



Discovering lethal alleles across the turkey genome using a transmission ratio distortion approach

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Summary

Deviation from Mendelian inheritance expectations (transmission ratio distortion, TRD) has been observed in several species, including the mouse and humans. In this study, TRD was characterized in the turkey genome using both allelic (specific- and unspecific-parent TRD) and genotypic (additive- and dominance-TRD) parameterizations within a Bayesian framework. In this study, we evaluated TRD for 23 243 genotyped Turkeys across 56 393 autosomal SNPs. The analyses included 500 sires, 2013 dams and 11 047 offspring (trios). Three different haplotype sliding windows of 4, 10 and 20 SNPs were used across the autosomal chromosomes. Based on the genotypic parameterizations, 14 haplotypes showed additive and dominance TRD effects highlighting regions with a recessive TRD pattern. In contrast, the allelic model uncovered 12 haplotype alleles with the allelic TRD pattern which showed an underrepresentation of heterozygous offspring in addition to the absence of homozygous animals. For regions with the allelic pattern, only one particular region showed a parent-specific TRD where the penetrance was high via the dam, but low via the sire. The gene set analysis uncovered several gene ontology functional terms, Reactome pathways and several Medical Subject Headings that showed significant enrichment of genes associated with TRD. Many of these gene ontology functional terms (e.g. *mitotic spindle assembly checkpoint*, *DRM complex* and *Aneuploidy*), Reactome pathways (e.g. *Mismatch repair*) and Medical Subject Headings (e.g. *Adenosine monophosphate*) are known to be related to fertility, embryo development and lethality. The results of this study revealed potential novel candidate lethal haplotypes, functional terms and pathways that may enhance breeding programs in Turkeys through reducing mortality and improving reproduction rate.

Keywords fertility, functional analysis, gene set enrichment, lethal haplotypes, transmission ratio distortion

Introduction

Owing to its considerable economic impact, reproduction has drawn the attention of turkey breeders and producers (Saif & Nestor 2002; Huff *et al.* 2005; Emamgholi Begli *et al.* 2019). Lethal alleles may cause mortality before, during or after the embryonic stage, and hence reduce reproductive performance. By their nature, livestock breeding programs

tend to increase inbreeding levels among individuals, and consequently the probability of mating parents carrying lethal alleles may increase (Granelle *et al.* 2015). Turkeys are not an exception, thus identifying genetic regions that influence reproductive efficiency and mortality is relevant and may enhance breeding programs in this species.

Many autosomal recessive lethal loci have been distinguished in livestock species such as cattle (e.g. Dong *et al.* 2019; Guarini *et al.* 2019) and the correct mate allocation is expected to reduce the economic losses (Cole *et al.* 2016). Several methods, such as screening for the absence of homozygous haplotypes (e.g. VanRaden *et al.* 2011; Hoff *et al.* 2017; Jenko *et al.* 2018) and transmission ratio distortion (TRD; Casellas *et al.* 2012, 2014), can be used to discover genomic regions with potentially lethal alleles. TRD

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is a process whereby the transmission of alleles from heterozygous parents to offspring deviates from Mendelian ratios, regardless of the cause (Crow 1999; Pardo-Manuel De Villena *et al.* 2000; Huang *et al.* 2013). Thus, TRD reveals locus-specific signals that provide insight into genetics and evolutionary processes of individual fitness variation, population divergence and speciation (Fishman & McIntosh 2019).

The availability of genomic markers has facilitated the task of investigating lethal alleles. The decline in reproductive performance and ability of parents to contribute equally to next generations may alter the expected Mendelian inheritance patterns, resulting in an observable TRD. For instance, it is possible to trace back the inheritance of each allele as well as the combination of two alleles inherited from parents to offspring using genotype trios with high accuracy. Several Bayesian models have been developed to detect and analyze all types of TRD based on allelic and genotypic parameterizations (Casellas *et al.* 2012, 2014). These two parameterizations have been already evaluated in a previous study (Casellas *et al.* In press) reporting the relevance of implementing and comparing the different parameterizations to capture all types of TRD. The objective of this study was to assess TRD based on allelic and genotypic parameterizations and perform a functional gene set enrichment analysis to uncover biological pathways associated with TRD in a purebred line of turkeys.

Materials and methods

Data

In this study, we used a turkey population with 23 243 (6867 males and 16 376 females) genotyped animals for 61 705 SNPs. The animals were hatched between late 2010 and early 2018, and genomic and pedigree information was provided by Hybrid Turkeys, Kitchener, Canada. The whole dataset combines 11 047 parent-offspring genotyped trios, including 500 sires and 2013 dams. All birds were genotyped with the same SNP genotyping platform array (65 000 SNP; Illumina, Inc.) and mapped to the Turkey 5.0 *Meleagris gallopavo* assembly (Dalloul *et al.* 2010). Quality control analyses were performed and resulted in the removal of non-autosomal SNP markers and those with a call rate below 90%. Whereas all birds had a call rate higher than 90% and passed the quality control criteria, the number of SNPs retained for analysis was 56 393 out of the 61 705 markers. BEAGLE 5.0 (Browning *et al.* 2018) was used to phase genotypes and impute the missing genotypes.

Statistical analyses

To trace the haplotype allele inheritance from parents to offspring in this turkey population, two parameterizations were considered in this study.

Allelic parameterization

Following Casellas *et al.* (2014, 2017), the probability of allele transmission (p) from heterozygous parents to offspring can be parameterized, including TRD effects on allelic basis, as:

$$p(A) = 1 - p(B) = 0.5 + \alpha_j$$

$$p(B) = 1 - p(A) = 0.5 - \alpha_j,$$

where A is the particular haplotype allele j being analysed, B represents the remaining haplotype alleles and α_j is the overall TRD for the allele j . To capture parent-specific TRD origin, a parent-specific model was also implemented on the basis of allelic parameterization, but including two different parameters:

$$p_s(A) = 1 - p_s(B) = 0.5 + \alpha_{sj}$$

$$p_d(A) = 1 - p_d(B) = 0.5 + \alpha_{dj},$$

where s and d represent sire and dam respectively, and α_{sj} and α_{dj} are sire- and dam-specific TRD for allele j . For all TRD parameters, flat priors were assumed within a parametric space ranging from -0.5 to 0.5 . Under a Bayesian implementation, the conditional posterior probabilities of the TRD parameters are defined as:

$$p(\alpha_j | \mathbf{y}) \propto p(\mathbf{y} | \alpha_j) p(\alpha_j)$$

$$p(\alpha_{sj}, \alpha_{dj} | \mathbf{y}) \propto p(\mathbf{y} | \alpha_{sj}, \alpha_{dj}) p(\alpha_{sj}) p(\alpha_{dj})$$

for overall and parent-specific TRD respectively, where \mathbf{y} is a column vector of genotypes of the offspring generation. The likelihood of data consists of a straightforward multiplication of the corresponding probabilities for each offspring (i.e. $\prod_n p_{\text{off}}(y_i)$), where n is the total number of offspring and p_{off} and y_i are the probability and the genotype of the i th offspring respectively. The software TRDSCAN version 1.0 (Id-Lahoucine *et al.* 2019) uses a multinomial process, hence the likelihood of the data becomes:

$$\prod_{i=1}^3 \frac{n_i!}{n_{AA,i}! n_{AB,i}! n_{BB,i}!} \times p_{\text{off},i}(AA)^{n_{AA,i}} \times p_{\text{off},i}(AB)^{n_{AB,i}} \times p_{\text{off},i}(BB)^{n_{BB,i}}.$$

In the above, n_i is the sum of $n_{AA,i}$, $n_{AB,i}$ and $n_{BB,i}$ offspring genotypes, $p_{\text{off},i}$ is the probability of an offspring genotype from the i th mating and $n_{AA,i}$, $n_{AB,i}$ and $n_{BB,i}$ are the number of AA, AB and BB offspring genotypes from the specific i th mating respectively. For the parent-specific TRD model, five kinds of matings were differentiated in the multinomial expression (i.e. $\prod_{i=1}^5$). For the sampling process, uniform proposal distributions (flat priors) were used for both α_g and δ_g within a deepened parametric space ranging from -1 to 1 (Casellas *et al.* 2012).

Genotypic parameterization

This parameterization captures the interaction between alleles of offspring genotypes. Additive (α_g) and dominance

(δ_g) or over-dominance, both positive or negative, TRD parameters are considered regardless of the origin of the allele. As described by Casellas *et al.* (2012, In press), the probability of observing offspring (p_{off}) from heterozygous-by-heterozygous mating can be estimated as follows:

$$P_{\text{off}}(\text{AA}) = \frac{(1 + \alpha_{gj} - \delta_{gj})}{4}$$

$$P_{\text{off}}(\text{AB}) = \frac{(1 + \delta_{gj})}{2}$$

$$P_{\text{off}}(\text{BB}) = \frac{(1 - \alpha_{gj} - \delta_{gj})}{4},$$

where α_{gj} and δ_{gj} are additive and dominance TRD parameters for the specific allele j respectively.

Under a Bayesian implementation, the conditional posterior probabilities of the TRD parameters are defined as:

$$p(\alpha_{gj}, \delta_{gj} | \mathbf{y}) \propto p(\mathbf{y} | \alpha_{gj}, \delta_{gj}) p(\alpha_g) p(\delta_{gj} | \alpha_{gj}),$$

where \mathbf{y} is a column vector of genotypes of the offspring generation. For the sampling process, uniform proposal distributions (flat priors) were used for both α_g and δ_g within an extended parametric space ranging from -1 to 1 (Casellas *et al.* 2012). Thus, as the parametric space for α_g is initially $[-1, 1]$, the parametric space for δ_g is restricted to $[-1, |\alpha_g|]$. Moreover, the parametric space of α_g itself is restricted to $[-1 + \delta_g, 1 - \delta_g]$ if $\delta_g > 0$ and this guarantees that the sum of offspring's genotypes probabilities for specific mating is equal to 1.

Transmission ratio distortion was evaluated using three sliding windows: 4, 10, and 20 SNP. The analyses were carried out using TRDSCAN version 1.0 software (Id-Lahoucine *et al.* 2019) based on a MCMC and the Metropolis–Hastings' algorithm (Hastings 1970). A single MCMC of 100 000 iterations was run for each analysis with the first 10 000 iterations being discarded as burn-in. Bayes factor (BF; Kass & Raftery 1995), which is a ratio of probabilities between full and null TRD models, was used to determine significant TRD (BF ≥ 100 ; decisive evidence according to Jeffreys' (1984) scale). To obtain a reasonable statistical power and to minimize false TRD as a result of genotyping errors, only haplotype alleles with a minimum number of 50 informative offspring (i.e. from heterozygous parents) and five heterozygous sires and/or dams were analyzed.

The identified TRD regions were then filtered to minimize genotypic errors and to eliminate regions with random TRD. First, an approximate empirical null distribution of TRD (Id-Lahoucine *et al.* 2019) at less than 0.001% margin error was used to remove TRD generated by chance. Also, a minimal number of informative parents (≥ 5 heterozygous sires and/or heterozygous dams) were considered to minimize possible false TRD from genotyping errors. Similarly, regions with few heterozygous parents fully explaining the observed TRD in the corresponding region were discarded as potential genotyping errors. Moreover, for the allelic

parametrization, an arbitrary minimum magnitude of TRD at least 0.20 and the number of underrepresented offspring at least 1000 were considered to identify haplotype alleles with strong allelic TRD patterns. The number of underrepresented offspring is the total number of offspring expected, but not observed for a particular allele, which is also approximately equal to the number of informative offspring multiplied by twice the magnitude of the TRD. These thresholds ensured the identification of target haplotypes with a moderate-to-high level of TRD (i.e. moderate-to-high penetrance) as well as a reasonable number of offspring that are expected, but not observed (i.e. a minimum frequency for the allele in the population). For the genotypic parametrization, an arbitrary minimum number of 10 non-observed homozygous offspring from heterozygous-by-heterozygous matings with additive TRD effect less than -0.50 and dominance TRD effect greater than 0.10 was considered to determine haplotype alleles with a substantial recessive TRD pattern. This threshold was considered to maintain the most important regions. It should also be emphasized that a well-known lethal haplotype in cattle industry (Holstein haplotype 3) was initially identified by VanRaden *et al.* (2011) with only seven non-observed homozygous offspring from heterozygous sires and heterozygous maternal grandsires matings. In addition, as different sliding windows were used, only the haplotype alleles with the largest BF within a region (with many physically linked haplotypes) were selected as the best candidates to explain the observed TRD in the region and potentially harbor the causal mutations. Thus, it is important to mention that the patterns of TRD observed in short windows are displayed in larger haplotypes including the same allele and also on other physically linked haplotypes, supporting the relevance of TRD in the corresponding particular locus.

Functional and gene set enrichment

Assignment of lethal haplotypes to genes

Genes associated with complex traits are expected to represent only a small fraction of the genetic variation and, hence, some genetic variants with small effects and disease risks may not ever be detected (Peng *et al.* 2010; Abdalla *et al.* 2016). To further investigate the potential lethal haplotypes identified with TRD, the coordinates of these haplotype regions were used to mine for annotated genes using the Turkey 5.0 (release 102) assembly (Dalloul *et al.* 2010). It has been reported that strong LD may extend up to 10–30 kb in chickens (Rao *et al.* 2008; Megens *et al.* 2009; Qanbari *et al.* 2010). Thus, haplotypes were assigned to genes if they were located within the genomic sequence of an annotated gene or within 15 kb of the 5' or 3' ends of the first and last exons respectively. The 15 kb distance was used to capture proximal regulatory regions and other

functional sites that may lie outside (e.g. promoter regions) but close to each gene. If a haplotype was found to be located within or close to more than one gene, all of these genes were included in the subsequent analyses.

Assignment of genes to functional categories

Gene Ontology (GO; Ashburner *et al.* 2000), Pathway Knowledgebase (Reactome; Fabregat *et al.* 2018) and Medical Subject Headings (MeSH; Coletti & Bleich 2001; Nelson *et al.* 2004) databases were used to define functional sets of genes. Biological descriptors, known as GO terms, fall into three categories: biological process, molecular functions and cellular components. Reactome, on the other hand, provides several biochemical networks including metabolic and regulatory pathways. Finally, MeSH is a collection of descriptors or headings representing key topics discussed in the papers indexed in the MEDLINE database. Whereas MeSH terms are classified into 19 categories, in this study, we were interested in only four: anatomy, disease, phenomena and processes, and lastly chemicals and drugs.

Pathway-based association analysis

The Fisher's exact test was used to declare the association of a given GO term, Reactome pathway and MeSH heading with TRD. This test was performed to search for an overrepresentation of significant genes in a given functional category among all genes. The *P*-value of observing *g* significant genes in the term was calculated as follows:

$$P\text{-value} = 1 - \sum_{i=0}^{g-1} \frac{\binom{S}{i} \binom{N-S}{k-i}}{\binom{N}{k}}$$

where *N* is the total number of genes analyzed in the study, *S* is the total number of genes that were deemed significantly associated with TRD and *k* is the total number of genes in the functional category in the database under consideration.

Owing to a lack of biological information related to turkeys, both the Turkey 5.0 assembly (Dalloul *et al.* 2010) and the GRCg6a chicken assembly (International Chicken Genome Sequencing Consortium 2004) were used to perform the pathway analysis. It is important to emphasize that all potential genes defined in this study are conserved across many organisms, including humans and chickens. The GO and Reactome enrichment analyses were carried out using GO (Ashburner *et al.* 2000) and PANTHER (Mi *et al.* 2016) respectively, whereas the MeSH enrichment analysis was performed using the MESH package (Tsuyuzaki *et al.* 2015) available in the R environment (R Core Team 2019).

Gene network

The overlaps among significant genes associated with GO terms and Reactome pathways and their functional networks were also examined. We retrieved close neighbor genes and then generated an aggregate interaction network based on physical protein interaction and co-expression of those genes using GENEMANIA software (Warde-Farley *et al.* 2010).

Results and discussion

Prevalence of TRD across the turkey genome

The prevalence of TRD was widely distributed across the turkey genome as shown in Fig. 1. The initial numbers of haplotype alleles detected with decisive significant evidence ($BF \geq 100$) of TRD, according to Jeffreys' scale (Jeffreys 1984), were 48 951, 52 896 and 54 287 for 4, 10 and 20 SNP haplotype windows respectively. Despite this high number of regions, some of them had a large BF (10^{100}) suggesting virtually 0 probability of the null TRD model. In addition, most of the detected TRD regions had low frequencies (i.e. rare variants); however, these haplotype alleles were supported by the large dataset. It is important to mention that it has been suggested that rare variants are more likely to be functional than common variants (Gorlov *et al.* 2008; Karaca *et al.* 2015), emphasizing the importance of TRD regions despite their low frequencies. It is noteworthy to mention that the majority of the regions were detected with more than one of the models applied, but with different fits and statistical significance. Thus, after the characterization of the TRD across the whole genome with the filtration criteria provided in the 'Materials and methods' section, the list of the most relevant haplotypes is provided in Tables 1 and 2 for regions with allelic and recessive TRD patterns respectively. These haplotypes indicate candidate regions potentially carrying deleterious alleles or genes affecting reproduction. However, these TRD findings were obtained under the assumption of no selection of offspring within a family to be genotyped. The violation of this must be taken into consideration by further investigating the source of the observed TRD signals. This is because the pre-selection of offspring to be genotyped within families (Id-Lahoucine & Casellas 2017) could be a source of bias on TRD analyses as discussed by Id-Lahoucine *et al.* (2019). It is well known that selection of data has been a concern and a limitation for many types of analyses. In particular, for TRD analyses, major genes that present crucial and large impact in reproductive performance are targeted. The genetic selection performed in turkey populations is based on selection indexes targeting a multiple-trait breeding objective (mainly highly polygenic production traits). Thus, the chance to observe TRD signals with absence of homozygous offspring as a result of selection is

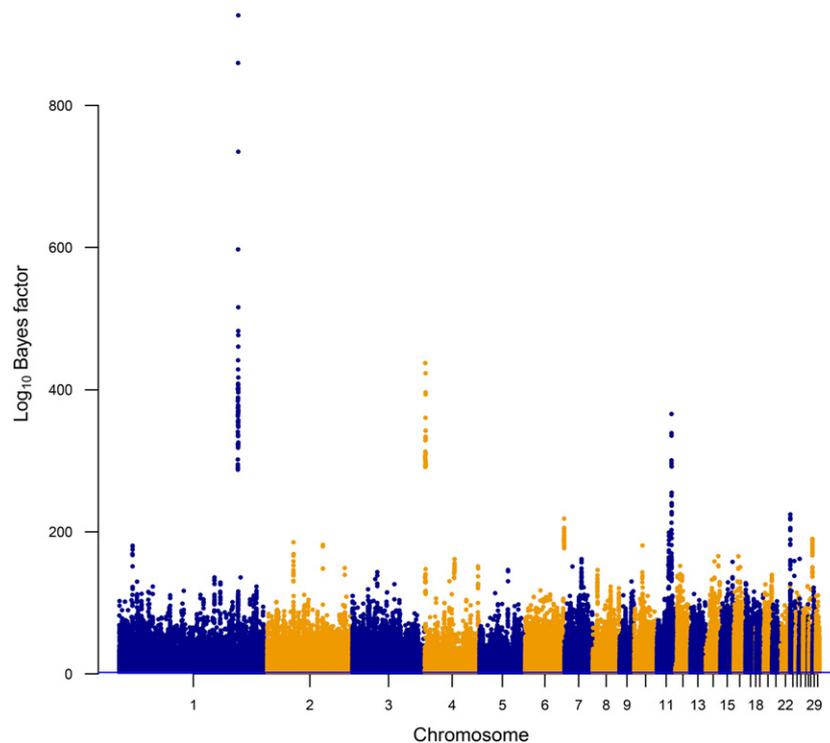


Figure 1 Bayes factor for haplotypes with transmission ratio distortion across the turkey genome. Significant haplotype alleles were determined based on \log_{10} Bayes factor ≥ 2 according to Jeffreys' scale (Jeffreys 1984)

less likely. Here, we are using the TRD method as an alternative strategy to first scan possible relevant regions harboring lethal alleles that will require further research to exclude both genotyping errors and biases from pre-selection in the offspring generation.

Haplotypes with allelic TRD pattern using the allelic parametrization model

Across the turkey genome, 12 potentially lethal haplotype candidates were detected with allelic TRD, as shown in Table 1. The number of informative offspring detected in those 12 potentially lethal haplotype regions ranged between 1400 and 3548, whereas the number of under-represented offspring reached 1564. Most of the haplotypes showed relatively small differences between male- and female-specific TRD, supporting their unspecific-parent TRD pattern. In contrast, one haplotype allele located on chromosome 16 showed parent-specific TRD, where it was high for the dam (-0.22) and low via the sire (-0.03). Although this region had some offspring (4 and 15) from homozygous-by-heterozygous ($AB \times AA$) matings (i.e. the homozygous parent carries two copies of the lethal allele), the numbers of offspring were extremely small compared with the expectations. Similarly, the matings of heterozygous-by-heterozygous ($AB \times AB$) in this region produced some (34) homozygous (AA) individuals. However, this

number is still substantially lower than expected, indicating lower viability. This could be due to the variation in specific TRD between males and females. As the probability of transmitting this lethal allele from heterozygous sires is close to the Mendelian expectation ($0.5 - 0.03 = 0.47$), the probability of observing live homozygous parents/offspring increases (4 instead of 0). Matings with either parent carrying the lethal allele in the homozygous state for the remaining 11 regions were not observed, supporting the lethal effect of this haplotype allele. On the other hand, it must be emphasized that these regions were also detected by the genotypic model. Nevertheless, the allelic model had better goodness-of-fit than the genotypic model in terms of deviance information criterion units and accurate TRD estimates with short credible intervals.

Haplotypes with recessive TRD pattern using the genotypic parametrization model

Based on the genotypic parametrization model, 14 potentially lethal haplotypes were identified with additive- and dominance-TRD resulting in lethal homozygous offspring from heterozygous-by-heterozygous matings as shown in Table 2. The additive TRD component of these regions ranged from -0.54 to -0.94 , and interestingly, the negative effects of these haplotypes are counteracted by the dominance TRD effects, which ranged between 0.11

Table 1 Potential candidate lethal or semi-lethal alleles identified with allelic transmission ratio distortion patterns by the allelic model

Chromosome	Region (kbp)	SNP ¹	Hetero sires ²	Hetero dams ³	Frequency (%)	AB ⁴ × AA		AB × BB		AB × AB		TRD effects ⁶			
						AA ⁵	AB	AB	BB	AA	AB	BB	α	α _s	α _d
1	173 127–173 367	10	17	360	4.54	0	0	641	1688	1	5	15	-0.23	-0.33	-0.23
2	71 543–71 622	4	21	305	2.93	0	0	397	1664	0	7	2	-0.31	-0.21	-0.32
2	99 815–99 884	4	67	384	4.44	0	0	654	1915	0	27	39	-0.25	-0.25	-0.25
5	36 746–36 854	4	15	262	2.77	0	0	403	1505	0	2	3	-0.29	-0.31	-0.29
6	50 141–50 184	4	22	182	1.37	0	0	146	1242	0	1	11	-0.40	-0.44	-0.39
7	10 128–10 171	4	61	277	2.66	0	0	342	1344	0	14	42	-0.31	-0.26	-0.32
10	10 424–10 465	4	15	235	2.34	0	0	327	1338	0	5	6	-0.31	-0.38	-0.30
11	15 529–15 551	4	25	410	4.41	0	0	639	2157	0	10	22	-0.28	-0.16	-0.29
14	16 726–16 743	4	32	287	2.96	0	0	383	1557	0	9	9	-0.31	-0.31	-0.31
16	10 429–10 450	4	95	457	8.21	4	15	1136	2025	34	189	145	-0.15	-0.03	-0.22
23	69–81	4	27	92	1.31	0	0	189	1271	0	8	30	-0.38	-0.42	-0.30
28	4507–4549	10	29	264	2.8	0	0	372	1597	0	8	21	-0.32	-0.27	-0.32

¹Number of SNP in a haplotype window.

²Number of heterozygous sires.

³Number of heterozygous dams.

⁴Genotypes of parents.

⁵Genotypes of offspring.

⁶α, α_s and α_d are overall, sire and dam allelic transmission ratio distortion respectively.

Table 2 Potential candidate lethal alleles identified with recessive transmission ratio distortion patterns by the genotypic model

Chr ¹	Region (Kbp)	SNP ²	Hetero sires ³	Hetero dams ⁴	Frequency (%)	AB ⁵ × AA		AB × BB		AB × AB		TRD effects ⁷		
						AA ⁶	AB	AB	BB	AA	AB	BB	α_g	δ_g
1	27 680–27 924	10	24	101	2.51	0	0	450	447	0	17	10	-0.58	0.29
1	29 253–29 484	10	30	108	4.33	0	0	305	265	0	20	11	-0.54	0.35
2	40 083–40 199	4	13	86	1.83	0	0	336	481	0	23	15	-0.77	0.14
3	29 955–30 188	10	5	39	0.67	0	0	106	142	0	15	10	-0.71	0.16
4	60 400–60 941	20	9	64	1.93	0	0	252	321	0	32	16	-0.72	0.21
4	65 823–65 908	4	10	48	0.91	0	0	153	232	0	20	11	-0.77	0.11
5	3900–3975	4	50	170	4.92	0	0	950	967	0	26	17	-0.62	0.30
6	8973–9097	10	19	81	2.48	0	0	431	458	0	21	11	-0.63	0.27
11	23 084–23 137	4	31	107	3.04	0	0	573	673	0	22	12	-0.67	0.23
12	15 689–15 829	20	9	49	1.84	0	0	285	304	0	15	10	-0.61	0.26
19	4054–4196	20	43	156	4.39	0	0	763	756	0	62	18	-0.63	0.33
21	9664–9680	4	38	129	3.03	0	0	525	573	0	52	22	-0.67	0.29
24	672–742	10	13	54	1.86	0	0	323	384	0	17	10	-0.66	0.22
28	1640–1794	20	20	107	2.96	0	0	484	476	0	15	10	-0.57	0.29

¹Chromosome.²Number of SNP on a haplotype window.³Number of heterozygous sires.⁴Number of heterozygous dams.⁵Genotypes of parents.⁶Genotypes of offspring.⁷ α_g and δ_g are additive and dominance transmission ratio distortion respectively.**Table 3** Biological process function terms significantly overrepresented with genes statistically associated with transmission ratio distortion

GO ID	Term (GO hierarchy level)	Number of genes in the GO term	Number of significant genes	P-value ¹
0051315	Attachment of mitotic spindle microtubules to kinetochore (10)	10	1	0.023
0033567	DNA replication, Okazaki fragment processing (11)	3	1	0.008
1902969	Mitotic DNA replication (11)	8	1	0.019
0007094	Mitotic spindle assembly checkpoint (14)	18	1	0.039
0071174	Mitotic spindle checkpoint (9)	18	1	0.039
2000697	Negative regulation of epithelial cell differentiation involved in kidney development (12)	2	1	0.006
2000094	Negative regulation of mesonephric nephron tubule epithelial cell differentiation (15)	1	1	0.004
0061218	Negative regulation of mesonephros development (11)	4	1	0.001
0045841	Negative regulation of mitotic metaphase/anaphase transition (12)	20	1	0.044
2000816	Negative regulation of mitotic sister chromatid separation (11)	21	1	0.046
0072183	Negative regulation of nephron tubule epithelial cell differentiation (14)	1	1	0.004
1903461	Okazaki fragment processing involved in mitotic DNA replication (12)	1	1	0.004
2000093	Regulation of mesonephric nephron tubule epithelial cell differentiation (12)	1	1	0.004

¹Significance declared at $P < 0.05$.

and 0.35. The interaction of additive and dominance TRD effects provides an equal chance for heterozygous (carriers) offspring to survive as non-carrier birds. From the heterozygous-by-heterozygous (AB × AB) matings, no homozygous (AA) individuals were observed, indicating the lethality of the haplotypes in the homozygous state. For these regions, the numbers of carrier parents were between five and 50 for sires and between 39 and 170 for dams. The number of informative offspring ranged from 273 to 1960, whereas the number of expected homozygotes that were not observed ranged from 10 to 22. For a fully recessive TRD

pattern, it is expected that there will be similar numbers of heterozygous (AB) and homozygous (BB) offspring from heterozygous-by-homozygous matings (AB × BB). Nevertheless, slightly more heterozygous offspring (AB) in heterozygous-by-homozygous matings were observed, which could be partly explained as a result of a random TRD (i.e. TRD generated by chance; Id-Lahoucine *et al.* 2019).

It is worth noting that most of these regions were only detected with the genotypic model whereas no statistical evidence was found using the allelic model. This is due to

Table 4 Cellular component function terms significantly overrepresented in genes statistically associated with transmission ratio distortion

GO ID	Term (GO hierarchy level)	Number of genes in the GO term	Number of significant genes	P-value ¹
0044444	Cytoplasmic part (7)	5503	18	0.038
0070176	DRM complex (15)	1	1	0.004
0034709	Methylosome (8)	6	1	0.014
0097431	Mitotic spindle pole (12)	18	1	0.039
0032021	NELF complex (14)	2	1	0.006
0090568	Nuclear transcriptional repressor complex (13)	19	2	0.000
0090571	RNA polymerase II transcription repressor complex (14)	4	2	0.000

¹Significance declared at $P < 0.05$.

Table 5 Molecular function terms significantly overrepresented in genes statistically associated with transmission ratio distortion

GO ID	Term (GO hierarchy level)	Number of genes in the GO term	Number of significant genes	P-value ¹
0005488	Binding (2)	9725	28	0.017
0015187	Glycine transmembrane transporter activity (13)	5	1	0.012
0015375	Glycine: sodium symporter activity (16)	1	1	0.004
0060090	Molecular adaptor activity (3)	124	2	0.028
0015175	Neutral amino acid transmembrane transporter activity (12)	23	1	0.048
0030674	Protein binding, bridging (4)	112	2	0.023
1990756	Protein binding, bridging involved in substrate recognition for Ubiquitination (11)	3	1	0.008

¹Significance declared at $P < 0.05$.

their different parameterizations, where the genotypic model includes the interaction between alleles, allowing detection of recessive TRD patterns. Specifically, the negative effect of the additive TRD component can be contrasted with the positive effect of the dominance TRD component in heterozygous offspring, allowing for capture of recessive TRD patterns. In contrast, the fact that the allelic parametrization is based on targeting the transmission of an allele from a specific parent to offspring, which is separate from possible interaction in offspring generation, prevents its ability to capture recessive TRD patterns.

Functional analysis and gene set enrichment

Sixty-seven biological process, 17 cellular components, 35 molecular function GO terms, in addition to 19 Reactome pathways and nine MeSH terms showed a significant overrepresentation (P -value < 0.05) of genes associated with TRD in turkeys. All of these significant terms and pathways are listed in Tables S1–S5. It is important to mention that these terms, pathways and genes should be further investigated and validated to avoid false positives. Four biological process GO terms, in a close GO hierarchy relationship and related to mitosis, showed a significant overrepresentation (P -value < 0.05) of genes statistically associated with TRD (Table 3). *Mitotic spindle assembly checkpoint* (GO:0007094) is a *mitotic spindle checkpoint* (GO:0071174) and a *negative regulation of mitotic*

metaphase/anaphase transition (GO:0045841), which is, in turn, a *negative regulation of mitotic sister chromatid separation* (GO:2000816). Mitosis, which is associated with TRD, is a specialized division of chromosomes that occur during the formation of reproductive cells (Nicklas 1971). A similar significant (P -value < 0.05) cellular component GO term, *Mitotic spindle pole* (GO:0097431), was also associated with TRD (Table 4).

Three GO terms detected in the analysis classified into the biological process domain showed significant association with TRD (Table 3). In addition to their similar functions in mitosis, these three terms are close in the GO hierarchy. *Okazaki fragment processing involved in mitotic DNA replication* (GO:1903461) is a *DNA replication, Okazaki fragment processing* (GO:0033567) and part of *mitotic DNA replication* (GO:1902969). In cell biology, Okazaki fragments (Sakabe & Okazaki 1966) comprise the processes involved in any mitotic cell cycle DNA replication, a necessary step in the cell cycle.

The *Negative regulation of mesonephric nephron tubule epithelial cell differentiation* (GO:2000094) term was detected as significantly enriched (P -value < 0.01) with genes associated with TRD and related to cell differentiation. This GO term is close in the GO hierarchy and in function to four other GO terms, which all can be linked to TRD: GO:2000093, GO:0061218, GO:0072183 and GO:2000697. Cellular differentiation is the process in which a simple cell changes from one cell type to a more

Table 6 Reactome terms significantly overrepresented in genes statistically associated with transmission ratio distortion.

Reactome ID	Reactome term name	Number of genes in the pathway	Number of significant genes	<i>P</i> -value ¹
R-GGA-5358508	Mismatch repair	11	1	0.025
R-GGA-5358606	Mismatch repair (MMR) directed by MSH2:MSH3 (MutSbeta)	3	1	0.008

¹Significance declared at $P < 0.05$.

Table 7 Disease and chemicals and drugs MeSH terms significantly overrepresented in genes statistically associated with transmission ratio distortion

Category	MeSH term ID	MeSH term name	<i>P</i> -value ¹
Chemicals and drugs	D000249	Adenosine monophosphate	0.012
Disease	D000708	Anaplasia	0.048

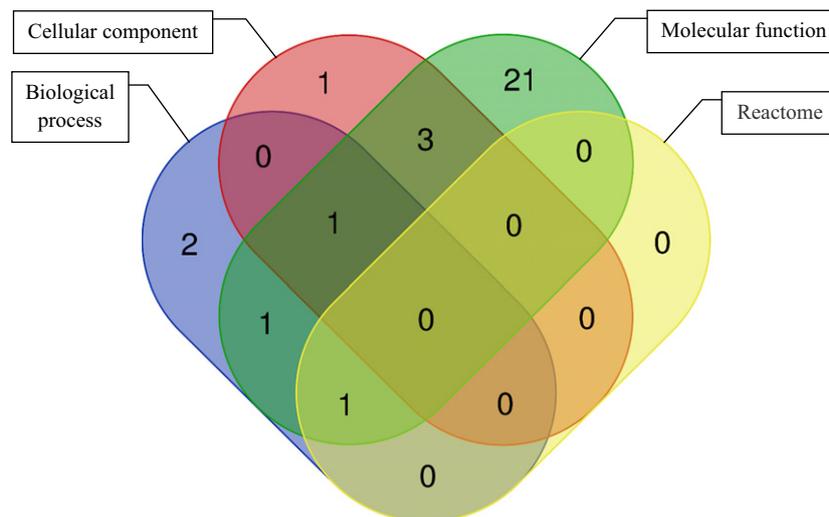
¹Significance declared at $P < 0.05$.

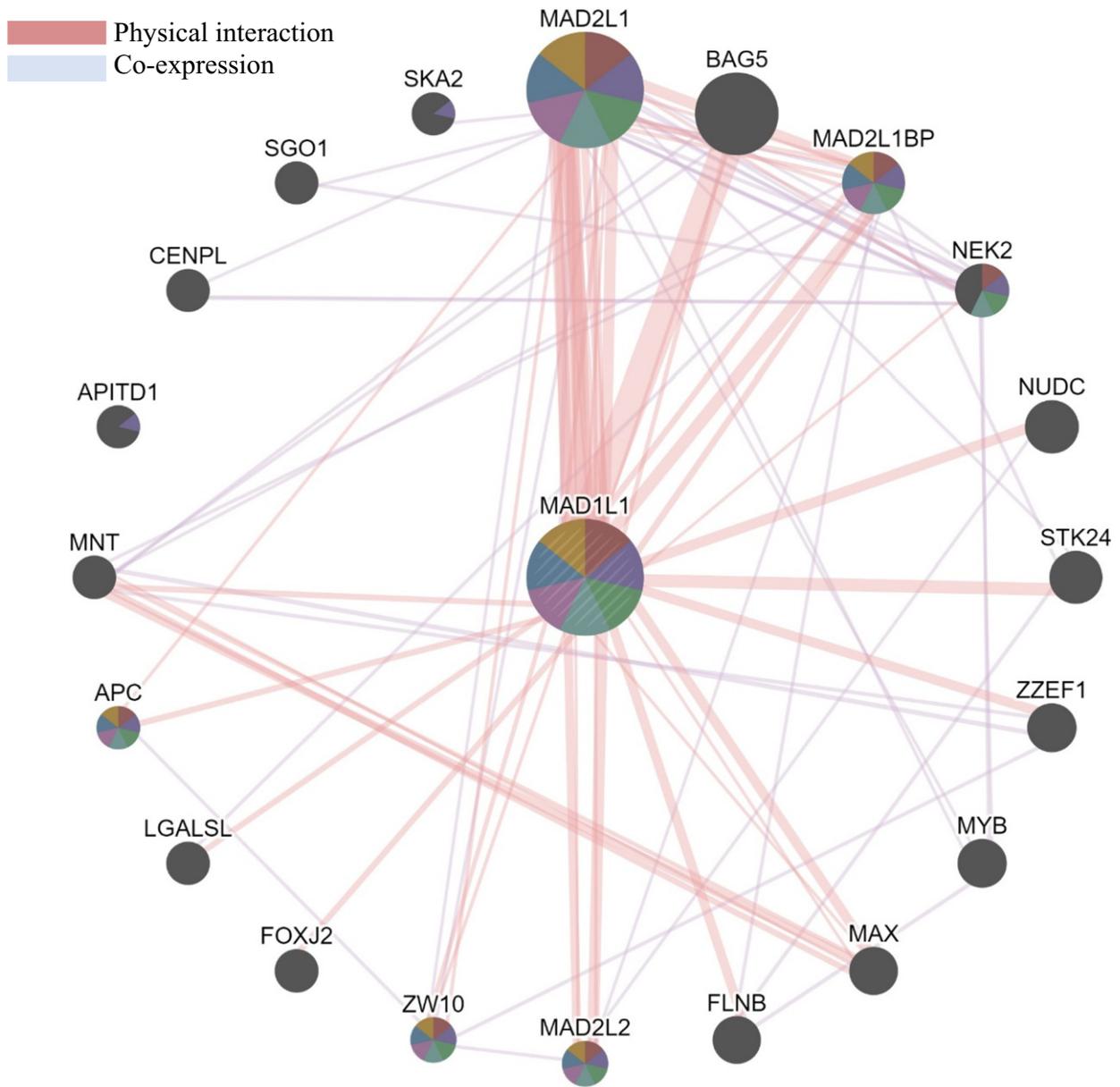
specialized type, which may occur in numerous steps (Jones & Taylor 1980; Slack 2007). In a study on the development of the kidney in mice, McCright *et al.* (2001) reported that animals died perinatally owing to the lack of normal capillary tufts, which is a result of defects in kidney development.

The terms *NELF complex* (GO:0032021) and two similar GO terms, *methylosome* (GO:0034709) and *cytoplasmic part* (GO:0044444), are classified under the cellular component domain and show a significant overrepresentation of genes statistically associated with TRD (Table 4). The *NELF complex* is a key regulatory step of the transcription cycle (Tamborrini & Piatti 2019). The GO term *DRM complex* (GO:0070176), located in the cellular component category,

is an *RNA polymerase II transcription repressor complex* (GO:0090571), which is, in turn, a *nuclear transcriptional repressor complex* (GO:0090568). Interestingly, a connection between RNA polymerase II and TRD has been previously reported (Paterson *et al.* 2009). Moreover, Harrison *et al.* (2006) reported that *DRM complex* is a transcriptional repressor complex and involved in cell fate specification.

Three GO terms, which are close in the GO hierarchy (Table 5), were classified into the molecular function category and showed a significant overrepresentation of genes statistically associated with TRD (Table 4). *Glycine:sodium symporter activity* (GO:0015375) is a *glycine transmembrane transporter activity* (GO:0015187), which is in turn a *neutral amino acid transmembrane transporter activity* (GO:0015175). Genetic factors that stop coding glycine cause late-stage embryo lethality and hence TRD (Seidel *et al.* 2011). *Protein binding, bridging involved in substrate recognition for ubiquitination* (GO:1990756) is a *protein binding, bridging* (GO:0030674). The molecular function of binding and sperm motility was reported by Bauer *et al.* (2007). As the authors mentioned, the binding partner for Ropporin and Ras proteins to the outer surface of the dense fiber proteins plays a crucial function in sperm motility.

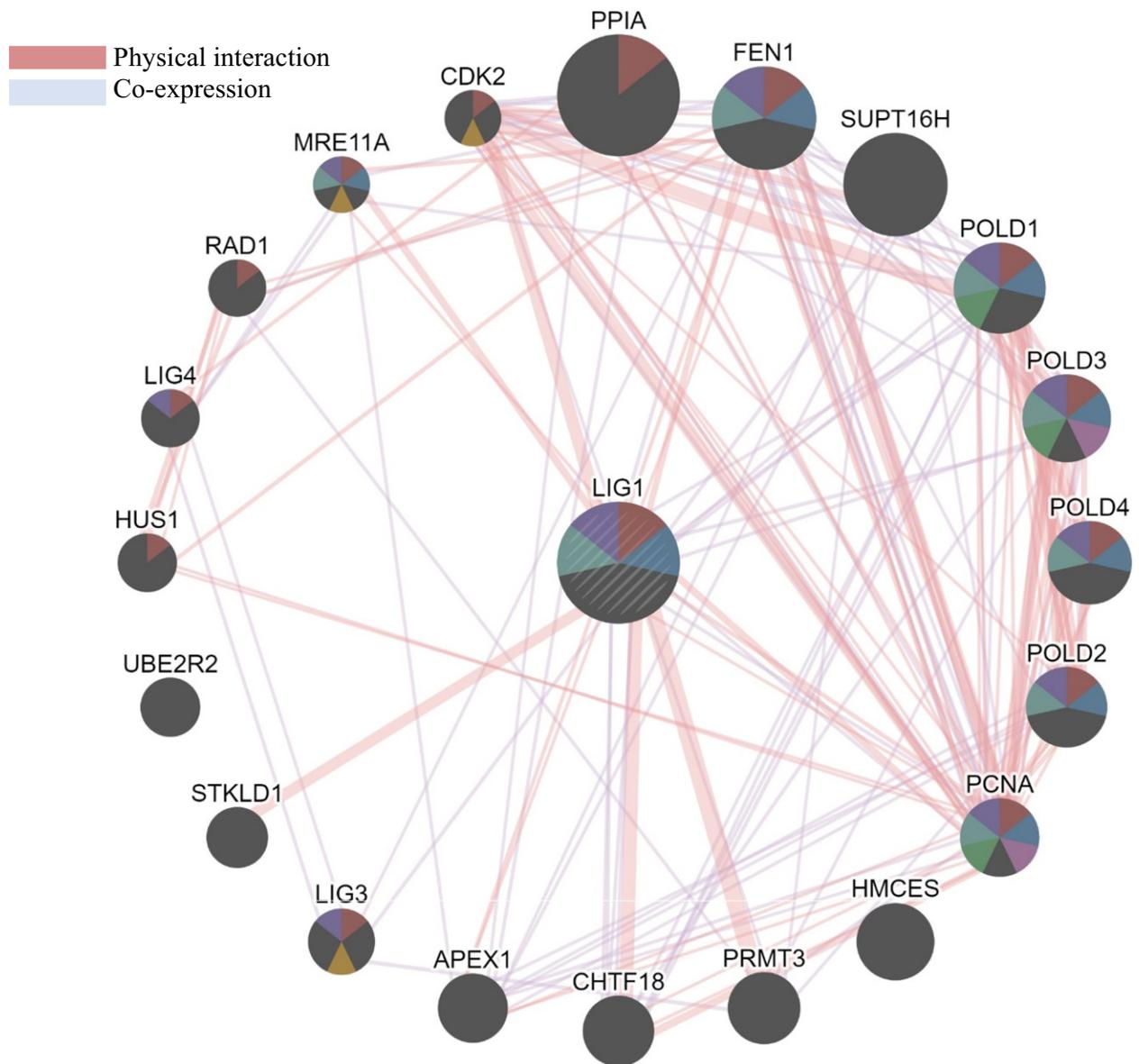
**Figure 2** Venn diagram showing overlaps between significant genes associated with the terms of three GO domains (presented in Tables 3–5) and the Reactome pathways (presented in Table 6)



Function:

- regulation of mitosis
- nuclear division
- regulation of nuclear division
- regulation of cell division
- mitotic cell cycle checkpoint
- negative regulation of mitotic cell cycle phase transition
- negative regulation of cell cycle phase transition

Figure 3 Network integration of gene MAD1L1 based on physical protein interaction and co-expression. The gene APITD1 is associated with MAD1L1 through pathways (links are not shown)



Function:

- DNA replication
- mitotic recombination
- mismatch repair
- meiotic cell cycle
- nuclear replication fork
- cell cycle DNA replication
- DNA-dependent DNA replication

Figure 4 Network integration of gene LIG1 based on physical protein interaction and co-expression. The gene UBE2R2 is associated with LIG1 through prediction (links are not shown)

Two Reactome pathways were detected as significantly (P -value < 0.01) enriched with genes related to TRD (Table 6). *Mismatch repair (MMR) directed by MSH2:MSH3 (MutSbeta)* (R-GGA-5358606) and *Mismatch Repair* (R-GGA-5358508) are similar in their activities to the GO term *Mitotic spindle assembly checkpoint* (GO:0007094) and represent the GO biological process *mismatch repair* (GO:0006298). Analogous to the GO terms related to mitosis and cell cycle, these two pathways are associated with DNA mismatch repair. It has been demonstrated that their activity increases and reaches the highest levels during the S phase of the cell cycle (Edelbrock *et al.* 2009). *Mismatch Repair* corrects single base mismatches and small insertion and deletion loops of unpaired bases. *Mismatch repair directed by MSH2:MSH3 (MutSbeta)* binds unpaired loops of two or more nucleotides (Palombo *et al.* 1996).

A MeSH term, *Adenosine Monophosphate* (D000249), classified into the chemicals and drugs category, showed a significant overrepresentation of genes statistically associated with TRD (Table 7). Interestingly, various reproductive functions, such as those requiring hormone synthesis and maintenance of fluid composition, are modulated by adenosine (Zhou *et al.* 2006; Aliagas *et al.* 2010). Another significant (P -value < 0.05) MeSH term, *Anaplasia* (D000708), is classified into the disease domain (Table 7). *Anaplasia* is related to neoplastic cells and refers to the loss of mature or specialized features of differentiated neoplastic cells (Rodriguez *et al.* 2010; Pujadas *et al.* 2019). Previous studies have shown that genetic variation among inbred mice as a result of continuous uploading and removal of rare variation (new mutations) may generate TRD (Casellas & Medrano 2008; Niu & Liang 2009).

Gene network

The Venn diagram (Fig. 2) depicted the intersections between the significant genes associated with the terms of three GO domains (Tables 3–5) and the Reactome pathways (Table 6). Notably, the gene *MAD1L1* is significant across the three GO domains and two of these GO domains (biological process and molecular function) overlap with the Reactome pathways in the gene *LIG1*. The aggregate interaction networks for *MAD1L1* and *LIG1* genes, based on physical protein interaction and co-expression, are shown in Figs 3 and 4 respectively. The functional networks of gene *MAD1L1* with 19 other genes indicate that this group of genes is involved in several activates related to mitosis such as regulation of mitosis and negative regulation of mitosis cell cycle phase transition. The gene *APITD1* was not linked to *MAD1L1* via physical protein interaction or co-expression networks, but through the pathways network (this connection is not shown in Fig. 3). Similarly, the functional network for gene *LIG1* revealed 19 genes associated with it through physical protein interaction and co-expression networks (Fig. 4) and one gene (i.e. *UBE2R2*)

through prediction. The functions for these genes include DNA replication and cell cycle DNA replication, which are among the most important cell activities concerning reproduction and cell deviation.

Conclusions

In this study, we applied allelic and genotypic parameterizations of TRD to detect potential lethal haplotypes in turkeys. The two methods revealed relevant regions across the turkey genome with either a classical recessive inheritance pattern (i.e. lethal only in the homozygous state) or allelic patterns (i.e. reduced viability of the carrier offspring). In addition to 19 Reactome pathways and nine MeSH terms, 67 biological process, 17 cellular components and 35 molecular function GO terms showed a significant (P -value < 0.05) overrepresentation of genes statistically associated with TRD. Functional networks among several significant genes also showed links to mitosis and cell replication. These highlighted pathways and gene ontologies, along with the overall findings of this study, will contribute in developing novel turkey breeding and management strategies, as well as to specific mating programs for turkeys.

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Conflict of interests

All authors declare no conflict of interest.

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Data availability statement

Data that support the findings of this study are available from Hybrid Turkeys upon reasonable request and were used under license for the current study, and thus are not publicly available.

References

- Abdalla E.A., Peñagaricano F., Byrem T.M., Weigel K.A. & Rosa G.J.M. (2016) Genome-wide association mapping and pathway analysis of leukosis incidence in a US Holstein cattle population. *Animal Genetics* **47**, 395–407.
- Aliagas E., Torrejón-Escribano B., Lavoie E.G., De Aranda I.G., Sévigny J., Solsona C. & Martín-Satué M. (2010) Changes in expression and activity levels of ecto-5'-nucleotidase/CD73 along the mouse female estrous cycle. *Acta Physiologica* **199**, 191–7.
- Ashburner M., Ball C.A., Blake J.A. *et al.* (2000) Gene ontology: tool for the unification of biology. *Nature Genetics* **25**, 25–9.
- Bauer H., Véron N., Willert J. & Herrmann B.G. (2007) The t-complex-encoded guanine nucleotide exchange factor Fgd2 reveals that two opposing signaling pathways promote transmission ratio distortion in the mouse. *Genes & Development* **21**, 143–7.
- Browning B.L., Zhou Y. & Browning S.R. (2018) A one-penny imputed genome from next-generation reference panels. *American Journal of Human Genetics* **103**, 338–48.
- Casellas J., Cañas-Álvarez J.J., González-Rodríguez A., Puig-Oliveras A., Fina M., Piedrafita J., Molina A., Díaz C., Baró J.A. & Varona L. (2017) Bayesian analysis of parent-specific transmission ratio distortion in seven Spanish beef cattle breeds. *Animal Genetics* **48**, 93–6.
- Casellas J., Gualarte R.J., Farber C.R., Varona L., Mehrabian M., Schadt E.E., Lusi A.J., Attie A.D., Yandell B.S. & Medrano J.F. (2012) Genome scans for transmission ratio distortion regions in mice. *Genetics* **191**, 247–59.
- Casellas J., Id-Lahoucine S., & Cánovas A. (In press). Discriminating between allele- and genotype-specific transmission ratio distortion. *Animal Genetics*. <https://doi.org/10.1111/age.13007>
- Casellas J., Manunza A., Mercader A., Quintanilla R. & Amills M. (2014) A flexible Bayesian model for testing for transmission ratio distortion. *Genetics* **198**, 1357–67.
- Casellas J. & Medrano J.F. (2008) Within-generation mutation variance for litter size in inbred mice. *Genetics* **179**, 2147–55.
- Cole J.B., Null D.J. & VanRaden P.M. (2016) Phenotypic and genetic effects of recessive haplotypes on yield, longevity, and fertility. *Journal of Dairy Science* **99**, 7274–88.
- Coletti M.H. & Bleich H.L. (2001) Medical subject headings used to search the biomedical literature. *Journal of the American Medical Informatics Association* **8**, 317–23.
- Crow J.F. (1999) Unmasking a cheating gene. *Science* **283**, 1651–2.
- Dalloul R.A., Long J.A., Zimin A.V. *et al.* (2010) Multi-platform next-generation sequencing of the domestic Turkey (*Meleagris gallopavo*): genome assembly and analysis. *PLoS Biology* **8**, e1000475.
- Dong C., Yuanjun H.U., Yurui W.U. & Xiaoying L.I. (2019) Risk factors of death in newborns with congenital diaphragmatic hernia. *Journal of Zhejiang University (Medical Science)* **48**, 83–8.
- Edelbrock M.A., Kaliyaperumal S. & Williams K.J. (2009) DNA mismatch repair efficiency and fidelity are elevated during DNA synthesis in human cells. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* **662**, 59–66.
- Emamgholi Begli H., Wood B.J., Abdalla E.A., Balzani A., Willems O., Schenkel F., Harlander-Matauschek A. & Baes C.F. (2019) Genetic parameters for clutch and broodiness traits in turkeys (*Meleagris gallopavo*) and their relationship with body weight and egg production. *Poultry Science* **98**, 6263–9.
- Fabregat A., Jupe S., Matthews L. *et al.* (2018) The reactome pathway knowledgebase. *Nucleic Acids Research* **46**, D649–55.
- Fishman L. & McIntosh M. (2019) Standard deviations: the biological bases of transmission ratio distortion. *Annual Review of Genetics* **53**, 347–72.
- Gorlov I.P., Gorlova O.Y., Sunyaev S.R., Spitz M.R. & Amos C.I. (2008) Shifting paradigm of association studies: value of rare single-nucleotide polymorphisms. *American Journal of Human Genetics* **82**, 100–12.
- Granleese T., Clark S.A., Swan A.A. & van der Werf J.H.J. (2015) Increased genetic gains in sheep, beef and dairy breeding programs from using female reproductive technologies combined with optimal contribution selection and genomic breeding values. *Genetics Selection Evolution* **47**, 70.
- Guarini A.R., Sargolzaei M., Brito L.F., Kroezen V., Lourenco D.A.L., Baes C.F., Miglior F., Cole J.B. & Schenkel F.S. (2019) Estimating the effect of the deleterious recessive haplotypes AH1 and AH2 on reproduction performance of Ayrshire cattle. *Journal of Dairy Science* **102**, 5315–22.
- Harrison M.M., Ceol C.J., Lu X. & Horvitz H.R. (2006) Some *C. elegans* class B synthetic multivulva proteins encode a conserved LIN-35 Rb-containing complex distinct from a NuRD-like complex. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 16782–7.
- Hastings W.K. (1970) Monte Carlo sampling methods using Markov chains and their applications. *Biometrika* **57**, 97–109.
- Hoff J.L., Decker J.E., Schnabel R.D. & Taylor J.F. (2017) Candidate lethal haplotypes and causal mutations in Angus cattle. *BMC Genomics* **18**, 799.
- Huang L.O., Labbe A. & Infante-Rivard C. (2013) Transmission ratio distortion: review of concept and implications for genetic association studies. *Human Genetics* **132**, 245–63.
- Huff G.R., Huff W.E., Balog J.M., Rath N.C., Anthony N.B. & Nestor K.E. (2005) Stress response differences and disease susceptibility reflected by heterophil to lymphocyte ratio in turkeys selected for increased body weight. *Poultry Science* **84**, 709–17.
- Id-Lahoucine S., Cánovas A., Jatón C., Miglior F., Fonseca P., Miller S., Sargolzaei M., Schenkel F., Medrano F. & Casellas J. (2019) Implementation of Bayesian methods to identify SNP and haplotype regions with transmission ratio distortion across the whole genome: TRDscan v. 1.0. *Journal of Dairy Science* **102**, 3175–88.
- Id-Lahoucine S. & Casellas J. (2017) Impact of incomplete pedigree data and independent culling level pre-selection on the genetic evaluation of livestock: a simulation study on lamb growth. *Livestock Science* **198**, 76–81.
- International Chicken Genome Sequencing Consortium (2004) Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. *Nature* **432**, 695–716.
- Jeffreys H. (1984) *Theory of Probability*. Oxford, UK: Clarendon Press.
- Jenko J., McClure M.C., Matthews D., McClure J., Johnsson M., Gorjanc G. & Hickey J.M. (2018) Analysis of a large data set reveals haplotypes carrying putatively recessive lethal alleles with pleiotropic effects on economically important traits in beef cattle. *Genetics Selection Evolution*, **51**, 9.

- Jones P.A. & Taylor S.M. (1980) Cellular differentiation, cytidine analogs and DNA methylation. *Cell* **20**, 85–93.
- Karaca E., Harel T., Pehlivan D., Jhangiani S.N., Gambin T., Akdemir Z.C., Gonzaga-Jauregui C., Erdin S., Bayram Y. & Campbell I.M. (2015) Genes that affect brain structure and function identified by rare variant analyses of Mendelian neurologic disease. *Neuron* **88**, 499–513.
- Kass R. & Raftery A. (1995) Bayes factors. *Journal of the American Statistical Association* **90**, 773–95.
- McCright B., Gao X., Shen L., Lozier J., Lan Y., Maguire M., Herzlinger D., Weinmaster G., Jiang R., & Gridley T. (2001) Defects in development of the kidney, heart and eye vasculature in mice homozygous for a hypomorphic Notch2 mutation. *Development*, **128**(4), 491–502.
- Megens H.-J., Crooijmans R.P.M.A., Bastiaansen J.W.M., Kerstens H.H.D., Coster A., Jalving R., Vereijken A., Silva P., Muir W.M. & Cheng H.H. (2009) Comparison of linkage disequilibrium and haplotype diversity on macro- and microchromosomes in chicken. *BMC Genetics* **10**, 86.
- Mi H., Poudel S., Muruganujan A., Casagrande J.T. & Thomas P.D. (2016) PANTHER version 10: expanded protein families and functions, and analysis tools. *Nucleic Acids Research* **44**(D1), D336–42.
- Nelson S.J., Schopen M., Savage A.G., Schulman J.-L. & Arluk N. (2004) The MeSH translation maintenance system: structure, interface design, and implementation. *Studies in Health Technology and Informatics* **107**(Pt 1), 67–9.
- Nicklas R.B. (1971) Mitosis. In: *Advances in Cell Biology* (Ed. by D. M. Prescott), pp. 225–97. Boston, MA: Springer US.
- Niu Y. & Liang S. (2009) Genetic differentiation within the inbred C57BL/6J mouse strain. *Journal of Zoology* **278**, 42–7.
- Palombo F., Iaccarino I., Nakajima E., Ikejima M., Shimada T. & Jiricny J. (1996) hMutSbeta, a heterodimer of hMSH2 and hMSH3, binds to insertion/deletion loops in DNA. *Current Biology* **6**, 1181–4.
- Pardo-Manuel De Villena F., De La Casa-Esperó E., Briscoe T.L., Sapienza C. (2000) A genetic test to determine the origin of maternal transmission ratio distortion: meiotic drive at the mouse Om locus. *Genetics*, **154**, 333–42.
- Paterson A.D., Waggott D., Schillert A., Infante-Rivard C., Bull S.B., Yoo Y.J. & Pinnaduwa D. (2009) Transmission-ratio distortion in the Framingham Heart Study. *BMC Proceedings* **3**(S7), S51.
- Peng G., Luo L., Siu H. *et al.* (2010) Gene and pathway-based second-wave analysis of genome-wide association studies. *European Journal of Human Genetics* **18**, 111–7.
- Pujadas E., Chen L. & Rodriguez F.J. (2019) Pathologic and molecular aspects of anaplasia in circumscribed gliomas and glioneuronal tumors. *Brain Tumor Pathology* **36**, 40–51.
- Qanbari S., Hansen M., Weigend S., Preisinger R. & Simianer H. (2010) Linkage disequilibrium reveals different demographic history in egg laying chickens. *BMC Genetics* **11**, 103.
- Rao Y.S., Liang Y., Na Xia M., Shen X., Du Jun Y., Glong Luo C., Hua Nie Q., Zeng H. & Quan Zhang X. (2008) Extent of linkage disequilibrium in wild and domestic chicken populations. *Hereditas* **145**, 251–7.
- R Core Team (2019) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.
- Rodriguez F.J., Scheithauer B.W., Burger P.C., Jenkins S. & Giannini C. (2010) Anaplasia in pilocytic astrocytoma predicts aggressive behavior. *The American Journal of Surgical Pathology* **34**, 147–60.
- Saif Y.M. & Nestor K.E. (2002) Increased mortality in turkeys selected for increased body weight following vaccination with a live Newcastle disease virus vaccine. *Avian Diseases* **46**, 505–8.
- Sakabe K. & Okazaki R. (1966) A unique property of the replicating region of chromosomal DNA. *Biochimica et Biophysica Acta* **129**, 651–4.
- Seidel H.S., Ailion M., Li J., van Oudenaarden A., Rockman M.V. & Kruglyak L. (2011) A novel sperm-delivered toxin causes late-stage embryo lethality and transmission ratio distortion in *C. elegans*. *PLoS Biology* **9**, e1001115.
- Slack J.M.W. (2007) Metaplasia and transdifferentiation: from pure biology to the clinic. *Nature Reviews Molecular Cell Biology* **8**, 369–78.
- Tamborini D. & Piatti S. (2019) Septin clearance from the division site triggers cytokinesis in budding yeast. *Microbial Cell* **6**, 296–8.
- Tsuyuzaki K., Morota G., Ishii M., Nakazato T., Miyazaki S. & Nikaido I. (2015) MeSH ORA framework: R/Bioconductor packages to support MeSH over-representation analysis. *BMC Bioinformatics* **16**, 45.
- VanRaden P.M., Olson K.M., Null D.J. & Hutchison J.L. (2011) Harmful recessive effects on fertility detected by absence of homozygous haplotypes. *Journal of Dairy Science* **94**, 6153–61.
- Warde-Farley D., Donaldson S.L., Comes O. *et al.* (2010) The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. *Nucleic Acids Research* **38**(Suppl. 2), W214–20.
- Zhou C.-J., Li J., Zou J.-C., Liang F.-S., Ye C.-J., Jin D.-M., Weng M.-L. & Wang B. (2006) Cloning and characterization of a second form of the rice adenine phosphoribosyl transferase gene (OsAPT2) and its association with TGMS. *Plant Molecular Biology* **60**, 365–76.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Biological process terms significantly overrepresented with genes statistically associated with TRD

Table S2 Cellular component function terms significantly overrepresented with genes statistically associated with TRD

Table S3 Molecular function terms significantly overrepresented with genes statistically associated with TRD

Table S4 Reactome pathways significantly overrepresented with genes statistically associated with TRD

Table S5 MeSH terms significantly overrepresented with genes statistically associated with TRD