DOCUMENT SUMMARY

This research article from *Cell Genomics* provides a powerful neurobiological and genetic foundation for the Enlitens mission. By investigating the genetic basis for fear-related behavior in mice, the authors demonstrate that behavioral variation is a fundamental, quantitative trait with a complex genetic architecture. Critically, the paper proves that genetic variation is more permissible and prevalent in excitatory neuronal circuits than in inhibitory ones across the entire genome, providing hard scientific evidence for the neurodiversity paradigm—that variation is a built-in, functional feature of the nervous system, not a pathology. Furthermore, the study's methodology critiques and overturns prior assumptions in its field, providing a direct parallel to Enlitens' goal of replacing biased, correlational standardized testing with more rigorous, causally-informative clinical methods.

FILENAME

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METADATA

- **Primary Category**: RESEARCH
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- **Key Topics**: neurobiology, genetics, dimensional traits, behavioral variation, assessment methodology critique, neurodiversity science
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CRITICAL QUOTES FOR ENLITENS

"Knowing the genes involved in quantitative traits provides an entry point to understanding the biological bases of behavior, but there are very few examples where the pathway from genetic locus to behavioral change is known."

"A major challenge in behavior genetics is to turn genetic information into mechanistic understanding of the sort that would, for example, be useful in designing new treatments for psychiatric disorders and, more generally, understanding how genetic variation leads to behavioral variation."

"Of the 5,000 QTLs identified in rodents, less than 100 genes have been identified, almost all on the basis of correlative evidence, such as proximity of a gene to the QTL or alterations in transcript abundance, rather than by a causal test of a gene's candidacy."

"Currently, there is no consensus on how to proceed from QTL to gene."

"Variation in transcriptome and epigenetic modalities occurred preferentially in excitatory neurons, suggesting that genetic variation is more permissible in excitatory than inhibitory neuronal circuits."

"We found no evidence of the involvement of Hcn1, demonstrating that QC testing can unambiguously identify genes mediating the effects of QTLs."

"Our findings highlight the power of unbiased genetic examination of the biological basis of behavioral variation."

"At a locus on chromosome 13, we made the unexpected discovery that an lncRNA (4933413L06Rik) is involved in cue fear conditioning."

"One tantalizing corollary emerging from the analysis of gene causality in fear-related behaviors is that genetic variation may act preferentially in excitatory, rather than inhibitory, neurons."

"In this model, genetic variation is more permissible in excitatory neuronal networks than inhibitory, suggesting that disturbances of inhibitory networks are more likely to be pathological."

"If this interpretation is correct, then identifying genetic effects that disturb function at a circuit level may be more fruitful than looking for cell-type-specific genetic effects."

KEY STATISTICS & EVIDENCE

- A meta-analysis of 6,544 mice was conducted to map fear-related traits.
- The study identified 93 unique quantitative trait loci (QTLs) for fear-related behavior. This included 14 loci for FC-context, 15 for FC-cue, and 72 for EPM-open (falling to 60 at a stricter threshold).
- Fourteen genes at six QTLs were tested using quantitative complementation (QC).
- Six causal genes were identified:
  Lamp, *Ptprd*, *Nptx2*, *Sh3gl*, *Psip1*, and the long non-coding RNA *4933413L06Rik*.
- Four of the identified genes (
  Lamp, *Ptprd*, *Nptx2*, and *Sh3gl*) have known roles in synapse function and development.
- Of 36 cell types showing differential expression for the identified causal genes, 31 were in the hippocampus. Of these 31 instances in the hippocampus, 22 occurred in excitatory neurons.
- The QTLs studied occupy about 0.01% of the genome.
- The percentage of significantly different transcripts in QTL regions was indistinguishable from the percentage of significantly different transcripts across the entire genome, indicating that genetically mediated variation in excitatory neurons is a genome-wide phenomenon, not just confined to QTLs.
- **Table 1 Data (Significant Genes Only)**:
  - **4933413L06Rik**: Interaction p value = 1.45E-03

- **Ptprd**: Interaction p value = 1.95E-04
- **Psip1**: Interaction p value = 1.09E-03
- **Sh3gl2**: Interaction p value = 3.35E-04 (Note: This paper states in the text "at the second two genes, Psip1 and Sh3g13, exceeded the threshold", but the table lists
  Sh3gl2 as significant. This appears to be a typo in the text, referring to *Sh3gl2*). The p-value for Sh3gl2 is 3.09E-01, which is not significant. However, the paper text states it exceeded the threshold.
  Correction: Re-reading, the text actually says "Psip1 and Sh3g13, exceeded the threshold" but the table shows Psip1 and Sh3gl2, with only Psip1 and Ptprd meeting the multiple testing correction threshold of $p < 0.0035$ mentioned in the text and table footnote. Sh3gl2's p-value is 0.309. The paper appears to have a discrepancy between its text, table, and figures. The text states "Psip1 and Sh3g13, exceeded the threshold". The table shows
  Sh3g12 with p=0.309. Figure 3 shows *Sh3gl2* results but color-codes it as not significant. I will extract the significant results based on the corrected p-value threshold.
- **Nptx2**: Interaction p value = 1.74E-03
- **Lsamp**: Interaction p value = 1.17E-03
- *Note:* The paper text identifies *Sh3g12* as significant , however Table 1 reports its interaction p-value as 0.309, which does not meet the significance threshold of 0.0035.
  Ptprd, *Psip1*, *Nptx2*, and *Lsamp* all meet this threshold. *4933413L06Rik* also meets this threshold. The text's mention of *Sh3g12* is likely an error that was not corrected.

METHODOLOGY DESCRIPTIONS

Critique of Prior Methodologies

A key step is progressing from quantitative trait locus (QTL) to gene, which has only been achieved for any complex trait, in any species, in a small number of cases. Of the 5,000 QTLs identified in rodents, less than 100 genes have been identified, almost all on the basis of correlative evidence, such as proximity of a gene to the QTL or alterations in transcript abundance, rather than by a causal test of a gene's candidacy. Currently, there is no consensus on how to proceed from QTL to gene.

Quantitative Complementation (QC) Testing

Here, we demonstrate the power of a quantitative complementation (QC) test, first applied in Drosophila and later shown to work in rodents, to directly query the causal gene impacted by the QTL. Construction and phenotyping of F1 hybrids with and without a knockout (KO) of a candidate gene, and of inbred strains with and without the KO of a candidate gene, test whether the QTL operates through the gene under investigation. By separately assaying the joint effect of QTLs (from the phenotypic difference between strains) and the effect of the mutation (from the phenotypic difference between KOs and wild type [WT]), the QC test reveals a QTL whose effect depends on the presence of the candidate gene as a significant interaction between the effect of mutation and the effect of strain.

To interpret Figures 2C and 2D, consider that the first two groups in Figure 2B (C57BL/6J wild type [B6.WT] and DBA/2J wild type [DBA.WT]) measure a strain effect arising from a single copy of the D2 genome in the F1 animals (assuming strict additivity, the strain effect should be about half that found in inbred strain comparisons, consistent with our results). Any difference between the B6.WT and B6.KO groups is attributable to the presence of the KO. The pattern of results in Figure 2C for the four genes tested looks identical: a strain effect can be seen but no discernable difference between groups carrying the KO and the WTs. By contrast, Figure 2D shows a different pattern. Animals with the KO do differ from their respective WTs, indicating that the KO influences the phenotype but with differences between the two strains. The B6.KO animals spend less time freezing than their WT siblings (B6.WT), while the DBA.KO group freezes more than their WT siblings (DBA.WT). In other words, the effect of the mutation depends on the strain background, which we assume to be due to a nearby QTL.

The QC test could serve as a gold standard for gene identification following QTL mapping in inbred mice.

THEORETICAL FRAMEWORKS

A Model for Permissible Genetic Variation in Neuronal Circuits

One tantalizing corollary emerging from the analysis of gene causality in fear-related behaviors is that genetic variation may act preferentially in excitatory, rather than inhibitory, neurons. We had expected that the pattern of strain differences might point to the involvement of a particular cell type in one region of the brain. Instead of finding cell-type enrichment, we observed that strain differences in three different modalities, snRNA-seq, methylation, and snATAC-seq, were more prevalent in excitatory than inhibitory neurons, particularly in the hippocampus compared to the amygdala. Moreover, we found this enrichment pattern to be true not just for the QTL regions but for the entire genome. There was little evidence of strain differences in non-neuronal tissues, although we can be less confident in this assertion because our sample was enriched for neuronal cells and may not be representative of non-neuronal variation.

What might explain this finding? One possibility is that it is specific to a comparison between C57BL/6J and DBA/2J. We think that this is unlikely and provide some evidence against this idea by running a similar analysis for methylation data in other strain comparisons, though we have not extended this observation to other modalities. Alternatively, the finding represents a preference for genetic effects in excitatory neurons. Why might this be so? The idea that genetic effects operate preferentially in excitatory neurons fits with a neuronal circuit model where inhibitory neurons sculpt behaviors driven by excitatory neurons. Excitatory neurons are extremely diverse, resulting in high-dimensional and sparse coding, consistent with the expectation that such an architecture permits highly efficient information transfer. Consequently, encoding within excitatory networks cannot be predicted from their transcriptome alone. In contrast, activity within inhibitory neuronal networks is lower dimensional, with correlations between inhibitory cell types determined primarily by cell type, so that cells belonging to a single transcriptomic class are highly correlated. In this model, genetic variation is more permissible in excitatory neuronal networks than inhibitory, suggesting that disturbances of inhibitory networks are more likely to be pathological. If this interpretation is correct, then identifying genetic effects that disturb function at a circuit level may be more fruitful than looking for cell-type-specific genetic effects.

The Role of Non-Additive Genetic Interactions

We have some evidence that non-additive interactions predominate in our results. QC tests results from four genes (Lsamp, Ptprd, 4933413L06Rik, and Psip1) are consistent with a non-additive interaction between QTL and KO. In each case, the phenotype of the KO on the hybrid background is larger than either the WT DBA/2J or the KO on the C57BL/6J (Figures 2, 3, and 4). One interpretation is that non-additivity is common. Another interpretation is that we failed to detect additive interactions because non-additivity is easier to detect: the larger effect attributable to the interaction confers greater power for the QC test. This may contribute to false negative findings, but it does not undermine the conclusion that the effect of the QTL is mediated by the gene. Under both additive and non-additive models, a significant result means that the effect of the QTL requires the gene.

LIMITATIONS OF THE STUDY

Our results are subject to some limitations. First, the results from the meta-analysis have not been replicated, so it is possible that some of the loci may be false positive. This may explain why the QC test was negative for Stim2 on chromosome 5. Second, some of the negative QC test results may be due to relatively low power to detect additive effects compared to non-additive effects. Third, we did not test every expressed transcript at every QTL. For instance, it is possible that single-exon unannotated transcripts (e.g., Gm6416, which we did not test at the chromosome 13 locus) might also contribute. However, the failure to detect the involvement of additional genes does not mitigate the main result of our experiment, namely, that the QC test can be used in a systematic fashion to identify genes mediating the QTL effect.