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Transcriptome Profiling in Rat Inbred Strains and Experimental Cross Reveals Discrepant Genetic Architecture of Genome-Wide Gene Expression

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Abstract

To test the impact of genetic heterogeneity on cis- and trans-mediated mechanisms of gene expression regulation, we profiled the transcriptome of adipose tissue in 20 inbred congenic strains derived from diabetic Goto-Kakizaki (GK) rats and Brown-Norway (BN) controls, which contain well-defined blocks (1Mb-183Mb) of genetic polymorphisms, and in 123 genetically heterogeneous rats of an (GKxBN)F2 offspring. Within each congenic we identified 73 to 1,351 differentially expressed genes (DEG), only 7.7% of which mapped within the congenic blocks, and which may be regulated in *cis*. The remainder localised outside the blocks, and therefore must be regulated in *trans*. Most trans-regulated genes exhibited approximately two-fold expression changes, consistent with mono-allelic expression. Altered biological pathways were replicated between congenics sharing blocks of genetic polymorphisms, but polymorphisms at different loci also had redundant effects on transcription of common distant genes and pathways. We mapped 2,735 eQTLs in the F2 cross, including 26% predominantly cis-regulated genes which validated DEG in congenics. A hotspot of over 300 eQTLs in a 10cM region of chromosome 1 was enriched in DEG in a congenic strain. However, many DEG among congenics, GK and BN did not replicate as eQTLs in F2 hybrids, demonstrating distinct mechanisms of gene expression when alleles segregate in an outbred population or are fixed homozygous across the entire genome or in short genomic regions. Our analysis provides conceptual advances in our understanding of the complex architecture of genome expression and pathway regulation and suggests a prominent impact of epistasis and mono-allelic expression on gene transcription.

Introduction

The analysis of expression quantitative trait loci (eQTL) can provide novel insights into the function of disease associated genes (CHEN *et al*. 2008; MONTI *et al*. 2008; PETRETTO *et al*. 2008; HEINIG *et al*. 2010) and gene pathways and networks (GHAZALPOUR *et al*. 2006; CHEN *et al*. 2008; EMILSSON *et al*. 2008; MONTI *et al*. 2008). The regulation of gene expression is orchestrated through complex local (cis-mediated) and distant (mediated in trans) mechanisms. eQTL studies in humans, which were until recently limited to cell systems, whole blood and biopsies from the most accessible organs (DIXON *et al*. 2007; EMILSSON *et al*. 2008; FAIRFAX *et al*. 2012; GRUNDBERG *et al*. 2012), have now been scaled up to genetic analysis of transcriptome regulation across a broad range of tissues of many healthy individuals (GTEX CONSORTIUM 2015). A key question is whether the predominantly cis-acting genetic architecture of gene regulation observed in studies of outbred populations, such as humans and heterogeneous stocks (HUANG *et al*. 2009), is the complete picture. These study designs are under-powered to detect trans-regulation, yet the reproducibility of gene expression is governed in large part by the extent to which it is controlled in cis as opposed to trans. Therefore experimental designs in which the genetic control of gene expression is forced to be in trans can reveal aspects of regulation that are normally hidden. The use of congenics, in which genetic variation is confined to specific genomic segments, but where variation in gene expression can be measured genome wide, is key to understanding trans effects.

eQTL experiments in animal models can contribute to improving knowledge of eQTL architecture and elucidating the function of disease susceptibility loci identified in genome-wide association studies (WILLIAMS and AUWERX 2015). A broad range of experimental mammalian systems developed in the laboratory mouse (BUCHNER and

NADEAU 2015) and rat (GAUGUIER 2005) allow the collection of organs from large cohorts of individuals maintained in strictly standardised conditions, thus limiting inter-individual phenotype variability, and provide powerful tools for eQTL mapping. The inbred Goto-Kakizaki (GK) rat model of type 2 diabetes mellitus was produced over many generations of breeding of rats from an outbred Wistar stock using glucose intolerance as the sole criterion for selecting breeders (GOTO *et al*. 1976). This process resulted in the isolation of the GK strain enriched for naturally occurring Wistar polymorphisms that contribute to diabetes and associated phenotypes, which we previously mapped by QTL analysis of pathophysiological phenotypes in F2 crosses between GK rats and Brown Norway (BN) controls (GAUGUIER *et al*. 1996; ARGOUD *et al*. 2006). Further physiological phenotyping and multi-tissue transcriptome profiling in a congenic strain designed to contain a large (~100Mb) QTL-rich region of the GK rat in a BN background validated QTL effects and suggested that congenics can be efficiently used to dissect out cis- and trans-mediated regulation of gene transcription (WALLIS *et al*. 2008).

Here we establish fundamental aspects of eQTL architecture and biology in adipose tissue, using three distinct but interrelated genetic settings namely (i) inbred GK and BN strains, (ii) a panel of 20 congenic strains that carry well-defined blocks of GK and BN haplotypes across a total of 35% of the rat genome and (iii) a large GKxBN F2 cross, in which we test congenic transcriptome results. Transcriptome profiling (Illumina bead arrays) is used to detect differentially expressed genes and pathways between congenics and control animals and to map cis- and trans-regulated eQTLs and eQTL hotspots in the cross. In the F2 cross, we are able to replicate only part of altered transcription regulation of genes and biological pathways detected in congenics. However, we show that the same biological pathways can be regulated by independent genomic loci,

suggesting redundant biological function of distinct gene sets. Differential transcriptional regulation in inbred strains and segregating populations supports the important role of gene x gene interaction (i.e. epistasis) in the control of genome expression.

Materials and Methods

Animals

A colony of GK/Ox rats bred at Biomedical Service Unit, University of Oxford, since 1995 from a GK/Par stock and BN rats obtained from a commercial supplier (Charles River Laboratories, Margate, UK) were used to produce a series of 20 BN.GK and GK.BN congenic strains using a genetic marker assisted breeding strategy (WALLIS *et al*. 2008). BN.GK congenics were designed to contain GK single genomic blocks introgressed onto the genetic background of the BN strain, whereas the reciprocal GK.BN congenics contain BN genomic blocks transferred onto a GK genetic background (Figure 1, Table S1). All congenic rats were genotyped as previously described (WALLIS *et al*. 2008) to monitor retention of donor alleles across the congenic interval and their elimination from the genetic background. Male GK, BN and congenic rats were used in all experiments. The F2 cross (n=123) between rats of the GK/Par colony and normoglycemic BN controls, previously derived to map QTLs for glucose tolerance, insulin secretion, adiposity and metabolomic variables(GAUGUIER *et al*. 1996; DUMAS *et al*. 2007), was used for eQTL mapping. The cohort consisted of 60 males and 63 females from two reciprocal crosses of 55 F2 rats originating from a GK female and 68 F2 rats originating from a BN female. Rats were allowed free access to tap water and standard laboratory chow pellets (B&K Universal Ltd, Grimston, Aldbrough, Hull, UK) and were maintained on a 12h light-dark cycle. At six months, animals were killed after an overnight fast and retroperitoneal fat pads (RFP) were rapidly dissected, snap frozen in liquid nitrogen and stored at -80C. Animal procedures were carried out under UK Home Office licences and approved by the ethical review panel of the University of Oxford.

RNA preparation

Total RNA was isolated from 100mg of frozen RFP using the RNeasy® 96 Universal Tissue kit (Qiagen, Crawley, UK). Briefly, frozen tissue samples were transferred into cooled RNeasy® 96 Universal Tissue plates, and homogenised in QIAzol Lysis Reagent using Qiagen's Tissue Lyser. Total RNA was purified using a spin technology according to the manufacturer's guidelines and eluted in 90µl of RNase-free water. RNA concentrations were determined using a NanoDrop spectrophotometer and RNA integrity was assessed using an Agilent 2100 Bioanalyser (Agilent Technologies, Waldbronn, Germany).

Illumina Bead Array hybridisation, scanning and data processing

Gene transcription profiling of RFP from F2 hybrids and from rats of the GK, BN and congenic strains (6 biological replicates per strain) was performed usingSentrix®BeadChipRatRef-12v1 Whole-Genome Gene Expression Arrays (Illumina Inc., San Diego, CA), which contain 22,523 oligonucleotide probes (replicated on average 30 times)allowing quantification of transcript levels for 21,910 genes. Biological replicates were individually hybridised to the arrays.

Double-stranded cDNA and purified biotin-labelled cRNA were synthesised from 300ng high quality total RNA using the Illumina® TotalPrep RNA amplification kit (Ambion Inc., Austin, TX). cRNA concentrations were determined using a NanoDrop spectrophotometer whilst cRNA quality and integrity were assessed using an Agilent 2100 Bioanalyser (Agilent Technologies, Waldbronn, Germany). Hybridisations onto the arrays were carried out using 750ng of each biotinylated cRNA. BeadChip arrays were scanned on the Illumia® Bead Array Reader (Illumina Inc., San Diego, CA). Data were analysed using the Illumina® BeadStudio Application software before undergoing comprehensive statistical analysis. Particular attention was given to the following

quality control parameters: $0 \leq$ G sat ≤ 1; Green 95 Percentile (GP95) for consistency between arrays (around 2000); GP5 background level in range of low 100 or below.

Whole genome sequencing of the GK/Ox strain (ATANUR *et al*. 2013) identified variants between GK and BN in 757 Illumina oligonucleotides (Table S2) which were excluded prior to array data analysis to avoid detection of spurious gene expression changes due to differences in binding between probes and oligonucleotides (ALBERTS *et al*. 2007). We verified absence of differential expression between GK, BN and relevant congenics for several of such genes (Figure S1). We also withdrew probes that detected only background signal (ie. Illumina detection score <0.5 in >50% of samples). Microarray data processing was carried out using normexp background correction and quantile normalization (SHI *et al*. 2010).

Microarray experiments were compliant with MIAME (Minimum Information About a Microarray Experiment) and both protocol details and raw data have been deposited in ArrayExpress (http://www.ebi.ac.uk/arrayexpress/) under the accession number E-MTAB-969 (F2 hybrids) and E-MTAB-1048 (BN, GK and congenic strains).

Genetic mapping of expression QTLs in the F2 (GKxBN) cross

eQTL analysis was performed using the R-qtl software package (BROMAN *et al*. 2003). We used genetic maps constructed in the cross with a combination of microsatellites and SNP markers (WILDER *et al*. 2004). Genome scans were carried out using the Haley-Knott regression method (HALEY and KNOTT 1992). To account for effects of sex and lineage on gene expression we used sex and cross direction as additive covariates in our models (SOLBERG *et al*. 2004). To obtain a genome wide significance threshold for each transcript, we conducted a permutation test with 1,000 permutations. QTLs with a genome-scan adjusted p-value <0.05 were retained as significant.

We used a regression model with sex and cross direction as additive covariants

176 $$(H_a) \quad y_i = \mu + \beta_c c_i + \beta_s s_i + \beta_g g_i + \varepsilon_i$$

177 With $\beta_c c_i$ denoting the effect of the cross, $\beta_s s_i$ denoting the effect of sex and $\beta_g g_i$
178 denoting the effect of the genotype.

179 **Statistical analysis of Illumina array data in the congenic strains**

180 Differential gene expression in the congenics was assessed by comparing array data in
181 each congenic strain to the relevant parental strain (GK for GK.BN congenics and BN
182 for BN.GK congenics). A linear model was fitted using LIMMA, accounting for effects
183 of experimental batches. Genes were tested for statistical differential expression using a
184 moderated t-statistic and a threshold of $p<0.05$.

185 **Pathway analyses**

186 To obtain functional categories that are enriched in the eQTL-controlled gene sets in the
187 F2 cross, we used a hypergeometric test on gene ontology terms and KEGG pathways
188 associated with these gene sets against the background of genes with detectable
189 expression (FALCON and GENTLEMAN 2007). For functional analysis of the congenic
190 transcriptomes, we used Gene Set Enrichment Analysis (GSEA) (SUBRAMANIAN *et al*.
191 2005). We detected pathways in the KEGG and reactome databases that are enriched
192 among the up- or down-regulated genes (KANEHISA and GOTO 2000; MATTHEWS *et al*.
193 2009). We used the standalone java version of GSEA 2.07 on the t-statistics of
194 differential expression with default parameters and n=1,000 permutations.

195 **Quantitative real-time PCR**

196 Total RNA was treated with Turbo DNA-free DNase kit, for removal of genomic DNA
197 (Ambion Inc., Austin, TX). First-strand cDNA synthesis was performed using
198 Superscript III First-strand Synthesis Supermix for qRT-PCR (Invitrogen, Paisley, UK).
199 Assays were performed on a Rotor-Gene 3000 system (Corbett Research, Milton, UK)
200 using the QuantiTect SYBR Green PCR kit (Qiagen Ltd., Crawley, UK). Analysis was

performed using the standard curve method (Rotor-Gene Software 5.0.47; Corbett Research, Milton, UK). This software allowed further verification that a single PCR product signal was quantified. Gene expression was normalised against the expression of either actin or acidic ribosomal phosphoprotein P0 (36B4). Experiments were performed in duplicate with samples prepared from six animals per group. Statistical significance was determined by the two-tailed independent sample t-test or univariate ANOVA, when testing more than two groups. Oligonucleotides designed to test gene expression are given in Table S3.

Results

Differential transcription regulation in adipose tissue in GK and BN strains

We initially surveyed genome-wide transcriptional patterns in adipose tissue of male GK and BN rats (n=6 per strain). We identified a total of 1221 genes showing evidence of significant differential expression (FDR<0.05) with an equivalent number of genes upregulated (514) and downregulated (707) in the GK (Table S4). The proportion of genes differentially expressed between strains was similar across chromosomes (between 3.1% of genes on chromosome 15 and 6.3% of genes on chromosome 10).

Transcriptional control by genetic polymorphism blocks in congenic strains

To establish the effects of blocks of linked genetic polymorphisms on gene transcription, we repeated fat transcriptome profiling in a series of reciprocal BN.GK and GK.BN inbred congenic strains (Figure 1, Table S4) using the same Illumina platform. The targeted genomic blocks in congenics collectively covered up to 950Mb (35%) of the rat genome length and contained over 9,900 protein coding genes (33.6% of the rat genes). BN.GK congenic strains each contain contiguous GK alleles within genomic blocks ranging in length from 1Mb to 183Mb from rat chromosomes 1 (10 congenic lines), 2 (1 congenic), 4 (1), 5 (2), 7 (2), 8 (1) and 10 (1), independently introgressed onto the genetic background of the BN strain. The reciprocal GK.BN strains contained BN genomic blocks of rat chromosomes 7 (1 congenic), 8 (1) and 10 (1) transferred onto a GK genetic background. Thus within a given congenic line, any difference in expression of a gene from the control line, if it is due to genetic differences, must be caused by polymorphisms within that congenic's variable block.

In pairwise comparisons between congenics and relevant GK or BN control, using six male rats per strain, we identified a total of 4,302 differentially expressed genes at FDR <0.05 per comparison, ranging from 73 (BN.GK7d) to over 1,000 (BN.GK1b, 1d, 1h,

234 1p, 1u) (Table 1). Gene density in congenic intervals had no apparent impact on the
235 number of differentially expressed genes between congenics and controls. For example,
236 congenics BN.GK1b, 1d, 1p and 1u have the highest numbers of differentially
237 expressed genes yet carry GK genomic blocks containing few genes (24 in BN.GK1d)
238 or many genes (1407 in BN.GK1p), suggesting that transcriptional regulation footprint
239 is related to the function rather than number of genes, A relatively small proportion
240 (7.7% on average) of differentially expressed genes were localised within the GK
241 congenic blocks themselves and may correspond to cis-regulated gene expression
242 (Table S4). The remainder, which mapped outside the congenic region, unambiguously
243 involve direct or indirect transcription regulatory mechanisms mediated in trans, as
244 illustrated in Figure S2.

245 To test the consistency of gene transcription patterns in congenic and control strains, we
246 analysed the set of 692 genes found significantly differentially expressed between
247 congenic rats and their relevant BN or GK controls and between GK and BN rats. Of
248 these, 469 (68%) showed consistent direction of expression changes in the two
249 comparisons (Table 1, Figure 2A). Remarkably, when only genes mapped within
250 congenic intervals were considered, we found almost complete concordance in
251 directions (159/165, 96%) in the two comparisons (Figure 2B), suggesting conservation
252 of cis-mediated control of gene transcription, but a lack of conservation of trans-
253 mediated control. The power to detect gene expression changes was independent of
254 whether a gene was inside a congenic block.

255 To assess robustness of differential gene expression in congenic rats, we compared
256 transcriptome data in congenic strains that contained overlapping genomic regions on
257 chromosome 1. Expression patterns of genes mapped to shared GK genomic blocks in
258 different congenics showed near complete consistency as illustrated in Figure 2C for

BN.GK1b and BN.GK1f. Data from reciprocal (BN.GK and GK.BN) congenics, which target the same >40Mb exchanged genomic segments on chromosomes 7 (BN.GK7a, GK.BN7a, GK.BN10_7a), 8 (BN.GK8b, GK.BN8a) and 10 (BN.GK10a, GK.BN10_7a) (Figure 1, Table S1), also supported consistent (but reversed, because of the reciprocity of the congenics) gene expression patterns. They provide strong evidence of robust transcriptional effects of BN and GK alleles at these loci when expressed in contexts of GK and BN genome background, respectively. Thus 24 of the 26 differentially expressed genes localised in the shared 40Mb exchanged region in BN.GK8b and GK.BN8a showed opposite expression trends, demonstrating conserved allelic effects on gene transcription (Figure 2D).

These results were further corroborated in the double congenics GK.BN10_7a, which contains two BN genomic blocks of chromosomes 7 and 10 introgressed onto a GK background. It combines the same regions of chromosome 7 as BN.GK7a and GK.BN7a, and the same region of chromosome 10 as BN.GK10a (Figure 1, Table S1). Gene transcription changes in GK.BN10_7a were remarkably consistent with BN.GK7a and GK.BN7a data for genes localised in chromosome 7 (e.g. *Pim3*, *Serhl2*, *Naprt1*, *Rbm9*) and BN.GK10a data for genes mapped to chromosome 10 (e.g. *Cd300lg*, *Cpb*, *Med13*, *Cbx*) (Figure 3, Table S4). To a lesser extent consistent transcriptional regulation in these congenics was also observed for genes mapped outside the exchanged genomic interval, demonstrating conserved trans-mediated gene expression patterns.

Importantly the majority of those genes outside of the congenic block that are differentially expressed exhibit changes very close to two-fold up or down (i.e. +/1 on the base-2 log scale in Figure 3, Table S4), allowing for the statistical uncertainties in the estimated expression levels. In contrast, differentially expressed genes within the

284 congenic block showed unconstrained changes. This suggests that the trans-effects are
285 largely confined to transitions between mono-allelic and bi-allelic expression, and thus
286 are both qualitatively and quantitatively distinct from the cis-effects.

287 The congenic series thus provide a powerful experimental system to discriminate cis-
288 and trans-mediated mechanisms of transcription of individual genes driven by
289 polymorphisms present in specific genomic blocks.

290 **Genomic blocks in congenic strains regulate specific and redundant biological**
291 **pathways**

292 To evaluate the biological consequences of coordinated gene transcription changes in
293 congenics, we carried out gene set enrichment analysis (GSEA). We identified 87
294 KEGG and 100 reactome pathways altered in at least one congenic strain (Tables S5
295 and S6). Analysis of KEGG pathways showed that the two reciprocal congenic series
296 containing GK genomic blocks on a BN background (BN.GK) and BN genomic blocks
297 on a GK background (GK.BN) can be separated, even though several pathways are
298 similarly affected in these two congenic series (Figure 4A). Concordant KEGG pathway
299 regulation was observed in congenics carrying overlapping GK genomic regions (Figure
300 4B; Tables S5). For example, significant upregulation of the citrate cycle pathway, as
301 defined by positive enrichment scores (ES) given by GSEA, was replicated in
302 BN.GK1p (ES=1.97; FDR q-value=0.02) and 1u (ES=2.14; FDR q-value=3.2 x 10^{-3})
303 (Figure 4B) and involved a common set of 12 genes (*Acly*, *Aco1*, *Aco2*, *Dlat*, *Dld*, *Dlst*,
304 *Idh1*, *Idh3b*, *Pc*, *Sdha*, *Sdhc*, *Sdhd*) (Table S7), which map outside the congenic
305 intervals and contribute to pathway enrichment among the 20 genes tested in the
306 pathway, suggesting a consistent effect of genetic polymorphisms localised in the
307 shared GK genomic block on distant genes.

As observed at the gene level, contrasting patterns of pathway regulation were found in reciprocal BN.GK and GK.BN congenics, indicating coherent effects of GK and BN alleles in the targeted region, when transferred onto BN and GK genomic backgrounds, respectively. For example, pathways downregulated in GK.BN8a (arachidonic acid metabolism, natural killer cell mediated cytotoxicity) and GK.BN7a (natural killer cell mediated cytotoxicity) were upregulated in BN.GK8b and BN.GK7a (Figure 4C-E). As noted above for the regulation of the citrate cycle in BN.GK1p and 1u, a core set of 6-7 genes contributed to pathway enrichment in reciprocal congenics, in addition to 4-15 strain specific contributing genes (Table S7).

Results from pathway analysis also provided evidence of alteration of the same biological mechanisms in BN.GK strains containing different GK genomic regions. For example the ribosome pathway was systematically upregulated in BN.GK2c (ES=2.38; FDR q-value<0.001), 5a (ES=2.18; FDR q-value<0.001), 5c (ES=2.53; FDR q-value<0.001), 7a (ES=2.45; FDR q-value<0.001) and 10a (ES=2.37; FDR q-value<0.001) (Table S5). Even though these strains carry different collections of GK variants (Figure 1), we identified a common set of 25 genes that contributed to pathway enrichment in all 5congenics (Table S8), demonstrating trans-effects of different regions converging on a common biological pathway. Along the same lines, consistent downregulation of natural killer cell mediated cytotoxicity in GK.BN7a and GK.BN8a (Figure 4D,E; Table S7), which target different chromosomes (Figure 1; Table S1), involved a common set of 15 genes (*Bid*, *Cd48*, *Chp2*, *Fcgr3a*, *Fyn*, *Icam2*, *Klrd1*, *Lat*, *Lck*, *Map2k1*, *Nras*, *Plcg1*, *Prf1*, *Rac1*, *Tnfsf10*) among the 40 genes of the pathway (Table S7). Interestingly, significant upregulation of this pathway in both BN.GK7a and BN.GK8b (Figure 4D,E) confirmed the contribution of 4 genes (*Bid*, *Lat*, *Lck*, *Prf1*), but involved a greater number of genes specific to these two BN.GK strains (*Hcst*, *Plcg2*,

Ppp3ca, *Ppp3r1*, *Ptpn6*, *Rac2*, *Vav1*) when compared to GK.BN strains (Table S7), suggesting interactions between alleles in the background and in the congenic intervals in the control of this pathway.

These results underline conserved and strain-specific functional consequences of genetic polymorphisms located in specific genomic blocks. They also demonstrate that distinct genomic regions have functionally redundant roles on biological pathways which may be regulated by different series of genetic polymorphisms or involve epistasis.

Genetic architecture of fat gene transcription regulation in (GKxBN) F2 hybrids

To validate architectural features of gene transcription identified in the GK rat and in congenic strains, we mapped eQTLs in white adipose tissue of rats from a GKxBN F2 cross. Genotypes at over 255 framework markers typed in the cross were used to impute allele probabilities and construct a map of 898 marker positions (2.5cM spacing between markers). Following withdrawal of oligonucleotides containing DNA variants, markers were tested for linkage to 15,822 detectable Illumina array signals in each of the 123 F2 rats. We identified a total of 2,735 eQTLs at FDR < 0.05, including 2,483 (90.8%) linked to transcripts corresponding to genes unambiguously localised in the rat genome assembly (RGSC3.4, Ensembl release 69) (Figure 5A, Table S9).

Results from transcriptome analyses in the F2 showed classical features of eQTL architecture, including local (cis) and distant (trans) genetic control and eQTL hotspots. A total of 1,500 eQTLs were linked to transcripts mapped to different chromosomes which unequivocally indicated the involvement of trans-mediated regulation of gene transcription (Figure 5B, Table S4). A high proportion of the remaining 1,159 eQTLs located to the same chromosomes as the linked transcripts were localised within 5Mb (n=698, 60%), 10Mb (n=910, 79%) or 20Mb (n=1,033, 89%) of the linked transcripts,

strongly suggesting cis-mediated transcription regulation (Figure 5C, Table S4). The most statistically significant of these eQTLs were localised in close vicinity to the linked transcripts (Figure 5D). eQTLs were evenly distributed across the genome, with an average of 4.8% of eQTLs per chromosome (1Mb average spacing between eQTLs), with the exception of chromosome 1 which showed a large excess (27.1%) of trans-regulating eQTLs (Figure 5B, Table S9), even though it only covers about 10% of the rat genome length and only contains 13% of rat genes. This eQTL excess can be explained by a hotspot of 319 eQTLs in a short region of chromosome 1 (49.4-59.4cM, 90.3-96.9Mb) (Figure 5E, Table S4). Other eQTL hotspots were detected on chromosomes 5 (57.5-62.5cM, 138.3-146.4Mb), 7 (70.0-72.5cM, 113.0-117.0Mb) and 17 (6.1-8.6cM, 11.4-14.6Mb). GK alleles at the eQTL hotspots on chromosomes 1, 7 and 17 were associated with downregulated expression of a high proportion of distant genes (64-73%), suggesting that eQTL clustering may be biologically relevant and reflect genuine mechanisms of gene transcription control driven by the eQTLs.

eQTLs in outbred F2 hybrids partially replicate transcriptome architecture in inbred strains

To assess the relevance of eQTLs to genome-wide transcription regulation in inbred strains, we initially compared gene differential expression between GK and BN with transcriptional effects of corresponding eQTLs in the cross. Only 50% of differentially expressed genes between these strains (614/1221) corresponded to eQTLs. Even though this only represents a small proportion (22%) of eQTLs, the effect of GK alleles on the direction of gene expression changes was consistent for the majority (86%; n=531) of these (Figure 6A). In addition, allelic effects on gene transcription in the F2 rats and inbred strains were consistent for all 362 cis-regulated eQTLs (Figure 6B) but for only 58% of trans-mediated eQTLs (Figure 6C).

We then compared genome-wide gene expression data in congenics and in F2 hybrids. The existence of several eQTLs was verified by transcription analysis of the linked genes in congenics and controls (Figure S1). We identified 1188 genes differentially expressed between congenics and controls that were linked to eQTLs, including 708 (60%) with concordant effects of GK alleles on the direction of gene expression changes in F2 and congenics (Figure 6D, Table S4). The resulting 26% replication rate in the F2 cross of differential gene expression in congenics is consistent with gene density in the congenic intervals (33.6% of all rat genes). When only eQTLs for the 358 differentially expressed genes localised in congenic intervals were considered, allelic effects on the direction of gene expression change in F2 and in congenics were consistent in the vast majority of cases (335, 94%) (Figure 6E, Table S4). Of note, both cis- and trans-acting eQTLs were equally validated in congenics when the corresponding genes were localised within the genomic blocks of the congenic strains (Figure S3).

To investigate in more detail trans-regulated eQTLs, we tested if the F2 eQTL hotspot detected in chromosome 1 was replicated in the congenic strain BN.GK1p, which contains GK alleles in this region. Over 36% (98/270) of trans-regulated genes in this eQTL hotspot were significantly differentially expressed between BN.GK1p and BN (Table S10). Remarkably, for all 98 genes, the effect of GK alleles on the direction of gene expression changes was consistent in F2 hybrids and in congenics. Congenic strains BN.GK1u and BN.GK1v which share large GK haplotypes with BN.GK1p, but distal to this eQTL hotspot, showed more limited differential expression of eQTL genes (14% and 8%, respectively). These data confirm the existence of this trans-eQTL hotspot driven by regulatory elements in the GK region specific to BN.GK1p.

Congenic transcriptome pathways do not predict F2 eQTL biological function

To test replication of biological features of fat transcriptomes identified in GK congenics, we repeated pathway enrichment analysis in the F2. Significantly affected pathways in the F2 included diabetes mellitus, digestion and absorption of fat, immunological processes (phagosome, allograft rejection, antigen processing and presentation, autoimmune diseases, cell adhesion molecules, natural killer cell mediated cytotoxicity, hematopoietic cell lineage) and metabolism of fatty acids and eleven amino acids (Table 2). Given the importance of the GK rat as a model for diabetes this suggests that many GK/BN alleles across the genome and that segregate in the cross contribute to metabolic and inflammatory mechanisms described in human diabetes.

The vast majority of pathways found altered in congenics, which may be directly or indirectly caused by genetic polymorphisms present in the congenic intervals, were not validated in F2 hybrids (Table S5), suggesting that the biological effects of genetic polymorphisms isolated in contiguous genomic blocks in inbred congenics may be counter-regulated by gene x gene interactions when alleles across the genome segregate in outbred individuals.

Discussion

We report the comprehensive genetic analysis of genome-wide fat gene transcription in a series of inbred congenic strains followed by replication in genetically heterogeneous F2 hybrids derived from the same combination of rat strains. We demonstrate conserved and discordant transcript regulation when genetic polymorphisms across the entire genome are fixed homozygous or segregate in a cross, and when they are dissected out in genomic blocks in congenic strains. Comparative analyses of transcriptomes in these systems provide an original experimental framework to assess the extent and impact of epistasis on the regulation of gene expression and biological pathways.

Transcriptome regulation in GKxBN F2 hybrids exhibits typical architectural features of eQTLs described in genetically heterogeneous populations in humans (DIXON *et al*. 2007; EMILSSON *et al*. 2008; GRUNDBERG *et al*. 2012; GTEXCONSORTIUM 2015) and in mapping panels in mice (SCHADT *et al*. 2003; BYSTRYKH *et al*. 2005; CHESLER *et al*. 2005) and rats (HUBNER *et al*. 2005; PETRETTO *et al*. 2006), including cis- and trans-mediated eQTLs and eQTL clusters controlling the transcription of large numbers of distant genes (SCHADT *et al*. 2003; EMILSSON *et al*. 2008). The definition of cis-eQTLs and eQTL hotspots in these genetic contexts is based on arbitrary estimates of physical distances separating genetic markers and linked transcripts (PETRETTO *et al*. 2006). Also, the existence of eQTL hotspots has been questioned (BREITLING *et al*. 2008) due to low effect size of trans-regulated eQTLs (ZHU *et al*. 2008). Assessment of these features in F2 cohorts is complicated by extensive linkage disequilibrium, preventing high resolution mapping needed to separate closely linked eQTLs.

In contrast, the genetic structure of congenic strains allows for a cruder measurement of gene differential expression caused by exchanging genetic polymorphisms present in well-defined genomic intervals in an otherwise uniform background, thereby

449 unambiguously distinguishing between cis- and trans-mediated regulations (BUCHNER
450 and NADEAU 2015). Differential expression of genes mapped within the genomic
451 regions exchanged in congenics should therefore correspond to cis-regulated effects
452 whereas differential expression of genes mapped outside these regions unambiguously
453 indicates trans-mediated regulation. Perhaps surprisingly, we find that almost all trans-
454 regulation in the congenics takes the form of two-fold changes in expression, which we
455 interpret as transitions between mono and bi-allelic expression. This is reminiscent of
456 expression changes observed in reciprocal crosses of mouse gene knockouts (CHESS
457 2012; MOTT *et al*. 2014) and suggests a different mechanism for trans regulation
458 compared to cis. This simple genetic architecture is not observed in the F2 cross,
459 presumably because many trans-loci regulate a given gene, smoothing out the individual
460 effects, which only become evident in the congenics. We suggest that trans effects in
461 outbred populations, including humans, are accumulations of multiple additive and non-
462 additive (epistatic) combinations of switches. This hypothesis deserves further
463 investigation, for example by building a series of combined GK.BN congenics in which
464 the fraction of the genome that is GK accumulates in a known manner. Similarly,
465 analysis of F1 hybrids of congenics (to generate heterozygosity) would demonstrate if
466 dominance effects mask the effects observed in pure congenics.

467 Congenic strains targeting overlapping genomic regions showed remarkably consistent
468 patterns of gene transcription, and we validated in congenics predominantly cis-
469 regulated eQTLs and an eQTL hotspot despite the eight year time-lag following the F2
470 hybrids (GAUGUIER *et al*. 1996) that was required to produce the congenic rats
471 (WALLACE *et al*. 2004; WALLIS *et al*. 2004). Although the congenic strains collectively
472 target about 30% of the rat genome, the total number of differentially expressed genes
473 among all congenics was far greater than the number of eQTLs and that of differentially

474 expressed genes between parental GK and BN strains (Figure 7). Even though animals
475 were thoroughly genotyped throughout the backcross breeding and inbreeding stages
476 required for the production of congenics, residual allele contaminants and gene
477 conversions may have remained undetected and may account for abundant gene
478 differential expression in congenics. Concordant transcriptional regulation between
479 congenics, F2 and parental GK and BN strains was proportional (25.9-38.4%) to the
480 fraction of the overall gene density and genomic length of the introgressed regions in
481 the congenic intervals, indicating that trans-mediated mechanisms of gene expression
482 can be efficiently uncovered in congenics. Comparison of transcription patterns in all
483 three experimental systems demonstrated consistent allelic effects on gene expression
484 for a subset of 232 genes (Figure 7). Transcription of a high proportion of these genes
485 (83.6%) was regulated in cis by eQTLs in the cross, as defined by transcripts mapped
486 within 10Mb of markers showing the strongest evidence of linkage. However, 39.3% of
487 these presumably cis-eQTL genes mapped outside congenic intervals (i.e. F2 data
488 suggested cis-regulation, but congenics demonstrated trans-regulation), indicating
489 additional contribution of trans-mediated control in the expression of these genes. eQTL
490 reports in humans suggesting that trans genetic variants can regulate multiple transcripts
491 (GRUNDBERG *et al*. 2012) support this observation.

492 We were able to validate in congenics only a fraction of trans-regulated eQTLs and
493 conversely identified an important proportion of differentially expressed genes in
494 congenics (83-89%) which were neither differentially expressed between GK and BN
495 strains nor detected as eQTLs in the cross (Figure 7). Our findings suggest that genetic
496 polymorphisms orchestrate gene expression regulation in different structures of gene x
497 gene and gene x environment interactions when fixed homozygous in the genome or
498 when segregating in an F2 cohort. However, due for example to statistical

considerations of methods applied to eQTL mapping and gene differential expression analysis between groups of individuals, a larger F2 cohort would be required to test this hypothesis. Transcriptional changes in our congenic series are crude functional consequences of homozygous genetic variants from the donor strain present in each of the genomic blocks, interacting with specific collections of fixed alleles from the recipient strain in the genetic background. Variations in phenotypic features among congenics (Figure S4) may also explain incomplete concordance of transcriptome data in these experimental systems. Congenic data suggest that epistatic regulations and gene x environment interactions may have a much more prominent impact on gene transcription than expected by estimates derived from eQTL experiments performed in genetically heterogeneous cohorts.

At pathway level, transcriptome data in the GKxBN F2 cross identified changes in inflammatory and metabolic mechanisms, which are relevant to diabetes pathogenesis and are caused by genome-wide segregation of genetic polymorphisms. In contrast, each congenic transcriptome provided biological signatures of a subset of linked genetic polymorphisms in a specific genomic interval, which can therefore be only partly replicated in F2 hybrids. An important finding in the congenics was that distinct genetic polymorphisms in disjoint genomic blocks control the same biological pathways, suggesting functional redundancy of genetic control. This phenomenon, which was also reported in the budding yeast where about 15% of genes affect growth rate (SOPKO *et al*. 2006), is proposed as a mechanism to ensure sustained maintenance of essential phenotypes. Our data also provided experimental support to the concept that mammalian syndromes can be caused by mutations at different loci in unrelated genes, which nevertheless share functional relationships with the disease phenotype (BRUNNER and VAN DRIEL 2004).

Conclusions

Results from our transcriptome analyses demonstrate the power of congenic series to dissect out cis- and trans-mediated mechanisms of gene expression, to attach biological functions to linked polymorphisms in genomic blocks and to validate eQTL hotspots. Our data underline the importance of systems genetics (CIVELEK and LUSIS 2014) to enhance knowledge of fundamental mechanisms, including functional epistasis and gene functional redundancy, that contribute to modulating the function of disease susceptibility genes and affect pathophysiological aspects of complex disorders.

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Authors' contributions PJK and DG conceived and designed the experiments. PJK, KA, SCC, RHW, ACYY, CH, SC and MTB performed the experiments. PJK, GWO, SPW, MTB, JBC, RM and DG analyzed the data. RM and DG wrote the manuscript.

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Legends to figures

Figure 1. Schematic representation of the exchanged genomic regions in congenic strains. Red bars show the GK genomic blocks introgressed onto the genomic background of the BN strain in BN.GK congenic series. Blue bars show the BN genomic blocks introgressed onto the genomic background of the GK strain in GK.BN congenic strains. Details of the genomic intervals targeted in each congenic strain are given in Table S1.

Figure 2. Patterns of gene transcription regulation in white adipose tissue by GK/BN polymorphisms in GK and BN rats and in congenic strains. Conserved gene expression patterns between BN.GK congenic rats and controls and between GK and BN rats are shown for all differentially expressed genes (A) and those localised in congenic intervals (B). Conservation of gene expression changes in congenics targeting overlapping genomic regions is illustrated by plotting expression ratio in strains BN.GK1b and BN.GK1f (C) and in reciprocal strains BN.GK8b and GK.BN8a (D). Full details of differentially expressed genes are given in Table S4.

Figure 3. Patterns of gene transcription regulation in congenic strains. Conserved allelic effects on genome-wide gene expression regulation in adipose tissue were tested in BN.GK7a and BN.GK10a congenics and in reciprocal strains GK.BN7a and GK.BN10_7a targeting largely overlapping genomic regions. Genomic positions of genes significantly differentially expressed ($P<0.05$) between BN.GK congenic and BN controls and between GK.BN congenic and GK controls are plotted along the X-axes and gene expression ratios are shown along the Y-axes. Chromosomal location of genes is colour-coded. Full details of differentially expressed genes, including genes that are localised in the congenic intervals and may be regulated in cis, are given in Table S4.

Figure 4. Global effects of GK/BN polymorphisms in congenic strains on biological pathways in white adipose tissue. Hierarchical clustering of differentially regulated pathways in congenic strains illustrate shared and strain specific biological mechanisms regulated by polymorphisms in genomic blocks (A). Pathways downregulated or upregulated in congenics are shown in blue and red cells, respectively. Statistically significant changes are indicated by – and +. Enrichment plots of genes contributing to significant alterations of biological pathways in congenics (B-E) illustrate conserved patterns of pathway regulation in congenics BN.GK1p and 1u (B) and contrasting patterns in reciprocal congenic strains (BN.GK and GK.BN) targeting overlapping regions of chromosomes 7 (D) and 8 (C,E). Output images of Gene Set Enrichment Analysis (GSEA) show the number of genes (vertical lines) upregulated (red bars) or downregulated (blue bars) contributing to overall stimulation or inhibition of the pathways (green curves). Full lists of altered KEGG pathways in congenics are shown in Table S5 and details of differentially expressed genes between congenics and controls are given in Table S4. Details of genes contributing to enrichment of pathways in B-E are shown in Table S7

Figure 5. Overview of eQTL features in adipose tissue of GKxBN F2 hybrids. Genetic positions of statistically significant eQTLs are plotted against the LOD scores (A). Local and distant eQTLs are illustrated by plotting genetic positions of statistically significant eQTLs and the genomic position of the linked transcripts (B). Data from pairs of eQTL and linked transcripts mapped to the same chromosomes were used to determine the distribution of genomic distances between transcripts and genetic markers showing the strongest evidence of statistically significant linkage in the cross (C) and relationships with the significance of genetic linkages (D). Data from pairs of transcripts and eQTLs mapped to different chromosomes were selected to illustrate distant (trans)

effects of genetic loci on gene transcription and trans-acting eQTL hotpsots (smaller circles) (E). Chromosomes are colour-coded on the circle. The colours of the lines illustrate the effects of eQTLs mapped to the same chromosomes on the expression of distant genes. Details of eQTLs are given in Table S4.

Figure 6. Correlation analysis of the effects of GK alleles on genome-wide gene expression in F2 hybrids and in inbred strains. The effects of GK alleles at statistically significant eQTLs (P-adjusted<0.05) in the GKxBN F2 cross are plotted against expression ratio of corresponding genes significantly differential expressed (P-adjusted<0.05) between GK and BN strains for all eQTLs (A) and cis- (B) and trans- (C) mediated eQTLs. The effects of GK alleles at eQTLs on gene expression regulation were compared in the F2 cross and in congenic strains for genome-wide gene expression data (D) and for genes mapped to congenic intervals (E). Details of differentially expressed genes between inbred strains and gene expression at eQTLs are given in Table S4.

Figure 7. Summary of transcriptome results among inbred strains and eQTL data in GKxBN F2 hybrids. The number of statistically significant eQTLs and differentially expressed genes between GK and BN and between congenics and controls are reported. Concordant data between the different experimental groups correspond to genes showing consistent allelic effects on the direction of expression changes.

Table 1. Overview of fat gene transcription profiling in congenic strains of the GK rat. Gene density indicates the number of genes localised in the congenic interval and in parentheses those represented on the Illumina Beadchip. Transcriptome data were analysed to identify differentially expressed genes (DEG) (P<0.05) in BN.GK and GK.BN congenic series when compared to BNandGK controls, respectively. The transcriptional regulatory footprint of each congenic region was calculated as the ratio of the total number of DEG between congenics and control to the number of genes localised in the corresponding congenic interval. The number and percentage of DEG localised in each congenic interval were determined. Replication of expression changes in parental strains and in congenics was evaluated by the number (N) and percentage of genes found consistently differentially expressed (same direction of expression change) between congenic and controls and between GK and BN, including those localised in congenic intervals (in parentheses). Replicated eQTL effects correspond to DEG between congenic and controls that are linked to eQTLs showing consistent direction of expression changes mediated by GK alleles in the F2 rats and in congenics. Cis eQTLs replicated in congenics are shown. Full details of individual DEG in inbred strains (GK, BN, congenics) and eQTLs are given in Table S4.

					Replicated GKvsBN		Replicated eQTL	
Congenic name	Gene density	Total DEG	Transcriptional footprint	DEG in congenic intervals (%)	N	%	N (%)	Replicated ciseQTL
BN.GK1b	203 (128)	1257	6.20	12 (1.0)	120 (3)	9.5 (2.5)	179 (14)	6
BN.GK1d	24 (11)	1239	51.71	1 (0.1)	76 (0)	6.1 (0)	171 (14)	0
BN.GK1f	394 (224)	874	2.24	21 (2.3)	77 (7)	8.7 (9.1)	148 (17)	8
BN.GK1h	994 (620)	1039	1.05	46 (4.4)	92 (15)	8.8 (16.3)	176 (17)	23
BN.GK1p	1407 (812)	1351	0.96	58 (4.3)	132 (20)	9.7 (15.2)	268 (20)	155
BN.GK1q	90 (45)	947	10.57	2 (0.2)	54 (0)	5.7 (0)	148 (16)	2
BN.GK1t	9 (4)	471	52.56	0 (0)	30 (0)	6.3 (0)	88 (19)	0
BN.GK1u	541 (363)	1337	2.48	19 (1.3)	114 (9)	8.5 (7.9)	196 (15)	11
BN.GK1v	210 (49)	931	4.45	6 (0.6)	55 (2)	5.9 (3.6)	122 (13)	28

BN.GK2c	1243 (627)	101	0.08	27 (26.7)	33 (16)	32.7 (48.5)	36 (36)	28
BN.GK4b	1578 (812)	232	0.15	26 (11.1)	45 (13)	19.2 (28.9)	49 (21)	26
BN.GK5a	511 (275)	94	0.18	9 (9.6)	18 (6)	19.1 (33.3)	17 (18)	10
BN.GK5c	259 (112)	557	2.15	12 (2.2)	44 (6)	7.9 (13.6)	67 (12)	5
BN.GK7a	576 (345)	91	0.16	13 (14.3)	30 (8)	33.0 (26.7)	30 (33)	14
BN.GK7d	275 (151)	73	0.27	4 (5.5)	9 (2)	12.3 (22.2)	13 (18)	5
BN.GK8b	448 (259)	312	0.71	8 (2.5)	36 (5)	11.3 (13.9)	25 (8)	7
BN.GK10a	1432 (870)	294	0.21	38 (12.9)	34 (12)	11.6 (35.3)	50 (17)	25
GK.BN7a	1014 (580)	54	0.05	11 (20.4)	19 (6)	35.2 (31.6)	16 (30)	12
GK.BN8	1149 (675)	486	0.42	32 (6.6)	74 (12)	15.2 (16.2)	71 (15)	19
GK.BN10_7a	2166 (1276)	140	0.07	39 (27.7)	49 (22)	34.8 (44.9)	54 (38)	40

Table 2. KEGG pathways underlying eQTL biological effects in the white adipose tissue transcriptome in the GKxBN F2 cross. Data were analysed with sex and cross as additive covariates. Congenic strains showing upregulation or downregulation in pathways identified in F2 hybrids are listed. Details of pathways can be found at www.genome.jp/kegg/.

Rank	KEGGID	P	Count	Size	Term	Congenics	
						Upregulation	Downregulation
1	5332	<0.01	17	34	Graft-versus-host disease	-	-
2	4145	<0.01	41	128	Phagosome	-	-
3	4940	<0.01	18	39	Type I diabetes mellitus	-	-
4	5416	<0.01	24	61	Viral myocarditis	-	GK.BN7a, 8a, 10_7a
5	4612	<0.01	22	58	Antigen processing and presentation	BN.GK7a	BN.GK1p; GK.BN10_7a
6	5330	<0.01	16	37	Allograft rejection	-	-
7	5320	<0.01	16	39	Autoimmune thyroid disease	-	-
8	4514	<0.01	29	92	Cell adhesion molecules	-	BN.GK1v,10a; GK.BN8a, 10_7a
9	1100	<0.01	167	798	Metabolic pathways	-	-
10	5140	<0.01	19	53	Leishmaniasis	BN.GK7a,7d	GK.BN10_7a
11	5322	<0.01	19	59	Systemic lupus erythematosus	-	-
12	380	<0.01	11	27	Tryptophan metabolism	-	-
13	5150	0.01	12	33	Staphylococcus aureus infection	-	-
14	250	0.01	9	22	Alanine, aspartate, glutamate metabolism	-	-
15	280	0.01	12	34	Valine, leucine and isoleucine degradation	BN.GK5a; GK.BN7a, 8a, 10_7a	BN.GK7d
16	360	0.01	5	9	Phenylalanine metabolism	-	-
17	350	0.01	7	16	Tyrosine metabolism	-	-
18	650	0.02	7	17	Butanoate metabolism	-	-
19	71	0.02	11	33	Fatty acid metabolism	GK.BN7a, 8a	-
20	790	0.02	4	7	Folate biosynthesis	-	-

21	5144	0.02	11	34	Malaria	-	-
22	511	0.02	6	14	Other glycan degradation	-	-
23	4650	0.02	19	70	Natural killer cell mediated cytotoxicity	BN.GK7a, 8b	GK.BN7a, 8a, 10_7a
24	4640	0.02	15	52	Hematopoietic cell lineage	-	GK.BN10_7a
25	982	0.03	13	44	Drug metabolism - cytochrome P450	GK.BN7a, 8a, 10_7a	-
26	270	0.03	9	27	Cysteine and methionine metabolism	BN.GK1v; GK.BN7a	-
27	4975	0.03	9	27	Fat digestion and absorption	-	-
28	450	0.04	5	12	Selenocompound metabolism	-	-
29	4970	0.05	15	57	Salivary secretion	-	-
30	4512	0.05	15	57	ECM-receptor interaction	-	BN.GK1b,1q,1v,5c,10a; GK.BN8a, 10_7a

749

Figure 1

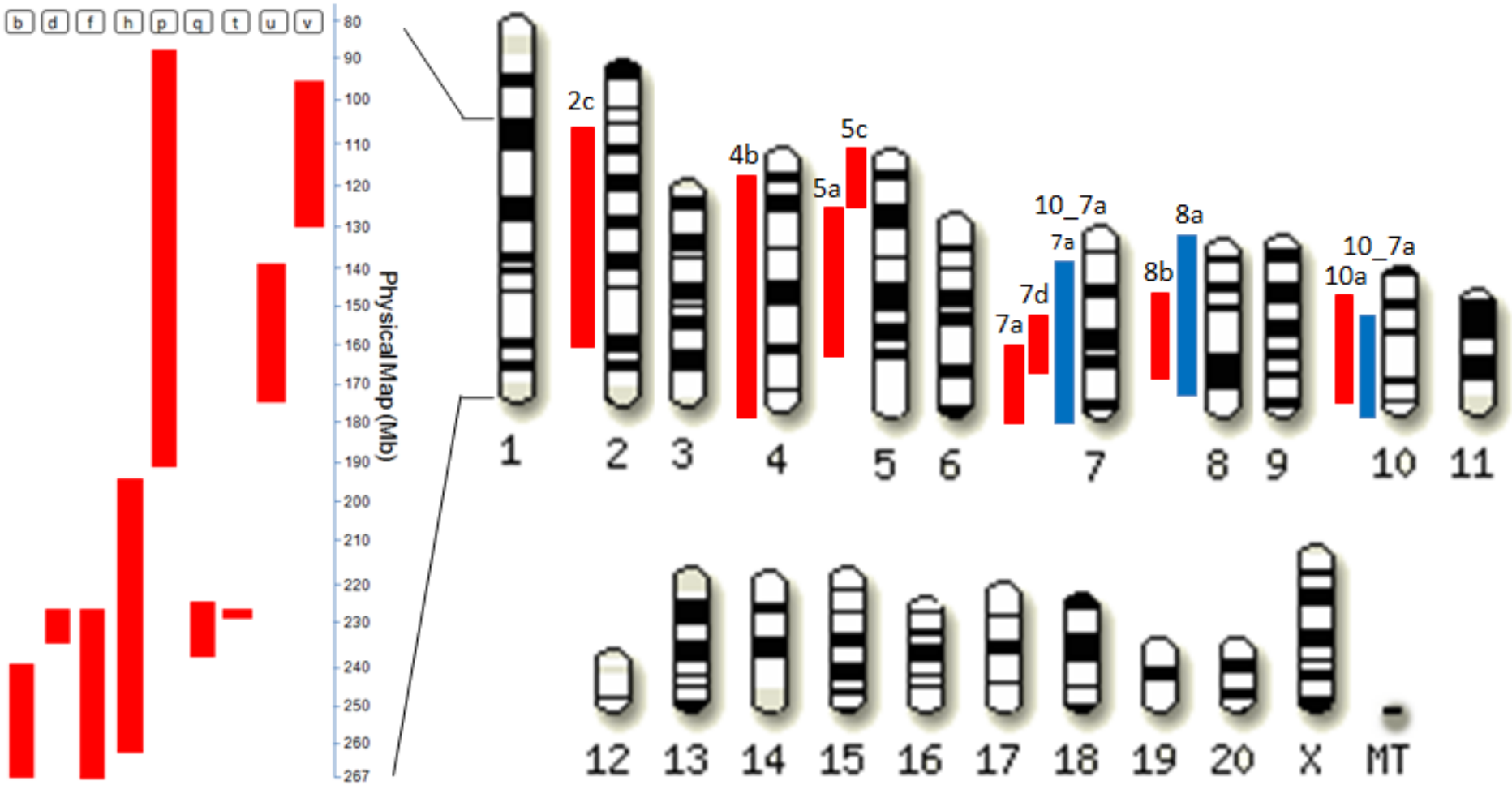
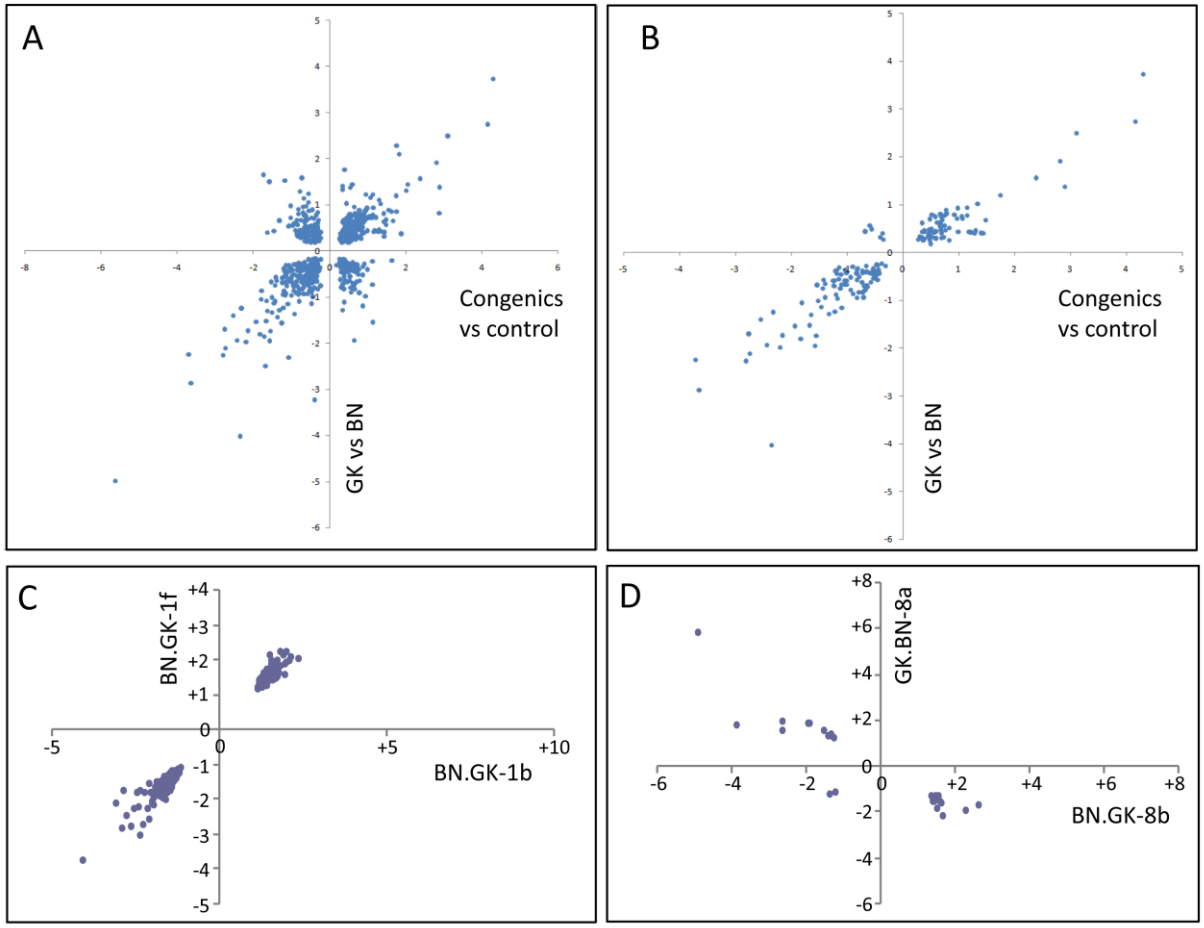
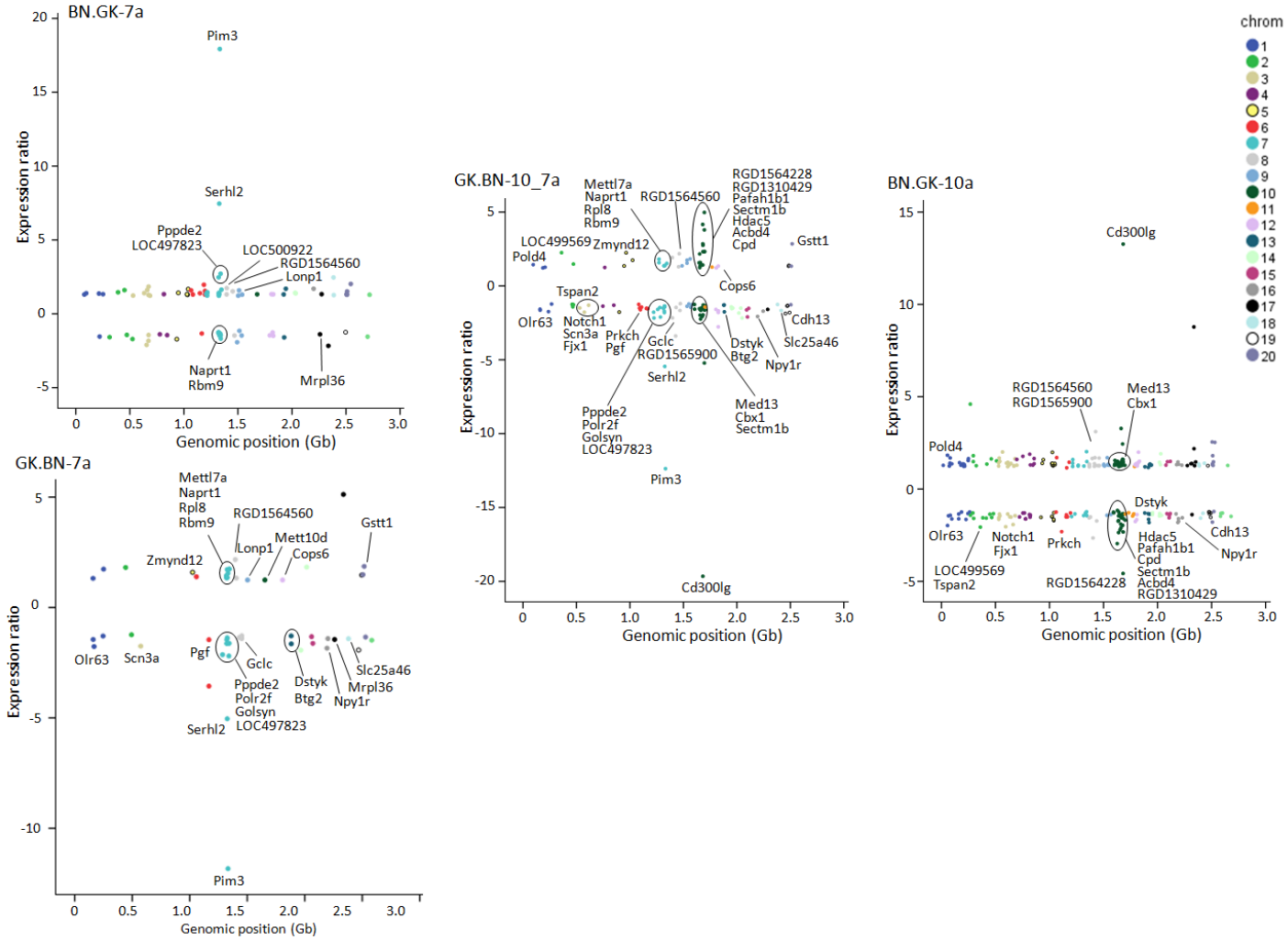

b
d
f
h
p
q
t
u
v
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240
250
260
267
Physical Map (Mb)
2c
4b
5a
5c
7a
7d
10_7a
7a
8b
8a
10a
10_7a
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
X
MT

Figure 2


A
Congenics
vs control
GK vs BN
B
Congenics
vs control
GK vs BN
C
BN.GK-1f
BN.GK-1b
D
GK.BN-8a
BN.GK-8b

753

Figure 3



754
755

Figure 4

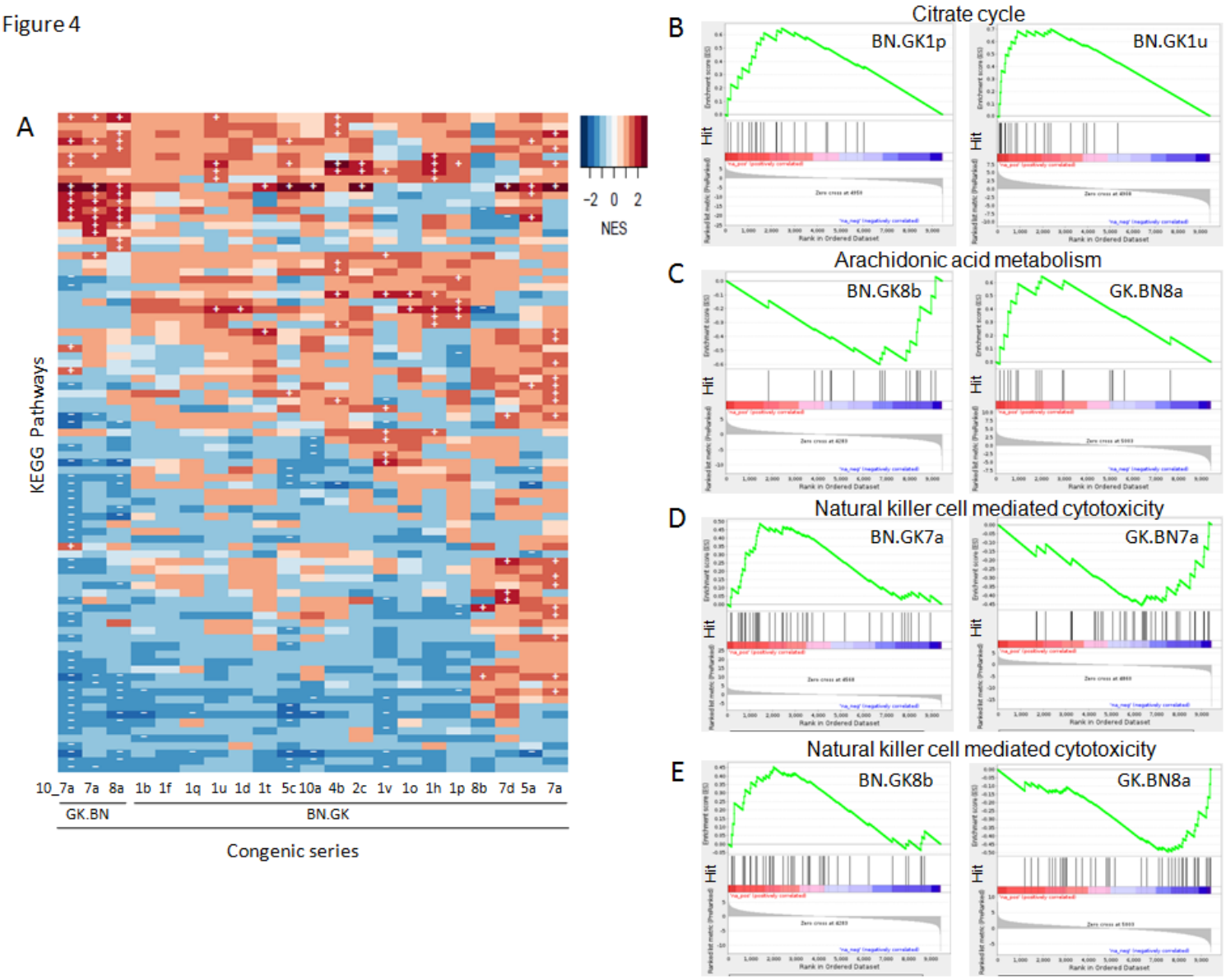
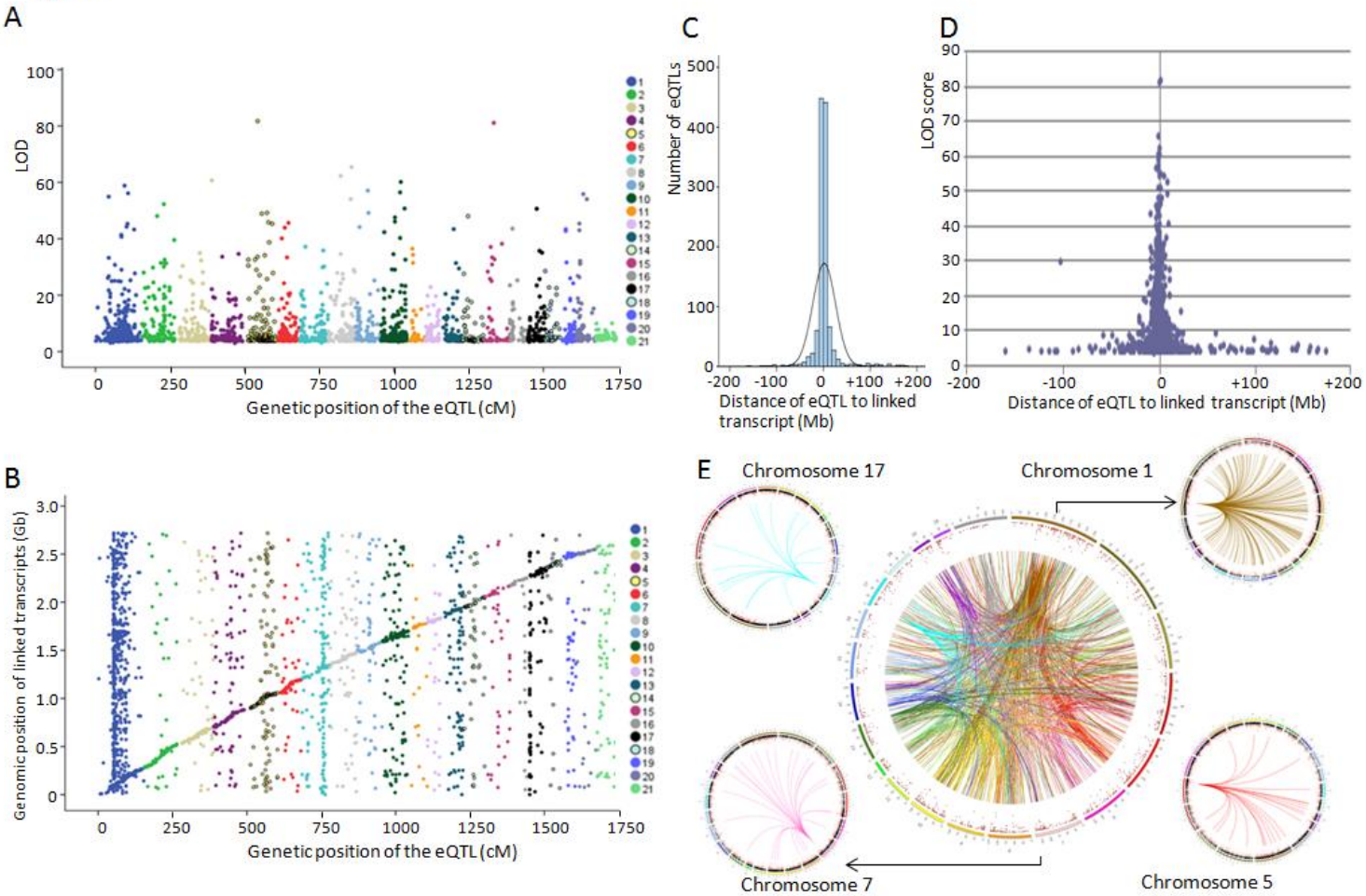
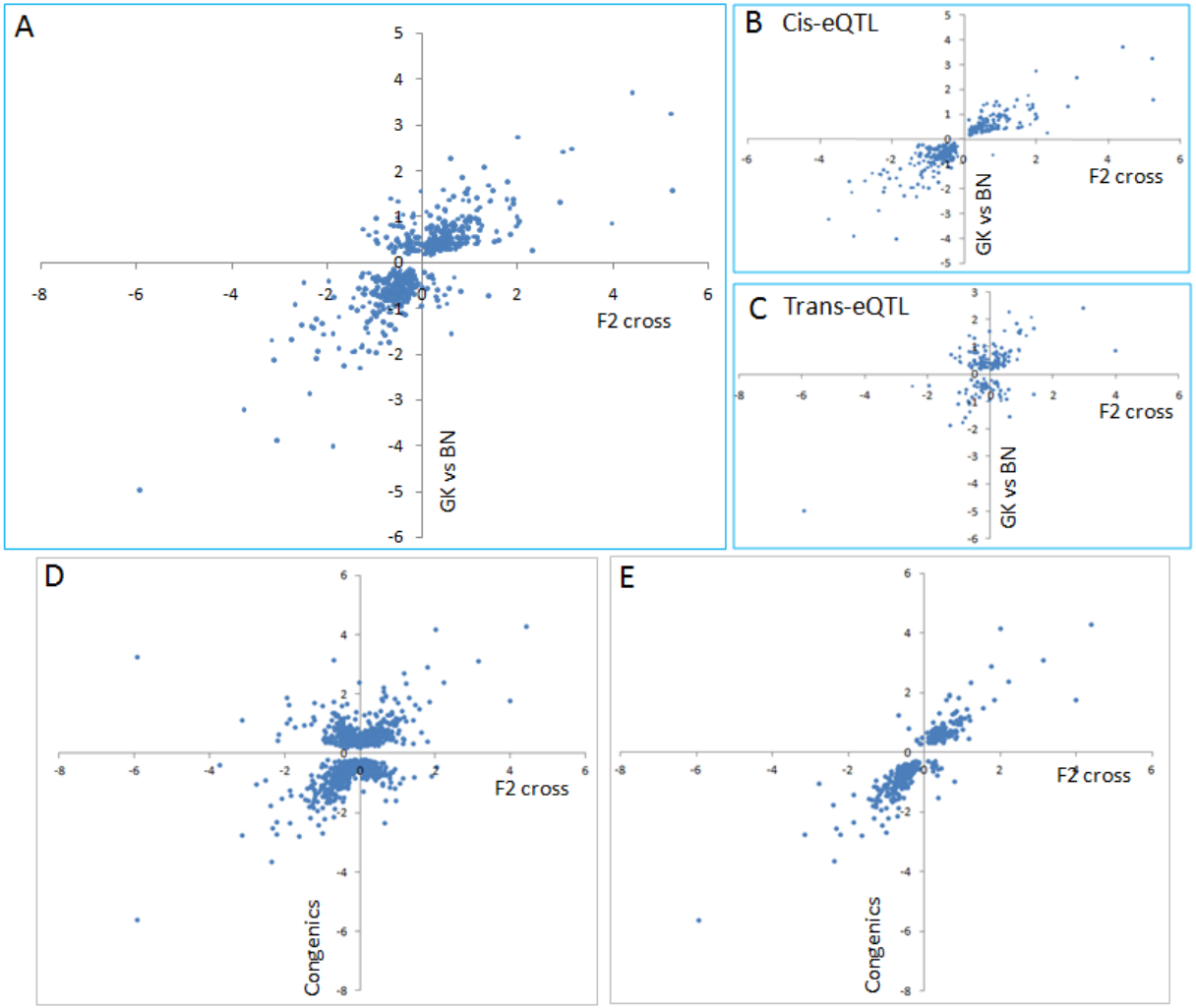

A
KEGG Pathways
-2 0 2
NES
10_7a 7a 8a 1b 1f 1q 1u 1d 1t 5c 10a 4b 2c 1v 1o 1h 1p 8b 7d 5a 7a
GK.BN
BN.GK
Congenic series
B
Citrate cycle
BN.GK1p
BN.GK1u
C
Arachidonic acid metabolism
BN.GK8b
GK.BN8a
D
Natural killer cell mediated cytotoxicity
BN.GK7a
GK.BN7a
E
Natural killer cell mediated cytotoxicity
BN.GK8b
GK.BN8a
Hit
Rank in Ordered Dataset

Figure 5


A
LOD
Genetic position of the eQTL (cM)
B
Genomic position of linked transcripts (Gb)
Genetic position of the eQTL (cM)
C
Number of eQTLs
Distance of eQTL to linked transcript (Mb)
D
LOD score
Distance of eQTL to linked transcript (Mb)
E
Chromosome 17
Chromosome 1
Chromosome 7
Chromosome 5

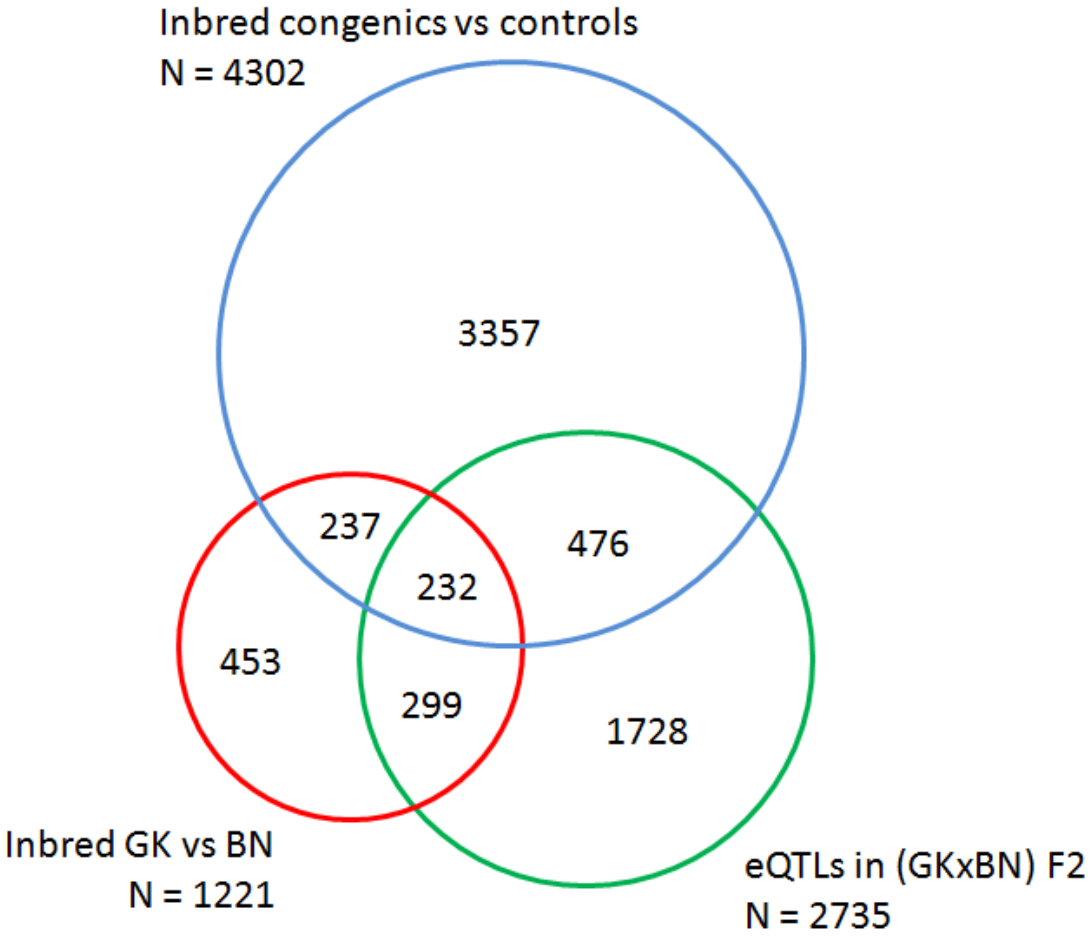
760

Figure 6



761

762 Figure 7



763