



Article Genomics of Dwarfism in Italian Local Chicken Breeds

Francesco Perini ^{1,†}^(b), Filippo Cendron ^{2,†}^(b), Zhou Wu ³, Natalia Sevane ⁴^(b), Zhiqiang Li ^{1,5}, Chunhua Huang ^{1,5}, Jacqueline Smith ³^(b), Emiliano Lasagna ^{1,*}^(b), Martino Cassandro ^{2,6}^(b) and Mauro Penasa ²^(b)

- ¹ Department of Agricultural, Food and Environmental Sciences, University of Perugia, 06121 Perugia, Italy
- ² Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padova, 35020 Legnaro, Italy
- ³ The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian EH25 9RG, UK
- ⁴ Department of Animal Production, Veterinary Faculty, Universidad Complutense de Madrid, Avenida Puerta de Hierro, 28040 Madrid, Spain
- ⁵ College of Animal Science and Technology, Chengdu Campus, Sichuan Agricultural University, Chengdu 611130, China
- ⁶ Federazione delle Associazioni Nazionali di Razza e Specie, 00187 Roma, Italy
- * Correspondence: emiliano.lasagna@unipg.it; Tel.: +39-075-58517102
- + These authors contributed equally to the work.

Abstract: The identification of the dwarf phenotype in chicken is based on body weight, height, and shank length, leaving the differentiation between dwarf and small breeds ambiguous. The aims of the present study were to characterize the sequence variations associated with the dwarf phenotype in three Italian chicken breeds and to investigate the genes associated with their phenotype. Five hundred and forty-one chickens from 23 local breeds (from 20 to 24 animals per breed) were sampled. All animals were genotyped with the 600 K chicken SNP array. Three breeds were described as "dwarf", namely, Mericanel della Brianza (MERI), Mugellese (MUG), and Pepoi (PPP). We compared MERI, MUG, and PPP with the four heaviest breeds in the dataset by performing genome-wide association studies. Results showed significant SNPs associated with dwarfism in the MERI and MUG breeds, which shared a candidate genomic region on chromosome 1. Due to this similarity, MERI and MUG were analyzed together as a meta-population, observing significant SNPs in the *LEMD3* and *HMGA2* genes, which were previously reported as being responsible for dwarfism in different species. In conclusion, MERI and MUG breeds seem to share a genetic basis of dwarfism, which differentiates them from the small PPP breed.

Keywords: genomic region; indigenous breed; chicken; SNP; GWAS

1. Introduction

With the development of poultry genetics and breeding, selection and crossbreeding have shaped the phenotypic diversity and demographic history of local breeds [1,2]. Moreover, their domestication has strongly limited the phenotypic differentiation of local breeds by promoting genetic variants favorable to productive traits [3]. Among them, animal body size is the paramount trait determining the profitability of poultry meat; indeed, optimizing this trait has been a major goal during domestication [3–5]. Hence, a heavy body weight and rapid growth have always been important traits within the poultry industry. In response to natural and/or artificial selection, chicken breeds vary enormously in size. In contrast, in response to natural and/or artificial selection, chicken breeds vary enormously in size, also allowing the development of much lighter chicken breeds called dwarf breeds. Dwarf animals are characterized by short body stature and light body weight [6]. However, the identification of dwarf animals based on phenotypic evaluation is not easy, as several "non-heavy" breeds only have small stature and are not actual dwarfs [6]. In chickens, the dwarf phenotype is evaluated by different traits including, but not limited to, body



Citation: Perini, F.; Cendron, F.; Wu, Z.; Sevane, N.; Li, Z.; Huang, C.; Smith, J.; Lasagna, E.; Cassandro, M.; Penasa, M. Genomics of Dwarfism in Italian Local Chicken Breeds. *Genes* 2023, 14, 633. https://doi.org/ 10.3390/genes14030633

Academic Editor: Klaus Wimmers

Received: 23 January 2023 Revised: 27 February 2023 Accepted: 1 March 2023 Published: 3 March 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). weight, height, and shank length [6,7]. Based on physiological and genetic characteristics, four distinct types of dwarfism in chickens have been identified: sex-linked dwarfism, which has been well-studied and is caused by mutations in the growth hormone receptor gene (*GHR*) [8,9]; autosomal dwarfism, which is caused by a nonsense mutation in the transmembrane protein 263 gene (*TMEM263*) [10]; the bantam phenotype, associated with the *HMGA2* gene on chromosome 1 [11]; and creeper dwarfism due to a creeper allele described as a 25 kb deletion containing the *IHH* and non-homologous end-joining factor 1 (*NHEJ1*) genes [12,13].

In Italy, the indigenous breed Mericanel della Brianza (MERI), from the Lombardia region, is the most popular dwarf breed and derives from dwarf chickens that were spread across small rural farms at the beginning of the last century [14]. The MERI is the only Italian bantam chicken breed with an official standard recognized by the Italian Association of Fancy Breeders and is mainly reared on fancy farms in the Lombardia region [15]. Indeed, the breed standard is based on qualitative traits such as comb type and plumage, shank, foot, skin, and eye color. Other Italian local breeds such as the Pepoi (PPP) and the Mugellese (MUG) are presumed to be dwarf according to their body size and the other quantitative traits reported in Table 1.

Table 1. Mean and standard deviation (SD) of the morphometric traits of Mericanel della Brianza, Mugellese, and Pepoi local chicken breeds (presumed dwarf), and the local heavy breeds used as the control population, namely, Ermellinata di Rovigo, Robusta Lionata, and Robusta Maculata (Broiler Ross 708 is not reported in the table as phenotypic characterization was conducted only for local breeds).

Breed	Sex	Body V (g	Veight ;)	Body L (cn	ength n)	Shank (cn	Length n)	Shank (cn	Width n)	Breast \ (cn	Width 1)	Wings (cn	span 1)
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Mericanel della Brianza	ਾ (n = 6)	962.1	175.3	33.0	2.9	4.9	0.6	3.8	0.6	26.0	3.4	28.8	2.4
	ç (n = 17)	731.7	91.3	28.1	1.9	4.8	0.4	3.1	0.2	24.4	2.3	25.5	1.6
Mugellese	o³ (n = 7)	1082.1	91.5	29.0	1.6	6.2	0.3	3.9	0.3	26.1	1.0	32.0	2.0
	ç (n = 17)	717.4	100.8	24.9	1.7	5.0	0.5	3.3	0.3	22.5	1.3	27.2	2.3

Breed	Sex	Body V (g	Veight ;)	Body L (cm	ength 1)	Shank l (cn	Length 1)	Shank (cn	Width 1)	Breast (cn	Width n)	Wings (cm	span 1)
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Pepoi	ೆ (n = 12)	1860.0	182.1	36.9	1.3	9.4	0.5	4.6	0.4	33.4	3.2	43.0	1.8
	ç (n = 12)	1293.3	219.2	32.3	1.6	7.5	0.4	3.8	0.3	28.9	1.9	37.2	1.8
Ermellinata di Rovigo	ೆ (n = 12)	3436.7	216.2	45.7	1.4	11.0	0.3	5.9	0.1	39.1	1.3	53.5	0.6
Robusta Lionata	ç (n = 12)	2322.5	152.5	40.0	1.9	9.1	0.6	4.8	0.2	34.8	1.6	45.9	1.3
	് (n = 12)	4035.8	1024.6	46.8	1.6	11.3	1.0	6.1	0.2	41.2	3.9	53.4	1.6
Robusta Maculata	ç (n = 12)	2753.3	378.0	41.4	2.4	9.3	0.7	4.7	0.2	36.9	2.9	46.7	1.7
	് (n = 12)	4221.7	253.2	38.7	2.2	9.5	0.7	4.7	0.2	37.3	3.5	46.2	1.8

Table 1. Cont.

Table 1. Cont.

Breed	Sex	Body V (g	Veight)	Body L (cn	ength 1)	Shank l (cn	Length 1)	Shank (cn	Width 1)	Breast (cn	Width n)	Wings (cm	span 1)
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	ç (n = 12)	2831.7	450.6	45.6	2.1	11.5	0.8	6.0	0.3	42.3	3.6	54.1	1.4

The PPP breed is mainly spread across the Veneto region and is reared for fancy but also for productive purposes. In fact, the PPP has good breast muscle mass, providing very tasty meat (https://www.pollitaliani.it/razze/pepoi/, accessed on 21 December 2022). The MUG chicken is a small breed that originated from the Mugello area in the northeast of the Tuscany region. The MUG is widespread in the region due to its excellent aptitude for brooding; indeed, it has been traditionally used as a putative hen for eggs of other chicken breeds. Currently, the MUG is under a recovery selection program due to its small effective population size [16,17].

The MERI, MUG, and PPP are similar in their genetics and background [18]; however, the body weight and morphological traits are more similar between MERI and MUG [15,19]. To the best of our knowledge, the genomic features linked to dwarfism of the three breeds have not been investigated thus far. The aims of the present study were to characterize the genome-wide sequence variation associated with the dwarf phenotype in MERI, MUG, and PPP, and to investigate the genes associated with their phenotype. Understanding the genetic differences underlying the dwarf phenotype is important to assess a marker-assisted genomic selection to maintain this phenotype.

2. Materials and Methods

2.1. Ethical Statement

Ethical approval was not required for the current study. Blood samples were collected in compliance with the European rules [Council Regulation (EC) No. 1/2005 and Council Regulation (EC) No. 1099/2009] during routine health controls by the public veterinary service.

2.2. Samples Collection

The dataset was previously described in Cendron et al. [18] and consisted of 582 samples: 20 to 24 animals (equal number of males and females) for each of the 23 local breeds, and nine to 13 animals for each of the four commercial lines. From the total dataset, we extracted three breeds (MERI, MUG, and PPP; Table 1) supposed to be dwarf, and hence used as the case groups. Moreover, a control population was chosen that included the three heaviest local breeds, namely, Ermellinata di Rovigo (PER), Robusta Lionata (PRL), Robusta Maculata (PRM) (Table 1), and the Broiler Ross 708 (Broiler, 13 animals). Blood samples (2 mL) were collected from the ulnar vein and stored in Vacutainers[®] tubes containing EDTA as an anticoagulant. All studied breeds were collected from at least three different poultry farms and conservation centers to ensure a representative sampling of the breed.

2.3. Genotyping

DNA extraction and genotyping were performed at Neogen (Ayr, Scotland, UK) using a commercial kit and the Affymetrix Axiom 600 K Chicken Genotyping Array, representing 580,961 SNPs. The Gallus_gallus 5.0 chicken assembly (accession number: GCA_000002315.3) was used in this study as the reference genome [20] including the

markers located on chromosomes 1 to 28 and on the sex chromosomes. SNPs with a call rate < 95%, minor allele frequency < 5% and animals with more than 10% of the missing genotypes were removed from the dataset. The software PLINK 1.9 [21] was used to process the data.

2.4. Population Structure Analysis

The population structure of the final dataset was estimated with principal component analysis (PCA) and multidimensional scaling plot (MDS). First, we used the PLINK command "–keep" to extract the MERI, MUG, and PPP breeds and the control population (PER, PRL, PRM, and Broiler) from the .ped file. This subset was used for the population structure analysis performed through PCA, MDS, and Neighbor-Joining Tree (NJ). PLINK 1.9 was used for this purpose and to generate eigenvectors and eigenvalues, and the ggplot2 (v3.1.0) package of R software was used to visualize the results of PCA [22]. The 1-ibs distance matrix was computed in PLINK 1.9, converted to a NJ tree using the VCF2Dis software (https://github.com/BGI-shenzhen/VCF2Dis, accessed on 1 November 2022), and visualized with R package "treeio" [23]. Pair-wise genetic relationships within and between breeds were estimated using a matrix of genome-wide identity-by-state genetic distances in PLINK 1.9 and plotted using MDS (components C1 and C2).

2.5. GWAS Analyses

A genome-wide association study (GWAS) was performed, comparing the case (supposed dwarf breeds) and control populations. The MERI, MUG, and PPP breeds do not have normal-size chicken counterparts. The breeds used in the control population were chosen based on phenotypic data such as live body weight, wingspan, body length, breast width, shank width, and shank length [19]. Particularly, the live body weight was used as a discriminant for choosing the control population. Breeds with higher mean live body weight were chosen (Table 1) [19]. Moreover, the genetic features were evaluated according to the PCA, MDS, and NJ results, and the most divergent genetic backgrounds from the case population were categorized as control population. All breeds in the present study had no genetic outliers and showed clear clustering.

We performed GWAS using a single dwarf breed as well as all of the dwarf breeds combined, compared against the control population. GWAS was performed in GEMMA software v0.94.128 with linear-mixed models, adjusting for the effect of sex and relatedness, using a previously calculated matrix [24]. Upon the separate analysis of males and females, we observed no difference in male/female SNPs identified by the GWAS approach. Thus, further analyses combined males and females together. The genomewide significance threshold was determined by the Bonferroni method, in which the conventional *p*-value (0.05) was divided by the number of tests performed as follows: cutoff = $-\log_{10}(0.05/\text{number of variants})$. A genomic control inflation factor, the lambda was calculated in each study to evaluate the confounding due to population stratification. Variants were then annotated using snpEFF version 5.1 with default parameters in vCard File (VCF) [25]. Only SNPs with significant *p*-values were taken into consideration for further analyses. The VCF file from the previous step was then used as input in Ensembl Variant Effect Predictor (VEP) to evaluate the effect of SNPs on the genes, transcripts, and protein sequence. Fst values for single SNP loci observed between the experimental and control populations were calculated with PLINK 1.9. In order to compare the linkage disequilibrium (LD) around the HMGA2 gene, we computed the r2 between the markers of this region against the highest associated variant. Moreover, Haploview software [26] was used for computing haplotypes around the HMGA2 gene in the studied populations. The SNPs used for this purpose had MAF >0.10 and the Haploview software calculated the block (alleles) if 95% of informative (i.e., non-inconclusive) comparisons were in strong LD.

3. Results

3.1. Population Structure

In Figure 1a and in Figure S1, the MUG and MERI birds were well-distinguished yet very close to each other. On the other hand, the PPP birds—the third breed categorized as dwarf—were distant to the other two dwarf breeds in both PCA and MDS (Figures 1a and S1). Additionally, according to the phylogenetic analysis, the relative genetic relationships within the breeds were estimated, which showed a similar separation of PCA and MDS results in both the dwarf breeds and control population, respectively. Figures representing the PCA and MDS results showed a similar distribution of the breeds along the graph, thus testifying to the capacity of the two methods to distinguish the breeds on a genomic basis. The clear separation among the breeds was confirmed in Figure 1b, where the three "dwarf" breeds shared a common ancestor before the PPP birds diverged from the other two breeds.



Figure 1. (a) Principal component analysis (PCA) and (b) Neighbor-Joining Tree (NJ) of the dwarf breeds and control population. Different breeds are highlighted with different shapes and colors: Mericanel della Brianza (MERI), Mugellese (MUG), Ermellinata di Rovigo (PER), Pepoi (PPP), Robusta Lionata (PRL), Robusta Maculata (PRM), and Broiler Ross 708 (Broiler).

3.2. GWAS

Based on the genetic information, we performed independent GWAS for each of the three dwarf breeds using genetic variants against the control population (Figure 2). The values of lambda for all GWAS analyses were close to one (1.074 to 1.146), suggesting a good control in the population stratification. With regard to sex-linked dwarfism, which is caused by mutations in the *GHR* gene located on chromosome *Z*, no significant SNPs in or around this gene were detected (Figure S2). Notably different signals were observed among the three "dwarf" breeds (Figure 2a–c). With regard to the comparison of the MERI breed with control population, 643 SNPs passed the significance threshold (5×10^{-8}), of which 215 were associated with known genes (Figure 2a). For the MUG breed, 256 variants with significant *p*-values and 96 genes associated with them were observed (Figure 2b). Finally, in Figure 2c, a large number of variants were significant (1379) for the PPP breed. Some of the genes detected in the MERI and MUG birds are known to be relevant to the dwarfism phenotype. Particularly in MUG birds, two main peaks on GGA1 and GGA5 were appreciable. For the peak on chromosome 5, the genetic variants were located in the intronic region of the *RGS6* gene. This was highlighted in all three supposed dwarf breeds.



Figure 2. Manhattan plot of (a) Mericanel della Brianza, (b) Mugellese, and (c) Pepoi against the control population. The red line represents the *p*-value $< -\log 10(1 \times 10^{-7})$. The blue line represents suggestive significance, with a value of $-\log 10(1 \times 10^{-6})$.

3.3. Chromosome 1 Candidate Region

First, we focused on the SNPs on chromosome 1 in each of the three breeds. Although the MUG and MERI chickens did not share the same variants, they both showed the most significant SNPs in regions of chromosome 1 containing genes of interest for dwarfism. These include *HMGA*, *GRIP1*, and *LEMD3* in the MUG birds, and *WIF1*, *RASSF3*, and *SRGAP1* in MERI (Table S1). For instance, the AX-75432737 SNP in MUG has been found at position 1:34378326 and it is responsible for an intronic variant within the *HMGA2* gene (*p*-value < 4.67^{-18} ; Table S1). This region (1:33701467–34603740) did not seem to be relevant in the PPP breed. For this reason, we carried out a meta-analysis combining both MUG and MERI as a unique dwarf "population" and compared them against the control population (Figure 3a). Meta-analysis showed 99 significant variants (*p*-value < 5×10^{-8}) annotated in 43 genes (Table 2).



Figure 3. Manhattan plot of (a) Mericanel della Brianza + Mugellese breeds tested against the control population and (b) details of the chromosome 1 region. The red line represents the *p*-value < $-\log_1 10(1 \times 10^{-7})$. The blue line represents the suggestive significance, with a value of $-\log_1 10(1 \times 10^{-6})$.

Gene ID	Gene name	Function	Reference
LEMD3	LEM domain containing 3	Responsible for bone density disorders	[27]
HMGA2	high mobility group AT-hook 2	Contributes to vascular development and sprouting angiogenesis by promoting IGFBP2 production	[28]
NTS	neurotensin	Induces hypotension, hyperglycemia, and cyanosis	[29]
MAEL	maelstrom spermatogenic transposon silencer	Involved in the development and progression of bladder, liver, and colorectal cancers	[30]
ILDR2	immunoglobulin-like domain containing receptor 2	Negative regulator for T cells	[31]
ERG	ETS transcription factor ERG	Member of the E-26 transformation-specific (ETS) family of transcription factors with roles in development that include vasculogenesis, angiogenesis, hematopoiesis, and bone development	[32]
MALRD1	MAM and LDL receptor class A domain containing 1	Positively affects FGF15/19 levels, a hormone that can modulate bile acid levels, repress gluconeogenesis and lipogenesis, and promote glycogen synthesis	[33]
CALCR	calcitonin receptor	Involved in regulating follicular maturation in the chicken ovary	[34]
USH2A	usherin	Can cause Usher syndrome type 2 and non-syndromic retinitis pigmentosa	[35]
PDE10A	phosphodiesterase 10A	Its repression brings a reduction in muscle pathology and improvement in locomotion, muscle, and vascular function	[36]
AMMECR1	AMMECR nuclear protein 1	Its inactivation is associated with growth, bone, and heart alterations	[37]
ENPEP	glutamyl aminopeptidase	Encodes glutamyl aminopeptidase, which is related to tumorigenesis and immune microenvironment	[38]
CASP6	caspase 6	Expressed in gastric and colorectal cancers	[39]
SGCZ	sarcoglycan zeta	A factor in the pathogenesis of muscular dystrophy and is expressed mainly in vascular smooth muscle	[40]
STX18	syntaxin 18	Physically interacts with proteins involved in cell cycle and apoptosis	[41]
MSX1	msh homeobox 1	Its interaction with p53 inhibits tumor growth by inducing apoptosis and inhibits angiogenesis	[42]
JAKMIP1	janus kinase and microtubule interacting protein 1	It is a microtubule-associated protein predominantly expressed in neurons and lymphoid cells, which contributes to the establishment of neuronal morphology	[43]
TACC3	transforming acidic coiled-coil containing protein 3	Functions in mitotic spindle assembly and chromosome segregation	[44]
ELP4	elongator acetyltransferase complex subunit 4	Associated with language impairment, autism spectrum disorder, and mental retardation	[45]
USH1C	USH1 protein network component harmonin	Mutations in the alternatively spliced exons of USH1C cause non-syndromic recessive deafness	[46]

 Table 2. Gene ID, name, function, and reference for genes annotated in the meta-analysis (MM).

Gene ID	Gene name	Function	Reference
ROM1	peripherin 2 like	It is a photoreceptor specific integral membrane protein with ~35% sequence identity to <i>PRPH2/RDS</i>	[47]
DPF3	double PHD fingers 3	Its overexpression in renal cell lines increases the growth rates and alters chromatin accessibility and gene expression, leading to the inhibition of apoptosis and the activation of oncogenic pathways	[48]
RGS6	regulator of G protein signaling 6	Regulator of <i>G-protein signaling 6 (RGS6)</i> is linked to autism spectrum disorder, bipolar disorder, major depression, and schizophrenia	[49]
SIPA1L1	signal induced proliferation associated 1 like 1	<i>CircSIPA1L1</i> upregulates <i>ALPL</i> through targeting <i>miR-204-5p</i> and promotes the osteogenic differentiation of SCAPs	[50]
PCNX1	pecanex homolog 1	Plays an important role in spermatogenesis	[51]
FASTKD2	FAST kinase domains 2	Metabolic phenotyping of <i>FASTKD2</i> -deficient cells reveals impaired cellular respiration with reduced activities of all respiratory complexes	[52]
SEMA5B	Semaphorin 5B	Promotes in vivo tumor growth	[53]
CC2D1B	coiled-coil and C2 domain containing 1A	Contributes to the regulation of developmental myelination in the central nervous system	[54]
PDE8A	phosphodiesterase 8A	May indicate a role for PDE8A in cAMP signaling related to motor function	[55]
IGF1R	insulin like growth factor 1 receptor	Important effect on growth, carcass. and meat quality traits in many species	[56]
TENM2	teneurin transmembrane protein 2	Its deficiency in human adipocyte precursors leads to the induction of brown adipocyte marker genes upon adipogenic differentiation	[57]
RRN3	RRN3 homolog, RNA polymerase I transcription factor	Plays a major role in the transcriptional regulation of ribosomal DNA and cell growth	[58]
MAD1L1	MAD1 mitotic arrest deficient like 1	Deregulation of cell proliferation in avian species	[59]
MYH11	myosin, heavy chain 11, smooth muscle	Involved in vascular contractility and several autosomal dominant mutations	[60]
TRAFD1	TRAF-type zinc finger domain containing 1	Involved in interferon (IFN)γ signaling and MHC I antigen processing/presentation	[61]
GSG1L2	GSG1 like 2	This gene would have been recruited to modulate <i>AMPAR</i> function early in vertebrate evolution	[62]
GLP2R	glucagon-like peptide 2 receptor	This gene and its ligand in vertebrates has a role in embryonic intestine development	[63]
STXBP4	syntaxin binding protein 4	Regulates APC/C-mediated p63 turnover and drives squamous cell carcinogenesis	[64]
HELZ	helicase with zinc finger	Directly interacts with <i>CCR4-NOT</i> and causes decay of the bound mRNAs	[65]
IFT22	intraflagellar transport 22	Binding of <i>IFT22</i> to the intraflagellar transport complex is essential for flagellum assembly	[66]
SNX19	sorting nexin 19	Restricts endolysosome motility through contacts with the endoplasmic reticulum	[67]

Table 2. Cont.

Gene ID	Gene name	Function	Reference
NTM	neurotrimin	Mediates estrogen-induced sympathetic pruning in some peripheral targets	[68]
PEX11G	peroxisomal biogenesis factor 11 γ	Implicated in various stages of peroxisome assembly	[69]

Table 2. Cont.

Genes of interest such as *LEMD3* and *HMGA2* were also observed in the meta-analysis. Figure 3b clearly highlights the relevance of GGA1 in the meta-population Mericanel della Brianza + Mugellese (MM) analysis, with significant *p*-values for the variants associated with the HMGA and LEMD3 genes. Clear implication of these two genes in dwarfism has been widely reported in the literature. In the same above-mentioned genomic region on GGA1, it was possible to find numerous genes linked with dwarfism, which were also observed in the MUG and/or MERI breeds [11,27,70]. For example, the AX-75431832 and AX-75432337 variants were commonly shared in MUG and MERI, and were mapped to intergenic regions between TBC1D30-WIF1 and MSRB3-HMGA2, respectively. Moreover, GRIP1 showed significance in the same genomic region in MUG and RASSF3 in the MERI birds. Hence, in both the single breed analysis and meta-analysis, we observed a potential candidate region on chromosome 1. To explore population differentiation and genetic distance based on the GWAS results, we calculated the Fst for each SNP in the dwarf breeds against the control population. We then took the significant SNPs associated with dwarf genes and filtered on the Fst results (Figure S3). It was encouraging to note that the Fst values of these SNPs were very close to 1, which indicated that these SNP loci and their associated dwarf genes had a great degree of differentiation in comparison to the control population. The LD data provided linkage information regarding the GGA1 locus. Figure S4 reports the *p*-values from the GWAS and the r2 values for each SNP, in both the MERI and MUG analyses as well as the MM meta-analysis. Moreover, an investigation of haplotypes in the MUG and MERI populations was carried out by Haploview around *HMGA2* (1:34284446–34472431). Only MUG showed a unique block in *HMGA2* (Figure S5a) and the frequency of this haplotype was 0.437 (Table S2). MERI birds showed four blocks (Figure S5b), of which the fourth was the longest haplotype and had a high frequency in population (i.e., 0.521) (Table S2).

3.4. Other Candidate Regions

The other interesting peak seen in Figure 3a was located on GGA5, with variants located around the RGS6 gene. Four intronic variants mapped to RGS6 exhibited p-values that ranged from 2.7 \times 10⁻¹⁶ to 6.41 \times 10⁻¹² (Table S1). Of considerable importance is also the IGF1R gene, which was detected in the MUG analysis. IGF1R is located on chromosome 10 and is notoriously associated with reduced growth and dwarfism. *IGF1R* was observed thanks to a significant intronic variant in MUG birds, and it was also corroborated by the MM meta-analysis where upstream gene variation was noted. In both cases, suggestively significant *p*-values were observed, relative to the previously mentioned variants on chromosome 1. It was interesting to see the genes that were shared between the different analyses in this study. The Venn diagram in Figure 4a shows the candidate genes shared between the single breeds and meta-analysis, while Figure 4b depicts the same data without the genes annotated for the PPP analysis, and all the genes from Figure 4 are listed in Table S3. Interestingly, only the calcitonin receptor (CALCR) gene was commonly shared among all groups (Figure 4a). In Figure 4b, however, eight genes were in common between the MERI, MUG and MM analyses. Among them, the gene currently known to be most related to the dwarf phenotype was HMGA2, followed by USH2A, USH1C, RGS6, PDE8A, RRN3, and STXBP4. Unfortunately, none of these genes are known to be related to dwarfism, although their function in relation to this phenotype has not been fully explored thus far.



Figure 4. Venn diagrams of the annotated genes with significant variants from the GWAS analyses. (a) Venn diagram from the meta-analysis (MM), Mericanel della Brianza (MERI), Mugellese (MUG), and Pepoi (PPP) breeds and the (b) Venn diagram representing genes from the MM, MERI, and MUG breeds.

4. Discussion

Chicken dwarf breeds were established in Europe from Greek and Roman times, only being used as fancy, luxury birds [71]. However, over time, there has been enormous development of these birds to act as brooder hens for similar birds such as pheasants and francolins. Unfortunately, for Italian dwarf chicken breeds, there are no documents ascertaining their history, origin, and which breeds were used for their establishment. Indeed, it is still difficult to categorize them as dwarf breeds. Only MERI is officially recognized as a dwarf breed, however, the genomic evidence for the dwarf phenotype is unclear. The MUG breed is recognized as an official breed with a standard phenotype; indeed, its morphometric measurements are similar to those of MERI birds. In contrast, PPP is not recognized either as an official breed or as a dwarf one. Additionally, the morphometric measurements of PPP are the most divergent among the three breeds in this study. To our knowledge, none of these three breeds has been studied for its genomic characteristics, apart from run-of-homozygosity and phylogenetic studies [18,72]. Even if the breeds taken into account have different backgrounds, the methods used for genomic distinction showed the ability to differentiate the breeds. Tight clustering reflects the genomic relationship within each breed, with no samples found as outliers.

4.1. Candidate Genes from GWAS

Single breeds showed similar signals on GGA5 compared to the control population. All GWAS analyses shared a common annotated gene (*RGS6*) in this region (5:26600000–27300000). The *RGS6* gene negatively regulates G protein signaling by activating GTPase activity. Although the precise physiological roles of *RGS6* are unknown, this gene is linked to cellular stress responses and induces cell cycle arrest and apoptosis [73]. In the same genomic region, another two genes were detected: *DPF3* in MM, MERI, and PPP, and *SIPA1L1* in MM and MERI. *SIPA1L1* is the signal induced proliferation associated 1-like 1 gene that has been implicated in osteogenesis in vitro [50] and has a strong association with maximal clutch length in chicken [74]. The most interesting gene related to the development of tissues in this region is double PHD fingers 3 (*DPF3*), which has a role in

heart development. The *DPF3* gene is specifically expressed during heart development in mice, chickens, and zebrafish, and the knockdown of *DPF3* in zebrafish resulted in myocardial contractility and incomplete heart formation; thus, *DPF3* was categorized as a major gene for heart development, but it is also involved in skeletal muscle [68,75]. Another important gene related to dwarfism and growth is *IGF1R*, which was annotated with upstream genetic variation in the MM and MUG analyses. Reduced *IGF1R* expression has been reported in dwarf Holstein calves [6] and also in chicken [56].

The most important findings in the present study come from the identified region on chromosome 1. The HMGA2 gene is significant in MERI, MUG and the MM meta-analysis, but not in PPP. This gene has a role in dwarfism in different species such as chicken [11], rabbit [70], and in humans [76]. Moreover, Plassais et al. [77] reported that HMGA2 is the main candidate gene for body size in dogs. HMGA2 controls the proliferation of myoblasts and skeletal muscle development by modulating the expression of IGF2-binding protein 2 (IGF2BP2), which regulates the downstream signaling of many genes related to cell growth [78]. In this study, none of the variants associated with the HMGA2 gene were responsible for modification in the translated protein. Indeed, they were all intronic or intergenic variants. In another study in humans, the role of *LEMD3* in the pathogenesis of osteopoikilosis and short stature was highlighted [79]. LEMD3 is located in the same genomic region as HMGA2 and was identified in MM and MUG analyses in the present study. Carneiro et al. [70] found these genes to be associated with dwarfism in rabbit. Moreover, the *LEMD3* functional mechanism is an antagonist to transforming growth factor-β signaling (TGF-β) [80]. LEMD3 has also been reported in the modulation of ear size in pigs [81], underlying the principal role in the modulation of growth in different species. Together with LEMD3, the WIF1 gene has been reported in pigs, rabbits, and dogs as a candidate gene for the modulation of growth [27]. WIF1 binds to Wnt proteins and inhibits the activity of the Wnt/b-catenin pathway. The Wnt/b-catenin pathway may regulate proliferation and differentiation in many tissues including controlling the growth of connective tissue by regulating connective tissue growth factor (CTGF) and fibroblast cells in the skin [70,77,81]. Regarding the present study, the WIF1 gene was reported with an intronic variant at position 1:34008227 in the MM and MERI analyses.

4.2. Genomic Convergence on Chromosome 1

Our results highlight that the Fst values of the variants on GGA1 segregate in the control population. The Fst analysis evaluated the important differences that exist between the populations for the studied genomic region on GGA1. A different view of what happens on chromosome 1 was provided by the LD results. Figure S4 depicts the LD levels of the most significant SNP compared with others from the same genomic region. High LD levels were found in the MERI genome, especially when compared to MUG and the MM meta-analysis. Moreover, the MUG birds showed distinct haplotypes around the *HMGA2* gene, suggesting a strong selection in that part of the genome. These results were in line with those reported by the Italian Association of Fancy Breeders for these breeds (https://www.fiavinfo.eu/nana-italiana-perche-no/ accessed on 10 January 2023). In fact, the MERI is the only breed to be officially recognized both as an official breed and at the same time as a dwarf. This result underlines that, over time, crossbreeding has led to selective pressure in MERI birds, precisely in the identified genes on chromosome 1. Regarding the PPP birds, these candidate genes were not identified in our analyses. In fact, it is reported that PPP is not an officially recognized breed because of its diverse phenotypic variability. Finally, the MUG breed seems to have different variants with respect to breeds with normal size. Based on the LD results, however, the chromosome 1 locus does not appear to be as significant as it seems to be for the MERI breed. Generally, the SNP arrays were designed with variants mostly mapped to non-coding genomic regions. Indeed, in the 600 K Affymetrix[®] Axiom[®] array for chickens, only 3.7% of SNPs out of 580,954, were in the coding regions [82]. This could explain why we have not found a causative mutation in an exonic region (e.g., in *HMGA2*), which may lead to a change in the final protein. However, this work has highlighted candidate genes for the dwarfism phenotype in MUG and MERI chickens. Further studies with deeper or targeted sequencing of the candidate regions could shed light on the main features of genes in the studied breeds.

5. Conclusions

In conclusion, we identified candidate genes for dwarfism in MUG and MERI chickens and highlighted the importance of genes on chromosomes 1 and 5, while we showed that the PPP population does not appear to be a true dwarf breed when we consider the candidate genomic loci and morphological data. Further studies should focus on the validation of results obtained in the present manuscript through a larger sample size and the genotyping of identified candidate genes.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/genes14030633/s1, Figure S1. Multidimensional scaling plot (MDS) of the dwarf breeds and control population. Different breeds are highlighted with different shapes and colors: Mericanel della Brianza (MERI), Mugellese (MUG), Ermellinata di Rovigo (PER), Pepoi (PPP), Robusta Lionata (PRL), Robusta Maculata (PRM), and Broiler Ross 708 (Broiler). Figure S2. GWAS analysis of sex chromosome in (a) Mericanel della Brianza, (b) Mugellese, and (c) Pepoi chicken breeds. Figure S3. Fst values associated with all SNPs used in the GWAS analysis for (a) Mericanel della Brianza, (b) Mugellese, (c) Mericanel della Brianza + Mugellese, and (d) Pepoi breeds. SNPs with *p*-value $< 5 \times 10^{-8}$ are highlighted in green. Fst values on chromosome 1 from 34 Mb to 35 Mb in (e) Mericanel della Brianza, (f) Mugellese, and (g) Mericanel della Brianza + Mugellese breeds. The dashed blue lines identify the gene *LEMD3* and the dashed red lines are the *HMGA2* gene. Figure S4. GWAS analysis of chromosome 1 (around 33 Mb-36 Mb) in (a) Mericanel della Brianza + Mugellese, (b) Mugellese, and (c) Mericanel della Brianza. The y-axis represents the log_{10} of *p*-value from the GWAS analysis. The colors show the level of linkage disequilibrium (red = 1, blue = 0) of the SNPs in the regions of the most significant SNPs (AX-75432737 in Mericanel della Brianza + Mugellese; AX-75432737 in Mugellese; AX-75431832 in Mericanel della Brianza). Figure S5. Linkage disequilibrium plot (from HaploView) of (a) Mugellese and (b) Mericanel della Brianza based on D' for the SNP. Key: when LOD < 2, D' < 1 is white, and D' = 1 is blue; when LOD > 2, D' < 1 is given in shades of pink/red and D' = 1 is given in bright red; the pair-wise D' values are given in the boxes. Table S1. The first four sheets show the annotation for all significant variants in the respective GWAS. The columns show the chromosome, the coordinate of the variant, identifier of SNP, the reference allele found in the reference genome, the alternative allele found in the studied sample, quality score, pass/fail filter, information on the variants, individual identifier as the genotype is in the form 0 or 1, where 0 indicates the reference allele and 1 indicates the alternative allele (i.e. "0|1" is the heterozygote). The last four sheets show all the SNPs with a *p*-value $< 5 \times 10^{-8}$ for each GAWS analysis. Table S2. Haplotypes derived from Haploview analysis and related to Figure S5 for each breed block and their frequencies in the population are reported. Table S3. List of genes from the Venn diagrams (Figure 4).

Author Contributions: Conceptualization, F.P., F.C., E.L., M.C. and M.P.; Methodology, F.P., Z.W., F.C. and N.S.; Software, F.P., Z.W., J.S. and C.H.; Formal analysis, F.P. and C.H.; Resources, M.C., J.S. and M.P.; Data curation, F.P., F.C., Z.L. and C.H.; Writing—original draft preparation, F.P., Z.W., F.C. and J.S.; Writing—review and editing, F.P., Z.W., Z.L., J.S., E.L., N.S., M.C. and M.P.; Visualization, F.P., C.H.; Supervision, N.S., E.L. and M.P.; Project administration, M.C. and M.P.; Funding acquisition, M.C. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the project "Protection of biodiversity of Italian poultry breeds", funded by the Ministry of Agricultural, Food and Forestry Policies (Italy) in the framework of the PSRN 2014-2020, sub-measure 10.2 "Support for sustainable conservation, use and development of genetic resources in agriculture". We also acknowledge funding from the China Scholarship Council, Grant/Award Number: 202206910011 and 202206910015.

Institutional Review Board Statement: Sampling was carried out by trained veterinarians within the frame of vaccination campaigns; hence, no permis-sion from the animal research ethics com-mittee was necessary. Veterinarians adhered to standard procedures and relevant international and national guidelines to ensure appropriate animal care.

Informed Consent Statement: Not applicable.

Data Availability Statement: All relevant data are included in the manuscript and its additional files. The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments: We acknowledge the technical support of I.I.S. "Duca degli Abruzzi" (Padova, Italy), I.S.I.S.S. "D. Sartor" (Castelfranco Veneto, Treviso, Italy), I.I.S. "A. Della Lucia" (Feltre, Belluno, Italy), the experimental farm "Sasse Rami" of the Veneto Agricoltura Agency (Ceregnano, Rovigo, Italy), the University of Milan (Italy), and the University of Florence (Italy). The authors wish to thank the three anonymous reviewers for their valuable comments to the manuscript and constructive suggestions.

Conflicts of Interest: The authors declare no conflict of interest.

References

- 1. Andersson, L. Genetic dissection of phenotypic diversity in farm animals. Nat. Rev. Genet. 2001, 2, 130–138. [CrossRef] [PubMed]
- Bruford, M.W.; Bradley, D.G.; Luikart, G. DNA markers reveal the complexity of livestock domestication. *Nat. Rev. Genet.* 2003, 4, 900–910. [CrossRef] [PubMed]
- 3. Andersson, L.; Georges, M. Domestic-animal genomics: Deciphering the genetics of complex traits. *Nat. Rev. Genet.* 2004, 5, 202–212. [CrossRef]
- 4. Mignon-Grasteau, S.; Boissy, A.; Bouix, J.; Faure, J.-M.; Fisher, A.D.; Hinch, G.N.; Jensen, P.; Le Neindre, P.; Mormède, P.; Prunet, P.; et al. Genetics of adaptation and domestication in livestock. *Livest. Prod. Sci.* 2005, *93*, 3–14. [CrossRef]
- 5. Zeder, M.A. Pathways to Animal Domestication; Cambridge University Press: Cambridge, UK, 2012; ISBN 9781139019514.
- 6. Boegheim, I.J.; Leegwater, P.A.; van Lith, H.A.; Back, W. Current insights into the molecular genetic basis of dwarfism in livestock. *Vet. J.* **2017**, 224, 64–75. [CrossRef]
- 7. Cole, R.K. An autosomal dwarfism in the domestic fowl. Poult. Sci. 2000, 79, 1507–1516. [CrossRef]
- 8. Agarwal, S.K.; Cogburn, L.; Burnside, J. Dysfunctional growth hormone receptor in a strain of sex-linked dwarf chicken: Evidence for a mutation in the intracellular domain. *J. Endocrinol.* **1994**, *142*, 427–434. [CrossRef]
- 9. Burnside, J.; Liou, S.S.; Zhong, C.; Cogburn, L.A. Abnormal growth hormone receptor gene expression in the sex-linked dwarf chicken. *Gen. Comp. Endocrinol.* **1992**, *88*, 20–28. [CrossRef]
- 10. Wu, Z.; Derks, M.F.L.; Dibbits, B.; Megens, H.-J.; Groenen, M.A.M.; Crooijmans, R.P.M.A. A Novel Loss-of-Function Variant in Transmembrane Protein 263 (TMEM263) of Autosomal Dwarfism in Chicken. *Front. Genet.* **2018**, *9*, 193. [CrossRef]
- Wu, Z.; Bortoluzzi, C.; Derks, M.F.L.; Liu, L.; Bosse, M.; Hiemstra, S.J.; Groenen, M.A.M.; Crooijmans, R.P.M.A. Heterogeneity of a dwarf phenotype in Dutch traditional chicken breeds revealed by genomic analyses. *Evol. Appl.* 2020, 14, 1095–1108. [CrossRef]
- Kinoshita, K.; Suzuki, T.; Koike, M.; Nishida, C.; Koike, A.; Nunome, M.; Uemura, T.; Ichiyanagi, K.; Matsuda, Y. Combined deletions of IHH and NHEJ1 cause chondrodystrophy and embryonic lethality in the Creeper chicken. *Commun. Biol.* 2020, *3*, 144. [CrossRef] [PubMed]
- 13. Jin, S.; Zhu, F.; Wang, Y.; Yi, G.; Li, J.; Lian, L.; Zheng, J.; Xu, G.; Jiao, R.; Gong, Y.; et al. Deletion of Indian hedgehog gene causes dominant semi-lethal Creeper trait in chicken. *Sci. Rep.* **2016**, *6*, 30172. [CrossRef] [PubMed]
- 14. Cerolini, S.; Madeddu, M.; Zaniboni, L.; Cassinelli, C.; Mangiagalli, M.G.; Marelli, S.P. Breeding performance in the Italian chicken breed *Mericanel della Brianza*. *Ital. J. Anim. Sci.* 2009, *9*, 382–385. [CrossRef]
- 15. Cozzi, M.C.; Colombo, E.; Zaniboni, L.; Madeddu, M.; Mosca, F.; Strillacci, M.G.; Longeri, M.; Bagnato, A.; Cerolini, S. Phenotypic and genetic characterization of the Italian bantam chicken breed Mericanel della Brianza. *Livest. Sci.* 2017, 205, 56–63. [CrossRef]
- Mannelli, F.; Franzoni, A.; Galigani, I.; Nistri, V.; Daghio, M.; Scicutella, F.; Marzoni, M.; Minieri, S.; Salvucci, S.; Cerolini, S.; et al. Characterization of morphological, reproductive, and productive performances of Mugellese breed: An update of knowledge. *Poult. Sci.* 2022, 102, 102259. [CrossRef]
- 17. Franzoni, A.; Gariglio, M.; Castillo, A.; Soglia, D.; Sartore, S.; Buccioni, A.; Mannelli, F.; Cassandro, M.; Cendron, F.; Castellini, C.; et al. Overview of Native Chicken Breeds in Italy: Small Scale Production and Marketing. *Animals* **2021**, *11*, 629. [CrossRef]
- Cendron, F.; Perini, F.; Mastrangelo, S.; Tolone, M.; Criscione, A.; Bordonaro, S.; Iaffaldano, N.; Castellini, C.; Marzoni, M.; Buccioni, A.; et al. Genome-Wide SNP Analysis Reveals the Population Structure and the Conservation Status of 23 Italian Chicken Breeds. *Animals* 2020, *10*, 1441. [CrossRef]
- 19. Perini, F.; Cendron, F.; Lasagna, E.; Cassandro, M. Morphological and genetic characterization of 13 Italian local chicken breeds. *Acta Fytotech. Zootech.* **2020**, 23, 137–143. [CrossRef]
- Warren, W.C.; Hillier, L.W.; Tomlinson, C.; Minx, P.; Kremitzki, M.; Graves, T.; Markovic, C.; Bouk, N.; Pruitt, K.D.; Thibaud-Nissen, F.; et al. A New Chicken Genome Assembly Provides Insight into Avian Genome Structure. *G3 Genes Genomes Genet*. 2017, 7, 109–117. [CrossRef]
- Chang, C.C.; Chow, C.C.; Tellier, L.C.; Vattikuti, S.; Purcell, S.M.; Lee, J.J. Second-generation PLINK: Rising to the challenge of larger and richer datasets. *GigaScience* 2015, 4, s13742-015. [CrossRef]
- 22. Wickham, H. ggplot2: Elegant Graphics for Data Analysis; Springer: New York, NY, USA, 2016; Volume 35, ISBN 978-0-387-98140-6.

- 23. Paradis, E.; Schliep, K. ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 2019, 35, 526–528. [CrossRef] [PubMed]
- 24. Zhou, X. GEMMA User Manual V0.91. Studies 2012, 44, 821–824.
- Cingolani, P.; Platts, A.; Wang, L.L.; Coon, M.; Nguyen, T.; Wang, L.; Land, S.J.; Lu, X.; Ruden, D.M. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. *Fly* 2012, *6*, 80–92. [CrossRef]
- Barrett, J.C.; Fry, B.; Maller, J.; Daly, M.J. Haploview: Analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005, 21, 263–265. [CrossRef] [PubMed]
- Liang, J.; Zhang, Y.; Wang, L.; Liu, X.; Yan, H.; Zhang, L. Molecular cloning of WIF 1 and HMGA 2 reveals ear-preferential expression while uncovering a missense mutation associated with porcine ear size in WIF 1. Anim. Genet. 2019, 50, 157–161. [CrossRef] [PubMed]
- Wang, J.; Chen, Y.; Zeng, Z.; Feng, R.; Wang, Q.; Zhang, Q.; Sun, K.; Chen, A.F.; Lu, Y.; Yu, Y. HMGA2 contributes to vascular development and sprouting angiogenesis by promoting IGFBP2 production. *Exp. Cell Res.* 2021, 408, 112831. [CrossRef]
- Hwang, J.-I.; Kim, D.-K.; Kwon, H.B.; Vaudry, H.; Seong, J.Y. Phylogenetic History, Pharmacological Features, and Signal Transduction of Neurotensin Receptors in Vertebrates. *Ann. N. Y. Acad. Sci.* 2009, *1163*, 169–178. [CrossRef]
- Zhang, X.; Ning, Y.; Xiao, Y.; Duan, H.; Qu, G.; Liu, X.; Du, Y.; Jiang, D.; Zhou, J. MAEL contributes to gastric cancer progression by promoting ILKAP degradation. *Oncotarget* 2017, *8*, 113331–113344. [CrossRef]
- Hecht, I.; Toporik, A.; Podojil, J.R.; Vaknin, I.; Cojocaru, G.; Oren, A. ILDR2 Is a Novel B7-like Protein That Negatively Regulates T Cell Responses. *Physiol. Behav.* 2018, 176, 139–148. [CrossRef]
- 32. Adamo, P.; Ladomery, M.R. The oncogene ERG: A key factor in prostate cancer. Oncogene 2016, 35, 403–414. [CrossRef]
- Wang, L.X.; Frey, M.R.; Kohli, R. The Role of FGF19 and MALRD1 in Enterohepatic Bile Acid Signaling. Front. Endocrinol. 2022, 12, 799648. [CrossRef] [PubMed]
- Krzysik-Walker, S.M.; Ocón-Grove, O.M.; Maddineni, S.B.; Hendricks, G.L.; Ramachandran, R. Identification of Calcitonin Expression in the Chicken Ovary: Influence of Follicular Maturation and Ovarian Steroids1. *Biol. Reprod.* 2007, 77, 626–635. [CrossRef] [PubMed]
- 35. Toualbi, L.; Toms, M.; Moosajee, M. USH2A-retinopathy: From genetics to therapeutics. Exp. Eye Res. 2020, 201, 108330. [CrossRef]
- Lambert, M.R.; Spinazzola, J.M.; Widrick, J.J.; Pakula, A.; Conner, J.R.; Chin, J.E.; Owens, J.M.; Kunkel, L.M. PDE10A Inhibition Reduces the Manifestation of Pathology in DMD Zebrafish and Represses the Genetic Modifier PITPNA. *Mol. Ther.* 2021, 29, 1086–1101. [CrossRef]
- Moysés-Oliveira, M.; Giannuzzi, G.; Fish, R.J.; Rosenfeld, J.A.; Petit, F.; Soares, M.D.F.; Kulikowski, L.D.; Di-Battista, A.; Zamariolli, M.; Xia, F.; et al. Inactivation of *AMMECR1* is associated with growth, bone, and heart alterations. *Hum. Mutat.* 2018, *39*, 281–291. [CrossRef] [PubMed]
- Wang, A.; Chu, H.; Jin, Z.; Gong, Z.; Jia, Q.; Zhu, B. ENPEP as a potential predictor of immune checkpoint inhibitor efficacy. *Cancer Med.* 2022, 11, 880–887. [CrossRef] [PubMed]
- Lee, J.W.; Kim, M.R.; Soung, Y.H.; Nam, S.W.; Kim, S.H.; Lee, J.Y.; Yoo, N.J.; Lee, S.H. Mutational analysis of the CASP6 gene in colorectal and gastric carcinomas. *Apmis* 2006, 114, 646–650. [CrossRef] [PubMed]
- Jin, P.; Wu, X.; Xu, S.; Zhang, H.; Li, Y.; Cao, Z.; Li, H.; Wang, S. Differential expression of six genes and correlation with fatness traits in a unique broiler population. *Saudi J. Biol. Sci.* 2017, 24, 945–949. [CrossRef]
- Bassett, T.; Harpur, B.; Poon, H.Y.; Kuo, K.-H.; Lee, C.H. Effective stimulation of growth in MCF-7 human breast cancer cells by inhibition of syntaxin18 by external guide sequence and ribonuclease P. *Cancer Lett.* 2008, 272, 167–175. [CrossRef]
- Son, M.J.; Rho, S.B.; Kim, K.; Oh, M.; Son, C.; Song, S.Y.; Park, K. Homeoprotein Msx1-PIASy Interaction Inhibits Angiogenesis. *Cells* 2020, *9*, 1854. [CrossRef]
- Vidal, R.L.; Fuentes, P.; Valenzuela, J.I.; Alvarado-Diaz, C.P.; Ramírez, O.A.; Kukuljan, M.; Couve, A. RNA interference of Marlin-1/Jakmip1 results in abnormal morphogenesis and migration of cortical pyramidal neurons. *Mol. Cell. Neurosci.* 2012, 51, 1–11. [CrossRef] [PubMed]
- 44. Burgess, S.G.; Peset, I.; Joseph, N.; Cavazza, T.; Vernos, I.; Pfuhl, M.; Gergely, F.; Bayliss, R. Aurora-A-Dependent Control of TACC3 Influences the Rate of Mitotic Spindle Assembly. *PLoS Genet.* **2015**, *11*, e1005345. [CrossRef]
- Addis, L.; Ahn, J.W.; Dobson, R.; Dixit, A.; Ogilvie, C.M.; Pinto, D.; Vaags, A.K.; Coon, H.; Chaste, P.; Wilson, S.; et al. Microdeletions of *ELP4* Are Associated with Language Impairment, Autism Spectrum Disorder, and Mental Retardation. *Hum. Mutat.* 2015, 36, 842–850. [CrossRef] [PubMed]
- 46. Ouyang, X.; Xia, X.; Verpy, E.; Du, L.; Pandya, A.; Petit, C.; Balkany, T.; Nance, W.E.; Liu, X. Mutations in the alternatively spliced exons of USH1C cause non-syndromic recessive deafness. *Hum. Genet.* **2002**, *111*, 26–30. [CrossRef] [PubMed]
- Stuck, M.W.; Conley, S.M.; Naash, M.I. Retinal Degeneration Slow (RDS) Glycosylation Plays a Role in Cone Function and in the Regulation of RDS·ROM-1 Protein Complex Formation. *J. Biol. Chem.* 2015, 290, 27901–27913. [CrossRef]
- Colli, L.M.; Jessop, L.; Myers, T.A.; Camp, S.Y.; Machiela, M.J.; Choi, J.; Cunha, R.; Onabajo, O.; Mills, G.C.; Schmid, V.; et al. Altered regulation of DPF3, a member of the SWI/SNF complexes, underlies the 14q24 renal cancer susceptibility locus. *Am. J. Hum. Genet.* 2021, 108, 1590–1610. [CrossRef]

- Ahlers-Dannen, K.E.; Yang, J.; Spicer, M.M.; Maity, B.; Stewart, A.; Koland, J.G.; Fisher, R.A. Protein Profiling of RGS6, a Pleiotropic Gene Implicated in Numerous Neuropsychiatric Disorders, Reveals Multi-Isoformic Expression and a Novel Brain-Specific Isoform. *Eneuro* 2022, 9, 1–17. [CrossRef]
- 50. Li, Y.; Bian, M.; Zhou, Z.; Wu, X.; Ge, X.; Xiao, T.; Yu, J. Circular RNA SIPA1L1 regulates osteoblastic differentiation of stem cells from apical papilla via miR-204-5p/ALPL pathway. *Stem Cell Res. Ther.* **2020**, *11*, 461. [CrossRef]
- 51. Geisinger, A.; Alsheimer, M.; Baier, A.; Benavente, R.; Wettstein, R. The mammalian gene pecanex 1 is differentially expressed during spermatogenesis. *Biochim. Biophys. Acta-Gene Struct. Expr.* **2005**, *1728*, 34–43. [CrossRef]
- Popow, J.; Alleaume, A.-M.; Curk, T.; Schwarzl, T.; Sauer, S.; Hentze, M.W. FASTKD2 is an RNA-binding protein required for mitochondrial RNA processing and translation. RNA 2015, 21, 1873–1884. [CrossRef]
- Kundu, A.; Nam, H.; Shelar, S.; Chandrashekar, D.S.; Brinkley, G.; Karki, S.; Mitchell, T.; Livi, C.B.; Buckhaults, P.; Kirkman, R.; et al. PRDM16 suppresses HIF-targeted gene expression in kidney cancer. *J. Exp. Med.* 2020, 217, e20191005. [CrossRef] [PubMed]
- Acheta, J.; Hong, J.; Jeanette, H.; Brar, S.; Yalamanchili, A.; Feltri, M.L.; Manzini, M.C.; Belin, S.; Poitelon, Y. Cc2d1b Contributes to the Regulation of Developmental Myelination in the Central Nervous System. *Front. Mol. Neurosci.* 2022, 15, 881571. [CrossRef] [PubMed]
- 55. Kruse, L.S.; Møller, M.; Kruuse, C. Distribution of PDE8A in the nervous system of the Sprague-Dawley rat. *J. Chem. Neuroanat.* **2011**, *42*, 184–191. [CrossRef] [PubMed]
- 56. Lei, M.; Peng, X.; Zhou, M.; Luo, C.; Nie, Q.; Zhang, X. Polymorphisms of the IGF1R gene and their genetic effects on chicken early growth and carcass traits. *BMC Genet.* **2008**, *9*, 70. [CrossRef] [PubMed]
- Tews, D.; Fromme, T.; Keuper, M.; Hofmann, S.; Debatin, K.; Klingenspor, M.; Wabitsch, M.; Fischer-Posovszky, P. Teneurin-2 (TENM2) deficiency induces UCP1 expression in differentiating human fat cells. *Mol. Cell. Endocrinol.* 2017, 443, 106–113. [CrossRef]
- Li, F.; Lin, J.; Li, T.; Jian, J.; Zhang, Q.; Zhang, Y.; Liu, X.; Li, Q. Rrn3 gene knockout affects ethanol-induced locomotion in adult heterozygous zebrafish. *Psychopharmacology* 2022, 239, 621–630. [CrossRef]
- Romanov, M.N.; Tuttle, E.M.; Houck, M.L.; Modi, W.S.; Chemnick, L.G.; Korody, M.L.; Mork, E.M.; Otten, C.; Renner, T.; Jones, K.C.; et al. The value of avian genomics to the conservation of wildlife. *BMC Genom.* 2009, 10, S10. [CrossRef]
- 60. Pucci, L.; Pointet, A.; Good, J.-M.; Davoine, E.; Cina, V.; Zanchi, F.; Deglise, S.; Duchosal, L.M.; Kirsch, M. A New Variant in the MYH11 Gene in a Familial Case of Thoracic Aortic Aneurysm. *Ann. Thorac. Surg.* **2020**, *109*, e279–e281. [CrossRef]
- 61. Van der Graaf, A.; Zorro, M.M.; Claringbould, A.; Võsa, U.; Aguirre-Gamboa, R.; Li, C.; Mooiweer, J.; Ricaño-Ponce, I.; Borek, Z.; Koning, F.; et al. Systematic Prioritization of Candidate Genes in Disease Loci Identifies TRAFD1 as a Master Regulator of IFNγ Signaling in Celiac Disease. *Front. Genet.* 2021, *11*, 562434. [CrossRef]
- 62. Ramos-Vicente, D.; Bayés, À. AMPA receptor auxiliary subunits emerged during early vertebrate evolution by neo/subfunctionalization of unrelated proteins: Evolution AMPAR Auxiliary Subunits. *Open Biol.* 2020, 10, 200234. [CrossRef]
- 63. Mo, C.; Zhong, Y.; Wang, Y.; Yan, Z.; Li, J. Characterization of glucagon-like peptide 2 receptor (GLP2R) gene in chickens: Functional analysis, tissue distribution, and developmental expression profile of GLP2R in embryonic intestine. *Domest. Anim. Endocrinol.* **2014**, *48*, 1–6. [CrossRef] [PubMed]
- 64. Rokudai, S.; Li, Y.; Otaka, Y.; Fujieda, M.; Owens, D.M.; Christiano, A.M.; Nishiyama, M.; Prives, C. STXBP4 regulates APC/Cmediated p63 turnover and drives squamous cell carcinogenesis. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, E4806–E4814. [CrossRef]
- 65. Hanet, A.; Räsch, F.; Weber, R.; Ruscica, V.; Fauser, M.; Raisch, T.; Kuzuoğlu-Öztürk, D.; Chang, C.-T.; Bhandari, D.; Igreja, C.; et al. HELZ directly interacts with CCR4–NOT and causes decay of bound mRNAs. *Life Sci. Alliance* **2019**, *2*, e201900405. [CrossRef]
- 66. Wachter, S.; Jung, J.; Shafiq, S.; Basquin, J.; Fort, C.; Bastin, P.; Lorentzen, E. Binding of IFT22 to the intraflagellar transport complex is essential for flagellum assembly. *EMBO J.* **2019**, *38*, e101251. [CrossRef] [PubMed]
- Saric, A.; Freeman, S.A.; Williamson, C.D.; Jarnik, M.; Guardia, C.M.; Fernandopulle, M.S.; Gershlick, D.C.; Bonifacino, J.S. SNX19 restricts endolysosome motility through contacts with the endoplasmic reticulum. *Nat. Commun.* 2021, *12*, 4552. [CrossRef] [PubMed]
- 68. Cho, S.; Manjula, P.; Kim, M.; Cho, E.; Lee, D.; Lee, S.; Lee, J.; Seo, D. Comparison of Selection Signatures between Korean Native and Commercial Chickens Using 600K SNP Array Data. *Genes* **2021**, *12*, 824. [CrossRef] [PubMed]
- Ebberink, M.S.; Koster, J.; Visser, G.; van Spronsen, F.; Stolte-Dijkstra, I.; Smit, G.P.; Fock, J.M.; Kemp, S.; Wanders, R.J.; Waterham, H.R. A novel defect of peroxisome division due to a homozygous non-sense mutation in the *PEX11β* gene. *J. Med. Genet.* 2012, 49, 307–313. [CrossRef] [PubMed]
- Carneiro, M.; Hu, D.; Archer, J.; Feng, C.; Afonso, S.; Chen, C.; Blanco-Aguiar, J.A.; Garreau, H.; Boucher, S.; Ferreira, P.G.; et al. Dwarfism and Altered Craniofacial Development in Rabbits Is Caused by a 12.1 kb Deletion at the *HMGA2* Locus. *Genetics* 2017, 205, 955–965. [CrossRef]
- 71. Pascal, T. Le razze della gallina domestica. Casa Editrice Nazionale; Roux e Viarengo: Torino, Italy, 1905; pp. 98–111.
- Cendron, F.; Mastrangelo, S.; Tolone, M.; Perini, F.; Lasagna, E.; Cassandro, M. Genome-wide analysis reveals the patterns of genetic diversity and population structure of 8 Italian local chicken breeds. *Poult. Sci.* 2021, 100, 441–451. [CrossRef]
- 73. Jeong, W.; Lim, W.; Ahn, S.E.; Lim, C.-H.; Lee, J.-Y.; Bae, S.-M.; Kim, J.; Bazer, F.W.; Song, G. Recrudescence Mechanisms and Gene Expression Profile of the Reproductive Tracts from Chickens during the Molting Period. *PLoS ONE* **2013**, *8*, e76784. [CrossRef]

- 74. Lien, C.-Y.; Tixier-Boichard, M.; Wu, S.-W.; Chen, C.-F. Identification of QTL and loci for egg production traits to tropical climate conditions in chickens. *Livest. Sci.* 2020, 234, 103980. [CrossRef]
- Lange, M.; Kaynak, B.; Forster, U.B.; Tönjes, M.; Fischer, J.J.; Grimm, C.; Schlesinger, J.; Just, S.; Dunkel, I.; Krueger, T.; et al. Regulation of muscle development by DPF3, a novel histone acetylation and methylation reader of the BAF chromatin remodeling complex. *Genes Dev.* 2008, 22, 2370–2384. [CrossRef] [PubMed]
- Alyaqoub, F.; Pyatt, R.E.; Bailes, A.; Brock, A.; Deeg, C.; McKinney, A.; Astbury, C.; Reshmi, S.; Shane, K.P.; Thrush, D.L.; et al. 12q14 microdeletion associated with *HMGA2* gene disruption and growth restriction. *Am. J. Med. Genet. Part A* 2012, 158A, 2925–2930. [CrossRef] [PubMed]
- Plassais, J.; Kim, J.; Davis, B.W.; Karyadi, D.M.; Hogan, A.N.; Harris, A.; Decker, B.; Parker, H.G.; Ostrander, E.A. Whole genome sequencing of canids reveals genomic regions under selection and variants influencing morphology. *Nat. Commun.* 2019, 10, 1489. [CrossRef]
- Li, Z.; Gilbert, J.A.; Zhang, Y.; Zhang, M.; Qiu, Q.; Ramanujan, K.; Shavlakadze, T.; Eash, J.K.; Scaramozza, A.; Goddeeris, M.M.; et al. An HMGA2-IGF2BP2 Axis Regulates Myoblast Proliferation and Myogenesis. *Dev. Cell* 2012, 23, 1176–1188. [CrossRef]
- Buysse, K.; Reardon, W.; Mehta, L.; Costa, T.; Fagerstrom, C.; Kingsbury, D.J.; Anadiotis, G.; McGillivray, B.C.; Hellemans, J.; de Leeuw, N.; et al. The 12q14 microdeletion syndrome: Additional patients and further evidence that HMGA2 is an important genetic determinant for human height. *Eur. J. Med. Genet.* 2009, 52, 101–107. [CrossRef]
- Lin, H.; Decuypere, E.; Buyse, J. Oxidative stress induced by corticosterone administration in broiler chickens (*Gallus gallus domesticus*): 1. Chronic Exposure. *Comp. Biochem. Physiol.-B Biochem. Mol. Biol.* 2004, 139, 737–744. [CrossRef]
- 81. Zhang, L.; Liang, J.; Luo, W.; Liu, X.; Yan, H.; Zhao, K.; Shi, H.; Zhang, Y.; Wang, L.; Wang, L. Genome-Wide Scan Reveals LEMD3 and WIF1 on SSC5 as the Candidates for Porcine Ear Size. *PLoS ONE* **2014**, *9*, e102085. [CrossRef]
- 82. Kranis, A.; Gheyas, A.; Boschiero, C.; Turner, F.; Yu, L.; Smith, S.; Talbot, R.; Pirani, A.; Brew, F.; Kaiser, P.; et al. Development of a high density 600K SNP genotyping array for chicken. *BMC Genom.* **2013**, *14*, 59. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.