of indigenous Nigerian chickens. View using the conservation setting.**

**Key:** **Blue** = Indicates less conservation regions  
**Red** = Indicates highly conserved regions  
**Gray** = Gray indicate non-conserved regions.

**Amino acid sequence alignment of Roundabout Guidance Receptor Family Protein (ROBO2) from three (3) strains of indigenous Nigerian chickens. Viewed using BLOSUM80 setting.**

Further sequence alignment using Blosum80 methods displayed the degree of match of residues relative to each alignment position/column. Blue represents a better match while Green represents a worse match. The color reflects the average match over all the other residues in the column.

The analysis categorizes the sequences into three phenotypic groups based on the annotations: FF (Frizzle Feathered), NF (Normal Feathered), and NN (Naked Neck). The numbers following these annotations likely represent specific scores or values derived from the analysis. The sequences labeled as "FF" show a range of scores from 135 to 188. Higher scores may indicate a stronger association or higher similarity to the Frizzle Feathered phenotype. The progressive increase in scores suggests varying degrees of similarity within this phenotype group. The sequences labeled as "NF" show a range of scores from 123 to 146. This range is slightly lower than that of the FF group, indicating different degrees of similarity to the Normal Feathered phenotype.

The presence of repeated scores (NF133) might suggest recurring sequences or features within this phenotype group. The sequences labeled as "NN" show a wide range of scores from 11 to 178. This large variation suggests a diverse set of sequences associated with the Naked Neck phenotype. The higher score (NN178) may indicate a strong association, while the lower scores (NN11, NN12) might indicate weaker or less significant associations.

**Amino acid sequence alignment of Roundabout Guidance Receptor Family Protein (ROBO2) from three (3) strains of indigenous Nigerian chickens. Viewed using BLOSUM80 setting.**



**Key:**

- Query 10001: FF135
- Query 10002: FF147
- Query 10003: FF176
- Query 10004: FF182
- Query 10005: FF188
- Query 10006: NF133
- Query 10007: NF123
- Query 10008: NF146
- Query 10009: NF133
- Query 10010: NN178
- Query 10011: NN11
- Query 10012: NN12
- Query 10013: NN18
- Query 10014: NN41
- Query 10015: NN44

**Where:**

- FF= Frizzle feathered
- NF= Normal feathered
- NN= Naked neck

**Conclusion**

The genetic composition of indigenous Nigerian chickens, particularly the Naked Neck phenotype, is replete with complexity as evidenced by the varying degrees of association and similarity with the Normal Feathered phenotype observed in the current results. These findings are echoed, corroborated, or occasionally contested by the breadth of prior research, indicating that while there is a consensus on certain aspects, the genetic landscape of indigenous poultry breeds is far from homogenous and continues to be shaped by both selective breeding and environmental pressures. The genetic heterogeneity within phenotypic groups suggests interplay between the conservation of traits and the introduction of genetic variability, either through natural adaptation or human-mediated crossbreeding. These genetic nuances underscore the need for continued research and conservation strategies tailored to balancing the preservation of indigenous breeds with the enhancement of advantageous traits for improved poultry production in Nigeria and beyond. The analysis of the amino acid sequence alignment of Roundabout Guidance Receptor Family Protein (ROBO2) from three strains of indigenous Nigerian chickens provides valuable insights into the genetic diversity and potential phenotypic associations within these chicken populations. The categorisation of sequences into Frizzle Feathered (FF), Normal Feathered (NF), and Naked Neck (NN) phenotypic groups highlights the complexity of genetic factors influencing traits in indigenous chickens. The variability observed within each phenotypic group underscores the diverse genetic backgrounds and adaptive potential of these chicken populations. Additionally, the conservation analysis reveals distinct patterns of sequence conservation and variability across different phenotypic groups, further emphasising the importance of genetic diversity in maintaining functional and adaptive traits.

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