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The developmental evolution of the seizure phenotype and cortical inhibition in mouse models of juvenile myoclonic epilepsy

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ABSTRACT

The $GABA_A$ receptor ($GABA_AR$) α1 subunit mutation, A322D, causes autosomal dominant juvenile myoclonic epilepsy (JME). Previous *in vitro* studies demonstrated that A322D elicits α1(A322D) protein degradation and that the residual mutant protein causes a dominant-negative effect on wild type $GABA_A$Rs. Here, we determined the effects of heterozygous A322D knockin ($Het_{α1}AD$) and deletion ($Het_{α1}KO$) on seizures, $GABA_AR$ expression, and motor cortex (M1) miniature inhibitory postsynaptic currents (mIPSCs) at two developmental time-points, P35 and P120. Both $Het_{α1}AD$ and $Het_{α1}KO$ mice experience absence seizures at P35 that persist at P120, but have substantially more frequent spontaneous and evoked polyspike wave discharges and myoclonic seizures at P120. Both mutant mice have increased total and synaptic α3 subunit expression at both time-points and decreased α1 subunit expression at P35, but not P120. There are proportional reductions in α3, β2, and γ2 subunit expression between P35 and P120 in wild type and mutant mice. In M1, mutants have decreased mIPSC peak amplitudes and prolonged decay constants compared with wild type, and the $Het_{α1}AD$ mice have reduced mIPSC frequency and smaller amplitudes than $Het_{α1}KO$ mice. Wild type and mutants exhibit proportional increases in mIPSC amplitudes between P35 and P120. We conclude that $Het_{α1}KO$ and $Het_{α1}AD$ mice model the JME subsyndrome, childhood absence epilepsy persisting and evolving into JME. Both mutants alter $GABA_AR$ composition and motor cortex physiology in a manner expected to increase neuronal synchrony and excitability to produce seizures. However, developmental changes in M1 $GABA_A$Rs do not explain the worsened phenotype at P120 in mutant mice.

Introduction

Juvenile myoclonic epilepsy (JME) is a common generalized epilepsy syndrome that accounts for 5–10% of all cases of epilepsy (Camfield et al., 2013). All JME patients experience myoclonic seizures, sudden, very brief jerks of a muscle or groups of muscles (Genton et al., 2013). In addition, most JME patients experience generalized tonic–clonic (GTC) seizures, and approximately 30% of JME patients exhibit absence seizures, brief episodes of staring and loss-of-awareness (Genton et al., 2013). On electroencephalography (EEG), myoclonic seizures are typically characterized as fast (4–6 Hz), polyspike-and-wave discharges (PSD), and absence seizures are associated with very rhythmic, 3 Hz spike-wave discharges (SWD). Different JME subsyndromes can be distinguished depending on the age of onset, rates of medical intractability, as well as the presence or absence of different seizure types (Martinez-Juarez et al., 2006).

JME is classified as a genetic generalized epilepsy syndrome and it is thought to be transmitted primarily by complex genetics. However, several genes have been identified that are associated with monogenic forms of JME (Delgado-Escueta et al., 2013). Although these monogenic forms of JME are much less prevalent than the polygenic forms, they serve as invaluable models that will help elucidate the pathophysiology of the more common forms of polygenic JME.

A highly penetrant form of monogenic JME arises from a heterozygous missense mutation of the $GABA_A$ receptor ($GABA_AR$) α1 subunit (Cossette et al., 2002). $GABA_A$Rs are the main inhibitory ligand-gated ion channels in mammalian brain (Benarroch, 2007). They are pentamers composed of five subunits that arise from eight gene families; many of the gene families contain multiple isoforms (α1–6, β1–3, γ1–3, δ, ε, θ, π, ρ1–3). *In vitro* studies demonstrated that the A322D mutation causes α1(A322D) protein misfolding with altered transmembrane topology (Gallagher et al., 2007) and rapid elimination by endoplasmic reticulum associated degradation (Bradley et al., 2008; Gallagher et al., 2007). This causes a 92% reduction of total α1(A322D) subunit expression (Ding et al., 2010). In addition, when expressed

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in vitro, the residual α1(A322D) subunit protein causes a small, but significant reduction of wild type $GABA_AR$ expression. These *in vitro* data suggested that the GABRA1(A322D) mutation produces epilepsy by causing a heterozygous loss of α1 subunit and also, possibly, by a dominant-negative effect on wild type $GABA_AR$ expression.

Previously, we reported that at postnatal day 35 (P35), heterozygous Gabra1 deletion ($Het_{\alpha1}KO$) caused SWDs and absence seizures (Arain et al., 2012) and altered $GABA_AR$ expression and physiology in the cortex and thalamus (Zhou et al., 2013, 2015). These data demonstrate that heterozygous loss of α1 subunit was sufficient to cause an epilepsy phenotype. However, we did not observe any PSDs, myoclonic seizures, or GTC seizures in the P35 $Het_{\alpha1}KO$ mice and thus they did not fully recapitulate a JME phenotype. This finding could suggest that the dominant negative effect from the α1(A322D) subunit and/or further brain development beyond P35 could be important for the production of these additional types of seizures. Therefore, we generated heterozygous Gabra1(A322D) knockin ($Het_{\alpha1}AD$) mice and directly compared their seizure phenotypes and $GABA_A$ receptor expression and function with those of $Het_{\alpha1}KO$ and wild type mice at two different times of development, P35 and P120.

Materials and methods

Animals and genotyping

All animal procedures were performed in accordance with protocols approved by the Vanderbilt University Institutional Animal Care and Use Committee (IACUC). Mice were housed in a temperature and humidity controlled environment, with a 12 hour light/dark schedule. Water and food was provided ad libitum. The $Het_{\alpha1}KO$ mice in the congenic C57BL/6 J strain were described previously (Arain et al., 2012). We collaborated with the Gene Targeting and Transgenic Facility at The University of Connecticut Health Center to design the $Het_{\alpha1}AD$ knockin mice (Fig. **1**A). Briefly, a bacterial artificial chromosome (BAC) construct containing exon 9 of the Gabra1 subunit was modified by mutating the GCC codon with GAC to encode the A322D missense mutation. The selection cassettes, PGKneo (surrounded by loxP sequences), and MC1-HSV-TK sequence were also inserted into the BAC vector. The BAC construct was transfected into embryonic stem cells (ESC). Correct homologous recombination was confirmed using selection with G148 and ganciclovir and further verification was done using PCR and DNA sequencing. The correctly-targeted ESC were injected into growing blastocysts and implanted into pseudo-pregnant females. The offspring exhibiting chimerism were used to test for germ line transmission. These $Het_{\alpha1}AD$ mice were then crossed with mice expressing Cre driven by the hypoxanthine–guanine phosphoribosyltransferase promoter to remove the loxP flanked PGKneo cassette. The resulting $Het_{\alpha1}AD$ mouse line was verified using PCR and DNA sequencing. We used speed congenic testing (DartMouse speed congenic facility at The Geisel School of Medicine) to rapidly backcross the $Het_{\alpha1}AD$ mice into a C57BL/6 J congenic line.

To directly compare the effects of heterozygous deletion and A322D knockin on epilepsy phenotypes and on $GABA_AR$ expression and function and to control for environmental effects, we used mice that had wild type, $Het_{\alpha1}KO$, and $Het_{\alpha1}AD$ mice within the same litter. Therefore, we mated $Het_{\alpha1}KO$ and $Het_{\alpha1}AD$ mice to produce wild type, $Het_{\alpha1}KO$ and $Het_{\alpha1}AD$ mice in a 1:1:1 ratio; as expected, the compound heterozygous $Het_{\alpha1}KO/Het_{\alpha1}AD$ mice died at approximately P15–P19 similar to the homozygous Gabra1 deletion mice ($Hom_{\alpha1}KO$) we reported previously (Arain et al., 2012). We also bred $Het_{\alpha1}AD$ mice to produce homozygous Gabra1(A322D) mice ($Hom_{\alpha1}AD$) that were used to determine the effects of the A322D mutation on α1(A322D) subunit protein expression; as a control, these experiments also included protein from $Hom_{\alpha1}KO$ which were bred from $Het_{\alpha1}KO$ parents.

In initial experiments, we verified that the correct codon was mutated by sequencing tail genomic DNA. We amplified the genomic DNA using

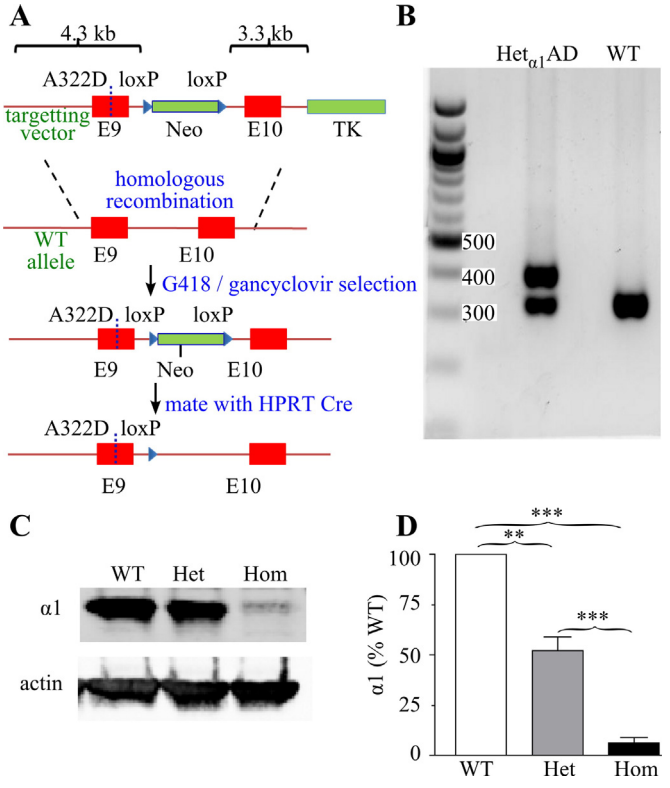

Fig. 1. Construction and characterization of $Het_{\alpha1}AD$ mice. A) Schematic of the construction of the $Het_{\alpha1}AD$ mice. Gabra1 exons are shown in red, selection cassettes (Neo = neomycin, TK = thymidine kinase) are green, and loxP sequences are blue triangles. The BAC targeting vector (top) shows the position of the A322D substitution (dotted line in exon 9). The targeting vector was introduced into the Gabra1 chromosome by homologous recombination and successful incorporations were selected by G418/ganciclovir treatment. The neomycin cassette was removed by Cre-lox recombination leaving a single loxP sequence. B) Agarose gel of genotyping PCR products showing 324 bp (wild type allele) and 407 bp (mutant allele) products in $Het_{\alpha1}AD$ (Het) mice and only 324 bp products in wild type (WT) mice. C–D) Western blots of cortical protein from P15 mice show that compared with wild type mice, $Het_{\alpha1}AD$ mice express 52 ± 7% ($P = 0.001$) and $Hom_{\alpha1}AD$ mice express 6 ± 3% ($P < 0.001$ vs WT and $Het_{\alpha1}KO$) the amount of α1 subunit protein. ** = $P < 0.01$, *** = $P < 0.001$.

the 5′-GGAGAGTACTCTATCTCTTCTG-3′ and 5′-GGTTATCTGTGTGGCTCACG-3′ primers, purified the amplified DNA on an agarose gel, and performed Sanger sequencing on the purified DNA with the same primers that were used for amplification. After we verified that the mutation was introduced correctly, we genotyped mice for all subsequent experiments using tail DNA with forward primer, 5′-CGTGAGCCACACAAATAACC-3′ and the reverse primer, 5′-ACCCTTTGATGGGTTACAGC-3′. Genotyping wild type and $Het_{\alpha1}AD$ DNA produced DNA bands of 324 bp and 407 bp, respectively. Genotyping the $Het_{\alpha1}KO$ mice was performed as described previously (Arain et al., 2012).

With this breeding strategy, we did not find any sex-dependent effect on epileptiform discharges or seizures and thus grouped males and females together for the EEG experiments. However we used females for the Western blots, immunofluorescence studies, and electrophysiology experiments in order to enable comparison with the prior studies (Zhou et al., 2013, 2015). We tested the mice in two age groups P33–38 (identified as "P35" in the text) and P120–P130 (identified as "P120" in the text).

Video/EEG monitoring of spontaneous and evoked epileptiform discharges

As described previously (Arain et al., 2012), we surgically implanted prefabricated mouse EEG headmounts that record two bipolar EEG channels and one subcutaneous nuchal EMG channel (Pinnacle Technologies). The surgeries were performed at least 48 h prior to the EEG.