High Throughput Functional Analysis of KCNB1 variants Associated with Epileptic Encephalopathy Type 26

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Background and Rationale

Mutations in KCNB1 have recently been implicated as the cause of epileptic encephalopathy type 26 (EIEE26) [1-3]. EIEE26 is characterized by multifocal seizures, global developmental delay, and hypotonia. KCNB1 encodes the Kv2.1 voltage-gated potassium channel alpha subunit, which is a major contributor to delayed rectifier potassium current in the brain. We previously demonstrated pathogenic effects of three EIEE26-associated KCNB1 mutations (S347R, T374I, G373R) on Kv2.1 protein function. CHO-K1 cells expressing the mutant channels exhibited loss of ion selectivity and voltage-dependent gating, and gain of a depolarizing inward cation conductance [1]. Similarly, a recent report of a novel EIEE26 mutation also demonstrated loss of ion selectivity and gain of a depolarizing cation conductance as major effects [3]. However, they did not observe loss of voltage-dependent gating. This suggests that a range of channel dysfunction can result in EIEE26 with similar clinical features. To determine the range of mutation effects, we studied a series of six variants from cases with a molecular diagnosis of EIEE26 ascertained by clinical exome sequencing.

Methods

To determine the effect of these KCNB1 variants on Kv2.1 channel function, we performed functional studies in a heterologous expression system. KCNB1 variants were introduced into a full-length human Kv2.1 cDNA expression construct by site-directed mutagenesis. Expression of wild-type (WT) or mutant Kv2.1 in CHO-K1 cells was achieved by electroporation. Following 72 hour recovery, automated planar array patch clamp recording was performed. Only cells with Rm>20 GΩ, Gm>150 MΩ and Ih/Io<1 were used for analysis. Cell surface biotinylation experiments were performed to determine the relative expression of WT and mutant channel protein at the plasma membrane.

Summary

- EIEE26 presents in infancy with multiple seizure types, developmental delay, hypotonia, and movement disorders.
- All KCNB1 mutations described to date have arisen de novo.
- Functional studies demonstrate that KCNB1 mutations associated with epileptic encephalopathy result in a spectrum of effects that result in lower activity and/or altered voltage-dependence of activation and inactivation.
- Future studies will test whether the mutations affect ion channel selectivity.
- Restoration of Kv2.1 function may be therapeutic.

References


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