Genetical Genomics of Behavior: A Novel Chicken Genomic Model for Anxiety Behavior

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ABSTRACT The identification of genetic variants responsible for behavioral variation is an enduring goal in biology, with wide-scale ramifications, ranging from medical research to evolutionary theory on personality syndromes. Here, we use for the first time a large-scale genetical genomics analysis in the brains of chickens to identify genes affecting anxiety as measured by an open field test. We combine quantitative trait locus (QTL) analysis in 572 individuals and expression QTL (eQTL) analysis in 129 individuals from an advanced intercross between domestic chickens and Red Junglefowl. We identify 10 putative quantitative trait genes affecting anxiety behavior. These genes were tested for an association in the mouse Heterogeneous Stock anxiety (open field) data set and human GWAS data sets for bipolar disorder, major depressive disorder, and schizophrenia. Although comparisons between species are complex, associations were observed for four of the candidate genes in mice and three of the candidate genes in humans. Using a multimodel approach we have therefore identified a number of putative quantitative trait genes affecting anxiety behavior, principally in chickens but also with some potentially translational effects as well. This study demonstrates that chickens are an excellent model organism for the genetic dissection of behavior.

KEYWORDS anxiety; behavioral genes; eQTL; QTL; causal genes; personality

THE identification of genes that harbor causal mutations underlying a quantitative trait (QTG) is rare for any trait (Flint 2003) and even more so for behavior. Successful examples of behavioral QTG identification are almost exclusively limited to mouse and rat models (Yalcin *et al.* 2004; Chiavegatto *et al.* 2008; Kim *et al.* 2009; Tomida *et al.* 2009; Gyetvai *et al.* 2011; Wang *et al.* 2012; Heyne *et al.* 2014), *Drosophila* (Anholt and Mackay 2004; Mackay 2004; Fitzpatrick *et al.* 2005), and the honeybee (Robinson *et al.* 2005, 2008). The ramifications for the identification of such behavioral genes are many and varied. For example, mood-based disorders are one of the top 10 causes of disability worldwide (Murray and Lopez 1996; Vos *et al.* 2015), yet the identification of susceptibility loci for such traits has been severely restricted (Kas *et al.* 2007), with only a handful identified. This is despite the often high heritability estimates for diseases such as schizophrenia (McGue and Bouchard 1998), bipolar disorder (Burmeister *et al.* 2008), and major depressive disorder (Burmeister *et al.* 2008). More generally, very little is known about what polymorphisms affect behavior in a nonmorbid fashion, *i.e.*, alleles that cause natural quantitative variation. Knowledge of such polymorphisms would surely assist in the identification of actual disease loci. With regard to evolutionary theory, behavioral personality studies now exist for a wide range of species (Sih *et al.* 2004); however, the genes underlying such traits still remain largely unexplored.

It has been suggested that interspecies trait genetics can help reveal alleles influencing susceptibility in humans (Kas *et al.* 2007), with the mouse model primarily considered for such a role, although others have been suggested (Kaluev and Cachat 2012). Domestic chickens exhibit a wide range of behavioral as well as morphological differences, as compared to their wild-derived progenitor, the Red Junglefowl (RJF). Behaviorally, for example, they differ in anxiety and antipredator behavior, as well as sociality (Schütz *et al.* 2001). These extreme changes in anxiety are most likely brought about by selection for tameness and reduced fear of humans (Price 1984). Chickens possess a compact genome (~1.09 Gb) and a high recombination rate (1 cM ~ 350 kb).

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Coupled with the extreme diversification brought about by domestication, they lend themselves to analyses designed to identify genes harboring variants underlying anxiety-like behavior. Several quantitative trait locus (QTL) studies of behavior have been performed (Schutz *et al.* 2002, 2004; Buitenhuis *et al.* 2004), yet no candidate genes have been reliably identified. Previous studies of anxiety QTG in chickens have been restricted to specific candidate genes or regions (Wiren *et al.* 2009; Wirén *et al.* 2013). If gene polymorphisms were identified, it would enable the vast knowledge of the neuroanatomy of chickens to be combined with the genetic mechanisms for a truly holistic examination of these traits.

Assessing anxiety in animals is potentially problematic, and various different tests have been devised to test anxiety in model organisms. Without doubt the most popular test is the open field test (Archer 1973; Ramos and Mormède 1997; Belzung 1999), which was devised by Hall (1934). This is a test of anxiety/emotionality in rats, using a brightly lit novel arena. A range of variables can be measured, but most typically locomotion (speed and distance), thigmotaxis (time spent close to the walls), and grooming behaviors are recorded (see review in Prut and Belzung 2003). A variety of modifications have been performed over the years, but always maintaining these basic themes (Prut and Belzung 2003). Anxiety in animals is generally considered to be triggered by two conditions—social isolation and agoraphobia, with the open field test now used in a wide variety of different animals ranging from chickens, pigs, lambs, and rabbits to primates (Forkman *et al.* 2007). Of the studies using rodents, perhaps the largest and most powerful (in terms of the size of the QTL identified) is the Heterogeneous Stock intercross (Valdar *et al.* 2006), which utilized >2000 mice and located QTL regions <3 Mb in size.

In humans, anxiety disorders include panic disorders, phobic disorders, generalized anxiety disorder (GAD), obsessive compulsive disorder (OCD), and post-traumatic stress disorder (PTSD) (Smoller *et al.* 2009). Despite the prevalence of anxiety disorders, there is a lack of knowledge regarding the causal genetic variants. A large genome-wide association study (GWAS) on major depressive disorder (MDD), a disorder that is closely linked with anxiety (Major Depressive Disorder Working Group Of The Psychiatric GWAS Consortium 2013), found no genome-wide significant SNPs, despite a sample size of ~18,000 subjects. Even more recently, a meta-analysis GWAS study for neuroticism (with a polygenic association with MDD) was performed and found a single significant SNP, which failed to replicate (Genetics of Personality Consortium 2015), while two significant loci were identified in a GWAS using a smaller sample size (9000 cases) but more stringent phenotyping of MDD (Cai *et al.* 2015).

The relationship between anxiety, bipolar disorder, and schizophrenia is a close and interesting one. Patients with bipolar disorder are found to have far higher incidences of anxiety-related traits (Cosoff and Julian Hafner 1998; Boylan *et al.* 2004; Harpold *et al.* 2005), and there is a strong association between the two (often >50% of bipolar patients have diagnosed anxiety disorders; for example, see Faraone *et al.* 1997). The link between schizophrenia and anxiety-related disorders is more complex, although several studies have shown a correlation between anxiety and both the negative symptoms (Kulhara *et al.* 1989; Sax *et al.* 1996; Norman *et al.* 1998; Huppert *et al.* 2001) and the positive symptoms (Norman and Malla 1991; Huppert *et al.* 2001) associated with schizophrenia. This must be taken into consideration with the complex interplay between quality of life, schizophrenia, anxiety, and depression (Huppert *et al.* 2001; Samsom and Wong 2015). Polygenic risk scores have been shown to possess cross-disorder associations, particularly with regard to adult-onset disorders (Psychiatric Genetics Consortium 2013). Therefore, although large anxiety-based GWASs in humans may not be readily available, large data sets for bipolar disorders (Psychiatric GWAS Consortium Bipolar Disorder Working Group 2011), schizophrenia (Schizophrenia Working Group of The Psychiatric Genomics Consortium 2014), and MDD (Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium 2013) are available and may also reveal associations with anxiety behavior.

In this study, we identify a number of QTGs underlying phenotypic differences in anxiety-related open field behavior between wild-derived RJF and domesticated White Leghorn chickens. We expanded an initial wild × domestic F_2 intercross up to an eighth-generation advanced intercross line (AIL). This AIL can generate far smaller confidence intervals for mapping than the initial F_2 (Darvasi 1998) and was combined with an expression QTL (eQTL) approach, using hypothalamus tissue from the same birds. Hypothalamus tissue was selected for this analysis due to its pivotal role in the hypothalmic-pituitary-adrenal (HPA) axis (integral to stress and anxiety responses), its known effects on anxiety-related behavior (File *et al.* 2000; McNaughton and Corr 2004; Kallen *et al.* 2008), and its control of the amygdala (McNaughton and Corr 2004) (also highly involved in the control of anxiety). Correlations between gene expression and behavior in shared QTL/eQTL regions identified 10 putative candidate genes that significantly correlated with anxiety-related open field behavior in chickens.

These candidate genes were then further assessed in four published data sets from humans and mice. Orthologous genes in three different human behavior-related GWASs were assayed—a human bipolar disorder data set (Psychiatric GWAS Consortium Bipolar Disorder Working Group 2011), the Psychiatric Genetics Consortium MDD GWAS (Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium 2013), and the most recent PGC schizophrenia data set (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014). These same genes were also assayed in a large Heterogeneous Stock (HS) mouse cross tested for anxiety behavior, using an open field arena (Valdar *et al.* 2006). Associations between QTG in chickens and orthologous genes in the human bipolar, MDD, and

schizophrenia GWAS data sets were detected for three of the candidate genes, while associations were also observed between four of these candidate genes in the mouse advanced Heterogeneous Stock intercross assayed for open field behavior. A complete summary of all steps taken, the findings from each step, and how they led to further experiments is presented in Figure 1.

Materials and Methods

Chicken study population and cross design

The intercross population used in this study was an eighth-generation intercross between a line of selected White Leghorn chickens maintained from the 1960s and a population of RJF originally from Thailand (Schutz *et al.* 2002, 2004). The intercross is based on 1 RJF male and 3 White Leghorn (WL) females. These were expanded into 41 F_1 and then 811 F_2 progeny and subsequently maintained at a population size of ~100 birds per generation until the F_7 generation. The F_2 intercross was previously measured for a variety of behavioral, morphological, and life history traits (Schutz *et al.* 2002; Kerje *et al.* 2003; Wright *et al.* 2008, 2010, 2012). A total of 572 F_8 individuals in six batches were generated from 118 families, using 122 F_7 individuals (63 females and 59 males), and assayed for behavioral measurements. Average family size was 4.76 ± 3.1 (mean, SD) in the F_8. A total of 129 of the 572 F_8's were used in an eQTL experiment, with the hypothalamus/thalamus dissected out at 212 days of age and RNA extracted. For further details on feed and housing see Johnsson *et al.* (2012). This study was approved by the local Ethical Committee of the Swedish National Board for Laboratory Animals.

Phenotyping

Animals were weighed at hatch, 8 days, 42 days, 112 days, and 212 days. Open field assessment is a standard anxiety measurement, performed in a variety of vertebrates and invertebrates (Prut and Belzung 2003). Trials were performed in a 100 × 80-cm arena at 4 weeks of age. The 60 × 40-cm area in the middle of each arena was considered to be the central zone. Individuals were placed in the corner of the arena in complete darkness, prior to the test starting, with the lights turned on immediately at the commencement of the test. Trials lasted 5 min. Four separate arenas were available, allowing up to four individuals to be tested simultaneously. Measurements were taken using the Ethovision software and continuous video recording (Noldus Information Technology, www.noldus.com). For each trial, total distance moved, velocity, proportion of time spent in the central zone, and frequency (number of times) that the central zone was entered were measured. Each trial was performed twice, with ~1 week between an individual's first and second test. Individuals were removed from the arena immediately upon the test finishing to reduce potential habituation, with exposure to the test arena therefore restricted to only the 5 min required for each trial. Correlations between the two trials were found to be significant (total movement Pearson's correlation coefficient = 0.55, t-value = 13, $P < 2.2 \times 10^{-16}$; velocity Pearson's correlation coefficient = 0.62, t-value = 19, $P < 2.2 \times 10^{-16}$; time in center correlation coefficient = 0.31, t-value = 8, $P = 9.2 \times 10^{-14}$); see Supporting Information, Figure S1. Additionally, maximum and minimum values were also calculated for distance moved in a further attempt to reduce environmental variation, by finding an individual's most fearful and least fearful scores. All behavioral phenotypes were correlated with one another ($P \leq 10^{-15}$ based on Pearson's correlation; see Table S1).

RNA isolation and gene expression microarrays

The last three batches of the F_8 generation (129 animals, *i.e.*, unselected with respect to behavioral phenotype) were culled at 212 days of age and the hypothalamus was immediately dissected out and snap frozen in liquid nitrogen, prior to storage at −80°. RNA was isolated with Ambion TRI Reagent (Life Technologies), according to the supplier's protocol. Reverse transcription was performed with the Fermentas Revert Aid Premium First-Strand cDNA Kit (Thermo Fisher Scientific) and oligo-$(dT)_{18}$ primers, followed by second-strand cDNA synthesis according to the supplier's protocol. All samples were quality checked on a Bioanalyzer chip (Agilent), and all had a RNA integrity number (RIN) value >8.1.

Gene expression was measured on NimbleGen 12 × 135K Custom Gene Expression Arrays. Array design, hybridization, scanning, and signal preprocessing were performed by Roche NimbleGen Services (Reykjavik, Iceland). The array included all Ensembl (Flicek *et al.* 2012) and RefSeq (Pruitt *et al.* 2009) chicken transcripts. In addition to the known transcripts, the array included probe sequences from a chicken brain cDNA library (Boardman *et al.* 2002). Probes based on the cDNA library were annotated by alignment against the chicken reference genome with Blat (Kent 2002). In total, this yielded an additional 10,686 probe sets. Each transcript was represented by three 60-mer oligonucleotide probes. To avoid SNPs in probe sequences, all known SNP positions derived from the recent resequencing of Red Junglefowl and domestic chickens (Rubin *et al.* 2010) were masked, so that probes could not be chosen from sequences with known SNPs. Array data were preprocessed with NimbleGen DEVA software (v. 2.6.0.0) and the robust multiarray average (RMA) algorithm (Irizarry *et al.* 2003). RMA comprises of a background correction, quantile normalization, and summarization of probes to probe set-level expression values with median polish and is the recommended normalization per the manufacturer's guidelines. Although 36,000 transcripts were tested, there was a high degree of overlap between the EST probes and the known RefSeq genes. The total number of unique probe sets was therefore ~17,000. All samples were handled in two batches at the Roche Nimblegen central facility in Iceland, with none being discarded. Including the array batch as a covariate did not change the eQTL or trait–expression correlations of the candidate genes. No further corrections for global effects on the expression phenotypes were included after the RMA.

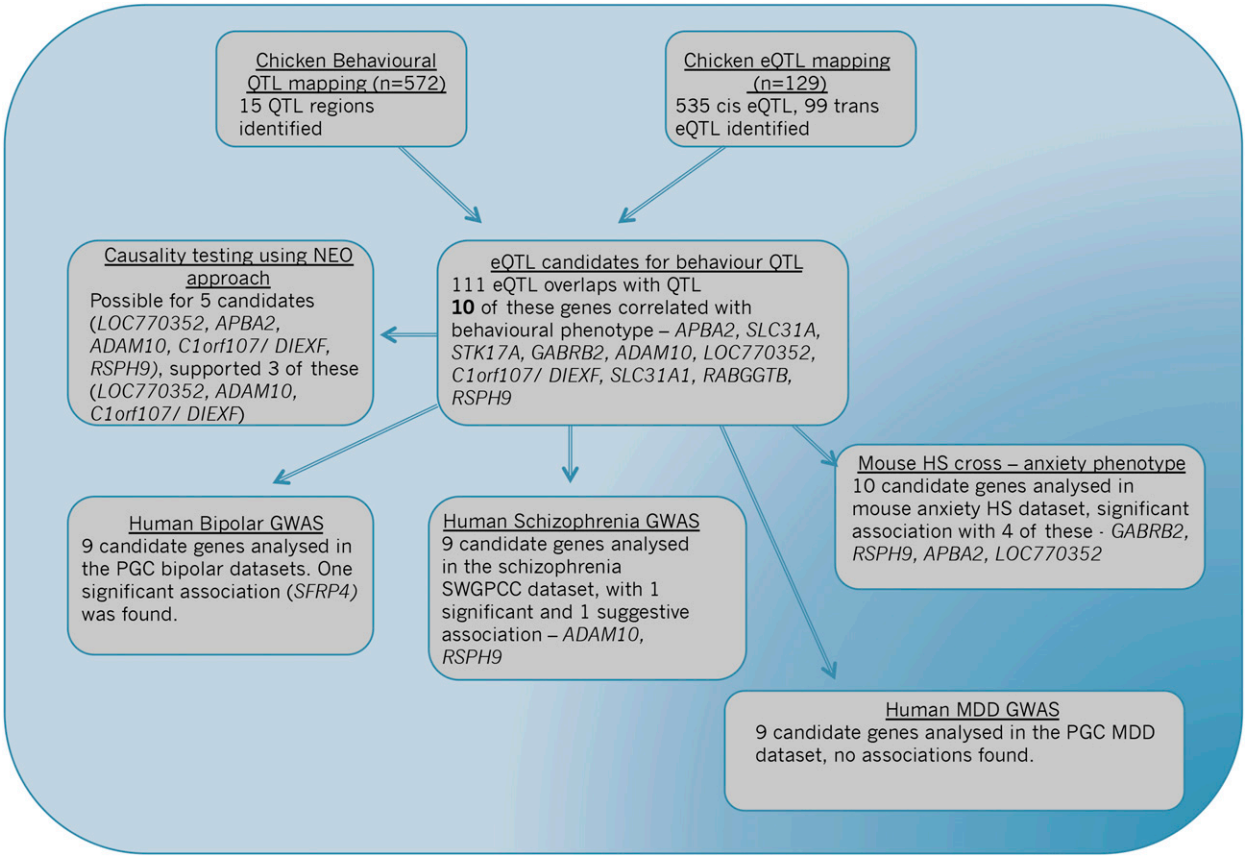

Figure 1 Flow-chart overview of experimental steps taken, plus the findings from each step.

Genotyping and QTL and eQTL mapping

DNA preparation was performed by Agowa (Berlin, Germany), using standard salt extraction, on all 572 F_8 individuals. A total of 652 SNP markers were used to generate a map of length ~9268 cM, with an average marker spacing of ~16 cM. A full list of marker locations and informativeness can be found in Johnsson *et al.* (2015). QTL analysis was performed using R/Qtl (Broman *et al.* 2003) for the standard interval mapping and epistatic analyses. Interval mapping was performed using additive and additive plus dominance models. Map generation was performed on the actual (F_8) data set, to account for the map expansion from the F_2 to the F_8. In this regard the map generation and QTL mapping are precisely like an F_2 analysis, only with more recombinations present (hence a larger overall map, with an increase from ~3000 cM to ~9300 cM). The increase in map size is approximately equal to that predicted by theory, with a maximum of fourfold increase hypothetically possible for an F_8 intercross (Darvasi and Soller 1995). Therefore, given our increased map size, we have on average a threefold increase in resolution compared to that for a standard F_2 intercross. This expanded map was also used to generate the significance thresholds, resulting in higher than normal thresholds compared to the F_2 (see later in article). In the behavioral QTL analysis batch, sex and arena were always included in the model as fixed effects, while body weight was included as a covariate. To account for a particular QTL varying between the sexes, a sex-interaction effect was added where significant. Digenic epistatic analysis was performed as per the guidelines given in Broman and Sen (2009). A global model that incorporated standard main effects, sex interactions, and epistasis was built up, starting with the most significant loci and working down for each trait.

eQTL mapping was also performed with R/qtl, using RMA preprocessed (Irizarry *et al.* 2003) expression levels as quantitative phenotypes with sex and batch as additive covariates. *Cis*-eQTL (defined as QTL that were located close to the target gene they affected) were mapped in an interval from the transcription start positions to the closest flanking markers spanning at least 100 cM (*i.e.*, 50 cM upstream and downstream of the gene, once again using the map generated using the F_8 data). A *cis*-eQTL was called if the LOD score reached above the empirical *cis* threshold (see below) at any marker in this interval. Note that it is potentially problematic to ascertain at what point a local eQTL is truly *cis* acting (*i.e.*, potentially on the same strand and acting putatively on a local enhancer to the gene in question), so more accurately our definition should be local rather than *cis*; however, in eQTL mapping this is a common problem and not one specific to our experiment, and therefore we have opted for the standard terminology used. Similarly, if the eQTL is 50 cM from

the gene, it could also be thought of as a more locally acting *trans* element. The *trans*-eQTL scan encompassed the whole genome and used a genome-wide empirical significance threshold.

In general an important caveat to mention in terms of estimating the r^2 effect of a QTL (*i.e.*, the percentage of variance explained by the QTL) is that this is less effective for smaller sample sizes (leading to an overestimation), with this being known as the Beavis effect (Beavis 1998) or the winner's curse. This typically starts occurring when the sample size is $<n = 1000$. The relatively large size of the behavioral QTL study used here ($n > 500$) reduces this effect. It does, however, pertain more strongly to the eQTL study and results in an overestimation of eQTL effect sizes.

Significance thresholds

Significance thresholds for behavioral QTL analysis were calculated for each trait, using permutation tests (Churchill and Doerge 1994, 1996), with 1000 permutations. As mentioned above, permutations were based on the full F_8 map data (~9000 cM rather than ~3000 cM). This is important, as the use of the original F_2 map would have resulted in far fewer tests being performed and artificially decreased the significance threshold. A suggestive significance level of a genome-wide 20% *P*-value cutoff threshold was used [principally due to being more conservative than the standard suggestive threshold (Lander and Kruglyak 1995)]. The approximate significance threshold was LOD ~ 4.4 (5% genome-wide), while the suggestive threshold was ~3.6 (20% genome-wide). Confidence intervals (C.I.) for each QTL were calculated with a 1.8-LOD drop method (*i.e.*, where the LOD score on either side of the peak decreases by 1.8 LOD); as this threshold gives an accurate 95% confidence interval for an intercross-type population (Manichaikul *et al.* 2006). The nearest marker to this 1.8-LOD decrease was then used to give the C.I. in megabases. Epistatic interactions were also assessed using permutation thresholds generated using R/qtl, with a 20% suggestive and a 5% significant genome-wide threshold used. In the case of epistatic loci, the approximate average significance thresholds for pairs of loci were as follows (using the guidelines given in Broman and Sen 2009): full model ~11, full *vs.* one ~9, interactive ~7, additive ~7, additive *vs.* one ~4.

Behavioral QTL significance thresholds were not modified based on the number of different phenotypes measured. In this case the phenotypes were correlated with one another (see Table S1) and were therefore not independent, thereby removing the need to multiple-test correct. It must be noted, however, that despite the *P*-values of the correlations being highly significant ($P < 10^{-15}$) some of the pairwise correlations were relatively modest in effect size (most notably between time spent in the central zone and velocity, with a correlation of 33%). This indicates that the traits are also not fully dependent on one another.

eQTL significance thresholds were also generated by permutation, although in this case there were two types of eQTL being mapped—*cis* (local)-QTL and *trans* (global)-QTL. In the case of *cis*-eQTL, only markers surrounding each gene were tested, therefore reducing the multiple-testing correction that is required. To account for both the full genetic map (in the case of *trans* effects) and the large number of phenotypes (~36,000) used, these permutations were based not on one phenotype at each time, but by using 100 randomly subsampled probe sets. In the case of *cis*-eQTL, this procedure was performed in a similar fashion, although only the limited region around each gene locus was used for extracting LOD scores (with 100 probe sets still used simultaneously for each permutation). This generated *cis* thresholds of LOD 4.0 and *trans* thresholds of LOD 6.0.

Family structure

Thresholds and analysis for an AIL are potentially problematic, as the family structure can be affected by nonsyntenic association, resulting in false positive results. To avoid this initially, we used a large number of families ($n = 118$) to generate the total number of individuals, to break down this substructure as much as possible. For instance, if only one offspring were used per family, there would be no family structure and the population would function exactly as recombinant inbred lines (Peirce *et al.* 2008). A principal components analysis approach was used to control for any residual family structure (Wu *et al.* 2011). We calculated the first 10 principal components (PCs) and then tested these for significance in each behavioral QTL regression. All significant PCs were then retained in the final model. This approach allowed us to both control for population substructure and test for epistatic interactions, which is impossible using other packages designed for advanced intercross QTL analysis. This was applied only to the behavioral QTL analysis, as the eQTL analysis utilized even smaller family sizes (129 individuals from 44 families), and nonsyntenic linkage (*i.e.*, that regions in linkage disequilibrium would provide false-positive signals) is less of an issue given that only specific loci were tested in the case of the *cis*-eQTL scan.

Analysis of candidate genes (eQTL genes falling within QTL intervals)

Significant eQTL were overlapped with behavioral QTL, with all significant eQTL genes then being considered candidate genes for the behavioral QTL they overlapped with. To further refine these candidate genes we then modeled the gene expression value on the behavioral trait for the QTL of interest (*i.e.*, if an eQTL overlapped a QTL for open field activity, the eQTL gene expression trait would be correlated with open field activity). For each eQTL overlapping a behavior QTL, a linear model was fitted with the behavior trait as a response variable and the expression traits as predictor, including sex and batch as factors. Weight at 42 days was included for traits where weight was used as a covariate in the QTL analysis (see Table S1). *P*-values for the regression coefficient were Bonferroni corrected for the number of uncorrelated eQTL in the QTL region. As the eQTL in a given region are often strongly correlated with one another [a typical feature of

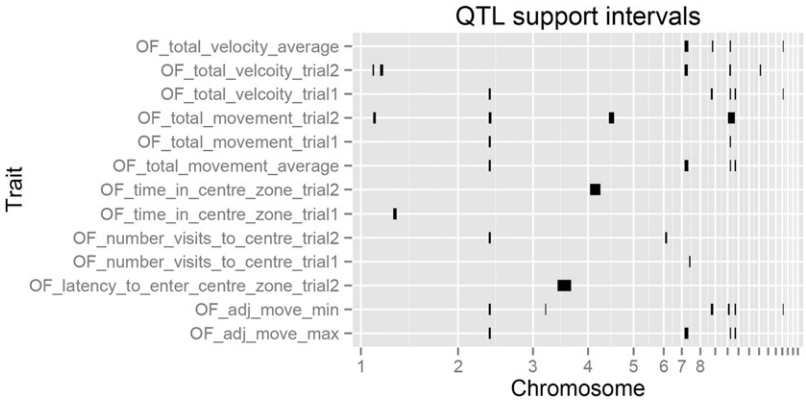

Figure 2 Genomic locations of all detected open field behavioral QTL. Bars indicate 1.8-LOD drop confidence intervals.

any genome—genes of similar expression are colocalized (Litvin *et al.* 2009)], it is required only to correct for the number of these eQTL genes that are independent (*i.e.*, the expression is not significantly correlated). This was especially true in our case, as we had numerous probes that were based on an EST library that frequently overlapped with the actual RefSeq genes (*i.e.*, they were the same genes tested). As we are testing a specific hypothesis (*i.e.*, that a given QTL is caused by the gene expression of an overlapping eQTL), we corrected only for the number of independent eQTL for that particular QTL, rather than for eQTL overlapping other QTL for that trait. The average multiple-testing correction was ~2 per QTL region. Any eQTL within a QTL C.I. that were significantly correlated with the QTL trait were therefore considered candidate causative genes and were then assessed for causality where possible. One issue with using this approach with this particular data set is that the behavioral QTL were based on up to 572 individuals, whereas the eQTL/expression phenotypes were available only for 129 individuals. Therefore the network edge orienting (NEO) method for causality testing was applied only where the behavioral QTL that a gene was potentially causative to was detectable in the smaller data set ($n = 129$).

Network edge orientation analysis

Causality analysis was performed using NEO software (Aten *et al.* 2008) to test whether the expression of correlational candidates was consistent with the transcript having a causal effect on the behavior trait. Single-marker analysis was performed with NEO fitting a causal model (marker → expression trait → behavior) and three other types (reactive, confounded, and collider). The NEO software evaluates the fit of the model with a χ^2-test, a higher *P*-value indicating a better fit of the model. The best-fitting model is chosen based on the ratio of the χ^2 *P*-value to the *P*-value of the next best model on a logarithmic scale (base 10), called local edge orienting against the next best model (leo.nb) scores. A positive leo.nb score indicates that the causal model fits better than any competing model. Aten *et al.* (2008) use a single-marker leo.nb score of 1, corresponding to a 10-fold higher *P*-value of the causal model, as their threshold. They also suggest that users inspect the *P*-value of the causal model to ensure the fit is good (in this case meaning the model *P*-value should be nonsignificant if the causal model fits the best). In effect this *P*-value is the probability of *another* model fitting the observed data. For each gene, we report the leo.nb score and *P*-value of the causal model.

Human and mouse GWAS comparisons

A description of the methods for the comparison of candidate genes with human GWAS and mouse QTL data is presented in File S1.

Data availability

Microarray data for the chicken hypothalamus tissue are available at E-MTAB-3154 in ArrayExpress. Full genotype and phenotype data are available on figshare with the following doi: 10.6084/m9.figshare.1265060.

Results

QTL mapping of fearful behavior ($n = 572$) in the AIL identified a total of 15 distinct QTL regions (comprising 34 QTL) for open field behavior, with the average confidence interval being ~3 Mb (see Table S2 and Figure 2). Average variance explained (R^2) for the behavioral QTL was 5.4%. Expression QTL mapping of hypothalamic gene expression in the AIL detected 535 *cis*-eQTL and 99 *trans*-eQTL for 537 genes (Table S3), with effect sizes of these eQTL ranging from 13% to 58% of the variance explained.

Candidate quantitative trait genes

A total of 111 eQTL probe sets were found to overlap behavior QTL, with these all then considered potential candidate genes. A regression between each candidate gene and the respective behavioral trait it overlapped with yielded a total of 10 significant correlational candidate genes, after a Bonferroni correction for the number of uncorrelated eQTL overlapping the behavior QTL region was applied (see Table 1, Figure 3, and Figure S2). These represented seven different QTL regions. Of the significant candidates, 2 genes were strongly significantly correlated ($P < 0.001$) with their corresponding behavioral trait: *GABRB2* and a novel gene *Hypothetical protein LOC770352*, with a further 4 also with a $P < 0.01$, including *APBA2*, *SLC31A*, *STK17A*, and *C1orf107/DIEXF*.

Table 1 Candidate gene expression levels predicting behavior

Chr	Trait	QTL C.I. (Mb)	eQTL	PhQTL pos	Gene *P*-value	NEO leo.nb score	Model *P*-value	Marker	Mouse HS open field LOD scores (additive model)			
									Latency	Total activity	Time in center	Marker
2	Time in center zone trial 2	45.5–51.9	SFRP4	2@426	0.04	NA	NA	rs2709114	—	—	—	rs13481716
2	Time in center zone trial 2	45.5–51.9	STK17A	2@426	0.004	NA	NA	rs1181601	—	—	—	rs6253368
3	Minimum total movement	25.1–27	RSPH9	3@277	0.03	0.823	5.48*E*-06	rs3778493	4.7	7.8	—	UT_17_45.56
3	Minimum total movement	25.1–27	C1orf107/DIEXF	3@277	0.003	0.368	0.804	rs7549523	—	—	—	rs3684501
8	Total velocity average	23.9–26.6	RABGGTB	8@194.0	0.05	NA	NA	rs7555627	—	—	—	rs3656403
10	Total velocity average	4.3–6.8	ADAM10	10@101	0.03	0.44	0.396	rs2242321	—	—	—	rs13480265
10	Total velocity average	4.3–6.8	APBA2	10@101	0.01	0.0421	0.0719	rs7680321	—	7.7	—	rs3717846
10	Total velocity trial 1	14.4–17.4	LOC770352	10@185.0	0.002	0.943	0.687	rs1480617	—	—	4.5	rs13476065
10	Total movement average	14.4–17.4	LOC770352	10@185.0	0.0002	1.07	0.956	rs1480617	—	—	4.5	rs13476065
10	Minimum total movement	14.4–17.4	LOC770352	10@182.0	0.003	0.874	0.925	rs1480617	—	—	4.5	rs13476065
10	Total movement trial 2	7.6–15.0	LOC770352	10@64.0	0.0012	0.747	0.878	rs1480617	—	—	4.5	rs13476065
10	Maximum total movement	14.4–17.4	LOC770352	10@185.0	0.0003	1.13	0.985	rs1480617	—	—	4.5	rs13476065
10	Total velocity trial 1	4.3–6.8	APBA2	10@99.0	0.004	0.977	0.131	rs7680321	—	7.7	—	rs3717846
10	Total velocity trial 1	4.3–6.8	ADAM10	10@99.0	0.04	0.515	0.37	rs2242321	—	—	—	rs13480265
10	Total movement trial 1	4.3–6.8	LOC770352	10@99.0	0.008	0.815	0.852	rs7680321	—	—	4.5	rs13476065
10	Total movement average	4.3–6.8	APBA2	10@99.0	0.02	−0.966	0.0002	rs7680321	—	7.7	—	rs3717846
13	Total velocity trial 2	1.5–4.1	GABRB2	13@32.0	0.0014	NA	NA	rs4302629	5.1	5.1	—	rs6225242
17	Total velocity average	1–2.5	SLC31A1	17@1	0.004	NA	NA	rs786978	—	—	—	rs3690581
17	Total velocity trial 1	1–2.5	SLC31A1	17@1	0.003	NA	NA	rs786978	—	—	—	rs3690581

Chromosome (Chr) and QTL confidence interval are given for the trait that each eQTL significantly correlated with. The phenotypic QTL position (PhQTL pos) is also given in centimorgans. The *P*-value of the correlation, after Bonferroni correction, is also provided (Gene *P*-value). The causality modeling scores are also indicated for each gene, as are the WTCCC *P*-values for each gene region. The association of each gene with the mouse HS cross is also given, with the LOD scores of the gene location for each of the three different open field tests provided.

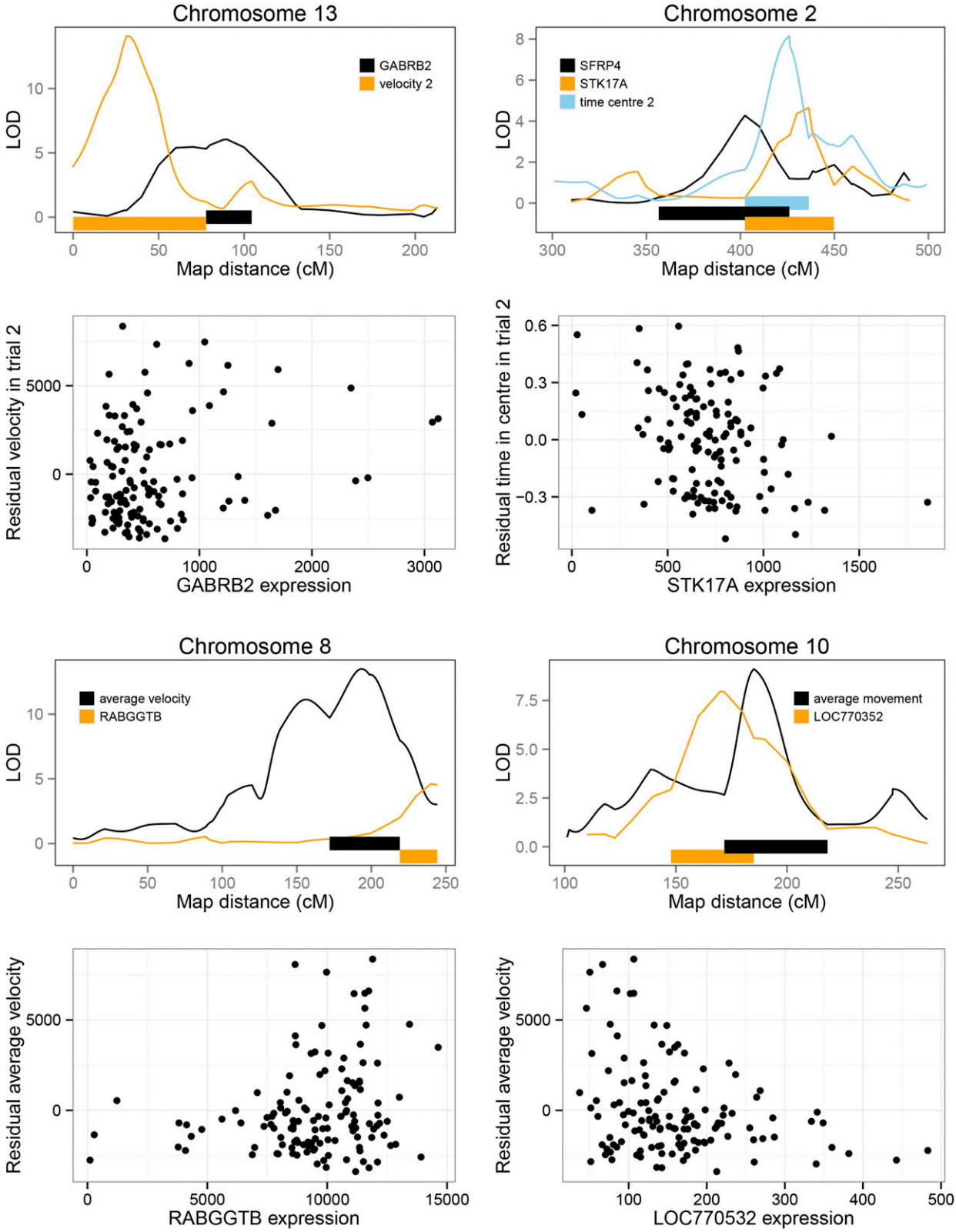

Figure 3 Part of LOD curves and scatterplots of trait *vs.* gene expression for selected candidates *GABRB2*, *RABGGTB*, *STK17A* and *SFRP4*, and *LOC770352*. Bars indicate 1.8-LOD intervals, extended to the closest marker.

To attempt to ascertain statistical causation a NEO approach (Aten *et al.* 2008) was used on 5 of the 10 candidate genes (*LOC770352*, *APBA2*, *ADAM10*, *C1orf107*, and *RSPH9*). The remaining 5 genes had an undetectable behavioral QTL in the reduced eQTL sample, preventing this analysis (see *Materials and Methods*). This approach is designed to identify statistical causation between the phenotype, genotype, and gene expression variables (*i.e.*, if the genotype is the causative locus for a QTL, this should then affect gene expression, which in turn affects the behavioral phenotype). The sole candidate gene *LOC770352* for the open field QTL at 14.4–17.4 Mb on chromosome 10 exhibited good statistical evidence for causality as assessed by its local edge orienting score (see *Materials and Methods*). In the case of the open field QTL at 4.3–7.8 Mb, of the two candidates *APBA2* and *ADAM10*, NEO calculations indicated that *ADAM10* was a better candidate (local edge orienting score was low for *APBA2* and higher for *ADAM10*, except for one of the open field variables). NEO also indicated that the gene *RSPH9* is unlikely to be causal to the chromosome 3 QTL, whereas there is more support for the gene *C1orf107*.

As a further assessment of both the 10 candidate genes and the method in general, all significant eQTL were correlated with the behavioral phenotypes and ranked in order. The average rank of these 10 genes was in the top 3% of all eQTL, with 2 of the genes (*STK17A* and *LOC770352*) being the top-ranked genes for time in the central zone and maximum total movement, respectively (see Table 2). The total number of eQTL correlated for each trait for which a significant candidate was obtained is given in Table S4.

Table 2 Candidate genes trait correlations and ranking *vs.* all eQTL

Gene	Trait	Overall rank (of 535 eQTL)	Trait–gene correlation *P*-value
ADAM10	Average velocity	52	0.027
ADAM10	Velocity trial 1	35	0.018
APBA2	Average total movement	34	0.024
APBA2	Average velocity	27	0.010
APBA2	Velocity trial 1	11	0.002
C1orf107/DIEXF	Minimum total movement	7	0.003
GABRB2	Velocity trial 2	7	0.006
LOC770352	Total movement trial 1	11	0.004
LOC770352	Total movement trial 2	2	0.001
LOC770352	Maximum total movement	1	<0.001
LOC770352	Minimum total movement	4	0.001
LOC770352	Average total movement	2	<0.001
LOC770352	Velocity trial 1	13	0.002
RABGGTB	Average velocity	23	0.008
RSPH9	Minimum total movement	39	0.033
SFRP4	Time in center trial 2	24	0.018
SLC31A1	Average velocity	17	0.004
SLC31A1	Velocity trial 1	15	0.003
STK17A	Time in center trial 2	1	0.002

Associations with MDD, bipolar disorder, and schizophrenia

To test for the presence of associations with human bipolar disorder, 9 of the 10 candidate genes (on closer examination *LOC770352* did not have a suitably conserved ortholog and was excluded) were examined in the PGC bipolar data set ($n \sim 7500$ cases) (Psychiatric GWAS Consortium Bipolar Disorder Working Group 2011). Of the genes tested, there was one significant association with *SFRP4* ($P = 0.0001$).

The PGC study for MDD is perhaps the closest human GWAS study to the anxiety phenotype measured here in chickens (Levinson 2006). However, this study identified no genome-wide significant SNPs in humans, indicating that the phenotype is less repeatable, has a different genetic architecture (smaller-effect loci), or is harder to accurately phenotype. It is also a more common disorder than the other behavioral disorders used here, with such disorders typically requiring a larger sample size than their less common counterparts. No associations were found with any of the genes tested.

Although farther from a standard anxiety phenotype, schizophrenia also has similar symptoms, making analysis of nine of the candidate genes also potentially relevant in a schizophrenia GWAS. The largest data set available has been collected by the PGC once again ($n \sim 36{,}000$ cases) (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014). Using this data set, a significant association was found between *ADAM10* and schizophrenia ($P = 1 \times 10^{-5}$), while a suggestive association was found with *RSPH9* ($P = 9 \times 10^{-5}$); see Table 3.

Associations with mouse models of anxiety

One of the largest studies performed using a mouse model to study anxiety, using an open field assay, utilized a Heterogeneous Stock cross to obtain far smaller confidence intervals than a standard F_2 intercross (average QTL C.I. <3 Mb in size) (Valdar *et al.* 2006). Testing for an association between the nearest SNPs to each of the 10 candidate genes under investigation and three open field variables (latency to move, total activity, and time spent in the center of the arena) revealed a total of four significant loci (see Table 2). Of the significant loci, several were replicated for the same behavioral measurement in both chickens and mice. *RSPH9* showed a significant correlation with total movement in chickens and for total activity in mice (LOD = 7.8). *APBA2* and *GABRB2* both showed a correlation with velocity in chickens and with total activity in mice (*APBA2* LOD = 7.7, *GABRB2* LOD = 5.1).

Discussion

In this study we use a combination of QTL and eQTL mapping in a novel anxiety model, chickens, and identify multiple candidate gene variants affecting anxiety behavior. We also present evidence that some of these gene polymorphisms may have some translational effects, although care must be taken

Table 3 Association of the nine genes tested in the GWASs

	Bipolar:		Schizophrenia:		Major depressive disorder:	
	PGCBD 2011 study		SWGPCC[a] 2014		PGCMDD	
Gene	*P*-value	Marker	*P*-value	Marker	*P*-value	Marker
SFRP4	0.0001*	rs7807315	NS	—	NS	—
STK17A	NS	—	NS	—	NS	—
RSPH9	NS	—	0.00009#	rs72859036	NS	—
DIEXF	NS	—	NS	—	NS	—
RABGGTB	NS	—	NS	—	NS	—
ADAM10	NS	—	0.00001*	rs6494035	NS	—
APBA2	NS	—	NS	—	NS	—
GABRB2	NS	—	NS	—	NS	—
SLC31A1	NS	—	NS	—	NS	—

Significant (*) and suggestive (#) *P*-values are given for each gene, along with the marker showing the highest association in the gene interval. In the case of *RSPH9* and *ADAM10* the distance of the marker from the gene is also given.
[a] Schizophrenia Working Group of the Psychiatric Genomics Consortium.

not to overstress these results. This approach is obviously costly in terms of the number of individuals required for the eQTL analysis and to date has to our knowledge been used only to identify QTG for startle-induced locomotion behavior (among other ecological phenotypes) in *Drosophila* (Ayroles *et al.* 2009), exercise behavior in mice (Kelly *et al.* 2012), and tameness in rats (Heyne *et al.* 2014), but has enormous potential. Here we use this technique to identify gene variants affecting anxiety for the first time in chickens. Chickens have many advantages, both genetic and phenotypic, making them attractive as a model for anxiety-related disorders. When this is coupled with the in-depth knowledge of the neural development of chickens (a classical model of such development), this represents a powerful resource.

Of the 10 candidate genes, 6 have been previously identified as having some bearing on behavior (mainly anxiety and stress related) or neuronal development, while 3 have no previous evidence (*SFRP4*, *C1orf107*, and *RSPH9*) and 1 is a novel gene (*LOC770352*). Such an enrichment gives us a great deal of confidence about the genes that have been identified, as well as providing far greater evidence for their effects on behavior, in many cases, than was available previously. The 6 genes with previous implications [*SLC31A* (Jones *et al.* 2008; Lagus *et al.* 2010; Goerlich *et al.* 2012), *ADAM10* (Postina *et al.* 2004; Schmitt *et al.* 2006; Bell *et al.* 2008; Jorissen *et al.* 2010), *GABRB2* (Moriarty 1995; Marín *et al.* 1997; Salvatierra and Arce 2001), *APBP2* (Kirov *et al.* 2008), and *STK17A* and *RABBGTB* (Kõks *et al.* 2004)] have a variety of effects in neural tissue or on behavior. *ADAM10* is required for embryonic brain development, (Jorissen *et al.* 2010), protects against amyloid plaque formation (Postina *et al.* 2004), increases synaptogenesis (Bell *et al.* 2008), and also causes behavioral differences in learning and memory (Schmitt *et al.* 2006). Similarly, *APBA2* has been implicated in schizophrenia by one study (Kirov *et al.* 2008), although this gene was not identified in the more recent PGC GWASs. *SLC31A* and *RABGGTB* have both been shown to have some links with anxiety behavior and memory. *SLC31A* (a copper transporter) has been linked with depression in rats (Lagus *et al.* 2010) and chickens exposed to social isolation and restraint stress (Goerlich *et al.* 2012), and genetic correlations suggest a link between copper and anxiety behaviors in BXD mice (Jones *et al.* 2008). *RABGGTB* has been linked with fear responsiveness in rats (Kõks *et al.* 2004). Although little has been characterized of *STK17A* [a serine/threonine kinase and a part of the death-associated protein kinase group (Kögel *et al.* 2001)], barring a link with p53 in cancer (Mao *et al.* 2011), there are several examples of other serine/threonine kinases affecting behavior, specifically learning and anxiety in *Drosophila* (Choi *et al.* 1991) and mice (Silva *et al.* 1992) (Hodge *et al.* 2002), with links to bipolar disorder (Klein and Melton 1996) and depression (Gould *et al.* 2004; O'Brien *et al.* 2004). In a parallel fashion, *GABRB2* has been found to have prior associations with schizophrenia (Lo *et al.* 2007; Pun *et al.* 2011) and bipolar disorder in German and Chinese cohorts, while the closely related *GABRB1* has associations with alcohol dependence (Parsian and Zhang 1999).

A point of note is how potentially translational the gene polymorphisms that can influence quantitative variation in behavior can be and the applicability between different model organisms and humans. It is striking how an open field assay in chickens and mice can show associations in the same genes for even the same aspects of the assay (activity in the case of *RSPH9*, *APBA2*, and *GABRB2*). Assessing the candidate genes in human data sets also gave some potential indications of translational effects in these gene polymorphisms. The data sets analyzed indicated putative effects for *ADAM10* and *RSPH9* with regard to schizophrenia and *SFRP4* with regard to bipolar disorder. Care must be taken in interpreting these results from human GWASs, however. It can be problematic to control for such variables as gene density and also recombination rate, the size of haplotype blocks, and so forth, when calculating suitable thresholds for significance for these selected-region scans. Similarly, the exact phenotype used must be chosen with care, with more stringent phenotyping criteria for the human disorder potentially leading to a more relevant trait comparison. Although the associations found here would be subthreshold values in a full GWAS analysis, for the targeted approach used here they offer indications that some of the genes may also play a role in human quantitative variation

and are worthy of further study to ascertain whether this is the case. Such an approach also offers a method of exploratory analysis to gain additional information from such large GWASs. Due to the issue of massive multiple-testing correction, it has been conjectured that many true significant associations are buried in the "noise" of such necessarily stringent thresholds. For example, the "missing" heritability that such GWASs fail to find has potentially been identified by considering the joint effect of all SNPs in linear mixed models [an excellent example of this is with human height (Yang *et al.* 2010), with this technique also used for psychiatric disorders (Cross-Disorder Group of the Psychiatric Genomics Consortium 2013)], in essence decreasing the significance threshold for inclusion into the genetic model. Assessing candidate genes supported by evidence from model organisms in such data sets offers a method of maximizing the potential from such studies

One potential issue with the use of chickens as model organisms for human behavior is the relatively compact nature of the chicken genome. It is possible that given the reduced genome size chickens are missing key regulatory elements that may have evolved in the noncoding DNA found in humans, which potentially harbor variants that affect the fine temporo-spatial regulation of the expression of such genes and may thus mask cross-species correspondences between risk loci. This is obviously true for all model organisms, however. Similarly, it is less likely the exact mutation affecting a gene is replicated between species, but the gene itself may still be functioning in a similar fashion. In this way, cross-species modeling is more likely to find candidate genes than candidate mutations.

In conclusion, we have used a combination of an advanced intercross, QTL, and eQTL analysis in a chicken model to identify 10 putative QTGs affecting anxiety behavior. These genes were then tested in published data sets using mice and humans and significant associations were identified. By using multiple forms of validation, this will hopefully provide greater weight of evidence for the effects of a particular gene. Similarly, in many cases not all of the different validation tests will positively associate with a given gene. The final form of validation for all of these genes would ideally be transgenic verification. This study represents the first of its kind for anxiety-related behavior in chickens and establishes them as a strong model for the genetic dissection of anxiety behavior and of personality in general.

Ethics statement

This study was approved by the Regional Committee for Ethical Approval of Animal Experiments (Jordbruksverket DNR 122-10). Birds were killed by cervical dislocation and decapitation, as per the guidelines of the permit.

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Genetical Genomics of Behavior: A Novel Chicken Genomic Model for Anxiety Behavior

Martin Johnsson, Michael J. Williams, Per Jensen, and Dominic Wright

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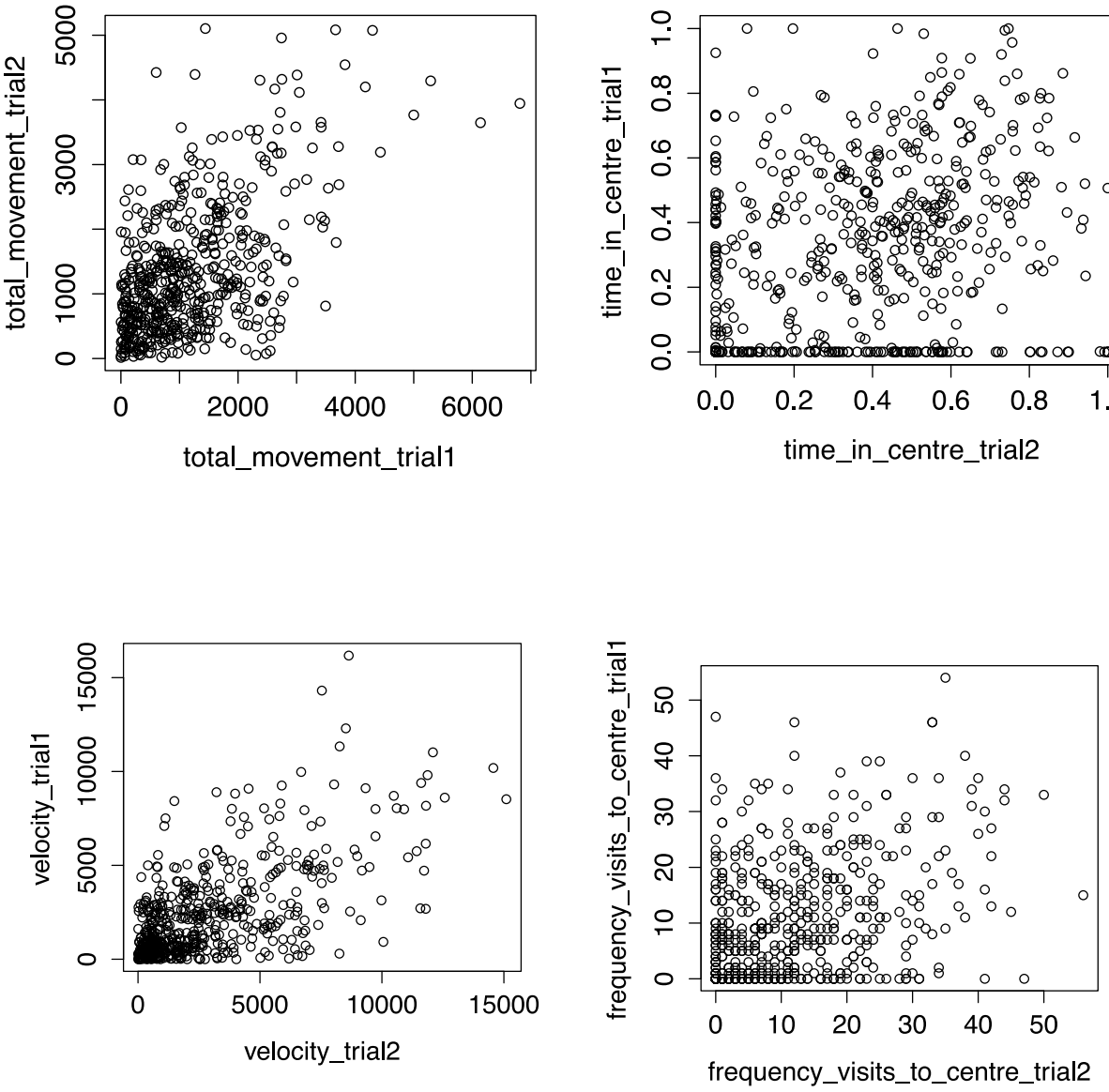

Figure S1. Scatter plots of behavioural phenotypes between the first and second trials

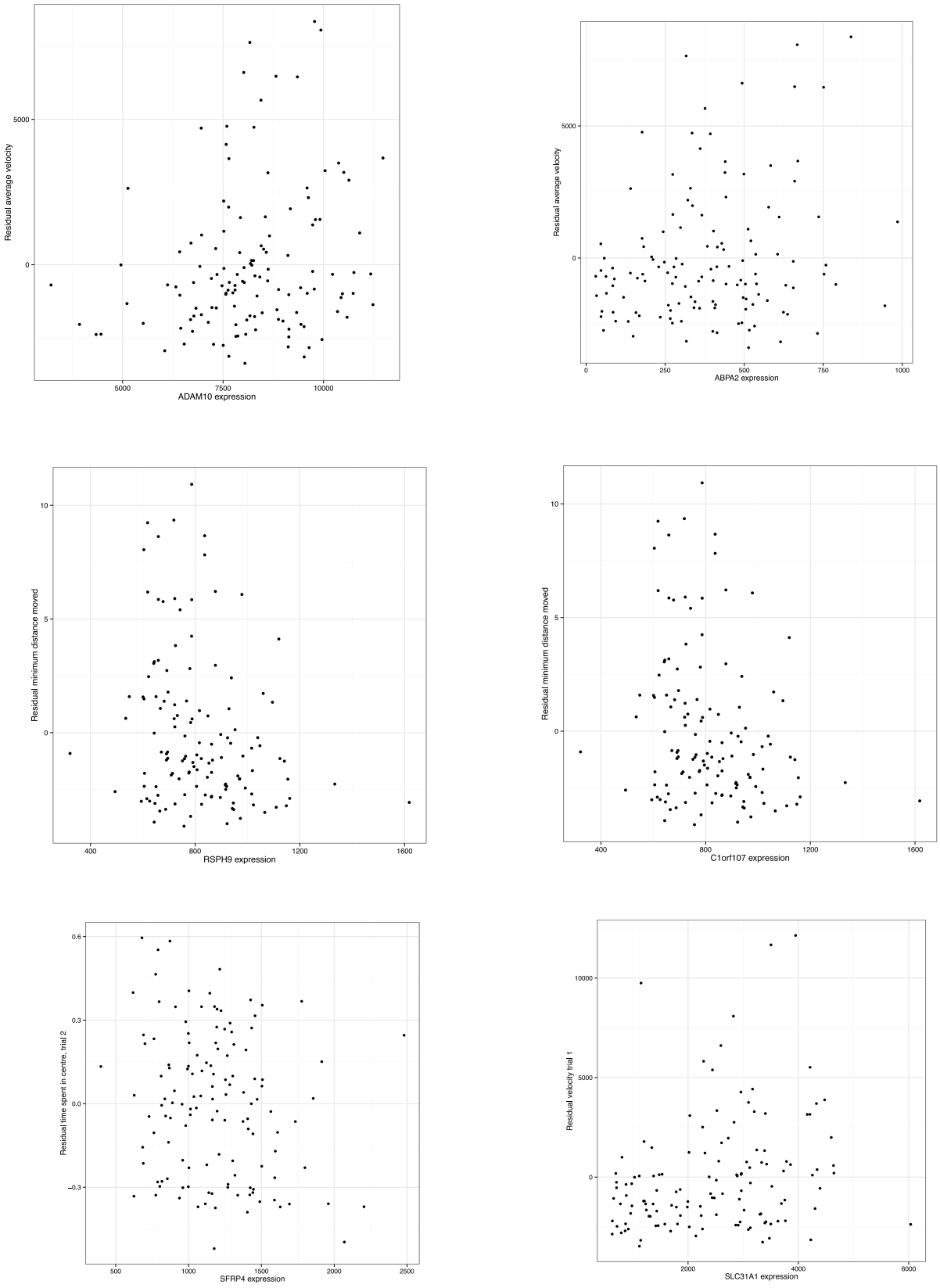

Figure S2. Scatter plots of the remaining candidate genes.

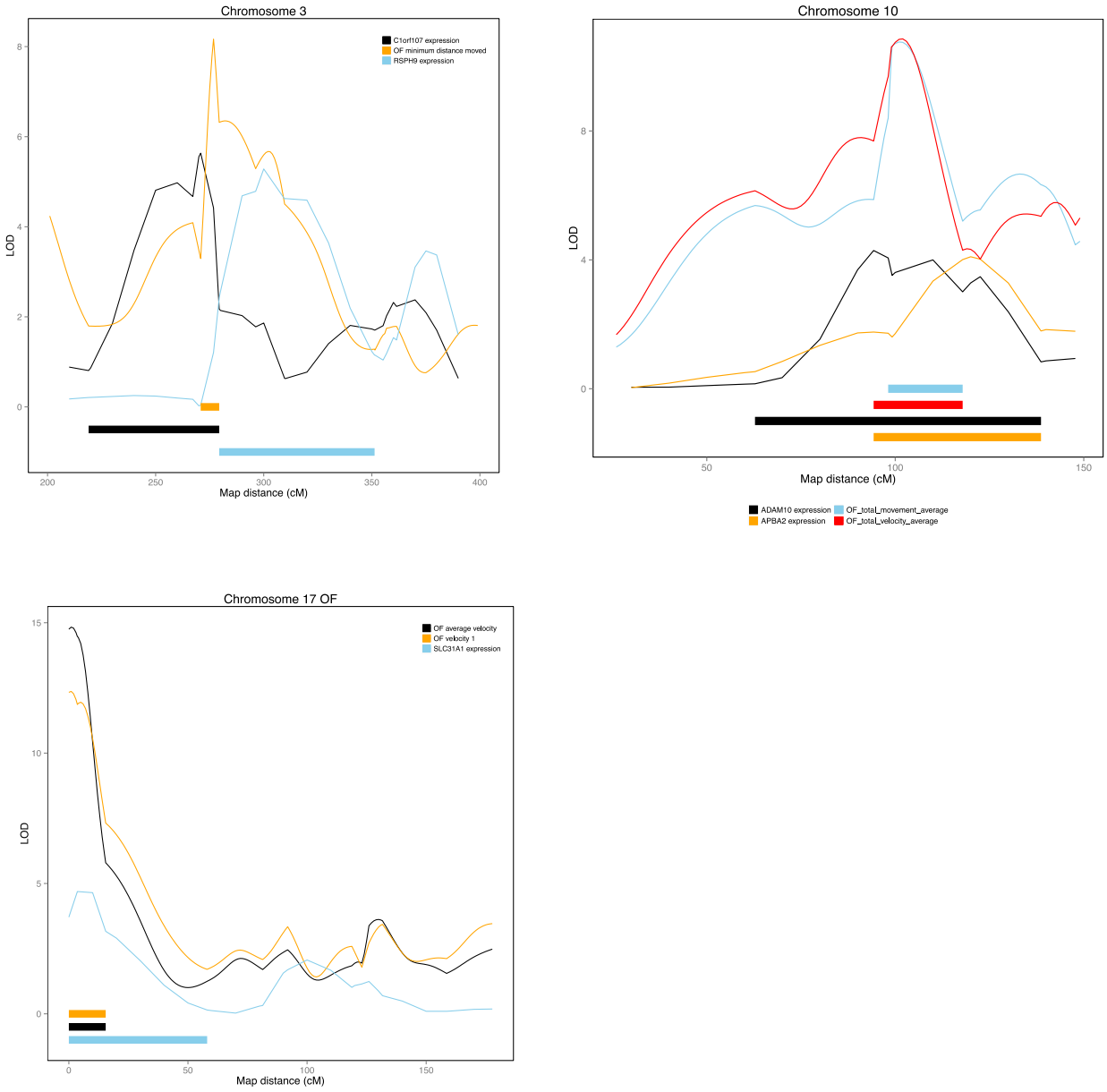

Figure S3. LOD graphs of overlapping candidate eQTL and QTL

Table S1. Pearson Correlations between open-field measurements from trial 1(A) and trial 2 (B) measurements. All correlations were strongly significant ($P < 3x10^{15}$).

(A) Trial 1 variables	total movement trial 1	velocity trial 1	time in centre trial 1
total movement trial 1	-	-	-
velocity trial 1	0.92	-	-
time in centre trial 1	0.4	0.33	-
frequency of visits to centre trial 1	0.8	0.8	0.43

(B) Trial 2 variables	total movement trial 2	velocity trial 2	time in centre trial 2
total movement trial 2	-	-	-
velocity trial 2	0.92	-	-
time in centre trial 2	0.36	0.32	-
frequency of visits to centre trial 2	0.79	0.76	0.38

Table S2. Behavioural QTL for open field behaviour. Chromosome, position (in cM), Lod score, r-squared (in %), additive and dominance values are given, as well as the confidence intervals (C.I.) as defined by a 1.8 LOD drop. Covariates for each trait, as well as any epistatic interactions are also provided (given as chromosome@position: chromosome@position).

trait	chr	position	LOD	R2	add+/-s.e.	dom+/-se	lower CI	upper CI	lower_marker	upper_marker	covariates	interaction
OF_total_velocity_trial2	1	387	13.2	6.2	985+/-209	37.50+/-285	379	409	1_24012566	1_27115232	sex, batch, arena, w42,	1@387:10@82
OF_total_movement_trial2	1	428	11.3	6.5	-12.96+/-9.35	-15.60+/-11.01	401	453	Gg_rs14799859	1_30756049	sex,batch,arena,w42	10@64:1@428, 1@428:sex
OF_total_velocity_trial2	1	591	15.7	7.4	-2611+/-427	-2839+/-683	580	595	Gg_rs15239304	Gg_rs14815974	sex, batch, arena, w42,	1@591:13@32
OF_time_in_centre_zone_trial2	2	426	8.1	6.0	0.00+/-0.03	-0.08+/-0.03	416	431	Gg_rs15094455	GG_rs15099683	sex, batch, arena, w42,	2@426:4@191
OF_maximum_total_movement	2	513	10.2	6.0	1.14+/-0.22	-0.40+/-0.29	504	521	Gg_rs15107655	Gg_rs15112090	sex, batch, arena, w42,	2@513:10@185
OF_total_movement_trial2	2	513	4.1	2.3	0.81+/-0.21	-0.04+/-0.27	503	541	Gg_rs15107655	Gg_rs14200463	sex,batch,arena,w42	-
OF_minimum_total_movement	2	513	9.8	5.2	0.61+/-0.17	-0.62+/-0.21	504	517	Gg_rs15107655	Gg_rs15112090	sex, batch, arena, w42,	2@513:10@182
OF_total_movement_average	2	513	10.3	6.0	0.94+/-0.19	-0.56+/-0.24	504	520	Gg_rs15107655	Gg_rs15112090	sex, batch, w42	2@513:10@185
OF_total_movement_trial1	2	515	21	8.8	-3.54+/-0.91	2.85+/-1.24	504	522	Gg_rs15107655	Gg_rs15112090	sex,batch,w42	10@99:2@515, 2@515:w42
OF_number_visits_centre_trial2	2	519	5.42	3.57	3.62+/-0.73	-1.27+/-1.07	505	568	Gg_rs15107655	Gg_rs14206130	sex, batch, arena, w42,	-
OF_minimum_total_movement	3	277	7.8	4.1	-0.29+/-0.21	-0.51+/-0.27	274	279	Gg_rs14327472	3_27044082	sex, batch, arena, w42,	3@277:10@63
OF_time_in_centre_zone_trial2	4	191	5.4	3.9	0.01+/-0.02	0.04+/-0.03	89	201	Gg_rs15481407	Gg_rs15522441	sex, batch, arena, w42,	2@426:4@191
OF_total_movement_trial2	4	360	7.1	4	-1.12+/-0.51	1.53+/-0.90	343	378	Gg_rs14457643	Gg_rs14470123	sex,batch,arena,w42	10@64:4@360
OF_number_visits_centre_trial2	6	69	5.65	3.72	7.65+/-2.01	-3.66+/-2.73	41	77	6_2930562	Gg_rs15765462	sex, batch, arena, w42,	6@69:sex
OF_maximum_total_movement	7	102	5.2	3.0	0.96+/-0.29	-0.78+/-0.36	94	113	Gg_rs15835348	Gg_rs15845344	sex, batch, arena, w42,	10@99:7@102
OF_total_movement_average	7	103	5.0	2.8	0.62+/-0.25	-0.54+/-0.31	92	110	Gg_rs15835348	Gg_rs15845344	sex, batch, w42	10@99.2:7@103
OF_total_velocity_average	7	104	6.8	3.1	387.30+/-179.98	-322.00+/-228	95	110	Gg_rs15835348	Gg_rs15845344	sex, batch, w42	7@104:10@101
OF_number_visits_centre_trial1	7	145	4.9	3.5	-15.26+/-11.13	33.33+/-12.48	137	153	7_15017822	Gg_rs15853763	sex, batch, arena, w42,	-
OF_minimum_total_movement	8	188	9.5	5.1	0.86+/-0.21	-0.75+/-0.36	140	203	8_21627386	Gg_rs15935538	sex, batch, arena, w42,	17@1:8@188
OF_total_velocity_average	8	194	13.6	6.5	744+/-167	-1070.00+/-297	183	207	Gg_rs14652282	Gg_rs15935538	sex, batch, w45	17@1:8@194
OF_minimum_total_movement	10	63	10.1	5.4	0.19+/-0.21	0.46+/-0.27	55	70	Gg_rs14941298	Gg_rs14001676	sex, batch, arena, w42,	3@277:10@63
OF_total_movement_trial2	10	64	15.1	8.9	13.43+/-8.52	-11.56+/-8.74	46	177	Gg_rs14941298	Gg_rs14949856	sex,batch,arena,w42	10@64:1@428, 10@64:sex
OF_total_velocity_trial2	10	82	11.2	5.2	-47+/-228	-42.70+/-347	67	111	Gg_rs14941656	Gg_rs14003134	sex, batch, arena, w42,	1@387:10@82
OF_maximum_total_movement	10	99	7.0	4.1	1.01+/-0.23	-0.95+/-0.36	98	107	Gg_rs14001865	Gg_rs14003134	sex, batch, arena, w42,	10@99:7@102
OF_total_movement_trial1	10	99	14.2	13.7	1.20+/-0.81	2.35+/-1.18	94	104	Gg_rs14001676	Gg_rs14003134	sex,batch,w42	10@99:2@515, 10@99:w42
OF_total_movement_average	10	99	7.2	4.1	0.73+/-0.19	-0.76+/-0.30	98	106	Gg_rs14001865	Gg_rs14003134	sex, batch, w42	10@99.2:7@103
OF_total_velocity_trial1	10	99	4.7	3.0	528.18+/-134.16	-619.00+/-192	95	110	Gg_rs15060526	Gg_rs14139143	sex, batch, w42	-
OF_total_velocity_average	10	101	9.9	4.6	649+/-150	-1006.00+/-230	96	107	Gg_rs14001676	Gg_rs14003134	sex, batch, w43	7@104:10@101
OF_minimum_total_movement	10	182	13.7	7.4	0.98+/-0.19	-0.59+/-0.26	176	188	Gg_rs14008254	GG_rs14951592	sex, batch, arena, w42,	2@513:10@182,10@182:17@1
OF_total_movement_average	10	185	8.2	4.7	0.72+/-0.19	-0.64+/-0.24	178	192	Gg_rs14008254	GG_rs14951592	sex, batch, w42	2@513:10@185
OF_maximum_total_movement	10	185	7.2	4.2	0.72+/-0.23	-0.67+/-0.29	178	192	Gg_rs14008254	GG_rs14951592	sex, batch, arena, w42,	2@513:10@185
OF_total_velocity_trial2	13	32	15.0	7.1	38.92+/-684.03	557.00+/-1083	28	40	GG_rs15676474	Gg_rs14991095	sex, batch, arena, w42,	1@591:13@32
OF_minimum_total_movement	17	1	14.0	7.6	0.55+/-0.17	-0.49+/-0.22	0	7	Gg_rs15035175	Gg_rs15033588	sex, batch, arena, w42,	10@182:17@1, 17@1:8@188
OF_total_velocity_average	17	1	15.0	7.2	611+/-129	-473.00+/-174	0	8	Gg_rs15035175	Gg_rs15033588	sex, batch, w44	17@1:8@194

Table S3. cis and trans eQTL

Probeset	Chromosome	LOD	Start	End
cis eQTL				
ENSGALT00000015891_LOC425783	1	4,9	0	406616
X603602527F1	1	5,3	366532	13314543
NM_001080210_FBXO18	1	4,4	366532	13314543
ENSGALT00000013813_EXOC4	1	5,0	366532	7500297
ENSGALT00000013814_EXOC4	1	4,8	366532	13314543
X603601503F1	1	5,7	366532	13314543
NM_204242_SEMA3E	1	4,0	681331	13314543
ENSGALT00000013515_LOC417722	1	5,5	7500297	14684440
X603868019F1	1	14,6	11301086	14684440
ENSGALT00000013087_PRKAR2B	1	5,0	11301086	20897163
X603143689F1	1	10,3	13314543	15697517
X603862742F1	1	5,5	14684440	17350997
ENSGALT00000000612_LOC769589	1	4,2	15697517	22215535
X603598166F1	1	4,0	15697517	22215535
ENSGALT00000030715_GPR37	1	4,1	17350997	25294185
ENSGALT00000014244_Q5ZKE1_CHICK	1	5,7	20897163	25294185
ENSGALT00000039114_Q5ZKE1_CHICK	1	5,6	20897163	25294185
X603597317F1	1	9,6	22215535	25294185
X603862804F1	1	4,1	34953886	37164711
ENSGALT00000016620_Q5F3D6_CHICK	1	4,6	37305405	43406820
X603595948F1	1	4,1	37305405	46086451
X603867954F1	1	4,4	37305405	46086451
NM_205208_CSRP2	1	4,1	37305405	46086451
ENSGALT00000038403_ACSS3	1	4,7	39668108	46086451
X603599019F1	1	4,5	39668108	46086451
ENSGALT00000019176_LOC769813	1	6,0	47775844	49952044
X603600031F1	1	5,9	50526061	53791052
ENSGALT00000030602_TOMM22	1	4,0	50526061	53791052
ENSGALT00000020279_NOL12	1	4,1	50526061	61816151
ENSGALT00000005090_LGALS2	1	5,8	52692420	61816151
ENSGALT00000037764_HMOX1_CHICK	1	6,8	52692420	61816151
ENSGALT00000030578_ENSGALG00000019306	1	4,4	52692420	61816151
NM_205344_HMOX1	1	5,6	55252063	61816151
ENSGALT00000037765_HMOX1_CHICK	1	4,9	55252063	61816151
X603142166F1	1	4,6	55252063	62215030
X603601419F1	1	6,4	55252063	62215030
X603601636F1	1	4,5	58811848	62215030
ENSGALT00000030545_ENSGALG00000019288	1	7,4	63570648	65535880
ENSGALT00000016631_TRY2_CHICK	1	4,9	71708614	81200026
ENSGALT00000037033_LOC768949	1	4,0	71708614	81200026
ENSGALT00000037035_LOC768949	1	4,6	71708614	81200026
ENSGALT00000037035_LOC768949	1	4,6	71708614	81200026
ENSGALT00000024768_ZPLD1	1	4,8	85140306	103169846
ENSGALT00000024772_CBLB	1	5,4	85140306	95241842
ENSGALT00000024920_GPA33	1	4,7	85140306	103169846
ENSGALT00000025763_DONSON	1	4,2	95241842	116783698
ENSGALT00000026212_SRPX	1	6,0	111555803	118226201
X603602790F1	1	4,8	114294426	118226201
ENSGALT00000026377_Q5F3B4_CHICK	1	4,6	117001710	123301222
ENSGALT00000036634_Q5F3B4_CHICK	1	5,2	117001710	123301222

NM_001012823_ACOT9	1	4,1	117001710	127009642
ENSGALT00000026387_PRDX4	1	5,4	117001710	127009642
ENSGALT00000026654_PHKA2	1	4,2	119902514	128374688
ENSGALT00000026744_B9A0U5_CHICK	1	6,1	125853070	128374688
ENSGALT00000026441_CNKSR2	1	4,3	127009642	134165693
ENSGALT00000036617_ARHGAP6	1	4,1	127009642	134165693
ENSGALT00000026830_STS	1	10,9	127009642	134165693
ENSGALT00000042197_SNORD89	1	4,5	127009642	134165693
ENSGALT00000027011_Q8AY37_CHICK	1	4,5	131662689	138107118
X603598304F1	1	10,2	134165693	138107118
ENSGALT00000022889_Q5ZL81_CHICK	1	12,9	138107118	142616238
ENSGALT00000027130_Q5ZL81_CHICK	1	8,6	138107118	145489901
X603142577F1	1	10,3	138107118	145489901
NM_001008681_ABHD13	1	4,1	140943462	149775518
ENSGALT00000027247_ITGBL1	1	7,4	145489901	149775518
X603002887F1	1	5,1	149775518	160055504
ENSGALT00000027361_TBC1D4	1	4,1	149775518	160055504
NM_001030821_IRG1	1	11,1	153797834	160055504
X603603033F1	1	6,4	166869980	172472876
ENSGALT00000027404_KIAA0564	1	4,1	166869980	172472876
X603863824F1	1	5,5	166869980	172472876
ENSGALT00000027507_NEK3	1	4,2	171456273	190334672
X603865290F1	1	6,3	174416546	180412143
NM_001006276_RFC3	1	4,5	174416546	180412143
X603863736F1	1	4,1	178512896	190334672
ENSGALT00000036449_VGFR1_CHICK	1	4,4	185988681	195271649
NM_001030837_RRM1	1	5,5	197685958	200400568
NM_001006284_POLD3	1	4,5	200157335	200400568
X603867141F1	2	5,4	8247809	14516677
ENSGALT00000011997_ARMC4	2	4,2	12757321	18391048
NM_001006356_ACBD5	2	4,5	12757321	18391048
X603866182F1	2	4,4	14516677	18391048
ENSGALT00000041376_ENSGALG00000024463	2	10,0	16687105	23979784
ENSGALT00000014467_ABCB1	2	9,8	19374198	23979784
NM_204894_ABCB1	2	12,7	19374198	23979784
ENSGALT00000038221_O93437_CHICK	2	11,2	19374198	23979784
X603143106F1	2	4,6	19374198	23979784
ENSGALT00000014705_GTPBP10	2	4,7	19374198	33358070
X603595549F1	2	4,2	19374198	23979784
NM_001012558_TMEM106B	2	15,9	21258215	28864817
ENSGALT00000017444_Q5F3Z0_CHICK	2	16,4	21258215	28864817
X603598188F1	2	11,2	21258215	28864817
X603600947F1	2	16,9	21258215	28864817
X603865923F1	2	10,1	21258215	33358070
X603868123F1	2	9,5	21258215	33358070
X603865064F1	2	9,2	23979784	33358070
ENSGALT00000018327_DPH3	2	4,2	28864817	38485435
X603596713F1	2	5,7	33358070	42847007
X603868164F1	2	4,1	33358070	45477693
X603866511F1	2	5,0	42847007	47702478
ENSGALT00000037515_SFRP4	2	4,3	42847007	47702478
NM_001007080_BMPER	2	4,5	45477693	55514291
ENSGALT00000020160_MRPL32	2	7,8	45477693	58012316

ENSGALT00000020160_MRPL32	2	7,8	45477693	58012316
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X603864267F1	2	4,6	45477693	58012316
X603866284F1	2	4,4	45477693	58012316
X603142604F1	2	5,8	45477693	58012316
ENSGALT00000035372_ENSGALG00000021829	2	7,5	61273711	65980620
X603866954F1	2	6,2	61273711	65980620
ENSGALT00000020690_MBOAT1	2	4,2	63190640	67567760
X603862212F1	2	4,8	66188071	77969669
X603596980F1	2	4,8	68904015	77969669
ENSGALT00000021239_ROPN1L	2	7,0	73601341	81254347
ENSGALT00000037061_C9orf4	2	4,5	73601341	91075653
NM_001031006_CCDC127	2	5,6	73601341	93026599
X603144121F1	2	4,8	79389272	88587412
ENSGALT00000030981_ENSGALG00000019522	2	8,3	87119086	93026599
X603599695F1	2	8,0	88587412	93026599
X603602441F1	2	4,4	91075653	95616928
X603597550F1	2	4,7	91075653	95616928
ENSGALT00000022184_TTRAP	2	7,4	91075653	101180990
X603595629F1	2	15,2	91075653	95616928
X603598785F1	2	4,5	91075653	95616928
ENSGALT00000022615_ENSGALG00000019502	2	5,7	95616928	108997666
ENSGALT00000023564_Q91429_CHICK	2	8,1	101180990	108997666
NM_205341_MYL12A	2	4,5	101180990	108997666
ENSGALT00000036879_Q90724_CHICK	2	7,4	101180990	108997666
X603865192F1	2	10,7	101180990	107005177
ENSGALT00000035370_ENSGALG00000021827	2	10,9	108997666	115374976
X603865613F1	2	6,6	114148246	122001442
X603143814F1	2	5,0	116617839	122001442
ENSGALT00000025015_LOC771318	2	6,5	116617839	122001442
ENSGALT00000030858_Q6VYQ9_CHICK	2	4,4	116617839	129748552
X603863179F1	2	6,1	116617839	122001442
ENSGALT00000024919_CA8	2	6,4	118557441	122001442
NM_001006345_RPL7	2	4,3	119095013	129748552
ENSGALT00000036651_Q6VYQ9_CHICK	2	4,5	122001442	131923745
NM_204290_FABP4	2	5,3	122001442	131923745
NM_001030943_RIPK2	2	5,7	122001442	131923745
ENSGALT00000025642_OSGIN2	2	4,7	125103948	131923745
X603864833F1	2	6,3	125103948	131923745
X603595861F1	2	7,7	125103948	131923745
NM_204337_NBN	2	8,4	125103948	131923745
ENSGALT00000025647_DECR1	2	5,0	125103948	131923745
ENSGALT00000026445_CALB1_CHICK	2	4,1	125103948	131923745
ENSGALT00000025702_CDH17	2	4,4	125103948	134658855
X603601310F1	2	4,8	125103948	131923745
ENSGALT00000025752_Q6V0P0_CHICK	2	4,3	125103948	131923745
NM_001030945_CCNE2	2	4,2	125103948	131923745
ENSGALT00000030772_MRPL13	2	5,1	131923745	143872110
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ENSGALT00000025947_LOC428379	2	6,8	134658855	141590046
X603144230F1	2	8,5	144240933	154682550
ENSGALT00000026023_LOC420300	2	15,0	145437818	154682550
X603865519F1	2	9,1	145437818	154682550

X603864309F1	3	12,4	4521634	8002840
ENSGALT00000041367_ENSGALG00000024454	3	5,4	16012304	21992770
ENSGALT00000035398_C1orf115	3	4,0	16519956	21992770
ENSGALT00000016072_DEF_CHICK	3	6,0	19002192	27044082
NM_001031051_C1orf107	3	5,6	19002192	27044082
ENSGALT00000016296_PLB1	3	5,0	27044082	35110420
ENSGALT00000016352_KIF6	3	7,7	27044082	35110420
ENSGALT00000016469_GLO1	3	5,0	27044082	31683357
X603597324F1	3	4,8	27044082	35110420
NM_205323_ACTN2	3	7,5	31683357	41002891
NM_001012405_EDARADD	3	5,0	31683357	45526535
ENSGALT00000023213_Q5EFZ6_CHICK	3	4,1	31683357	45526535
X603599479F1	3	6,9	35275260	41002891
ENSGALT00000017618_ERO1LB	3	4,0	41002891	56172535
ENSGALT00000017896_GNG4	3	4,6	41002891	45526535
ENSGALT00000004102_GGPS1	3	4,5	41002891	45526535
X603600684F1	3	4,4	41002891	45526535
X603141543F1	3	8,0	41002891	45526535
ENSGALT00000018844_QKI_CHICK	3	4,0	41002891	49519821
ENSGALT00000018853_PACRG	3	4,0	41002891	50524750
ENSGALT00000018198_Q6JLA9_CHICK	3	4,1	42383185	56172535
ENSGALT00000018703_RPS6KA2	3	4,9	42383185	56172535
ENSGALT00000018845_QKI_CHICK	3	4,6	42383185	49519821
ENSGALT00000037580_AKAP12	3	4,8	42383185	57107507
ENSGALT00000037561_ENSGALG00000023060	3	4,9	42383185	56172535
ENSGALT00000022317_SERAC1	3	4,9	42383185	61258323
ENSGALT00000024711_SCML4	3	4,4	42383185	58914157
X603599288F1	3	6,2	42383185	49519821
ENSGALT00000021996_C6orf98	3	4,2	45526535	56172535
ENSGALT00000018087_KIAA0133	3	4,0	46585550	57107507
ENSGALT00000031806_Q5ZL46_CHICK	3	9,0	46585550	50524750
X603598254F1	3	5,8	46585550	54435208
ENSGALT00000022192_TIAM2	3	4,1	46585550	56172535
ENSGALT00000037550_TIAM2	3	4,6	46585550	50524750
X603595625F1	3	4,3	46585550	50524750
ENSGALT00000022346_PLAGL1	3	4,7	46585550	61258323
ENSGALT00000020095_GRM1	3	7,2	47228401	50524750
NM_001031069_EPM2A	3	9,3	47228401	50524750
X603863949F1	3	4,4	50524750	68395467
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ENSGALT00000004569_ENPP1	3	4,2	50524750	68395467
X603868124F1	3	4,4	50524750	74929724
X603868124F1	3	4,4	50524750	74929724
X603866058F1	3	4,0	50524750	56172535
ENSGALT00000022536_MAP7_CHICK	3	4,6	54435208	68395467
ENSGALT00000022584_FAM54A	3	4,8	54435208	70785013
X603862470F1	3	5,0	56172535	68395467
X603866243F1	3	5,1	57107507	70785013
X603866243F1	3	5,1	57107507	70785013
ENSGALT00000024129_FAM26F	3	5,1	57107507	68395467
ENSGALT00000024129_FAM26F	3	5,1	57107507	68395467
X603601231F1	3	5,6	58914157	68395467
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ENSGALT00000022704_LAMA2	3	8,5	58914157	65383400
ENSGALT00000022704_LAMA2	3	8,5	58914157	65383400
ENSGALT00000024014_MCM9	3	6,7	58914157	70785013
X603864382F1	3	6,7	61592118	74929724
X603867930F1	3	6,8	81906220	89309106
ENSGALT00000026596_HS1BP3	3	5,7	102997484	108000014
ENSGALT00000036857_HS1BP3	3	4,8	102997484	108000014
X603863855F1	4	7,0	1267185	1816999
X603596676F1	4	10,2	1267185	3665369
X603863273F1	4	9,1	1267185	3665369
ENSGALT00000007868_RAB9B	4	4,6	1267185	3665369
X603865711F1	4	9,9	1267185	3665369
X603595821F1	4	6,3	1267185	3665369
NM_001031115_DKC1	4	5,2	1267185	3665369
ENSGALT00000008105_DKC1_CHICK	4	6,5	1267185	3665369
ENSGALT00000010323_GPR112	4	6,8	1267185	4098763
X603864651F1	4	15,1	3665369	5071148
X603597044F1	4	4,8	3665369	5071148
NM_001031118_FAM122A	4	4,8	3665369	5071148
ENSGALT00000010691_ATP11C	4	4,0	4098763	7019896
X603142811F1	4	8,8	11442136	15351471
ENSGALT00000014414_CXorf39	4	4,4	12433131	25656420
ENSGALT00000016043_ZNF330	4	4,1	30479695	34600626
ENSGALT00000038470_Q5ZJF2_CHICK	4	8,2	30479695	37860292
ENSGALT00000017213_ENSGALG00000010577	4	4,5	30479695	52996490
NM_001012404_EPGN	4	4,2	30479695	34600626
ENSGALT00000018503_IGFBP7	4	4,0	43210609	53839212
ENSGALT00000020122_CISD2	4	4,4	57822163	68340521
ENSGALT00000021879_PDLI3_CHICK	4	4,4	57822163	68340521
ENSGALT00000032107_PDGFRL	4	4,0	59340250	70644714
ENSGALT00000022204_ZDHHC2	4	4,1	59340250	71833345
NM_001004401_PPAT	4	4,1	59340250	70644714
ENSGALT00000022312_PUR1_CHICK	4	5,9	62983124	70644714
X603866410F1	4	4,7	66719658	71833345
ENSGALT00000037420_N4BP2	4	6,1	66719658	71833345
X603864570F1	4	4,6	70644714	76801898
X603602358F1	4	11,3	70644714	76801898
ENSGALT00000023261_ENSGALG00000014396	4	4,4	70644714	82659753
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ENSGALT00000023419_CD38	4	6,0	73138498	83961289
X603599417F1	4	6,7	73138498	82659753
X603863527F1	4	4,0	73138498	83961289
ENSGALT00000024208_STX18	4	4,3	73138498	83961289
X603598729F1	4	15,3	76801898	82659753
X603863258F1	4	22,1	76801898	82659753
ENSGALT00000024175_OTOP1	4	9,5	76801898	82659753
NM_001004429_TACC3	4	5,3	84031497	87177230
ENSGALT00000025333_Q5ZLP9_CHICK	4	5,8	84031497	87177230
NM_204403_BUB1B	5	4,0	83465	3871281
ENSGALT00000039501_USH1C	5	10,5	5571125	16303985
ENSGALT00000010667_LOC770392	5	4,8	5571125	16303985
ENSGALT00000014000_LOC771349	5	5,4	21008191	32709570
NM_001004405_CAPN3	5	4,9	24177749	32709570

ENSGALT00000022453_ENSGALG00000013838	5	7,2	27607021	32709570
X603865840F1	5	6,1	29578341	39187331
X603601654F1	5	12,6	29578341	39187331
X603596306F1	5	5,4	29578341	41050901
X603868077F1	5	4,7	29578341	39187331
X603143975F1	5	14,3	34951482	39187331
NM_001007935_BRMS1L	5	5,6	38043869	41050901
NM_001039302_SIP1	5	5,0	38043869	41050901
ENSGALT00000016505_Q5ZJH1_CHICK	5	4,4	38043869	41050901
ENSGALT00000016593_C14orf45	5	7,8	38043869	41050901
X603600251F1	5	5,9	39187331	47913881
X603868322F1	5	4,1	39187331	52405269
X603867292F1	5	7,3	45899976	52405269
X603598306F1	5	4,0	47913881	53310912
X603863866F1	5	5,7	54756251	59333159
ENSGALT00000037279_MDGA2	5	4,7	58169099	59333159
X603598388F1	6	4,2	1545609	8141307
ENSGALT00000041354_LOC423636	6	4,5	1545609	13429082
X603596512F1	6	4,0	2327260	6939525
ENSGALT00000003834_DYDC1	6	4,3	2327260	11071977
ENSGALT00000004257_DNAJC9	6	4,8	2327260	6939525
X603597362F1	6	4,6	2930562	6939525
X603865854F1	6	4,6	6939525	11071977
X603599919F1	6	5,0	6939525	11071977
NM_001031222_SLC16A9	6	10,7	6939525	11071977
ENSGALT00000004715_PLEKHK1	6	4,1	8141307	11071977
ENSGALT00000005033_MOT9_CHICK	6	11,9	8141307	11071977
X603600511F1	6	4,5	11071977	18893102
X603602109F1	6	4,3	11071977	17574768
X603866766F1	6	8,0	13429082	18893102
ENSGALT00000009577_ANXA8	6	8,6	15310578	18893102
ENSGALT00000040000_Q52P71_CHICK	6	4,9	17574768	25762392
ENSGALT00000010634_TMEM20	6	5,4	17574768	25762392
ENSGALT00000011313_TDT_CHICK	6	4,9	17574768	25762392
X603862991F1	6	4,2	17574768	25762392
X603599830F1	6	6,0	22733878	25762392
X603568048F1	6	4,8	23508327	33406702
X603862885F1	6	4,0	23508327	31897003
X603601127F1	6	6,2	25762392	31897003
ENSGALT00000014156_RBM20	6	4,4	25762392	31897003
ENSGALT00000039061_ENSGALG00000023527	6	8,6	25762392	31897003
ENSGALT00000014431_VTI1A	6	5,2	25762392	31897003
ENSGALT00000014801_TRUB1	6	4,4	25762392	33406702
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X603863661F1	6	7,6	28521405	33406702
ENSGALT00000015227_SFXN4	6	6,9	28521405	33406702
X603599016F1	6	5,5	28521405	33406702
ENSGALT00000015809_Q5F351_CHICK	6	4,6	31897003	33406702
ENSGALT00000003720_Q5F3K3_CHICK	7	4,2	1635843	3641233
ENSGALT00000034035_ENSGALG00000021145	7	4,4	1635843	3641233
ENSGALT00000006250_CO6A3_CHICK	7	4,2	2430814	5572688
X603868103F1	7	4,3	5179026	13578026
ENSGALT00000013654_ALS2CR4	7	5,5	12322416	14469650

X603601250F1	7	4,5	12322416	14469650
ENSGALT00000014791_O42096_CHICK	7	5,2	16301838	19669162
ENSGALT00000018729_XRCC5	7	11,3	23444134	27877398
X603864485F1	7	5,8	35109841	37306479
X603863672F1	7	7,2	36362022	37306479
X603599147F1	8	4,5	4126408	21627386
ENSGALT00000033698_LOC424428	8	4,3	4126408	8371160
X603601261F1	8	4,6	4126408	18297294
ENSGALT00000009468_CCDC18	8	4,8	4126408	18297294
ENSGALT00000009470_CCDC18	8	4,7	4126408	18297294
X603599396F1	8	5,3	4126408	18297294
ENSGALT00000014128_SYDE2	8	4,0	4126408	21627386
X603141831F1	8	4,4	4126408	18297294
X603141830F1	8	12,8	4126408	12853035
X603142079F1	8	8,4	7547459	18297294
NM_001012596_FAM129A	8	16,0	7547459	14397546
X603868076F1	8	5,4	7547459	18297294
ENSGALT00000008936_CCHL_CHICK	8	4,3	7547459	21627386
X603866233F1	8	5,0	7547459	18297294
NM_204481_HS2ST1	8	4,5	7547459	21627386
X603602157F1	8	4,1	12853035	23936961
X603862689F1	8	5,1	25513686	29856293
X603597242F1	8	8,4	25513686	29856293
ENSGALT00000017754_LOC772391	8	7,0	25513686	29856293
NM_001031291_PDE4B	8	4,3	26648181	29856293
X603599542F1	8	4,8	26648181	29856293
ENSGALT00000032905_C1orf173	8	5,0	26648181	29856293
ENSGALT00000018575_RABGGTB	8	4,5	26648181	29856293
NM_204441_PROC	9	4,2	2544843	4332354
ENSGALT00000041251_ENSGALG00000024385	9	7,5	2544843	4332354
X603598875F1	9	9,7	2544843	4332354
ENSGALT00000035455_5S_rRNA	9	4,0	3277617	5142892
ENSGALT00000035451_5S_rRNA	9	4,9	3277617	5142892
ENSGALT00000035483_5S_rRNA	9	6,0	3277617	5142892
X603598638F1	9	7,1	5142892	14845755
X603144142F1	9	4,7	5142892	10175136
ENSGALT00000010106_ILKAP	9	4,2	5142892	10175136
X603144063F1	9	4,5	5142892	14845755
ENSGALT00000008844_VAMP2	9	4,4	5142892	14845755
X603577149F1	9	4,3	5142892	17633781
X603866863F1	9	5,0	8447508	14845755
ENSGALT00000011255_BDH_CHICK	9	5,5	8447508	14845755
X603862895F1	9	4,3	8447508	17633781
ENSGALT00000040228_ENSGALG00000023912	9	4,3	8447508	17633781
X603867866F1	9	5,5	10175136	15673485
ENSGALT00000011075_NCBP2	9	5,3	10175136	17633781
NM_001006547_BDH1	9	6,1	10175136	14845755
X603596685F1	9	15,7	10175136	14845755
ENSGALT00000011090_Q92062_CHICK	9	7,6	12748848	17633781
X603866968F1	9	4,9	12748848	14845755
ENSGALT00000011701_ENSGALG00000007231	9	5,1	12748848	17633781
X603865631F1	9	4,0	12748848	23249432
ENSGALT00000011741_IL1RAP	9	4,8	13218506	17633781

X603600966F1	9	6,8	13218506	17633781
ENSGALT00000014363_ATP11B	9	4,8	15673485	20745674
NM_205174_SKIL	9	6,8	17633781	23249432
ENSGALT00000015236_Q05951_CHICK	9	5,7	17633781	23249432
ENSGALT00000015237_Q05951_CHICK	9	6,0	17633781	23249432
X603867113F1	9	8,5	17633781	23249432
X603864983F1	9	15,8	17633781	23249432
X603598984F1	9	6,4	17633781	23249432
X603863957F1	9	7,4	17633781	23249432
ENSGALT00000015530_ARL14	9	8,7	17633781	23249432
ENSGALT00000039093_MLF1	9	4,6	17633781	23249432
ENSGALT00000034778_LOC415312	10	5,3	764133	4342469
ENSGALT00000006440_LARP6	10	4,2	3610700	6830363
X603862632F1	10	4,3	3610700	12602118
X603863210F1	10	4,8	3610700	6830363
X603862030F1	10	4,0	4342469	12602118
X603600179F1	10	7,9	13319695	15038239
X603599320F1	10	5,3	17399683	20781391
X603601549F1	10	5,3	19430079	20781391
X603603027F1	11	11,2	5192111	9911245
X603864355F1	11	10,2	5192111	9911245
NM_001005843_UQCRFSL1	11	6,5	5192111	9911245
ENSGALT00000007212_TSHZ3	11	4,2	8487357	12069070
ENSGALT00000042240_gga.mir.1634	11	5,0	9911245	14396075
NM_001001760_CDH13	11	5,9	15190886	20762093
NM_001134359_FANCA	11	6,7	18677745	21105796
ENSGALT00000002333_STAB1	12	4,5	1215858	5341982
ENSGALT00000003879_LOC770732	12	4,4	1215858	5341982
X603863412F1	12	4,0	1215858	5341982
ENSGALT00000007193_PARP3	12	5,2	1215858	5341982
ENSGALT00000036286_ENSGALG00000022656	12	5,2	1649965	5341982
ENSGALT00000003330_TUSC4	12	5,0	2181145	5341982
ENSGALT00000036273_CCDC48	12	4,7	2483263	6113162
ENSGALT00000008165_HDAC11	12	5,0	2483263	6113162
X603603373F1	12	5,2	2483263	6113162
X603595621F1	12	5,9	11017459	14051161
ENSGALT00000036229_ENSGALG00000022639	12	4,6	12040054	16879345
X603862915F1	12	4,7	14051161	18023160
X603568189F1	13	4,1	875751	2424019
X603597807F1	13	4,2	2424019	10105991
ENSGALT00000011745_Q90591_CHICK	13	6,0	4116054	8702725
ENSGALT00000041081_Q90591_CHICK	13	7,2	4116054	8702725
X603598354F1	13	7,8	4116054	8702725
X603866983F1	13	8,3	4116054	8702725
ENSGALT00000004213_GRPEL2	13	5,0	4116054	10105991
ENSGALT00000004517_ERGIC1	13	5,7	5555459	11093750
X603868001F1	13	4,2	11093750	17914800
X603868338F1	13	4,1	11093750	17914800
NM_205095_MAPK9	13	6,4	11093750	17914800
X603863190F1	14	4,0	742709	5105858
ENSGALT00000005510_BHLHB8	14	7,1	742709	2699489
NM_001030636_TOP3A	14	6,6	3600574	8716372
ENSGALT00000033826_ENSGALG00000021043	14	7,3	3600574	8716372

X603863473F1	14	7,5	3600574	8716372
X603865473F1	14	4,3	3600574	10172497
X603596248F1	14	7,3	3600574	10172497
NM_001012522_ABCC1	14	4,3	3600574	8716372
X603600762F1	14	5,2	5105858	8716372
X603868033F1	14	8,4	5716424	10172497
ENSGALT00000009821_DCTN5	14	6,7	5716424	10172497
X603599183F1	14	5,2	5716424	10172497
ENSGALT00000010357_Q5F3U4_CHICK	14	5,0	5716424	10172497
X603144657F1	14	6,3	5716424	10172497
ENSGALT00000039948_TMC7_CHICK	14	4,1	5716424	10172497
X603595988F1	14	4,2	5716424	10172497
X603603312F1	14	10,4	5716424	10172497
X603863911F1	14	8,5	5716424	10172497
ENSGALT00000011592_RMI2_CHICK	14	6,7	5716424	10172497
NM_001006174_C16orf75	14	7,8	5716424	10172497
ENSGALT00000011682_FAM18A	14	9,7	5716424	10172497
ENSGALT00000011879_Q5F3R3_CHICK	14	4,1	5716424	10172497
ENSGALT00000012057_Q8UWH0_CHICK	14	4,1	5716424	10172497
X603862792F1	14	4,1	5716424	10172497
ENSGALT00000015211_CRAMP1L	14	4,1	5716424	10172497
X603597475F1	14	12,5	5716424	10172497
X603866743F1	14	4,6	12196898	15410006
X603862496F1	14	5,8	12196898	15410006
X603867381F1	14	4,2	12196898	15410006
X603598221F1	15	6,6	103493	1588362
X603144222F1	15	4,3	103493	1588362
NM_001007836_C22orf25	15	4,1	103493	4043393
X603601550F1	15	5,1	103493	1588362
X603603039F1	15	7,1	1949871	6012728
X603598106F1	15	4,4	4043393	9388341
X603862791F1	15	4,1	4043393	9388341
X603601138F1	15	5,3	4043393	9388341
ENSGALT00000038278_CCDC60	15	4,5	4043393	9388341
X603599406F1	15	4,9	8451459	10677054
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ENSGALT00000000234_Q5PY04_CHICK	16	5,3	93395	161670
NM_213582_CLEC2D	16	5,3	93395	161670
ENSGALT00000001628_Q3LUJ2_CHICK	16	5,3	93395	161670
ENSGALT00000000176_ENSGALG00000000126	16	4,5	93395	161670
NM_001099356_LOC427095	16	8,5	93395	161670
ENSGALT00000031517_KIFC1	16	8,5	93395	161670
ENSGALT00000041177_ENSGALG00000024359	16	7,8	93395	161670
ENSGALT00000041163_ENSGALG00000024352	16	10,5	93395	161670
ENSGALT00000041162_ENSGALG00000024351	16	5,0	93395	161670
NM_001030673_B.G	16	6,0	93395	161670
X603142468F1	16	4,2	93395	161670
ENSGALT00000014372_SLC31A1	17	4,7	989540	4068238
ENSGALT00000011523_TENA_CHICK	17	5,0	989540	4068238
ENSGALT00000040050_Q50L61_CHICK	17	4,5	1502377	4068238
ENSGALT00000008132_FAM102A	17	5,8	4068238	6076178
NM_001197034_C9orf16	17	5,9	6076178	8788041
ENSGALT00000007637_C9orf16	17	5,4	6076178	8788041

X603865387F1	17	4,1	6076178	8788041
NM_001013395_ASS1	17	4,6	6076178	8788041
ENSGALT00000006389_ASSY_CHICK	17	4,4	6076178	8788041
X603596287F1	17	10,8	6076178	7278120
X603577223F1	17	9,5	6076178	7278120
ENSGALT00000034763_KIAA1529	17	5,7	6076178	7278120
X603867214F1	17	5,4	6076178	8788041
X603596177F1	17	4,7	6076178	10218896
X603602420F1	17	7,5	7854285	10218896
ENSGALT00000038911_B3GALT4	17	4,4	7854285	10218896
NM_205240_ST6GALNAC1	18	7,4	3045583	4925696
X603602435F1	18	4,2	3045583	7417296
X603862865F1	18	10,5	4226902	5855624
X603864422F1	18	5,5	6201651	9437165
ENSGALT00000018981_ENSGALG00000011617	18	4,2	7417296	9437165
ENSGALT00000012452_MRPL27	18	4,9	7417296	9437165
ENSGALT00000040475_LOC431205	20	5,2	1192309	3161038
ENSGALT00000040467_ENSGALG00000023997	20	6,3	1192309	3161038
X603597894F1	20	4,1	1192309	3161038
X603598011F1	20	5,7	1192309	3161038
X603600905F1	20	4,6	3161038	6177275
X603595816F1	20	4,6	4136908	7847356
X603601366F1	20	4,3	6904824	9630882
ENSGALT00000039008_ENSGALG00000020920	20	4,8	9630882	13068468
X603862244F1	20	6,0	9630882	13201606
X603142182F1	20	4,8	9630882	13201606
ENSGALT00000012637_PFDN4	20	4,7	10678946	13201606
ENSGALT00000042433_gga.mir.1687	20	7,8	10678946	13201606
X603867077F1	21	8,2	630420	1784095
X603866295F1	21	4,9	2684266	6694869
ENSGALT00000006507_PRDM2	21	4,4	2684266	6694869
X603864183F1	21	4,2	4622435	6694869
ENSGALT00000029001_gga.mir.200a	21	5,1	4622435	6694869
X603864620F1	21	6,4	4622435	6694869
X603598688F1	21	5,1	4622435	6694869
NM_205209_SLC2A1	21	7,2	5546680	6708327
X603866361F1	23	5,2	182018	2444101
ENSGALT00000000878_Q800V4_CHICK	23	5,8	182018	2444101
ENSGALT00000000879_Q800V4_CHICK	23	5,1	182018	2444101
ENSGALT00000041206_Q800V4_CHICK	23	6,1	182018	1032588
ENSGALT00000041207_Q800V4_CHICK	23	5,4	182018	1032588
NM_001030889_FABP3	23	5,5	182018	2444101
NM_001030891_PPP1R8	23	4,6	182018	2444101
ENSGALT00000001485_Q5ZLA7_CHICK	24	5,5	1135292	2603372
X603867427F1	24	5,9	1812729	3041589
X603600958F1	24	4,7	4228274	6273428
ENSGALT00000040673_Q2TV23_CHICK	26	4,0	219282	2320301
ENSGALT00000040182_CAZA1_CHICK	26	4,6	2320301	3822138
ENSGALT00000002879_RSBN1	26	4,8	2320301	4567958
X603597281F1	26	6,2	3048852	4567958
ENSGALT00000040923_LOC431352	27	4,5	376706	1861637
ENSGALT00000040915_LOC770241	27	5,4	376706	1861637
ENSGALT00000012755_LOC770204	27	5,4	376706	1861637

ENSGALT00000040905_LOC770241	27	4,1	376706	1861637
X603866012F1	27	5,4	1205103	3253573
ENSGALT00000002026_ATP5G1	27	5,3	2322999	4341846
X603862580F1	28	8,4	991556	1608048
X603862171F1	28	4,8	991556	1608048
X603862231F1	28	4,4	991556	2008685
X603601715F1	28	4,1	991556	2008685
X603595508F1	28	7,8	991556	2008685
X603143365F1	28	7,0	991556	2008685
X603599785F1	28	13,4	2008685	2818024
ENSGALT00000004744_SF4	28	4,3	2144111	3803332
X603142039F1	28	4,7	2144111	3803332
X603864916F1	28	7,8	2144111	3803332
X603862309F1	28	14,1	2144111	3803332
X603865163F1	28	15,2	2144111	3803332
ENSGALT00000005236_CRTC1	28	5,2	2144111	3803332
X603600130F1	28	7,4	2818024	4212418
X603600130F1	28	7,4	2818024	4212418

trans eQTL

ENSGALT00000031996_ENSGALG00000008198	1	6,0	0	406616,00
X603599147F1	1	6,1	366532	2129951,00
ENSGALT00000041345_ENSGALG00000024432	1	6,7	34953886	37164711,00
ENSGALT00000030858_Q6VYQ9_CHICK	1	6,4	53791052	61816151,00
X603599638F1	1	6,6	71708614	81200026,00
ENSGALT00000009242_ENSGALG00000005756	1	7,3	73974022	81200026,00
ENSGALT00000018474_NEK10	1	8,6	79869493	84853091,00
ENSGALT00000026712_TMEM27	1	6,2	158224386	171456273,00
ENSGALT00000014634_STEAP4	1	7,4	158224386	171456273,00
X603599277F1	1	7,3	171456273	177200150,00
ENSGALT00000036647_ENSGALG00000022793	1	6,3	182023225	190334672,00
X603602450F1	1	6,2	182023225	190334672,00
X603598770F1	1	8,6	182023225	190334672,00
X603142727F1	1	6,1	185988681	192029819,00
X603142727F1	1	6,1	185988681	192029819,00
X603865923F1	1	6,3	200157335	200400568,00
ENSGALT00000014188_Q08515_CHICK	2	6,5	19374198	23979784,00
ENSGALT00000031934_VASH2	2	6,6	19374198	23979784,00
ENSGALT00000012402_C10orf76	2	6,4	19374198	23979784,00
X603602192F1	2	6,1	19374198	23979784,00
X603142838F1	2	6,2	19374198	23979784,00
X603866246F1	2	6,2	21258215	122001442,00
X603865293F1	2	9,1	45477693	51905237,00
NM_001033643_CR1L	2	6,0	63190640	67567760,00
ENSGALT00000037058_RAP1GAP	2	6,5	116617839	122001442,00
ENSGALT00000026026_PSCA	2	6,1	118557441	122001442,00
ENSGALT00000028942_gga.mir.29a	2	6,9	154026560	154682550,00
X603600958F1	3	6,5	19002192	25988814,00
ENSGALT00000015254_ACCN5	3	6,5	27044082	31683357,00
ENSGALT00000037384_ENSGALG00000022999	3	6,5	29563951	35110420,00
X603577242F1	3	7,8	106743551	110258389,00
ENSGALT00000016016_FOXG1_CHICK	3	6,9	106743551	110258389,00
ENSGALT00000001708_LOC419390	3	12,8	106743551	110258389,00

ENSGALT00000035200_LOC419390	3	7,7	106743551	110258389,00
NM_001006276_RFC3	4	8,2	1267185	3665369,00
ENSGALT00000037384_ENSGALG00000022999	4	7,8	1267185	1816999,00
NM_205343_ASMT	4	7,1	9694471	11442136,00
X603862324F1	4	6,7	9694471	12433131,00
ENSGALT00000021664_GYS2	4	6,4	15351471	29505460,00
X603862676F1	4	6,1	30479695	34600626,00
X603600966F1	4	6,8	53839212	59340250,00
ENSGALT00000036505_ENSGALG00000022741	4	7,8	79090159	83961289,00
ENSGALT00000014634_STEAP4	5	6,7	39187331	45899976,00
ENSGALT00000037384_ENSGALG00000022999	5	6,5	54756251	59333159,00
X603864553F1	7	6,5	12322416	14469650,00
ENSGALT00000037680_C4orf32	7	7,3	12322416	14469650,00
NM_001030636_TOP3A	7	6,5	13578026	16301838,00
NM_001012880_PRIM2	7	9,6	15017822	17907290,00
NM_204438_CCL20	7	15,2	15017822	17907290,00
NM_204765_MEOX1	7	8,4	15017822	17907290,00
ENSGALT00000031027_SPB10_CHICK	7	10,2	16301838	19669162,00
ENSGALT00000037186_FANCM	9	7,4	17633781	23249432,00
X603142454F1	10	6,1	3610700	6830363,00
ENSGALT00000026377_Q5F3B4_CHICK	10	6,5	9525779	13319695,00
ENSGALT00000036634_Q5F3B4_CHICK	10	7,9	9525779	13319695,00
NM_205159_MYL3	10	8,3	17399683	20781391,00
ENSGALT00000007043_PIGB	10	6,2	17399683	20781391,00
X603599029F1	11	6,2	1303458	5192111,00
ENSGALT00000039200_PIM3	12	6,9	1215858	5341982,00
ENSGALT00000040727_PSMD11	12	6,1	1215858	5341982,00
ENSGALT00000041040_LOC426373	12	6,1	1215858	5341982,00
ENSGALT00000040323_C11orf2	12	6,2	1649965	5341982,00
ENSGALT00000016866_LRRC41	12	6,4	1649965	5341982,00
ENSGALT00000004187_ENSGALG00000021472	12	6,1	1649965	5341982,00
ENSGALT00000008014_SMCR7	12	6,2	1649965	5341982,00
ENSGALT00000012813_RNF215	12	6,3	1649965	5341982,00
ENSGALT00000014848_ENSGALG00000009124	12	6,2	1649965	5341982,00
ENSGALT00000039033_Q90689_CHICK	12	7,3	1649965	5341982,00
ENSGALT00000039242_ENSGALG00000023592	12	7,1	1649965	2483263,00
ENSGALT00000041153_ENSGALG00000024345	12	6,1	1649965	5341982,00
NM_001012842_BSDC1	12	6,2	1649965	5341982,00
X603864687F1	12	6,3	1649965	5341982,00
X603142827F1	12	6,6	2181145	5341982,00
ENSGALT00000005827_NTNG2	12	6,8	2181145	5341982,00
ENSGALT00000038359_SLC25A10	12	6,4	2181145	5341982,00
X603864922F1	12	6,1	14051161	18023160,00
ENSGALT00000023113_SLC25A22	12	6,0	14051161	18023160,00
ENSGALT00000031336_ENSGALG00000019731	13	6,4	1519484	4116054,00
ENSGALT00000021111_Q5ZKV2_CHICK	13	6,5	2424019	8702725,00
ENSGALT00000006763_A4L9I7_CHICK	14	7,2	5716424	10172497,00
X603597584F1	14	6,2	5716424	10172497,00
NM_204897_SERPINB10	15	7,1	4043393	9388341,00
X603597236F1	15	6,1	4043393	9388341,00
X603862655F1	15	9,5	4043393	9388341,00
X603868108F1	15	7,1	4043393	9388341,00
NM_206990_UTS2	15	11,5	4043393	9388341,00

X603862324F1	18	7,1	536515	4226902,00
X603602064F1	18	6,1	4226902	7417296,00
X603867663F1	25	6,8	1382096	2000000,00
X603866524F1	25	6,1	1382096	2000000,00
ENSGALT00000041377_LOC428499	25	6,5	1382096	2000000,00
ENSGALT00000031581_ENSGALG00000016493	25	6,2	1382096	2000000,00
ENSGALT00000032066_ENSGALG00000014401	25	7,4	1382096	2000000,00
ENSGALT00000042595_U6	25	6,4	1382096	2000000,00
ENSGALT00000034985_LRRC38	25	6,5	1382096	2000000,00
ENSGALT00000010544_NCAPG2	25	6,0	1799755	2000000,00
X603862865F1	28	6,5	991556	1608048,00
X603142079F1	8_random	8,1	33415	387677,00
NM_001012596_FAM129A	8_random	15,4	33415	379929,00
NM_001012596_FAM129A	8_random	15,4	33415	379929,00
X603141830F1	8_random	12,0	33415	387677,00

Table S4. Number of eQTL correlated with traits for which candidate genes identified.

Trait	number of eQTL correlated with trait	% of eQTL correlated with trait
time in centre trial2	52	0.10
minimum total movement	59	0.11
average velcoity	83	0.16
maximum total movement	68	0.13
total movement trial1	53	0.10
average total movement	67	0.13
velocity trial1	68	0.13
average total movement	42	0.08
velocity trial2	54	0.10

File S1. SUPPLEMENTAL METHODS

Associations with MDD, bipolar disorder and schizophrenia

The summary statistics for the PGC GWAS study on bipolar disorder, the PGC GWAS study on major depressive disorder (MDD) and the PGC GWAS study on schizophrenia were obtained and used to test for putative associations between nine of the ten candidate genes identified (on further investigation the gene *LOC770352* was found to have only a relatively weak human orthologue and hence excluded). The summary statistics contained all the p-values calculated in the original paper, meaning it was not necessary to recalculate these scores. Associations were assessed at the orthologous gene positions +/- 120kb in the case of the bipolar dataset (the total average interval size for each gene was ~248kb, with these 120kb regions up and down-stream also being included to ensure local cis-eQTL control regions would also be analysed). In the case of the schizophrenia and MDD datasets as these were both meta-analyses and the number of individuals were so large (over 20k individuals in each schizophrenia study and 18k in the MDD study) and from so many diverse populations that the search was restricted solely to the orthologous gene position +/- 50kb due to the LD being greatly reduced in these datasets. To find a significance threshold, we randomly selected 1000 sections of the genome each time selecting the lowest p-value from each segment. As nine separate regions were being tested, the total interval size to select was 250kb x 9 = 2.25Mb for the bipolar studies, and 120kb x 9 = 1080kb for the schizophrenia and MDD studies. In this way, a 5% significance (P=0.0001 for the PGC bipolar study, $P=1x10^{-5}$ for the schizophrenia study, P=0.0002 for the MDD study) and a 20% suggestivity threshold (P=0.0004 for the PGC bipolar study, $P=1x10^{-4}$ for the schizophrenia study, P=0.001 for the

MDD study) was found, tailored to each individual study and the size and number of the regions tested.

Associations with Mouse HS cross

The mouse HS cross dataset was used to assess the ten candidate genes. This cross consists of repeated crossing between eight founder strains (with the repeated intercrossing between the different founders giving the short haplotype blocks and increased resolution). SNPs were selected in the orthologous gene regions, using the mus genome build-37, with the closest SNP to each gene selected. Three different open field behaviours – latency to move, total activity and time spent in the centre of the arena were used. Associations were tested using the freely available website (http://mus.well.ox.ac.uk/gscandb/), with the standard additive model option used. . Assessing significance is somewhat problematic as the significance threshold is only provided for a whole genome scan, rather than 10 individual SNPs as assessed here. A LOD score of ~4.8 was considered suggestive at a whole genome level and ~5.6 was significant for the full scan. We attempted a relatively crude randomisation test by randomly selecting 100 genes and recording the LOD score for each trait at the gene. This gave a significance threshold ~LOD 4 for each behavioural trait.