Genome-wide detection and analysis of copy number variation in Italian indigenous chicken breeds

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XII. European Symposium on Poultry Genetics 2023

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Progetto collettivo beneficiario per il Comparto Avicoli con il sostegno del Fondo Europeo Agricolo per lo Sviluppo Rurale (FEASR) https://ec.europa.eu/agriculture/rural-development-2014-2020_it

FONDO EUROPEO AGRICOLO PER LO SVILUPPO RURALE: l'Europa investe nelle zone rurali

Introduction

Copy number variants (CNV) are structural genomic alterations distributed across the entire genome in all species, with a mean size of at least 50 bp [1, 2], and they are caused by insertions, deletions, duplications, and translocations of DNA fragments [2-6]. In all species, CNV can intersect genes, altering their structure and expression, and causing phenotypic variability and disease susceptibility in humans [7, 8] and animals [6, 9, 10]. The CNV can explain a large portion of the loss of heritability in genome-wide studies for some traits [11, 12]. Although CNV are less prevalent in the genome than other molecular markers, they cover a larger portion of the genome and thus can have powerful effects on phenotypic variability [13-15]. Several studies in chickens have pinpointed quantitative trait loci (QTL) and positional candidate genes marked by significantly associated SNP for economically important traits, including growth performance, carcass characteristics, and abdominal fat deposition [15, 16].

Aim

To investigate the type and amount of CNV and CNV regions (CNVR), and the genes that undergo the effect of their presence with an unprecedented resolution using a high-density SNP chip in a large sample of Italian local chickens.

Results

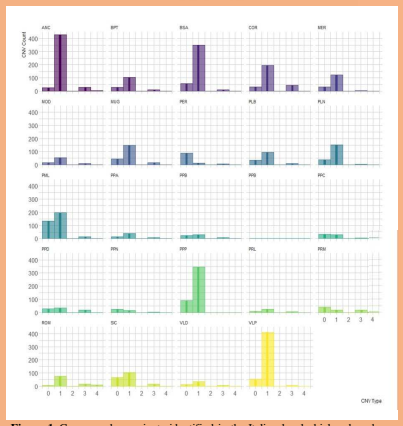

Figure 1. Copy number variants identified in the Italian local chicken breeds.

Breed	Type					Length	Mean	Min Length	Max Length	Genome Coverage (%)
	0	*1*	*3*	*4*	*Total*					
ANC	24	427	27	2	480	30,895,153	64,365	2,378	918,621	2.71
BPT	27	104	8	0	139	9,930,942	71,446	5,580	1,862,447	0.87
BSA	58	348	11	0	417	44,933,947	107,755	6,464	803,891	3.94
COR	31	196	43	0	270	21,300,170	78,890	857	923,575	1.87
MER	32	124	4	0	160	15,414,093	96,338	5,157	900,960	1.35
MOD	15	54	9	0	78	2,324,960	29,807	4,494	100,019	0.20
MUG	46	150	15	0	211	10,431,587	49,439	2,726	506,421	0.91
PER	90	22	7	0	119	5,647,643	51,813	7,526	472,494	0.49
PLB	36	96	9	0	141	8,284,013	58,752	4,389	304,806	0.73
PLN	38	152	5	0	195	10,091,542	51,752	1,643	347,761	0.88
PML	134	198	13	0	345	24,815,055	71,928	4,431	412,846	2.17
PPA	15	39	8	0	62	3,095,619	49,929	9,035	153,514	0.27
PPB	24	31	8	1	64	1,847,570	28,868	3,023	115,628	0.16
PPC	34	39	5	0	78	2,284,986	33,603	3,023	221,819	0.20
PPD	27	35	18	0	80	2,276,698	28,459	3,023	221,819	0.20
PPN	23	26	2	0	51	527,861	12,875	3,023	45,506	0.05
PPP	91	344	0	0	435	75,993,667	174,698	4,904	2,863,848	6.66
PRL	9	25	6	0	40	941,152	23,529	3,614	68,519	0.08
PRM	40	30	17	1	88	2,075,529	27,310	2,378	69,072	0.18
ROM	6	74	16	8	104	4,068,436	39,120	6,264	217,674	0.36
SIC	65	104	15	0	184	8,853,192	48,115	5,105	256,437	0.78
VLD	13	33	7	0	53	4,929,325	94,795	3,499	1,059,613	0.43
VLP	52	409	7	0	468	79,647,292	170,187	3,249	2,849,628	6.98
Unique CNVR	930	3,060	260	12	4262	370,610,432	1,463,770	95,785	15,696,918	32

Table 1. Descriptive statistics of Copy Number Variation Regions identified in the Italian chicken breeds.

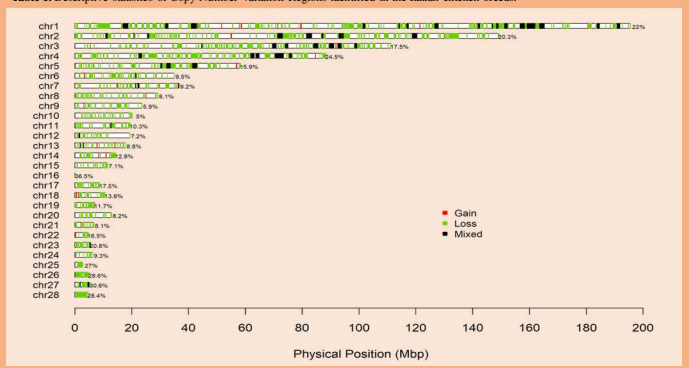

Figure 3. Physical distribution of Copy Number Variation Regions on chromosomes, according to state (gain, loss, and mixed).

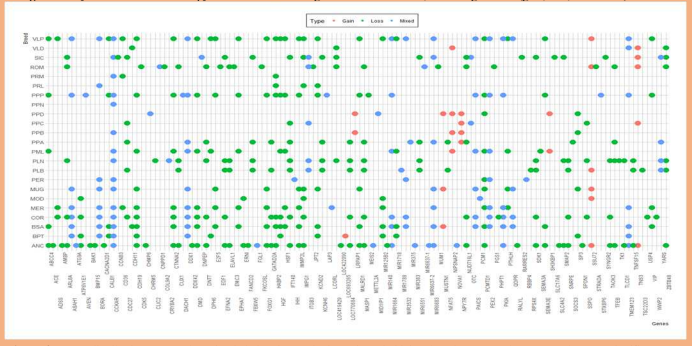

Figure 4. Genes distribution among the animals belonging to the investigated chicken breeds. Reported genes are the most significant as present in at least 4 animals across breeds. The colours indicate the status of CNVR in which the genes were annotated (red = gain, green = loss, blue = mixed).

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This work was supported by the project: "Protection of biodiversity of Italian poultry breeds" – TriBAvI - 2014 – 2020, – PSRN – Support for the conservation, use and sustainable development of genetic resources in agriculture. Sub-measure 10.2. - MASAF

Ancona (ANC)
Bianca di Saluzzo (BSA)
Bionda Piemontese (BPT)
Cornuta di Caltanissetta (COR)
Livorno Bianca (PLB)
Livorno Nera (PLN)
Mericanel della Brianza (MER)
Modenese (MOD)
Mugellese (MUG)
Ermellinata di Rovigo (PER)
Millefiori di Lonigo (PML)
Padovana Argentata (PPA)
Polverara Bianca (PPB)
Padovana Camosciata (PPC)
Padovana Dorata (PPD)
Polverara Nera (PPN)
Pepoi (PPP)
Robusta Lionata (PRL)
Robusta Maculata (PRM)
Romagnola (ROM)
Siciliana (SIC)
Valdarnese (VLD)
Valplatani (VLP)

Figure 2. Schematic representation of chicken breeds distribution in Italy.

Discussion

- Large number of CNV has been reported (Figure 1) compared to previous studies [6]; however, the number of CNV is very heterogeneous between breeds.
- The distribution of CNV is more prominent in the first six autosomes and linked with the types 0 and 1 (Figure 3).
- Approximately 40% of the CNVR (480 out of 1,172) are conserved and the remaining 692 are new and represent single regions (Table 1).
- Chromosome 16 stood out with a high proportion of its length covered by CNVR (36.5%; Figure 3); indeed, chromosome 16 is the shortest autosome of the genome of *Gallus gallus* and the MHC, here present, is subject to genomic copy number variation [17, 18].
- On chromosome 1, CNVR include *CACNA2D1* (calcium voltage-gated channel auxiliary subunit alpha 2 delta 1) which is related to muscle contraction [19]; *DMD* (dysthrophin), one of the most important factors for muscle development and structural stability of the tissue [20]; and *DACH1*, involved in skeletal development and inhibitor of growth factor beta [21]. Some other genes were *BORA*, related to cell growth and divisions and consequently influences on whole growth traits [22]; *IMMP2L*, involved in the reproduction traits and fertility [23].
- On chromosome 3, the most relevant is *DDX1* that strengthens the immunity response and therefore it may have played a role in the acquired resistance of local breeds to environmental stimuli [24].
- On chromosome 4, *CCKAR* is important for body weight and its variants have a central role in the diversification of gene expression [25].
- The genes *IFT140* and *ARL8A* were identified on chromosomes 14 and 26, respectively; these genes are associated with eggs and fertility [26]. These findings are important due to the low efficiency of these local breeds in terms of fertility and egg production [27].
- Several genes were found across breeds, and they include *SLC4A2* (solute carrier family 4 member 2) on chromosome 2, *CCNB3* (cyclin B3) on chromosome 4, and *DNPEP* (aspartyl aminopeptidase) on chromosome 7. These genes have been linked to muscle development and tissue-specific biological processes in muscle [28].
- Noteworthy, the gene encoding the miRNA *MIR6683* is present in CNVR 621 which has been identified in the BSA, MER, MUG, PPA, SIC and VLP breeds, and is associated with sex determination [29].
- KEGG analysis identified the MAPK signalling pathway, which plays an important role in complex cellular programs like proliferation, differentiation, development, transformation, and apoptosis [30].

Material and methods

- 508 individuals belonging to 23 local Italian chicken breeds were analyzed using the Affymetrix Axiom 600 K Chicken Genotyping Array.
- SNPs with call rates below 97% and Dish Quality Control values under 82% were removed.
- PennCNV software was used to CNV calling.
- Multiple Hidden Markov Models (HMMs - agre.hmm, affygw6.hmm, and hh550.hmm) were used in PennCNV software (Figure 5).
- Genomic wave adjustments were applied using a chicken GC model file generated based on GC content.
- To validate the CNV calls, an optimal segmenting module from SVS 8.7.0 (Golden Helix Inc.) was utilized, and samples with outliers were removed following quality assurance procedures (Figure 5).
- The final CNV data were summarized and Copy Number Variation Regions (CNVR) were defined using the HandyCNV R package.
- Genes within CNVR were considered if observed in more than five individuals across the breeds and on annotated *Gallus gallus 6.0* using Database for Annotation, Visualization, and Integrated Discovery (DAVID) (Figure 4).

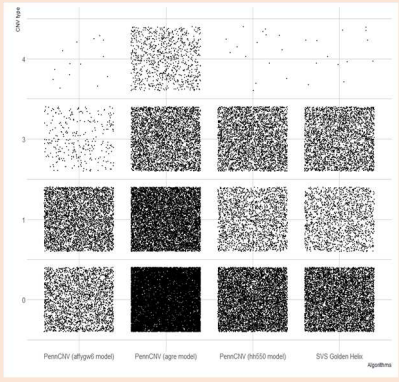

Figure 5. Number of CNV called using different algorithms.

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