Iqsec2 knockout mice recapitulate the intellectual disability and epilepsy phenotype of patients with loss-of-function mutations

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The IQ motif and SEC7 domain-containing protein 2 (IQSEC2) is an X-chromosome gene mutated in both males and females leading to Intellectual Disability (ID) and severe early-onset seizures. The pathogenesis underpinning these mutations remains unknown. Utilizing CRISPR/Cas9 targeted editing, we have generated an Iqsec2 KO mouse model to investigate the molecular and cellular deficits in this gene resulting in disease outcomes, a fundamental step towards the design and implementation of potential treatment options. We confirmed the loss of Iqsec2 mRNA expression and the lack of Iqsec2 protein detected within the brain of founder and progeny mice. Recapitulating the human setting, both male (48%) and female (45%) Iqsec2 KO mice present with frequent and recurrent seizures. There was an increased occurrence of seizures, reabsorption and unsuccessful nurturing of live young in breeding females. Developmentally, the KO mice exhibit significantly increased hyperactivity, altered anxiety and fear responses, decreased social interactions, delayed learning capacity and decreased memory retention/novel recognition; recapitulating the psychiatric issues, autistic-like features and cognitive deficits present in patients with loss-of-function IQSEC2 mutations. Interestingly, the loss of Iqsec2 function not only causes severe ID and seizures in KO male mice, but in agreement with the patient setting, similar severity is also noted in females despite being in a heterozygous state for this X-chromosome gene. We contend this newly generated mouse model provides a highly relevant biological tool required to interrogate IQSEC2/Iqsec2 function in the brain.

IQSEC2 is a guanine nucleotide exchange factor, which catalyzes exchange of GDP for GTP in a number of ARF superfamily of proteins. IQSEC2 is highly expressed in the forebrain, specifically localized to excitatory synapses as part of the N-methyl-D-aspartate receptor(NMDAR) complex (13, 14). The exact role IQSEC2 plays at excitatory synapses remains unclear. Limited studies indicate a role in the activity-dependent removal of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR) and activity-dependent synaptic plasticity (15, 16). Our own studies have shown that IQSEC2 also has a fundamental role in controlling neuronal morphology (17). However, there is currently no published research investigating the impact of loss or altered Iqsec2 function on the development and resulting cognitive outcomes in any animal model. It is not certain if severe loss-of-function mutations in IQSEC2 can be transmitted in the human setting, with only missense variants giving rise to milder non-syndromic features being maternally inherited. Hence, it was unclear if the loss of Iqsec2 function modelled in mice would survive into postnatal life, be reproductively viable or useful to model disease pathogenicity observed in humans. Here, we show that mice with the complete loss of function of Iqsec2 by successfully targeting exon 3 using CRISPR/Cas9 technology survive into postnatal life and are viable. In this study, we investigate the effect of severe loss-of-function mutations driving the phenotype in patients, including the emerging female-specific phenotype using a mouse modelling the KO of Iqsec2.

Animals were humanely killed by cervical dislocation. Brain was dissected from the skull and cut into two halves sagitally along the cerebral fissure. The right-hand side brain was separated and minced into cortex (n = 2, one each for protein and RNA) and cerebellum, snap-frozen in liquid nitrogen, and stored at −80°C pending analysis. RNA was extracted from 40 mg homogenised brain cortical tissue in TRIzol reagent and converted to cDNA using SuperScript RT (Thermo Fisher Scientific) as described previously Each individual Iqsec2 KO hemizygous male or heterozygous female sample was normalised to the averaged wild-type data for their respective sex, with resulting data displayed as relative Iqsec2 expression to their respective sexed wild-type controls. Protein extraction, SDS–PAGE, and Western blot analysis of protein levels were performed as described previously. The primary antibodies were rabbit anti-IQSEC2 (1:2,000) as previously described, rabbit anti-IQSEC1 and rabbit anti-IQSEC3, both used at 1:1,000 (Invitrogen), and mouse β-actin Sigma-Aldrich A2228). Secondary antibodies from DAKO (Santa Clara) were goat antimouse HRP and goat antirabbit HRP Images were imported into Image Studio (Li-Cor Biosciences), and band intensities of Iqsec proteins were normalised to their respective β-actin loading control, and where required were harmonized across multiple immunoblots using a consistent control sample. Each individual Iqsec2 KO hemizygous or heterozygous sample was normalised to the averaged pooled wild-type data for their respective sex, with relative intensities presented (n for each as described in figure legends).

Review of the female patients with complete loss-of-function mutations in IQSEC2 shows that comorbid behavioural and psychiatric features are frequently present in addition to ID. Hence, it was not surprising that mice modelling Iqsec2 KO in the heterozygous female state displayed a range of phenotypic traits across a series of behavioural tests corresponding to these additional features. Notably, the Iqsec2 KO heterozygous females recapitulated

a reduction in intellectual functioning and autistic-like behaviours, demonstrated through a loss of novel recognition on the Y-maze, a reduction in learning and memory during the Barnes maze trial, and an overall reduction in interaction time during the sociability test. Altered anxiety-like/fear responses and hyperactivity in the Iqsec2 KO heterozygous female mice on multiple apparatus correlate with an increase in hippocampal volume, which has been associated with mental retardation and psychiatric issues such as autism, attention-deficit disorder, and schizophrenia. We also observe a thinning of the corpus callosum in the Iqsec2 KO heterozygous female mice, a phenotype emerging in several cases. Taken together, these findings highlight that our Iqsec2 KO mouse model recapitulates the complex phenotypic spectrum observed in female patients with loss-of function IQSEC2 variants. Interestingly, the emergence of a speech phenotype is noted in the proband reported in this study and 26 of the 38 published female cases with loss-of-function variants Although we have not yet addressed this clinical feature in mice, it would be interesting to investigate it, particularly in view of the observations of reduced mothering skills of the breeding heterozygous females.

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