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Functional Effects of Schizophrenia-Linked Genetic Variants on Intrinsic Single-Neuron Excitability: A Modeling Study

Tuomo Mäki-Marttunen, Geir Halnes, Anna Devor, Aree Witoelar, Francesco Bettella, Srdjan Djurovic, Yunpeng Wang, Gaute T. Einevoll, Ole A. Andreassen, and Anders M. Dale

ABSTRACT

BACKGROUND: Recent genome-wide association studies have identified a large number of genetic risk factors for schizophrenia (SCZ) featuring ion channels and calcium transporters. For some of these risk factors, independent prior investigations have examined the effects of genetic alterations on the cellular electrical excitability and calcium homeostasis. In the present proof-of-concept study, we harnessed these experimental results for modeling of computational properties on layer V cortical pyramidal cells and identified possible common alterations in behavior across SCZ-related genes.

METHODS: We applied a biophysically detailed multicompartmental model to study the excitability of a layer V pyramidal cell. We reviewed the literature on functional genomics for variants of genes associated with SCZ and used changes in neuron model parameters to represent the effects of these variants.

RESULTS: We present and apply a framework for examining the effects of subtle single nucleotide polymorphisms in ion channel and calcium transporter-encoding genes on neuron excitability. Our analysis indicates that most of the considered SCZ-related genetic variants affect the spiking behavior and intracellular calcium dynamics resulting from summation of inputs across the dendritic tree.

CONCLUSIONS: Our results suggest that alteration in the ability of a single neuron to integrate the inputs and scale its excitability may constitute a fundamental mechanistic contributor to mental disease, alongside the previously proposed deficits in synaptic communication and network behavior.

Keywords: Biophysical modeling, Functional genomics, Genome-wide association studies, Layer V pyramidal cell, Neuron excitability, Schizophrenia

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Schizophrenia (SCZ) is a severe mental disorder with heritability estimates ranging from .6 to .8 (1). A recent genome-wide association study (GWAS) identified more than a hundred genes exceeding genome-wide significance, confirming the polygenic nature of this psychiatric disorder (2). This remarkable success in gene discovery brings up the next big challenge for psychiatric genetics—translation of the genetic associations into biological insights (3). Attaining this goal is supported by the development of biophysically detailed neuron models, boosted by the recent launch of mega-scale neuroscience projects (4). These models make it possible to investigate SCZ disease mechanisms by computational means, ultimately aiming toward achieving better clinical treatments and disorder outcomes (5,6).

The 108 recently confirmed SCZ-linked loci span a wide set of protein-coding genes (2), including numerous ion channel-encoding genes. The disorder is associated with genes affecting transmembrane currents of all major ionic species, sodium (Na^+), potassium (K^+), and calcium (Ca^{2+}). In addition, some of the SCZ-linked genes are involved in regulation of the Ca^{2+} concentration in the intracellular medium (2), which is another great contributor to excitability. It is thus reasonable to hypothesize that the SCZ-linked genes should have an impact on the excitability at the single-neuron level.

We focused our study on cortical layer V pyramidal cells (L5PCs) as a principal computational element of the cerebral circuit. An L5PC extends throughout the cortical depth with the soma located in layer V and the apical dendrite branching into the apical tuft in layer I, and its long axon may project to nonlocal cortical and subcortical areas. The tuft serves as an integration hub for long-distance synaptic inputs and is often considered a biological substrate for cortical associations providing high-level context for low-level (e.g., sensory) inputs to the perisomatic compartment (7). Therefore, the ability of L5PCs to communicate the apical inputs to the soma has been proposed as one of the mechanisms that could be impaired in the mental disease (7). In agreement with this hypothesis, recent psychiatric GWASs consistently reported association of genes coding for the subunits of

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voltage-gated Ca^{2+} channels as risk factors in SCZ and bipolar disorder (8–10).

In the present proof-of-principle study, we applied a model (11) of L5PCs to explore how genetic variants in SCZ-linked genes affect the single-cell excitability. We carried out our study by linking a documented effect of a genetic variant in an ion channel or Ca^{2+} transporter-encoding gene to a change in the corresponding neuron model parameter. It should, however, be noted that information does not generally exist for the effect of single nucleotide polymorphism (SNP) variants identified through GWASs on the biophysical parameters required for the computational models. We instead used information obtained from in vitro studies of more extreme genetic variations, including loss-of-function mutations. A central assumption of this approach is that the effects of SNP variants can be represented as scaled-down versions of those of the more extreme variants and that the emergence of the full psychiatric disease phenotype results from the combined effect of a large number of subtle SNP effects (12,13). A deficit in synaptic communication is likely to contribute to SCZ (2,14–16) but is outside the scope of the present work.

METHODS AND MATERIALS

The L5PC Model

The multicompartmental neuron model used in this work was based on a reconstructed morphology of a layer V thick-tufted pyramidal neuron [cell #1 in (11)]. The model includes the following ionic currents: fast inactivating Na^+ current, persistent Na^+ current, nonspecific cation current, muscarinic K^+ current, slow inactivating K^+ current (I_{Kp}), fast inactivating K^+ current, fast noninactivating K^+ current, high-voltage activated Ca^{2+} current, low-voltage activated Ca^{2+} current, small-conductance Ca^{2+}-activated K^+ current (I_{SK}), and finally, the passive leak current. See Supplement 1 for the model equations, and for the simulation codes, see ModelDB entry 169457 (http://senselab.med.yale.edu/ModelDB/showModel.cshtml?model=169457).

Genes Included in the Study

Since we did not aim to provide a comprehensive evaluation of a representative fraction of the genetic risk factors of SCZ but rather to provide a proof of principle of the computational modeling approach, we selected the genomic loci using the following approach. We based our study on a recent GWAS (2), which reported significantly associated SNPs that were scattered across hundreds of genes with a variety of cellular functions. We concentrated on those genes that encoded either ionic channels or proteins contributing to transportation of intracellular Ca^{2+} ions.

We used the SNP-wise p value data of Ripke *et al.* (2), and for each gene of interest we determined the minimum p value among those SNPs that were located in the considered gene. We performed this operation for all genes encoding either subunits of voltage-gated Ca^{2+}, K^+, or Na^+ channel; subunits of a small-conductance calcium-activated potassium (SK), leak, or hyperpolarization-activated cyclic nucleotide-gated channel; or Ca^{2+}-transporting ATPase. The genes *CACNA1C*, *CACNB2*, *CACNA1I*, *ATP2A2*, and *HCN1* possessed a small minimum p value each ($p < 3 \times 10^{-8}$)—these genes were also highlighted in the locus-oriented association analysis as performed in Ripke *et al.* (2). To extend our study to explore a larger set of genes, we used a more relaxed threshold ($p < 3 \times 10^{-5}$) for the minimum p value and obtained the following genes in addition to the previously mentioned ones: *CACNA1D*, *CACNA1S*, *SCN1A*, *SCN7A*, *SCN9A*, *KCNN3*, *KCNS3*, *KCNB1*, *KCNG2*, *KCNH7*, and *ATP2B2*. Of these, we omitted the genes that are not relevant for the firing behavior of an L5PC. It should be noted that we used the SNPs reported in Ripke *et al.* (2) only to name the above SCZ-related genes, and due to lack of functional genomics data, we could not include the actual SCZ-related SNPs in our simulation study. Instead, we searched in PubMed for functional genomic studies reporting the effects of any genetic variant of the above genes. For details, see Supplement 1.

RESULTS

A New Framework for Bridging the Gap Between GWASs of SCZ and Computational Neuroscience

In this work, we reviewed the literature on effects of variants in SCZ-related genes on ion channel behavior and intracellular Ca^{2+} dynamics and interpreted the reported effects in the context of our neuron model parameters. An overview of the relevant studies is given in Table 1, while the effects of each variant on the L5PC model parameters are given in Table 2. These data gave us a direct interface for linking a change in the genomic data, such as an SNP or an alternative splicing, into a change in neuron dynamics. The reported data, however, often corresponded to variants with large phenotypic consequences that, in general, are absent in SCZ patients. To simulate subtle cellular effects caused by the common SNPs related to SCZ (17), we downscaled the variants of Table 2 by bringing the changed parameters closer to the control neuron values when the reported change caused too large an effect in the neuron firing behavior. Our approach is illustrated in Figure 1.

The variants in Table 1 were 23 in total, although some of them represented a range of effects of different variants (Table S1 in Supplement 1). The entries corresponded to variants of genes encoding for Ca^{2+} channel subunits (*CACNA1C*, *CACNB2*, *CACNA1D*, *CACNA1I*), intracellular Ca^{2+} pumps (*ATP2A2*, *ATP2B2*), Na^+ channel subunits (*SCN1A*, *SCN9A*), K^+ channel subunits (*KCNS3*, *KCNB1*, *KCNN3*), and a nonspecific ion channel subunit (*HCN1*). In the following, we present simulation results for the L5PC model equipped with some of these downscaled variants (we refer to these model neurons as variant neurons or simply as variants). As we did not know the quality of the effects of the actual SCZ-related polymorphisms, we performed the simulations for a range of differently scaled variants, including negative scalings (i.e., opposite effects with regard to the effects reported in Table 2). We concentrated our study on a representative sample of six variants, highlighted in Table 2. These variants represented six genes with different roles in L5PC electrogenesis and a wide range of observed effects (Table S2 in Supplement 1).

Variants Show Altered Intracellular (Ca^{2+}) Responses to Short Stimuli

To characterize the implications of SCZ-related genes on the neuron excitability, we started by analyzing the effects of the