Genetically-encoded impairment of KCC2 cotransporter functional regulation in human idiopathic generalized epilepsy

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Introduction

The genetic architecture of idiopathic generalized epilepsy (IGE) is not well established, and novel pharmacotherapeutic strategies are needed based on specific molecular alterations driving disease pathogenesis. The KCC2 cotransporter establishes the low intraneuronal Cl- levels required for GABAA and glycine receptor-mediated inhibition of neuronal firing. KCC2 deficiency in model organisms results in network hyperexcitability and seizures, but disease-associated KCC2 variants or mutations have not been described in humans. We hypothesized genetic mutation and/or functionally relevant variation in KCC2 might contribute to IGE by altering neuronal Cl- homeostasis, and consequently, GABA/glycine activity.

Methods

We utilized DNA sequencing of a large (>300) French-Canadian patient cohort of severe IGE, along with greater than 4000 matched French-Canadian controls; gramicidin perforated patch clamp electrophysiology and ratiometric Cl-sensing in neurons to measure EGABA, ECl, and Cl- extrusion capacity, to assess transporter function; surface expression studies using immunohistochemistry to monitor transporter plasmalemmal localization; and biochemistry with phospho-specific antibodies to monitor transporter phosphorylation states at specific regulatory residues.

Results

We report two non-synonymous functional variants in KCC2, R952H and R1049C, exhibiting clear statistical association with severe idiopathic generalized epilepsy (IGE) in a large French-Canadian patient cohort. These variants reside in evolutionarily conserved residues near important regulatory domains in the KCC2 cytoplasmic C-terminus, and are predicted to be highly pathogenic in silico. Relative to WT KCC2, both IGE variants exhibit significantly impaired Cl--extrusion capacities that result in less hyperpolarized glycine equilibrium potentials (EGly), as well as decreased stimulatory phosphorylation at the regulatory site serine 940.

Conclusions

These data are the first to describe KCC2 mutations significantly associated with human disease, and suggest genetically-encoded impairment of KCC2 functional regulation may be a risk factor for the development of human IGE. Given the recent development of KCC2 activators, targeting this molecule in treatment-resistant IGE may be a novel treatment approach.

Learning Objectives

-To understand the unmet clinical need of IGE patients

-To learn about a new target with therapeutic potential in IGE, the KCC2 cotransporter

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References

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