Genome-Wide Run of Homozygosity Analysis Reveals Candidate Genomic Regions Associated with Environmental Adaptations of Tibetan Native Chickens

Jingwei Yuan  
Chinese Academy of Agricultural Sciences
Shijun Li  
Huazhong Agricultural University
Zheya Sheng  
Huazhong Agricultural University
Meikun Zhang  
DQY Ecological Co. Ltd.
Xuming Liu  
DQY Ecological Co. Ltd.
Zhengdong Yuan  
DQY Ecological Co. Ltd.
Ning Yang  
China Agricultural University
Jilan Chen (chen.jilan@163.com)  
Chinese Academy of Agricultural Sciences Institute of Animal Science  https://orcid.org/0000-0002-9400-009X

Research

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Abstract

Background

In Tibet, two most important breeds are Tibetan chicken and Lhasa white chicken, and the duo exhibit specific adaptations to high altitude and produce indispensable protein for humans living in the plateau. These breeds were partly included in conservation plans as their biodiversity is important as a genetic resource. However, population genetic analysis of the chickens is rarely investigated. Based on whole-genome sequencing data of 113 chickens from 4 Tibetan chicken population including Shigatse (SH), Nyemo (NM), Dagze (DZ) and Nyingchi (LZ), as well as Lhasa white (LW) chicken population, we performed genetic diversity and differentiation, run of homozygosity (ROH), genomic inbreeding and selection signature analyses.

Results

Our results showed high genetic diversity across the five chicken populations. The linkage disequilibrium decay was highest in LZ, and moderate level of genetic differentiation was found between LZ and other populations (Fst ranging from 0.05 to 0.10). Furthermore, the highest ROH-based inbreeding estimate ($F_{ROH}$) was 0.11 in LZ, whereas it ranges from 0.04 to 0.06 in the other four chicken populations. In total, 74, 111, 62, 42 and 54 ROHs containing SNPs with concurrency ranked top 1% were identified for SH, NM, DZ, LZ and LW, respectively. BDNF, CCDC34, LGR4, LIN7C, GLS, LOC101747789, MYO1B, STAT1 and STAT4 were shared genes harbored by these ROHs in the five populations, suggesting their important roles in adaptation of the chickens. Combined with selection signature analysis, we also identified a common candidate genomic region harboring AMY2A, NTNG1 and VAV3 genes. These genes have been reported to contribute to digestion, neurite growth and high-altitude adaptation, which could be involved in selection during evolution process.

Conclusions

High genetic diversity was observed in Tibetan native chickens. Nyingchi population, possessing highest $F_{ROH}$, is genetically distant from other chicken population. Candidate genes in ROH islands could aid the genetic characterization of the five Tibetan native chicken populations. Our findings contribute to the understanding of genetic diversity and offer valuable insights for the genetic mechanism of adaptation, as well as provide veritable tools that can help in the design and implementation of breeding and conservation strategies for Tibetan native chickens.

Background

The Tibetan plateau, which is the largest high-altitude area on earth with an average altitude exceeding 4000m, represents 25% of the landmass of China. The high-altitude environment has produced many unique highland animal genetic resources. Tibetan chicken was one of domestic avian breed found in this area dating to at least 7th century A.C from historical literature [1]. Tibetan chickens are widely distributed in farming areas of Tibet, including Shigatse, Lhasa, Lhoka and Nyingchi. Besides Tibetan chicken, Lhasa white chicken was another predominantly native breed, which was bred for several decades by intercrossing male White leghorn and female Tibetan chicken. The basis of physiological and genetic adaptations to the extreme environmental conditions of the Tibetan plateau have recently been partly explored in Tibetan chicken [1−3], and genomic analysis indicated that Tibetan chicken might be composed of multiple distinct populations [2, 4]. However, population genomic analysis was rarely conducted on diverse chicken populations reared on the Tibetan area. The importance of genetic resource conservation and utilization prompt us to analyze the genetic diversity and adaptation of native chickens in Tibet from the perspective of population genetics.

The availability of high-throughput affordable sequencing techniques enabled genome-wide analysis of the genetic structure and relationships in animal populations. Thousands of omics data have opened new perspectives for a more accurate animal genetic analysis. As reviewed in [5], runs of homozygosity (ROH), which is a kind of long continuous homozygous stretches in the genome are formed by the combination of two identical haplotypes in an individual. ROH was found to be ubiquitous even in outbred populations, and is usually considered to be an index of autozygosity. Long homozygous regions throughout the genome result from demographic events, mating between close relatives (population bottleneck), reduction in population size (genetic drift), selection (breeding) and small inversions that suppress recombination. Thus, population demography, structure and diversity can be explored based on the distribution and location of ROHs. Moreover, previous studies have shown that characterizing inbreeding based on ROH provides a better measurement of individual autozygosity than estimating overall inbreeding based on pedigree information, in which kinships between base animals are not accounted for [6]. The prevalence of ROH in individual genome has also been a factor for understanding the genetic
basis of complex phenotypes. ROH facilitated the investigation of highly inbred genomic regions, which were first referred to as ROH islands [7] within a population. These ROH islands can provide important insights into the population genetics and are also likely to be signatures of positive selection due to linkage disequilibrium (LD) [8]. Since ROH islands are potential signatures of selection, overlapping ROH islands across populations and species are a valuable tool in comparative genomic studies and may reveal important genetic regions for complex traits. ROH analyses are becoming complementary to genome-wide association studies and detection of population-specific major genes in humans and animals [9].

In chickens, ROH has been applied to assess the genome-wide diversity in local and imported genetic resources, which contributes to maintain an effective program for conservation of endangered populations [10–13]. The long-term selection molded the presence of ROHs and their associated genomic regions resulting in unique population adaptation to environment-imposed challenges in broilers, suggesting that ROHs are likely the result of selection events and not attributable only to demographic and population history [14]. Moreover, multiple candidate genes involved in growth, egg production, disease resistance and behavior were identified to be associated with ROH islands in different chicken breeds [15, 16].

Improving our knowledge about within-breed diversity and the population structure in livestock species is fundamental for understanding environmental adaptation, implementing conservation programs and designing selection plans [10, 17, 18]. While most efforts are dedicated to studying cosmopolitan breeds, a growing attention has been paid to the local breeds which are important genetic resources for their potential to solving problems in agriculture related to environmental changes [19, 20]. Local chickens in Tibet have evolved over centuries under extremely natural condition. They may serve as a great reservoir of genetic pool for identification of genes under natural and artificial selection particularly those harboring putative signatures of environmental adaptation in ROH regions. Herein, four Tibetan chicken populations from Shigatse, Nyemo, Dagze and Nyingchi were collected and genotyped, and a local cultivated breed, Lhasa white was also included in the analysis. All the Tibetan chicken populations were raised traditionally by local farmers, and Lhasa white was kept by Institute of Animal Husbandry and Veterinary, Tibetan Academy of Agricultural and Animal Husbandry Sciences. The objectives of the present study were to (i) evaluate the genetic diversity of Tibetan chickens from different areas of Tibetan plateau using whole genome sequence data, (ii) detect ROH within each chicken population's genome and evaluate genomic inbreeding and (iii) reveal the genomic regions characterized by ROH islands and their possible roles in environmental adaptation of Tibetan native chicken. The results are expected to provide valuable information for the gene-phenotype association, as well as for the conservation of chicken genetic resources in Tibetan.

Methods

Ethics statement

All birds were treated following the guidelines established by the Council for Animal Welfare of China. The experimental protocols were approved by the Science Research Department of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (CAAS).

Whole-genome sequencing and data processing

Genomic DNA was extracted from blood of 114 female chickens. After purification and integrity verification of DNA, whole genome sequencing was performed using the Illumina HiSeq 2500 Sequencer (Illumina, Inc., SanDiego, CA, USA) to generate 150 bp paired-end reads. To minimize mapping errors, low-quality reads were removed using FastQC software following Yan et al [21]. The clean reads from each bird were aligned to the chicken reference genome (Gallus gallus5.0) using the Burrows–Wheeler Alignment (BWA) tool [22] with the default parameters. Picard toolkit was subsequently used to sort the alignment results and filter out potential PCR duplicate reads. The resulting alignments were indexed using SAMtools [23] and processed according to the best practices for the Genome Analysis Toolkit (GATK) [24]. To obtain high-quality SNPs, we set a minimum quality score of 20 for both bases and mapped reads to call variants. Finally, the SNPs of each bird were combined to obtain a common set of SNP data, which was subjected to filtering with the following rigorous criteria using the GATK VariantFiltration module; (a) quality by depth > 5.0 (b) mapping quality score > 40.0 (c) FS < 60.0 (d) MQRankSum > −12.5 (e) ReadPosRankSum > −8.0 and (f) Filtering out any three SNPs clustered in a 10 bp window [25]. The qualified SNPs were annotated using chicken reference genome. Finally, annotated SNP data was filtered using PLINK v1.90 software [26] with the following parameters: sample call rate > 90%, SNP call rate > 95%, and Hardy–Weinberg equilibrium p-value < 10−5. After these steps, a total of 20,385,015 SNPs distributed across 33 autosomes in 106 birds were obtained for subsequent analyses. Among these birds, 20, 33, 25, 10 and 18 were sampled from Shigatse, Nyemo, Dagze, Nyingchi and Lhasa white population, respectively.

Genetic diversity analysis
The filtered SNPs were further pruned to obtain independent SNP markers using the ‘-indep-pairwise’ option, with a window size of 50 SNPs, a step of 10 SNPs, and r^2 threshold of 0.2. Principal components (PC) analysis was carried out with pruned data in PLINK, and first 2 PCs were extracted and plotted using R software. The proportion of observed heterozygosity (Ho) and the expected heterozygosity (He) were estimated from the SNP data following Visser et al. [27]. The number of observed homozygous loci, the number of expected homozygous loci and the number of non-missing loci were calculated using ‘-het’ option. The expected heterozygosity and observed heterozygosity estimate for all individuals within each population were averaged over all SNPs. Ho and He calculation were performed with pruned data with all SNPs. SNPs with minor allele frequency (MAF) ≥ 0.01 and MAF ≥ 0.05, respectively. Further, PopLDDecay [28] was used to estimate linkage disequilibrium (LD) using SNPs with MAF ≥ 0.01, and the LD decay was plotted in R software. Moreover, fixation index (Fst) value of SNPs across the genomes were estimated using VCFtools [29], and then averaged to evaluate the relatedness among populations.

**Runs of Homozygosity (ROH) detection**

Prior to the ROH detection, the eligible SNPs with MAF ≥ 0.01 were separately filtered for each population. Long homozygous fragments were scanned in the pruned data using PLINK according to the following parameters: minimum number of 50 SNPs in a ROH (homoyzg-snp 50), sliding windows of 50 SNPs (homoyzg-window-snp 50), allowance for not more than 5 missing SNPs (homoyzg-window-missing 5) and 3 heterozygous SNPs per window (homoyzg-window-het 3). The minimum length of an ROH segment was 300 kb (homoyzg-kb 300). The minimum SNP density was 1 SNP per 50 kb (homoyzg-density 50), and the maximum gap between two consecutive SNPs was 1000 kb (homoyzg-gap 1000). Finally, the rate in which an SNP was included in the total of sliding windows was at least 0.05 (homoyzg-window-threshold 0.05). After the run, the identified ROHs were classified into three classes according to their size, < 1 Mb, 1 ~ 3 Mb and > 3 Mb, as previously reported in chickens [10].

**Genomic inbreeding analysis**

Genomic inbreeding based on ROH (F_{ROH}) was estimated using PLINK according to previous methods [30]. The F_{ROH} for each bird was calculated as \( \sum_{i=1}^{n} \frac{L_{ROH_i}}{L_{aut}} \), where \( L_{ROH_i} \) is the length of ROH of \( i \)th individual, and \( L_{aut} \) is the genome length of autosome covered by the SNPs in the sequence data. The inbreeding coefficient for an individual based on homozygous SNPs (F_{HOM}) was computed as \( \frac{(O-E)}{(L-E)} \), where \( O \) is the number of observed homozygotes, \( E \) is the number expected by chance, and \( L \) is the number of non-missing autosomal SNPs. Genomic SNP-by-SNP inbreeding coefficient (F_{GRM}) and the correlation between uniting gametes (F_{UNI}) estimates were computed in GCTA software as previously reported [31]. The correlation of the genomic inbreeding coefficients estimated by the above four methods were compared by Pearson correlation.

**Identification of common ROH and gene annotation**

To identify the genomic regions that harbored common ROHs across the five chicken populations, we estimated the occurrences of SNPs in ROH by counting the number of times when the SNP was detected in those ROHs using detectRUN package [32] in R. The genomic regions commonly associated with ROHs were screened by selecting the top 1% SNPs observed in ROHs. Adjacent SNPs that met this threshold were merged into genomic regions named ROH islands. Based on these consensus regions, we annotated QTL based on chicken regions commonly associated with ROHs were screened by selecting the top 1% SNPs observed in ROHs. Adjacent SNPs that met this threshold were merged into genomic regions named ROH islands. Based on these consensus regions, we annotated QTL based on chicken regions commonly associated with ROHs were screened by selecting the top 1% SNPs observed in ROHs. Adjacent SNPs that met this threshold were merged into genomic regions named ROH islands. Based on these consensus regions, we annotated QTL based on chicken SNPs. Genomic SNP-by-SNP inbreeding coefficient (F_{GRM}) and the correlation between uniting gametes (F_{UNI}) estimates were computed in GCTA software as previously reported [31]. The correlation of the genomic inbreeding coefficients estimated by the above four methods were compared by Pearson correlation.

**Selection signatures analysis**

To detect selection signatures in each ROH island, the integrated haplotype score (iHS) was calculated within each population. The iHS is a measure of the amount of extended haplotype homozygosity at a given SNP, that uses phased genotypes to identify putative regions of recent or ongoing positive selection in genomes [35]. Herein, the haplotype was phased using SHAPEIT [36] with recombination rate 0.01 as previously reported for chicken genome [37]. The derived haplotypes were then analyzed using the reh (v2.0) R package [38]. The iHS score was computed for each SNP and further standardized to a \( P \) value with following formula \( p_{iHS} = \frac{1}{2} \Phi^{-1}(1 - \frac{2}{\sqrt{2\pi}} \Phi^2(iHS) - 0.5) \), where \( \Phi^2(iHS) \) represents the Gaussian cumulative distribution function, and \( p_{iHS} \) is the two sided \( P \) value associated with the neutral hypothesis of no selection [38]. The \( p_{iHS} \) of SNPs higher than threshold of 0.1% of total SNPs were considered as putative signatures of selection.

**Results**
Summary of population genetic statistics

Genetic diversity was evaluated by observed heterozygosity (Ho) and expected heterozygosity (He) using eligible SNPs under Hardy–Weinberg equilibrium for Shigatse (SH), Neymo (NM), Dagze (DZ), Nyingchi (LZ) and Lhasa white (LW) chicken population, respectively. Ignoring the minor allele frequency (MAF) of SNPs, the Ho ranged from 0.215 to 0.306. When we removed SNPs with MAF < 1%, Ho remained consistent, and Ho increased just slightly ranging from 0.271 to 0.307 when only SNPs with a MAF ≥ 5% were considered. Mean MAF for each population ranges from 0.106 to 0.130 when MAF filter was set at 0, and it went up from 0.207 to 0.224 when only SNPs with MAF ≥ 5% were used. The results of diversity indices are shown in Table 1. The linkage disequilibrium (LD) decay pattern was different for each of the five populations (Figure S1a). LD was observed in all populations between SNPs within a short range. For SNPs up to 5 kb apart, the average $r^2$ values were 0.08, 0.07, 0.08, 0.20 and 0.09 for SH, NM, DZ, LZ and LW populations, respectively. There was a clear difference between LZ and the other four chicken populations. All SNPs without filtering for MAF were further pruned for principal component analysis (PCA). PCA revealed a cluster separation between LZ and the other 3 Tibetan chicken population. NM and DZ were also clearly separated by PC1 and PC2 excepted for few scattered individuals. Chickens from SH and LW population mixed together forming one cluster, occupying an intermediate position between DZ and NM populations (Figure S1b). Furthermore, population fixation index was calculated to evaluate the population relatedness (Table S1). We found that Fst values were small for all pairwise comparison, ranging from 0.001 (SH vs. LW) to 0.095 (DZ vs. LZ). Concordantly, LZ population were moderately distant from other populations with larger Fst value ranging from 0.052 to 0.095, while LW population was a synthesized line that which seems to be genetically closest to the all Tibetan chicken population.

Phenotypic traits were used to study the genetic distance among populations (Table S1). In general, the Fst values were small for all pairwise comparison, ranging from 0.001 (SH vs. LW) to 0.095 (DZ vs. LZ). Concordantly, LZ population were moderately distant from other populations with larger Fst value ranging from 0.052 to 0.095, while LW population was a synthesized line that which seems to be genetically closest to the all Tibetan chicken population.

| Population | N | ALL SNPs | SNPs with MAF ≥ 0.01 | SNPs with MAF ≥ 0.05 |
|------------|---|----------|----------------------|----------------------|
|            |   | Ho       | He                   | MAF                  | Ho       | He                   | MAF                  | Ho       | He                   | MAF                  |
| SH         | 20 | 0.226 ± 0.021 | 0.242 ± 0.000 | 0.130 ± 0.143 | 0.226 ± 0.021 | 0.242 ± 0.000 | 0.170 ± 0.141 | 0.271 ± 0.025 | 0.291 ± 0.000 | 0.207 ± 0.135 |
| NM         | 33 | 0.215 ± 0.023 | 0.225 ± 0.000 | 0.129 ± 0.142 | 0.215 ± 0.023 | 0.225 ± 0.000 | 0.157 ± 0.142 | 0.300 ± 0.023 | 0.313 ± 0.000 | 0.224 ± 0.131 |
| DZ         | 25 | 0.234 ± 0.035 | 0.237 ± 0.000 | 0.129 ± 0.143 | 0.234 ± 0.035 | 0.237 ± 0.000 | 0.167 ± 0.142 | 0.307 ± 0.049 | 0.311 ± 0.000 | 0.223 ± 0.132 |
| LZ         | 10 | 0.306 ± 0.014 | 0.299 ± 0.000 | 0.106 ± 0.146 | 0.306 ± 0.014 | 0.299 ± 0.000 | 0.216 ± 0.141 | 0.306 ± 0.014 | 0.299 ± 0.000 | 0.216 ± 0.141 |
| LW         | 18 | 0.248 ± 0.045 | 0.254 ± 0.000 | 0.129 ± 0.144 | 0.248 ± 0.045 | 0.254 ± 0.000 | 0.179 ± 0.141 | 0.292 ± 0.054 | 0.300 ± 0.000 | 0.214 ± 0.134 |

Note: SH, NM, DZ, LZ and LW denote Shigatse, Nyemo, Dagze, Ningychi and Lhasa white chicken population, respectively. Ho, He and MAF denote observed heterozygosity, expected heterozygosity and minor allele frequency, respectively. Data was shown as mean ± standard deviation.

Table 1: Observed (Ho), expected (He) heterozygosity and minor allele frequency (MAF) for each chicken population.

Runs of homozygosity (ROH) within each population

In current study, we identified 1269, 2438, 1366, 1284 and 1342 ROHs for SH, NM, DZ, LZ and LW chicken population, respectively. ROH were identified in all sampled birds. The average number of ROH segments was highest in the LZ (128.4 ROH/bird) compared to the remaining population. The lowest and highest average length of ROHs were observed in DZ (54.6 Mb) and LZ (102.5 Mb), respectively. SNP number harbored in ROH varied between the study populations. The maximum number of SNPs in ROH (22,386) was located on GGA1 of SH population, while the minimum number of SNPs in ROH (50) was located on GGA1 chromosome found in LZ chicken population (Table 2). As shown in Fig. 1a and b, ROHs identified in 106 birds were mainly distributed across the chromosome 1 ~ 15, 17 ~ 28 and 33 (GGA1 ~ GGA15, GGA17 ~ GGA28 and GGA33) of the chicken genome, although more of ROH regions were clustered in GGA1 ~ GGA8. The classification shown that short ROHs (< 1Mb) were predominant, accounting for 79.75–86.09% of ROHs across all populations (Fig. 1c). For a better view, we plotted ROH number against ROH length for each bird (Fig. 1d). We observed that the correlation between ROH number and ROH length was consistently high in NM (r = 0.87), DZ (r = 0.92), LZ (r = 0.91) and LW (r = 0.84), while it considerably varied in SH (r = 0.71). Moreover, the only bird with extremely long ROHs covering a length of 277.112 Mb was found in SH, while the bird with the shortest ROH length of 0.831 Mb belongs to DZ population.
Table 2
Descriptive statistics of run of homozygosity for 5 chicken populations

| Population | N  | Number of ROH | Average number per bird | Length of ROH (Mb) | Average length per bird (Mb) | SNP number range |
|------------|----|----------------|-------------------------|--------------------|------------------------------|-----------------|
| SH         | 20 | 1269           | 63.45 ± 41.94           | 1116.19            | 55.81 ± 62.57                | 52 ~ 22386      |
| NM         | 33 | 2438           | 73.88 ± 25.87           | 1680.94            | 50.94 ± 23.61                | 52 ~ 18809      |
| DZ         | 25 | 1366           | 54.64 ± 32.71           | 974.25             | 38.97 ± 34.86                | 50 ~ 17675      |
| LZ         | 10 | 1284           | 128.40 ± 12.69          | 1025.36            | 102.54 ± 15.05               | 50 ~ 2024       |
| LW         | 18 | 1342           | 74.56 ± 55.92           | 1089.51            | 60.53 ± 54.94                | 50 ~ 13096      |

Note: SH, NM, DZ, LZ and LW denote Shigatse, Nyemo, Dagze, Ningychi and Lhasa white chicken population, respectively. Data were shown as mean ± standard deviation.

Genomic inbreeding coefficients

Genomic inbreeding was evaluated by the proportion of the genome within ROH (F_{ROH}), genomic SNP-by-SNP inbreeding coefficient (F_{GRM}), excess of homozygosity (F_{HOM}) and correlation between uniting gametes (F_{UNI}). As shown in Fig. 2a, the four genomic inbreeding coefficients (F_{ROH}, 0.060 ± 0.047; F_{HOM}, 0.006 ± 0.136; F_{GRM}, 0.007 ± 0.168; F_{UNI}, 0.007 ± 0.077) were small and varied across the five populations. These coefficients revealed a similar trend within each population except for LZ, which showed high F_{ROH} (0.110 ± 0.016), low F_{HOM} (-0.029 ± 0.055), low F_{GRM} (-0.027 ± 0.046) and low F_{UNI} (-0.027 ± 0.052). Therefore, Tibetan native chickens were diverse and were less affected by inbreeding. We further analyzed the correlation among four inbreeding coefficients, in which overall correlation coefficients were 0.55 between F_{ROH} and F_{HOM}, 0.51 between F_{ROH} and F_{UNI}, 0.91 between F_{HOM} and F_{UNI}, 0.39 between F_{GRM} and F_{UNI}, respectively (Fig. 2b), while the correlation between F_{ROH} and F_{GRM}, and between F_{HOM} and F_{GRM} were not significant. Moreover, birds were most consistent within LZ population regarding the four indices, whereas large inter-bird variability existed within LW population, which is consistent with its population history (Lhasa white has undergone a recent hybrid process).

Gene Annotation of ROHs

We detected 74, 111, 62, 42 and 54 ROH islands containing SNPs with concurrency ranked top 1% (top 1% ROH islands) for SH, NM, DZ, LZ and Lhasa LW chicken populations, respectively (Fig. 3). Annotated genes of ROH islands were retrieved from the Ensembl genome browser, resulting in a list of 316, 491, 259, 197 and 166 Ensembl genes for SH, NM, DZ, LZ and LW, respectively. Gene ontology (GO) analysis revealed that these genes were significantly enriched in several biological process including positive regulation of synapse assembly, positive regulation of I-kappaB kinase/NF-kappaB signaling, osteoblast differentiation, cellular response to amino acid stimulus, cell adhesion, endodermal cell differentiation and so on (Table S2). Among these genes, 11 genes located on GGA5 and GGA7 were common to all the five populations. These genes include BDNF, CCDC34, LGR4, LIN7C, GLS, LOC101747789, MYO1B, STAT1 and STAT4 (Fig. 3). In addition, the chicken QTL database was interrogated to map the top 1% ROH islands. Result of the search identified 26, 29, 21, 19 and 17 QTLs that overlapped with the ROH islands in SH, NM, DZ, LZ and LW populations, respectively (Table S3). The common QTLs that overlapped with top 0.1% ROH islands in study populations were for comb and ileum weight. This suggests that comb and ileum may play important role in chicken adaptation to high altitude. The ROH islands that harbored QTL for ovary weight and percentage were specifically detected in Tibetan chicken (Table 3).
Table 3
Top 0.1% ROH islands overlapped with reported QTLs for 5 populations.

| Population | Chromosome | Start (bp) | End (bp) | Number of SNPs | Associated QTL trait |
|------------|------------|------------|----------|----------------|-----------------------|
| SH         | 1          | 190913541  | 191008990| 46             | Feed intake          |
|            | 2          | 144110859  | 144201790| 45             | Wattles weight       |
|            | 3          | 37006451   | 37110698 | 54             | Comb weight          |
|            | 3          | 85264887   | 85339273 | 23             | Comb weight          |
|            | 3          | 85362178   | 85530321 | 89             | Comb weight          |
|            | 7          | 7051986    | 7293162  | 59             | Ovary weight         |
|            | 7          | 7917536    | 8273027  | 131            | Ovary weight         |
|            | 8          | 31868      | 702746   | 267            | Ileum weight         |
|            | 8          | 742239     | 797216   | 54             | Ileum weight         |
|            | 8          | 11310511   | 11656850 | 81             | Ovary percentage     |
|            | 11         | 2781379    | 3782297  | 125            | Feed intake          |
| NM         | 1          | 127562685  | 127670930| 62             | Bursa of Fabricius weight |
|            | 1          | 148097189  | 148502945| 271            | Fear-tonic immobility duration |
|            | 2          | 93414138   | 93804523 | 192            | Feather-crested head |
|            | 3          | 37019870   | 37444374 | 202            | Comb weight          |
|            | 3          | 8516461    | 8531108  | 79             | Comb weight          |
|            | 3          | 8533481    | 8547712  | 77             | Comb weight          |
|            | 5          | 2234882    | 2605876  | 141            | Body weight (28 days) |
|            | 7          | 8089149    | 8139910  | 12             | Ovary weight         |
|            | 7          | 7239376    | 8061927  | 104            | Ovary weight         |
|            | 8          | 198456     | 643706   | 177            | Ileum weight         |
|            | 11         | 3367829    | 3766928  | 130            | Feed intake          |
|            | 14         | 31804      | 780922   | 364            | Wattles length       |
| DZ         | 1          | 41890128   | 42183082 | 103            | Yolk weight          |
|            | 2          | 82252562   | 82590119 | 120            | Feather-crested head |
|            | 2          | 82995651   | 83236647 | 99             | Feather-crested head |
|            | 2          | 143417810  | 143575154| 88             | Wattles weight       |
|            | 3          | 36964612   | 37202503 | 113            | Comb weight          |
|            | 3          | 37203796   | 37293935 | 33             | Comb weight          |
|            | 8          | 37270      | 401505   | 108            | Ileum weight         |
|            | 8          | 9055060    | 9347374  | 117            | Ovary percentage     |
|            | 8          | 9712707    | 10369442 | 222            | Ovary percentage     |
|            | 8          | 10503250   | 11473553 | 108            | Ovary percentage     |
|            | 8          | 11475230   | 11998950 | 129            | Ovary percentage     |

Note: SH, NM, DZ, LZ and LW denote Shigatse, Nyemo, Dagze, Ningchi and Lhasa white chicken population, respectively. ROHs highlighted in bold denote the shared QTL on chromosome 8 in 5 chicken populations.
| Population | Chromosome | Start (bp) | End (bp) | Number of SNPs | Associated QTL trait |
|------------|------------|------------|----------|----------------|---------------------|
| 28         | 2          | 4669120    | 4969625  | 72             | Abdominal fat weight |
| LZ         | 2          | 134425921  | 134528295| 29             | Wattles weight      |
| 4          | 2          | 41794287   | 41979935 | 33             | Ileum weight        |
| 7          | 2          | 8252320    | 8252320  | 1              | Ovary weight        |
| 7          | 2          | 9960252    | 10065745 | 42             | Ovary weight        |
| 7          | 2          | 8284975    | 8814836  | 65             | Ovary weight        |
| 7          | 2          | 8252320    | 8252320  | 1              | Comb weight         |
| 7          | 2          | 9960252    | 10065745 | 42             | Comb weight         |
| 7          | 2          | 8284975    | 8814836  | 65             | Comb weight, Receiving feather pecking |
| 7          | 2          | 9960252    | 10065745 | 42             | Pectoralis minor weight |
| 8          | 2          | 781122     | 1127960  | 67             | Ileum weight, Body weight (36 days), Body weight (46 days), Spleen weight |
| 8          | 2          | 1443038    | 1580622  | 40             | Ileum weight, Body weight (36 days), Body weight (46 days) |
| LW         | 1          | 127516561  | 127828483| 102            | Bursa of Fabricius weight |
| 1          | 1          | 150304925  | 150605645| 120            | Fear-tonic immobility duration |
| 1          | 1          | 160765702  | 160956991| 87             | Fear-tonic immobility duration |
| 2          | 2          | 73319835   | 73368670 | 11             | Feather-crested head |
| 2          | 2          | 82462750   | 83287108 | 429            | Feather-crested head |
| 3          | 2          | 60530655   | 60739692 | 107            | Comb weight         |
| 4          | 2          | 71334142   | 71797285 | 181            | Gizzard weight      |
| 8          | 2          | 357766     | 657445   | 112            | Ileum weight        |
| 12         | 2          | 19551518   | 19936175 | 171            | Breast muscle pH    |

Note: SH, NM, DZ, LZ and LW denote Shigatse, Nyemo, Dagze, Ningychi and Lhasa white chicken population, respectively. ROHs highlighted in bold denote the shared QTL on chromosome 8 in 5 chicken populations.

**Selection signature analysis**

Notably, the genomic region containing common QTLs, ranges from 0.03 Mb to 1.13 Mb of GGA8, and harbors six top 0.1% ROH islands across the study populations. Given that the five chicken populations were cultivated in Tibetan plateau for many decades, we speculated that this genomic region on GGA8 may be under natural or artificial selection. By focusing on GGA8, we calculated integrated haplotype homozygosity (iHS) for each population. Excepted for DZ birds, SNPs harbored in the above 6 top 0.1% ROH islands were strongly selected (Fig. 4), and the average $|iHS|$ values of SNPs in each ROH island were higher than average value of the total SNPs on GGA8. There were 1, 1, 8 and 2 SNPs ($P$ value ranked top 0.1%) that harbored signatures of selection in SH, NM, LZ and LW population, respectively. Further mining of annotated genes in this region revealed that AMY2A, NTNG1 and VAV3 were the only three candidate genes. The candidate ROH islands, SNPs and genes were listed in Table 4.
Table 4
The extended homozygosity of ROH island detected on chromosome 8.

| Populations^1 | Start (bp) | End (bp) | Length (bp) | Region iHS value^2 | Chromosome iHS value^3 | SNP position (bp)^4 | Percentage of SNP in ROH (%) | P value | Nearby genes | Location (kb)^5 |
|---------------|------------|----------|-------------|--------------------|------------------------|---------------------|--------------------------|---------|--------------|----------------|
| SH            | 31868      | 702746   | 670878      | 1.30               | 0.75                   | 87637               | 50                       | 7.24e-05| AMY2A        | D 37.10        |
|               | 742239     | 797216   | 54977       | 1.72               |                        |                     |                          |         |              |                |
| NM            | 198456     | 643706   | 445250      | 1.78               | 0.73                   | 553538              | 54.55                    | 6.76e-05| NTNG1        | U 274.61       |
| DZ            | 37270      | 401505   | 364235      | 0.68               | 0.71                   |                     |                          |         |              |                |
| LZ            | 781122     | 1127960  | 346838      | 2.99               | 0.81                   | 781122              | 70                       | 2.01e-04| NTNG1        | U 47.02        |
|               |            |          |             |                    |                        | 960534              | 80                       | 5.14e-04| NTNG1        | Intron         |
|               |            |          |             |                    |                        | 1019114             | 80                       | 5.57e-04| VAV3         | Intron         |
|               |            |          |             |                    |                        | 1040079             | 80                       | 5.61e-06| VAV3         | Intron         |
|               |            |          |             |                    |                        | 1090605             | 80                       | 3.43e-04| VAV3         | Intron         |
|               |            |          |             |                    |                        | 1104514             | 80                       | 3.69e-04| VAV3         | Intron         |
|               |            |          |             |                    |                        | 1107611             | 80                       | 1.08e-04| VAV3         | Intron         |
|               |            |          |             |                    |                        | 1112244             | 80                       | 2.72e-04| VAV3         | Intron         |
| LW            | 357766     | 657445   | 299679      | 1.73               | 0.75                   | 612663              | 50                       | 3.41e-07| NTNG1        | U 215.49       |
|               |            |          |             |                    |                        | 630438              | 44.44                    | 4.37e-05| NTNG1        | U 197.71       |

1: SH, NM, DZ, LZ and LW denote Shigatse, Nyemo, Dagze, Ningychi and Lhasa white chicken population, respectively;
2: Region iHS value denotes mean iHS value of SNPs in the studied region;
3: Chromosome iHS value denotes mean iHS value of all SNPs in the chromosome 8;
4: SNP with highest iHS value;
5: D and U indicates that the SNP is in the downstream and upstream of the gene, respectively.

Discussion

In current study, whole genome sequences of four Tibetan chicken populations reared in Tibetan plateau were analyzed by focusing on genetic diversity, run of homozygosity, genomic inbreeding and selection signature. A composite Tibetan local breed, Lhasa white was also included in the analysis to compare results among populations. Lhasa white is a synthetic breed generated by crossing male White Leghorn and female Tibetan chicken, which has more than sixty years of history in Tibetan plateau.

Observed (Ho) and expected (He) heterozygosity were effective indices to evaluate the genetic diversity within populations. We calculated Ho as mostly previous studies using SNPs with MAF ≥ 0.05, and found values close to 0.3 in all populations, which are similar to values estimated in modern chicken populations using sequence data [15] and are higher than values estimated in Italian local chickens [12]. Moreover, when we kept all SNPs in the analysis to avoid any bias [39], we found similarly lower Ho for all populations compared to Ho reported in Dutch local chickens evaluated by chicken 60K SNP arrays [10]. In our study, we observed slightly lower heterozygosity than expected heterozygosity in SH, NM, DZ and LW, suggesting subtle inbreeding in these populations. However, a little heterozygosity excess...
(Ho > He) existed in LZ population. This may indicate a recent bottleneck or an isolate-breaking effect [40] which may likely be due to the recent domestication and selection process. According to Wright's interpretation of $F_{ST}$ [41], pair-wise $F_{ST}$ among SH, NM and DZ were less than 0.05, indicating a little genetic differentiation. LZ was moderately distant from SH, NM and DZ, suggesting that LZ population was bred with little or no admixture in a relatively isolated environment. These findings confirmed that two or more Tibetan chicken populations live in the plateau [2, 4].

In present study, number and the distribution of ROHs identified for Tibetan native chickens were comparable to that previously reported in broiler [14]. Most ROHs identified in our study belonged to the short class, indicating that little deleterious inbreeding happened in five chicken populations [42]. The relationship between total number of ROH and total length of the genome covered by ROH showed considerable variation among animals within and across populations. Similar distributions were also reported in other livestock species, such as indigenous sheep [17] and cattle [43]. Genomic data is the only reliable source to estimate inbreeding and relatedness of marginalized populations in the absence of other data sources, such as pedigree. It is commonly accepted that the proportion of the genome within ROH ($F_{ROH}$), genomic SNP-by-SNP inbreeding coefficient ($F_{GRM}$), excess of homozygosity ($F_{HOM}$) and correlation between uniting gametes ($F_{UNI}$) were indicators for inbreeding assessment [44]. Herein, the ROH-based genomic inbreeding coefficients of Tibetan chicken were similar to estimates in other Chinese local chickens that were under conversation [11], while the values were much lower than those in modern chickens [15, 16] and other local chickens from Italy [12]. This suggests that Tibetan chickens maintained their natural diversity in the plateau. The correlation between $F_{ROH}$ and $F_{HOM}$ as well as $F_{UNI}$ were significantly high, similar to those estimated in cattle [45], pig [46], horse [47] and modern chicken [15], indicating that $F_{ROH}$ can be used as an accurate estimate of the proportion of IBD (identity by descent). However, the correlation between $F_{GRM}$ and $F_{ROH}$ and that between $F_{GRM}$ and $F_{HOM}$ close to 0 may probably be due to the number of SNPs used in this study, since sequences generated much more SNPs than SNP arrays, which significantly affected $F_{ROH}$ estimates [42, 48]. Similar situation was reported in other domestic animals [49, 50]. Moreover, the $F_{HOM}$ measurements that employed homozygous sites in the genome were more sensitivity to allelic frequencies [42].

Whole genome sequencing allows ROH to be more reliably detected, and analyzing the effect of common ROHs may reveal the demographic history of animal populations and allows exploration and testing of new approaches to understand complex traits [5]. The ROH islands of Tibetan chickens and Lhasa white chickens harbored many QTLs and candidate genes controlling economically important traits, including conformation, production, egg and meat quality, digestion and absorption, reproduction and growth traits. Regarding common genes located on GGA5, leucine-rich repeat-containing G protein-coupled receptor 4 (LGR4), enriched in biological process of osteoblast in GO database, contributes to regulation of energy metabolism including food intake and energy expenditure [51]. Brain-derived neurotrophic factor (BDNF), enriched in positive regulation of synapse assembly is considered important for the temperature perception in chicken [52]. In rats, BDNF administration in the paraventricular nucleus reduced energy intake and decreased body weight [53]. STAT1 and STAT4 are members of Janus kinase (JAK)-signal transducer and activators of transcription (STAT) pathway that plays critical roles in facilitating various cellular reactions to diverse forms of cellular stress, including hypoxia/reperfusion, endotoxin, ultraviolet light, and hyperosmolarity [54]. Moreover, these genes were previously identified as ROH islands-associated genes in Italian [12] and Mexican native chickens [20], suggesting that ROH-related homeostasis and STATs pathway are important in highland chickens. Particularly, metal ion binding was enriched in 34 genes. Although the process of how metal ion binding affect animal's physiology and production is rarely reported, some genes enriched in the term including VAV3, NOS2, COL3A1 and PRKD1 were putative candidate genes associated with highland adaptation [55], implying that metal ion binding may be associated with highland adaptation.

ROH islands might be indicative of genomic regions underwent natural and artificial selection [56]. The iHS approach appears to be the most powerful for detecting ongoing selection processes for which the target allele has a moderate to high frequency within a population. If the iHS method detects a genomic region, this region can contain several loci that may be undergoing selection within the breed. Therefore, the iHS method can detect breed-specific candidate genes under selection [57]. Our iHS analysis revealed that the common genomic region with different ROH islands on GGA8 were overlapped with putative selection signature in SH, NM, LZ and LW populations, indicating selective forces were undergoing in the regions. Commonly identified regions by both iHS and ROH analysis harbored AMY2A, NTNG1 and VAV3 genes. While AMY2A encodes a member of the alpha-amylase family of proteins, which is involved in carbohydrates and glycogen metabolism, affecting growth, carcass traits and feed intake efficiency in chicken [58]. Previous report shown that AMY2A was under selection for metabolism, energy availability and response to thermal stress in African chickens [59]. Similar to African village conditions, chicken feeding is mainly based on scavenging, household waste and some grain supplementation in the Tibetan plateau. Therefore, carbohydrate metabolism, energy generation and transport are important traits for feeding adaptation. NTNG1 is a responsible gene for axon and neurite growth [60] and has been linked to body weight gain of beef cattle in a meta-analysis [61], as well as birth weight of pigs in a GWAS research [62]. The gene was also differentially expressed in chicken hepatocellular cell line in response to stress [63]. VAV3 is a member of the VAV gene family that play vital roles as guanosine nucleotide exchange factors for Rho GTPases and
signaling adaptors downstream of protein tyrosine kinases [64], and it regulates osteoclast function, bone mass, hypothyroidism and renal systems [65]. Specifically, VAV3 has been identified as candidate gene associated with highland adaptation in Ethiopians [66] and Ethiopian sheep [67]. This probably resulted from the role it plays in homeostasis of the cardiovascular [68]. We therefore suggest that VAV3 also functions putatively in the adaptation to high altitude of Tibetan chicken.

Conclusions

In present study, we used whole genome sequence data to characterize the genetic diversity and investigate the distribution of ROH across the genomes of five Tibetan indigenous chicken populations. Different LD, diversity and ROH patterns were observed in the five populations. Genetic diversity evaluated by observed heterozygosity was high for the five populations. The Nyingchi population, which is distant from other populations had the highest proportion of long ROH fragments and ROH-based genomic inbreeding which reflect recent inbreeding events. We identified a total of 343 ROH islands harboring 1429 genes and 112 QTLs, in which five common genes were involved in energy metabolism and STATs pathway. Moreover, a genomic region on GGA8 houses AMY2A, NTNG1 and VAV3. This region is suggested as a candidate genomic region for adaptation to high-altitude environment, which should further be validated. Our findings contribute to the understanding of genetic diversity, population inbreeding and the underlying genetic mechanism of high-altitude adaptation, and may help in the design and implementation of breeding and conservation strategies for highland chickens.

Abbreviations

SH: Shigatse; NM: Nyemo; DZ: Dagze; LZ: Nyingchi; LW: Lhasa white; ROH: run of homozygosity; FROH: ROH-based inbreeding coefficient; LD: Linkage disequilibrium; BWA: Burrows–Wheeler Alignment; GATK: Genome Analysis Toolkit; Ho: Observed heterozygosity; He: Expected heterozygosity; FGRM: Genomic SNP-by-SNP inbreeding coefficient; FHOM: Excess of homozygosity; FUNI: correlation between uniting gametes; MAF: minor allele frequency; iHS: integrated haplotype score; GO: Gene ontology; LGR4: leucine-rich repeat-containing G protein-coupled receptor 4; BDNF: Brain-derived neurotrophic factor;

Declarations

Ethics approval and consent to participate

All birds were handled following the guidelines established by the Council of China for Animal Welfare. The experimental protocols were approved by the Science Research Department of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (CAAS) (Beijing, China).

Consent for publication

Not applicable.

Availability of data and materials

The data and computing programs used in this manuscript are available from the corresponding authors on request.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

JY contributed to performing of the study and the analysis of data, and writing of the manuscript. ZS and SL contributed to interpretation of data. SL, MZ, XL & ZY contributed to the sample and data collection. SL, NY & JC contributed to the design of the study, interpretation of data, and reviewing of the manuscript. All authors submitted comments on the draft, read, and approved the final manuscript.

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**Figures**

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Figure 1

Descriptive graphics of run of homozygosity (ROH) in 5 Tibetan native chicken populations. a. The average number of ROHs per chromosome (bars) and average percentage of each chromosome covered by ROHs (lines) for all chickens. b. The distribution of ROHs in the whole genome for all chickens. c. The percentage of total ROH within each ROH length category, including short (< 1 Mb), medium (1–3 Mb), and long (> 3 Mb) per chicken population. d. Total number of ROHs and total length of genome (Mb) covered by ROH segments per birds for each chicken population.
Figure 2

Genomic inbreeding and their correlation in 5 Tibetan native chicken populations. a. Genomic inbreeding coefficients inferred from the proportion of the genome within ROH (FROH), genomic SNP-by-SNP inbreeding coefficient (FGRM), excess of homozygosity (FHOM) and correlation between uniting gametes (FUNI). b. The correlation between each of 2 genomic inbreeding coefficients across birds. The scatter plot was distinguished by chicken population.
Figure 3

Circular Manhattan plot incidence of each SNP in run of homozygosity (ROH) for 5 Tibetan native chicken populations. From inside to outside, circles denote Shigatse, Nyemo, Dagze, Ningyichi and Lhasa white population, respectively. The outermost circle denotes the chromosome. The shared genes harbored in top 1% ROH islands by 5 populations were shown in red. The y axis denotes frequency (%) of SNP occurred in ROH.
Figure 4

Chromosome-wide distribution of selection signatures detected by iHS on Chromosome 8 for 5 Tibetan native chicken populations. The red line represents the threshold levels of top 0.1% SNPs. SH, NM, DZ, LZ and LW denote Shigatse, Nyemo, Dagze, Ningychi and Lhasa white chicken population, respectively.

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