Genetic and epigenetic pathways affected in lines divergently selected on feather pecking

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Introduction

The Grouphousenet synergies to prevent damaging behaviour in pigs and laying hens enabled me (Dr. Elske N de Haas) to visit the Avian Biology Group, at Linköping University, Sweden in end of November 2019. From an earlier STSM (2017) we continued our data analysis and interpretation of results. Dr Fábio Pertillé conducted the statistical analysis with use of genetic sequenced data of 2 laying hen lines.

Material and method

The lines were divergently selected on gentle and severe feather pecking for 7 generations and maintained to differ in their expression of this damaging behaviour (Piepho et al., 2016). We used 20 female chicks of 10 weeks old. From theses chickens we collected the brains and dissected the thalamus. This area is important for regulation of stress responses, and we hoped to link gene-expression with alteration in gene-expression, as known to be affected by stressful conditions (Ericcson et al., 2016) and transgenerational (Goerlich et al., 2012). Furthermore, we wanted to have behavioural data of these lines, so as to link genetic or epigenetic markers to the expression of the behaviour. In 2017, we conducted a immunoprecipitation method combined with genotype by sequencing technique. This technique developed by Dr. Fábio Pertillé and Dr. Carlos Guerrero-Bosanga is a novel technique in which 2% of the genome remains which is further assessed for methylation by an antibody binding to these sites. This technique allows to sequence also relatively small genomic regions which would normally been overlooked. Despite the many genetic studies in these genetic lines (for an overview see for example De Haas and van der Eijk, 2019) this technique has yet not been applied. It thus provide the possibility to find new regions involved in this behaviour, and has further the potential to assess the pathways involved rather than targeting specific quantitative trait loci (QTL), been found earlier. However, we did link our genetic information to the known QTLs so as to ascertain these regions were found and overlapped with our data. As the data of this STSM is currently being processed in a manuscript only preliminary Figures can be shown, due to publication issues.

Results

We found over 100.000 single nucleotide polymorphisms (SNPs). With using a specific coverage level we ended up with 70.000 SNPs, losing a few individuals with low coverage. With this information we conducted a Principal Component Analysis (PCA) which roughly clustered the two groups. Interestingly one LFP individual was clustered under the HFP group, and looking at the behavioural data it appears this individual performed the highest level of damaging severe feather pecking as observed.

Further processing the data taking into account P-values for repeated testing, we ended up with +700 SNPs. Always our analysis contained HFP (treated) as compared to LFP (case control, untreated). Now we could differentiate where in the genomic region the SNPs were located. This is important so as to known their relative importance. For the top SNPs around 25% were in exon regions, of which three genes has a missense variant, in our case it meant that they could have a moderate impact on the protein which this gene is transcribing.

To assess pathways influenced we used an online platform Gene Ontology Resource (GO) (http://geneontology.org/) where we could file the SNPs to compare to the reference genome of Gallus gallus 5.0. See Figure 1 for an overview of pathways affected with P-value < 0.0005 in the top SNPs. See Figure 2 for an overview of pathways affected with P-value <0.05 in all significant SNPs but not adjusted for repeated testing. When taking a closer look at the extent in which a particular pathway was affected, I looked at the number of genes within the pathway to be affected. Interestingly, we found that most genes within the pathway locomotion were affected. This is in accordance with other studies in these lines looking at locomotion (Kjaer, 2009; Rodenburg et al., 2017; de Haas et al., 2018)

We found 340.000 differently methylated regions (DMRs). Again we corrected for repeated testing and ended up with a little over 200 DMRs. Most of the DMRs were in promotor regions, and most were either from C to T or vice versa. This is important for methylation, as this means either a gain or loss in location for methylation to occur. As our epigenetic information is lined with the genetic information, and expressed as such we cannot show figures yet. Our manuscript is aimed to be submitted in January 2020, where all results are combined to understand which changes are from genetic and which are from epigenetic.

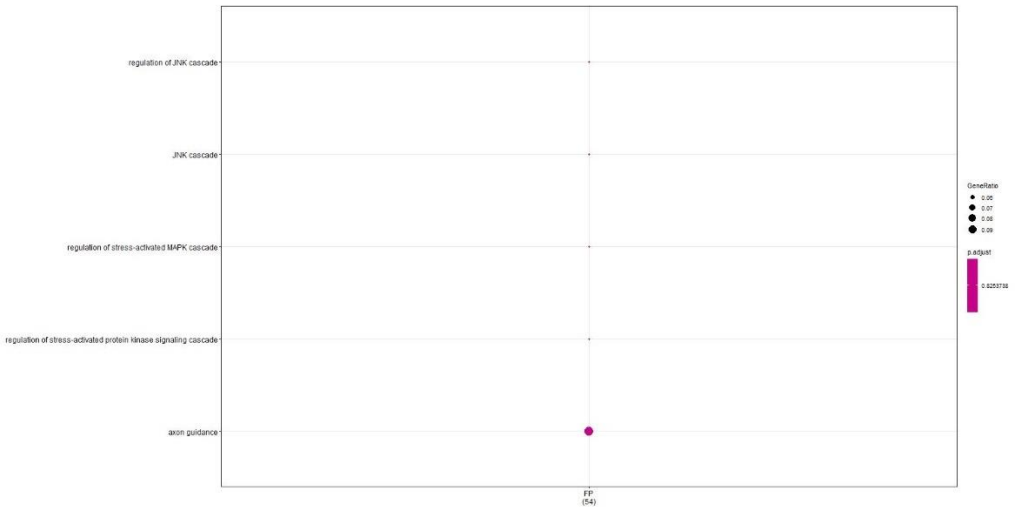

Figure 1. Top SNPs associated with pathways by GO

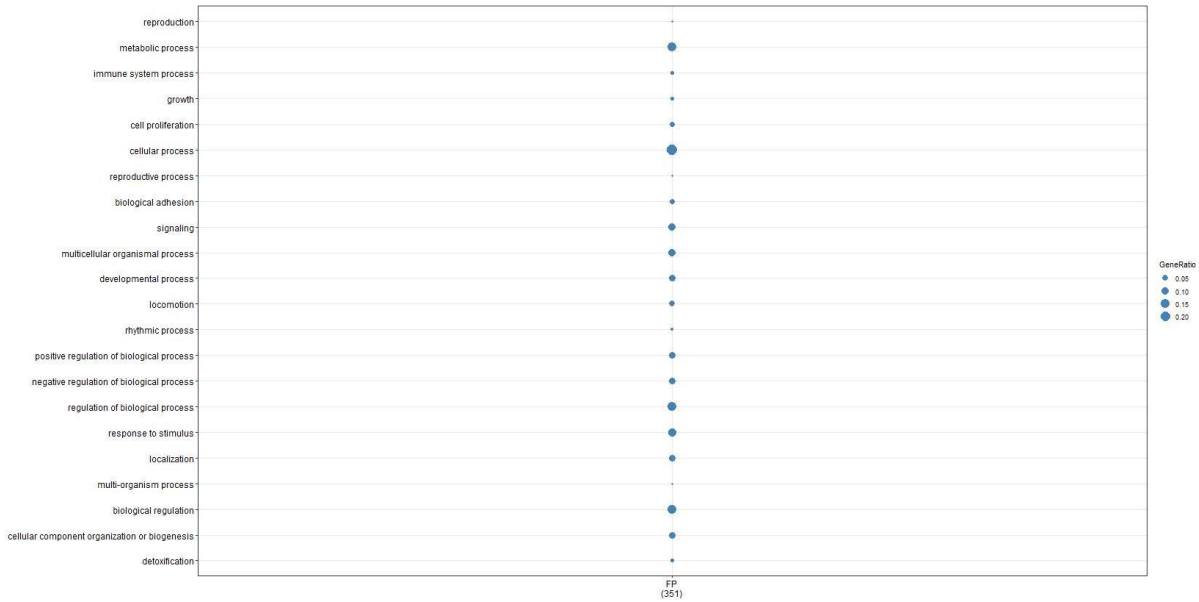

Figure 2. Significant SNPs associated with pathways by GO