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Gain-of-Function Effects of *KCNMA1*-N999S Mutation on Human BK Channel Properties

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Mutations in several types of ion channels are associated with seizure and paroxysmal dyskinesia, including the D434G and N999S (N995S) mutations in *KCNMA1*, the gene encoding the alpha pore−forming subunit of the BK voltage− and Ca^{2+}−activated K^+ channel. While the association of D434G with neurological dysfunction is supported by familial inheritance, N999S mutations in *KCNMA1* have been identified only as *de novo* variants in 5 patients by whole exome sequencing. To investigate the potential for N999S to alter excitability, mutant channels were studied in inside-out patches from HEK293T cells, in the context of a human brain isoform. N999S channels produced currents that were activated by depolarizing voltage steps and increasing intracellular Ca^{2+}. N999S current-voltage relationships were 40−90mV more hyperpolarized compared to wildtype in the presence of Ca^{2+}_i (N999S $V_{1/2}$: 93±3mV, −30±8mV, −35±5mV; WT: 180±2mV, 60±5mV, −22±3mV at 1, 10, 100μM, respectively). This shift was also observed in the absence of Ca^{2+}_i (N999S $V_{1/2}$: 173±3mV; WT: 212±4mV), but reduced in magnitude. In addition, N999S currents activated faster and deactivated slower than WT. In physiological K^+, action potential voltage commands elicited larger currents from N999S channels compared to WT. These data classify N999S as a gain-of-function (GOF) mutation in *KCNMA1* across multiple conditions. Consequently, BK channel inhibition, either via selective antagonists or inhibition of the intracellular Ca^{2+} sources, is predicted to mitigate the increased current produced by this mutation *in vivo*. Finally, the N999S effect on BK channel properties exceeded the GOF alterations produced by D434G (180±5mV, 143±3mV, 24±5mV, −43±3mV at 0, 1, 10, 100μM Ca^{2+}_i), suggesting a biophysical basis for the clinical heterogeneity and variable penetrance among patients harboring GOF genetic alterations in *KCNMA1.* Taken together, these data provide new insight into the mechanistic basis and therapeutic options for *KCNMA1*-linked channelopathy.