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Preview

Better together: Non-additive interactions between schizophrenia risk genes

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Zhang, Zhang, Forrest *et al* combine allele-specific open chromatin (ASoC) mapping and CRISPR-editing to evaluate the functional impact of schizophrenia risk variants on human neuronal gene expression, synaptic development, and function. In doing so, they uncover surprising non-additive effects between target genes regulated by the same risk variant.

Genetic liability associated with neuropsychiatric disorders most frequently arises from common genetic variation that confers small individual effects and contributes to phenotypes only when considered in aggregate.[1] These risk variants, termed single nucleotide polymorphisms (SNPs), are typically non-coding, likely acting by regulating the expression of one or more gene targets. Definitively identifying the precise disease SNP(s) and molecular mechanisms at hundreds of risk loci is a major unsolved challenge. In earlier work, this team demonstrated that by uncovering differential allelic chromatin accessibility in heterozygous individuals, allele specific open chromatin (ASoC) could be used as a functional readout of SNP activity in human neurons.[2] In this issue of *Cell Genomics,* Zhang, Zhang, Forrest *et al* extend this work by empirically resolving new causal SNPs associated with risk for schizophrenia, validating effects on neuronal gene expression and neurodevelopment (**Figure 1**).

Overall, the authors further dissect the relationship between genetic variation, gene expression, and cellular phenotypes in human glutamatergic neurons. Using human induced pluripotent stem cell (hiPSC)-derived *NGN2*-induced glutamatergic neurons, ASoC identified 31 putative causal risk SNPs at 26 schizophrenia risk loci. For four of these loci, CRISPRi confirmed the regulatory activity and resolved cis-target genes. Precise CRISPR-editing at one schizophrenia risk SNP (rs2027349) revealed complex cis-regulation of multiple proximal (*VPS45, AC244033.2*) and distal genes (*C1orf54*) that together altered dendritic complexity, synapse development, and neuronal activity.

In total, here the team identified 8,205 ASoC SNPs in *NGN2*-neurons (induced via transcription factor overexpression in hiPSCs) from 20 individuals, comparable to the 3547 ASoC SNPs they previously reported in donor-matched hiPSC-derived neural progenitor cells and 5611 ASoC SNPs in NPC-derived neurons (generated by directed differentiation).[3] There was a remarkably robust correlation (R = 0.81) in ASoC SNPs between the current *NGN2*-neuron and previous NPC-neuron dataset. Altogether, ~16% of ASoC SNPs were previously reported as eQTLs in human brain or hiPSC-derived neurons. When narrowing down to just the credible SNPs at 108 GWAS risk loci,[4] there was a significant overlap in ASoC of schizophrenia SNPs across the previous and current reports: 7 of the 31 ASoC schizophrenia SNPs in *NGN2*-neurons replicated in NPC-neurons, and reciprocally, 9 of the 17 ASoC schizophrenia SNPs in NPC-neurons replicated in *NGN2*-neurons. SNPs were most enriched for schizophrenia GWAS variants relative to other neuropsychiatric or neurodegenerative disorders.

The authors applied pooled CRISPR droplet sequencing (CROP-seq) analysis to confirm the regulatory activity of ASoC SNPs at four schizophrenia GWAS loci: rs2027349 (*VPS45*), rs12985055 (*BCL11B*), rs10933 (*PBRM1/GNL3*), and rs7148456 (*BAG5*). In doing so, they revealed *cis*-target genes of these SNPs in *NGN2*-neurons. Indeed, consistent with their previous finding that ASoC variants regulate both adjacent and distal genes through chromatin contacts,[2] here the team showed that CRISPRi of rs2027349 decreased expression of two adjacent genes, *VPS45* and a long non-coding RNA (*AC244033.2*), as well as a distal gene that encodes an open reading frame (*C1orf54*). Only those differentially expressed genes identified following CRISPRi for rs2027349, but not rs10933 or rs7148456, were enriched for gene ontology terms related to neuronal processes, function, and components, suggesting the specific biological relevance of rs2027349 to schizophrenia risk.

Next, to definitively connect rs2027349 with its transcriptomic and phenotypic impacts, the team applied CRISPR-editing to generate isogenic AA, AG, and GG hiPSCs that differed only at this single non-coding SNP. Consistent with their CRISPRi results, the authors found that the A allele was associated with increased expression of *VPS45*, *AC244033.2,* and *C1orf54* in *NGN2*-neurons derived from two independent donors across neuronal maturation. Genome-wide, down-regulated genes in AA (vs GG) neurons were strongly enriched for synaptic gene ontology terms and schizophrenia risk genes, whereas up-regulated genes were related to neurodevelopment. Moreover, AA (vs. GG) neuronal transcriptional differences were correlated with those observed in post-mortem brain tissues of major psychiatric disorders and down-regulated genes were enriched for those associated with risk for schizophrenia and other neuropsychiatric disorders. Finally, AA (vs GG) neurons showed increased dendritic complexity, synaptic puncta density, and

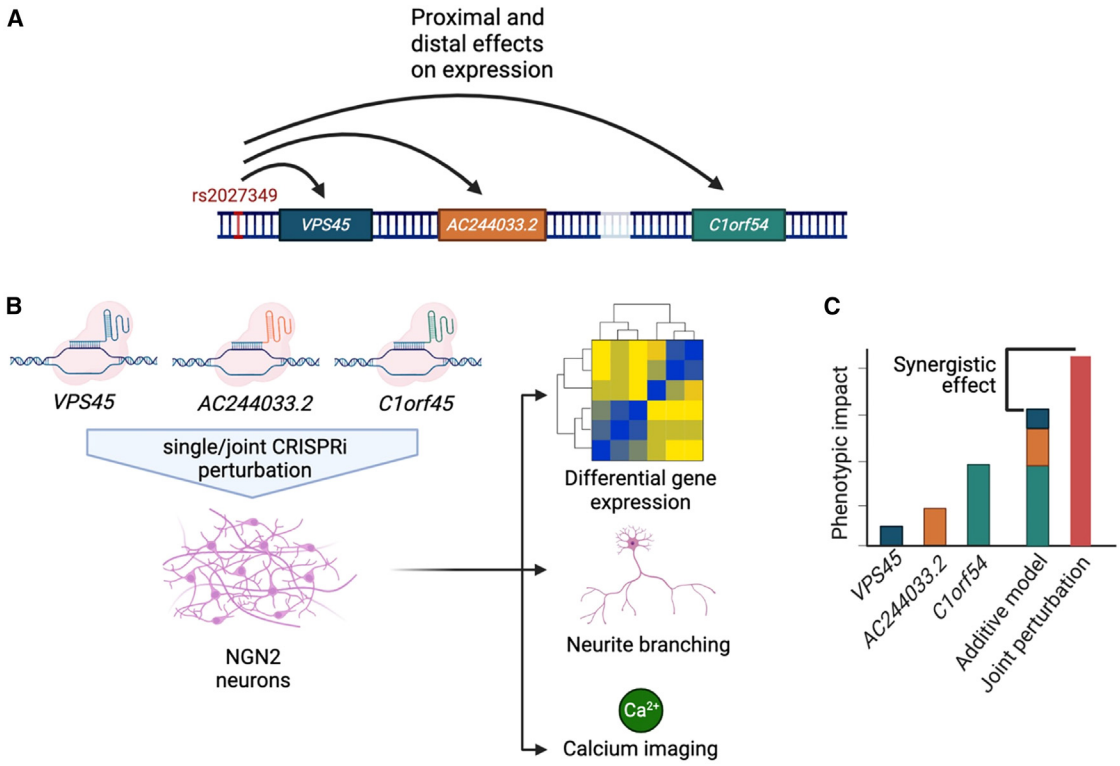

Figure 1. Elucidation of non-additive risk gene interactions

A: The schizophrenia risk-associated SNP rs2027349 regulates expression of multiple proximal (*VPS45*, AC244033.2) and distal genes (*C1orf54*).

B: Individual and joint knockdown of *VPS45*, AC244033.2 and *C1orf54* expression in *NGN2*-neurons results in altered gene expression, neurite branching, and neuronal activity.

C: Joint perturbations can result in more or fewer synergistic (non-additive) effects than predicted by the additive model.

hyperactivity, reversed by knocking-down distinct *cis*-regulated genes (*VPS45, AC244033.2,* or *C1orf54*), suggesting a phenotypic contribution from all three genes.

A series of individual and combinatorial knockdown experiments further dissected the impact of *VPS45, AC244033.2,* and *C1orf54*. Targets of *VPS45* and AC244033.2 were enriched in those (predominantly synaptic) genes down-regulated in AA (vs GG) neurons, while targets of *C1orf54* were predominantly up-regulated. Altogether, all three genes seem to contribute to the phenotypic changes associated with rs2027349, but by altering the expression of different downstream genes. The authors fit a linear regression model to the individual knockdown studies, towards exploring the extent that each individual gene knockdown explained the differential expression observed between isogenic rs2027349 AA vs GG neurons; introduction of interaction terms between the individual knockdowns markedly improved model performance, which was further improved by fitting empirical data from a combinatorial knockdown experiment. Finally, the authors compared the effects of an expected additive model created from the individual gene knockdowns to a measured combinatorial perturbation. Those genes whose changes in expression did not fit the additive model were strongly enriched for genes associated with psychiatric disorders, while those with additive effects were not. Likewise, knockdown of these three genes, alone and in combination, revealed that they contributed to altered dendritic complexity and calcium signaling in a non-additive and synergistic fashion. Altogether, the authors surprisingly reveal that independent target genes regulated by the same risk SNP function together in a synergistic manner to impact neuronal function.

Likewise, for four top-ranked schizophrenia GWAS genes, we reported that combinatorial perturbation resulted in downstream effects that could not be predicted from individual perturbations alone. Non-additive effects were enriched for genes involved in synaptic function and schizophrenia risk.[5] More recently, across fifteen schizophrenia risk genes, we described how non-additive effects occur to a substantially greater extent following combinatorial perturbations of genes from within a shared biological pathway[6] and that shared neuronal impacts converge on synaptic function.[7] Altogether, we conclude that the effects of polygenic risk cannot yet be extrapolated from experiments testing one risk gene at a time. Further studies are need to clarify how the additive model observed at the population-level[8] is influenced by interactions that alter phenotypic outcomes at the individual-level, particularly in the context of core genes/pathways with specific roles in disease etiology.[9] It is critical to explore the clinical relevance of these *in vitro* findings in terms of predicting clinical outcomes and druggable targets.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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