Effect of an autism-associated KCNMB2 variant, G124R, on BK channel properties

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ABSTRACT

BK K⁺ channels are critical regulators of neuron and muscle excitability, comprised of a tetramer of pore-forming α-subunits from the KCNMA1 gene and cell- and tissue-selective β subunits (KCNMB1-4). Mutations in KCNMA1 are associated with neurological disorders, including autism. However, little is known about the role of neuronal BK channel β subunits in human neuropathology. The β2 subunit is expressed in central neurons and imparts inactivation to BK channels, as well as altering activation and deactivation gating. In this study, we report the functional effect of G124R, a novel KCNMB2 mutation obtained from whole-exome sequencing of a patient diagnosed with autism spectrum disorder. Residue G124, located in the extracellular loop between TM1 and TM2, is conserved across species, and the G124R missense mutation is predicted deleterious with computational tools. To investigate the pathogenicity potential, BK channels were co-expressed with β2 WT and β2 G124R subunits in HEK293T cells. BK/β2 currents were assessed from inside-out patches under physiological K⁺ conditions (140/6 mM K⁺ and 10 μM Ca²⁺), during activation and inactivation (voltage-dependence and kinetics). Using β2 subunits lacking inactivation (β2IR) revealed that currents from BK/β2IR G124R channels activated 2-fold faster and deactivated 2-fold slower compared with currents from BK/β2IR WT channels, with no change in the voltage-dependence of activation (V½). Despite the changes in the BK channel opening and closing, BK/β2 G124R inactivation rates (τinact and τrecovery), and the V½ of inactivation, were unaltered compared with BK/β2WT channels under standard steady-state voltage protocols. Action potential-evoked current was also unchanged. Thus, the mutant phenotype suggests the β2 G124R TM1-TM2 extracellular loop could regulate BK channel activation and deactivation kinetics. However, additional evidence is needed to validate pathogenicity for this patient-associated variant in KCNMB2.

1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental condition linked to inherited and de novo genetic changes that includes repetitive behavior, impaired social interaction, and impaired verbal/non-verbal communication (El-Fishawy and State, 2010; Wisniowiecka-Kowalnik and Nowakowska, 2019). To date, more than 102 gene mutations have been linked to ASD (Satterstrom et al., 2020) including ion channels and their regulatory subunits (Schmunk and Gargus, 2013). Among the K⁺ channels associated with ASD are KCNMA1 and KCNMB4, genes encoding the large conductance ‘BK’ calcium- and voltage-activated K⁺ channel and its modulatory subunit β4, respectively. Both KCNMA1 mutations and single nucleotide polymorphisms (SNPs) have been linked to ASD or related phenotypes, such as intellectual disability and developmental delay (Bailey et al., 2019; Miller et al., 2021; Launomnier et al., 2006; Staisch et al., 2016; Song et al., 2018; Plante et al., 2019; Wu et al., 2020; Vesil et al., 2018; Perche et al., 2022). BK channel activity is altered in several Fragile X and Angelman human patient and animal ASD models, associated with increased neuronal excitability that can be reversed via BK channel modulation (Contractor et al., 2015; Deng and Klyachko, 2016; Deng et al., 2013; Sun et al., 2019; Valerie Lemaire-Mayo et al., 2020; Hurley et al., 2021). One such modulation occurs through the β4 subunit, and both BKα and BK/β4 channels directly interact with Fragile X mental retardation protein (FMRP) to

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alter activation and deactivation gating kinetics and channel open probability (Deng et al., 2013; Deng and Klyachko, 2016; Kshatri et al., 2020). Thus, changes in BK channel activity via a KCNMA1 mutation, or through an accessory β subunit (β4), are associated with ASD-like features. However, little is known about β2, a neuron and endocrine-expressed β subunit modulating BK channel activity that is widely expressed in brain (Brenner et al., 2006).

Both β2 and β4 subunits provide unique phenotypes to the BK channel that alter BK channel activity (Gonzalez-Perez and Lingle, 2019). BK channel expression overlaps with both β2 and β4 in central neurons (Latorre et al., 2017; Tseng-Crank et al., 1996), with β4 typically more highly expressed compared with β2 (Brenner et al., 2006). While both subunits slow gating kinetics, β2 predominantly confers an inactivation via pore occlusion from the intracellular N-terminus, reducing BK channel activity (Brenner et al., 2006b; Jaffe et al., 2001). Additionally, β2 and β4 affect the calcium sensitivity of BK channels, but in different ranges (Brenner et al., 2006a). Thus, both accessory subunits are able to reduce the BK channel activity through different mechanisms, leading to multiple possibilities for alteration of neuronal excitability. While only β4 function is implicated in ASD-related learning disability at present, genome-wide association studies have recently identified KCNMB2 SNP associations in autism case trios and other neuropathological states (Beecham et al., 2014; Kimbrel et al., 2018; Xia et al., 2020). β2 expression has been detected in several areas of the brain implicated in ASD (Brenner et al., 2006b; Nordahl et al., 2012; Schumann et al., 2004; Stoodley, 2014).

In this study, we characterize a new patient-derived KCNMB2 variant (G124R) from a child diagnosed with ASD as a primary clinical presentation. Electrophysiological investigation of β2G124R was performed on a brain-expressed BK channel isoform, using voltage protocols designed to assess the effect of G124R on steady-state BK/β2 channel activation and inactivation properties and action potential-evoked BK current levels. Our results show that G124R alters the BK/β2 activation and deactivation kinetics, without affecting the inactivation phenotype, defining a functional consequence for the mutation on BK current properties.

2. Methods

2.1. Genetic and bioinformatic analysis

Exome sequencing was performed using genomic DNA isolated from whole blood from proband and parents (MagnaPure, Roche). Libraries were prepared using the Ion AmpliSeq™ Exome Kit (Life Technologies) and quantified by qPCR. The enriched libraries were prepared using Ion Chef™ and sequenced on PI™ Chip in the Ion Proton™ System (Life Technologies) to provide >90% of amplicons covered by at least 20X. Signal processing, base calling, alignment and variant calling were performed on a Proton™ Torrent Server using the Torrent Suite™ Software. Variants were annotated using Ion Reporter™ Software, and pedigree analysis was performed using the Genetic Disease Screen (GDS) trio workflow. Variant filtering and prioritization were performed using an in-house software program and a local database. Candidate variants were visualized using IGV (Integrative Genomics Viewer) and confirmed by Sanger sequencing. Candidate variants were evaluated at both the gene and variant levels, based on patient phenotype and the inheritance pattern and classified following the American College of Medical Genetics and Genomics (ACMG) guidelines. A molecular clinical geneticists board evaluated each variant for classification as pathogenic, likely pathogenic, or variant of uncertain significance (VUS) for reporting. The study was carried out in accordance with the Declaration of Helsinki of the World Medical Association and approved by the Local Ethics Committee (Madrid, Spain; Ref. 30062019). Informed consent was obtained from parents, after full explanation of the procedures.

The β2 homology model QCZM9 was obtained from the Swiss-model protein repository (https://swissmodel.expasy.org/repository/) and is made using the p4 cryoEM structure 6V35 (Tao and MacKinnon, 2019) as a template. The primary sequence alignment was performed in Uniprot (https://www.uniprot.org/align/) between the β2 sequences: human Q9Y691, rat Q811Q0, mouse Q9CZM9, pig F1SGC9 and cow Q1RW7. Pathogenicity predictor tools REVEL (Rare Exome Variant Ensemble Learner), CADD (Combined Annotation Dependent Depletion), MetaLR (Meta Logistic Regression) and Mutpred were obtained online at the Ensembl website (https://www.ensembl.org/Tools/VEP) (Dong et al., 2015; Ioannidis et al., 2016; Pejaver et al., 2020; Rentsch et al., 2019).

2.2. Patch-clamp electrophysiology

The human KCNMA1 (BKα subunit) (MG279689) and human KCNB2 (β2WT) cDNA sequences were expressed from the pcDNA3.1+ mammalian expression vector (Brenner et al., 2000a; Whitt et al., 2016). Inactivation was removed by deleting the Phe-Ile-Tyr residues (ΔFIW), in the resulting construct called β2IR (IR: Inactivation Removed) (Wallner et al., 1999; Wang et al., 2002; Whitt et al., 2016). Gly124Arg (rs201338193; GGG to AGG) was introduced into the β2WT and β2IR backgrounds and verified by sequencing. The BK channel contained a N-terminal extracellular Myc tag and EYFP in RK2, used to identify transfected cells. The Myc and EYFP inserts do not affect the BK channel properties (Plante et al., 2019). The BK channel and β2 plasmids were transfected into HEK293T cells as previously described (Moldenhauer et al., 2020; Whitt et al., 2016), in a ratio 1:2 (0.9 μg BKα and 1.8 μg β2, respectively). Electrophysiological recordings were performed 24 h post-transfection.

Macroscopic BK currents were obtained in the inside-out patch-clamp configuration at room temperature (RT: 22–24 °C) (Moldenhauer et al., 2020). Recordings were performed in physiological K+ conditions, with the pipette solution (in mM): 134 NaCl, 6 KCl, 1 MgCl2, 10 glucose, and 10 HEPES (pH7.4 with NaOH), and the internal (bath) solution (in mM): 110 KMeSO4, 10 NaCl, 30 KCl, 10 HEPES, 1 MgCl2, 5 HEDTA and adding Ca2+ to have 10 μM CaCl2 (pH 7.2 with KOH), Ek was −75 mV. The free Ca2+ concentration was calculated with WebMaxC (http://web.stanford.edu/~cpatton/webmaxcS.htm). Voltage protocols were applied using a MultiClamp700B amplifier and CLAMPEX v10.3 (Molecular Devices, Sunnyvale, CA). Current traces were acquired at 50 kHz and filtered online at 10 kHz (pClamp 10.6, Molecular Devices). Two protocols were used to assess activation and deactivation, respectively, from BK + β2IRWT or β2IRG124R. The activation protocol stepped from a Vhold of −120 mV, then from −100 to +100 mV (Δ +10 mV increments) for 30 ms, and stepping back to −150 mV for 15 ms to generate tail currents. Normalized conductance (G/Gmax) was calculated from the current level 150–200 μs after the peak of the tail current, divided by the highest conductance value calculated for each patch. Conductance-voltage (G/Gmax-V) curves were fitted with a Boltzmann function, constrained between 0 and 1, to obtain the half-maximal voltage of activation (V1/2) (Prism v9.0.0, GraphPad Software, San Diego, CA) as described in Moldenhauer et al. (2020). Only patches with maximal V1/2 shifts (>2 S.D. from the first quartile average) were included. Activation time constants (τact) were obtained single exponential fits of the current rise to the steady-state (SS) phase. The second voltage protocol was used to obtain τdeact: From −150 mV (Vhold), the protocol steps to +200 mV for 20 ms for activation, followed by steps from −200 mV to −50 mV (Δ +10 mV increments) for 15 ms. τdeact was obtained from single exponential fits of the peak of the tail current to the SS.

Inactivation was assessed from BK + β2WT or β2IRG124R co-expression. Inactivation kinetics (τinact) and the half maximal voltage of the steady-state inactivation (V1/2_inact) were determined from square voltage steps from −120 mV (Vhold), stepping to −200 to +200 mV (Δ +20 mV increments) for 150 ms, followed by +160 mV post-pulse, and back to −120 mV for 15 ms. The inter-sweep time was 2–3 s at the Vhold. τinact was obtained from a single exponential fit of the macroscopic current.
decay. $V_{1/2,\text{inact}}$ was obtained from a Boltzmann fit to the normalized maximum current obtained from the post-pulse, $I_{1-160/\text{inact}}$. A two-pulse protocol was used to determine the kinetics of recovery from inactivation, $T_{\text{recovery}}$. From $-120$ mV ($V_{\text{hold}}$), the voltage was stepped to $+160$ mV for 150 ms, stepping back to $-150$ mV, followed by a $+160$ mV post-pulse 10 ms duration at increments of 2 ms. The post-pulse maximal current was normalized by the maximum current from the activation step ($I_{\text{max}}$) and was plotted against time to obtain a single exponential fit for $\tau_{\text{recovery}}$. For action potential (AP)-activated currents, patches were first evaluated to determine the stoichiometry of the BK/β2 channel complex using an initial step to $+160$ mV for 150 ms. In patches where the $I_{\text{inact}}$ was $20 \pm 5$ ms (Wang et al., 2002), corresponding to full stoichiometry with 4 β2 subunits, a representative hypothalamic neuronal AP waveform was applied as the voltage command (Shelley et al., 2013). The area under the curve (AUC) was calculated from 4 ms before and after the maximum peak current evoked by the AP command and normalized to the maximum peak current evoked by the initial square voltage step to $+160$ mV. In all protocols, leak current was subtracted with a P/5 protocol using a $V_{\text{hold}}$ sub sweep between $-120$ and $-150$ mV.

Statistical analysis was performed in Prism v9.0.0. BK channel $\pm$ BK/β2IR (WT and G124R) $V_{1/2}$, activation, and deactivation values were compared using a one-way ANOVA with Bonferroni post-hoc test. For BK/β2 (WT and G124R), $V_{1/2,\text{inact}}$, inactivation kinetics, and AUC (AP-evoked current) comparisons were made with an unpaired t-tests. All data in figures are presented as mean $\pm$ SEM (lines and whiskers), with the individual data points for each patch.

3. Results

3.1. Patient case description

The proband is a 12-year-old male diagnosed with autism spectrum disorder (ASD) and mild intellectual disability, with predominant impairment of language and attention abilities. Partum occurred via uncomplicated vaginal delivery after 40-weeks of pregnancy. Birth weight was 3600 gr (60th percentile), length 53 cm (90th percentile), occipitofrontal diameter (OFD) 36.5 cm (90th percentile). Family history was not relevant; his parents are healthy. His first single syllable utterances and first verbal words were said at 1.5 and 6 years of age, respectively. He walked at 1.5 years old.

At 5 years, the patient showed verbal and nonverbal communication deficits, lack of joint attention, avoidance of mutual eye contact, stereopathy, and idiosyncratic language associated with hyperkinetic behavior. He received risperidone to improve these behaviors. The patient was placed on atomoxetine at age 8 for attention deficits. This therapy, at doses of 1.2 mg/kg/d, was associated with clear improvement in attention impulsiveness and social adaptation at school. He thus continues 1.2 mg/kg/d of atomoxetine daily and was enrolled in a conventional school system with supports. He maintained good behavior and average school performance.

At 11 years old, the clinical examination disclosed a weight of 35 kg (45th percentile), a height of 145 cm (70th percentile), and an OFD of 54 cm (60th percentile), without dysmorphic features. Routine laboratory screening including thyroid function and neumoetabolic tests were within the normal range. Sleep video-EEG test and auditory evoked potentials displayed normal results. Brain 3T MRI did not reveal any structural abnormalities. Further information was obtained using the Autism Diagnostic Interview-Revised (ADI-R) and the Autism Diagnostic Observation Scale (ADOS) (Connelly et al., 2013; Fitzgerald, 2010). He met the criteria for autism spectrum disorder on both instruments.

Whole-exome sequencing (WES) revealed a heterozygous missense mutation in exon 4 of the KCNMB2 gene on human chromosome 3q26.3 (Gene ID: 10242): NM_181361.3; c.370G > A; p.Gly124Arg (rs201338193). The allele frequency of this variant in the general population is 0.0092% (gnoMAD v2.1.1), with a higher frequency in Asian population (0.055%). No homozygous variant carriers have been described. In the WES trio analysis, the G124R variant was not present in either parent. No other missense variants with a CADD score over 15 (Rentzsch et al., 2019), nor LOF variants with a clear phenotypic association, were identified in the patient DNA.

3.2. In silico structural analysis of the G124R mutation in the j2 subunit

Genomic computational analyses predicted G124R as a pathogenic variant, with a CADD score of 29.6. This prediction was further supported by tools incorporating additional structural and functional properties, including Mutpred (score of 0.917), REVEL (0.543), and CADD PHRED (31), but not MetaLR (0.111) (Dong et al., 2015; Ioannidis et al., 2016; Pejaver et al., 2020; Rentzsch et al., 2019) (Fig. 1C). A Mutpred score of 0.8 yields a 5% probability of a false positive, thereby a score of 0.917 had a 0.5% probability of false positive (Pejaver et al., 2020). In the case of REVEL, a score above 0.5 means that the 75.4% of disease mutations are detected as pathogenic and only the 10.9% of neutral variants (Ioannidis et al., 2016). The CADD PHRED score of 31 indicates that, of all the possible substitutions of at residue 124, G124R is in the top 1% for deleterious effects. Although the MetaLR score did not directly hypothesize pathogenicity, this could be related with the lack of maximum minor allele frequency (MMAF) parameter of 1% or higher (Dong et al., 2015).

The BK channel j2 protein encoded by the KCNMB2 gene is comprised of two transmembrane domains, TM1 and TM2, with an intervening extracellular loop and intracellular N- and C- termini facing the cytosol (Fig. 1A). Most notably, the inactivation particle (1-45aa) is located at the N-terminus, where the motif Phe-Ile-Thr (FIW) blocks the conduction pathway through the inner vestibule of the BK channel, a process that primarily occurs in the open state (Fan et al., 2020; Solaro and Lingle, 1992; Wallner et al., 1999; Wang et al., 2002; Xia et al., 1999, 2000, 2003). G124 is located in the extracellular loop (Fig. 1A) in a highly conserved region between different mammalian species (>97%) (Fig. 1B). This extracellular loop contains three asparagine residues targeted for N-glycosylation, N88, N96 and N119, relevant for the association kinetics between BK and j2 (Huang et al., 2017). Additionally, the amino acid sequence from 137 to 147 (KINQKCSYIPK) is rich in basic residues and has been proposed to be responsible for the outward rectification through an electrostatic mechanism (Chen et al., 2008; Lv et al., 2008). Finally, several cysteine residues in the extracellular loop form disulfide bonds between them in the other β subunits (Wu et al., 2013; Zeng et al., 2003), providing a structural frame for the charybdotoxin blockage protection in which the N-glycosylations and the lysine rich sequence contribute. Thus, the location of the G124 mutation in this extracellular loop region has the potential to affect several aspects of BK/j2 channel properties.

3.3. Functional effect of the G124R mutation on non-inactivating BK current

β2 affects multiple aspects of BK channel gating, most notably conferring rapid inactivation via the hydrophobic N-terminal ‘ball’, but also shifting the voltage-dependence of activation to more hyperpolarized potentials and slowing the kinetics of activation (at low Ca$^{2+}$ concentration) and deactivation (Brenner et al., 2000a; Gonzalez-Perez and Lingle, 2019). To first evaluate the inactivation properties, the initial three amino acids of the ‘inactivation particle’ were removed from the N-terminus (G2IR) (Wang et al., 2002; Whitt et al., 2016). HEK293T cells were transfected with BK α subunits alone, or co-transfected with β2IR WT or mutant β2IRG124R subunits. Inside-out patches were recorded in physiological Na$^{+}$ and K$^{+}$ gradients in the presence of 10 μM intracellular Ca$^{2+}$. These conditions mimic the K$^{+}$ driving force and intracellular Ca$^{2+}$ concentration during neuronal
H.J. Moldenhauer et al.

**Figure 1**

**A** shows a molecular structure, with **B** listing the consensus sequence for various species:
- **HUMAN** 116 VYVNLTSSEKLLYYHTEETIKIN 146
- **RAT** 116 VYVNLTSSEKLLYYHTEETIKIN 146
- **MOUSE** 116 VYVNLTSSEKLLYYHTEETIKIN 146
- **PIG** 149 VYVNLTSSEKLLYYHTEETIKIN 179
- **CHICKEN** 147 VYVNLTSSEKLLYYHTEETIKIN 177
- **COW** 116 VYVNLTSSEKLLYYHTEETIKIN 146

**C** displays voltage-clamp experiments on BKα, BKβ2|RWT, and BKβ2|RG124R channels.

**D** represents the normalized current-voltage relationship, with G/Gmax shown at ±100 mV and ±150 mV.

**E** illustrates the change in V1/2 with BKβ2|RWT and BKβ2|RG124R, showing the shift in voltage for half-maximal activation.

**F** and **G** depict the time constants (τact and τdeact) as a function of voltage (V(mV)).

*(caption on next page)*
activity (Fakler and Adelman, 2008; Marrion and Tavalin, 1998). Using a square voltage step protocol, currents activated from BK/β2WT or BK/β2G124R were analyzed. Because expression can vary in transfected heterologous cells, only patches that elicited the maximal V1/2 shift, indicative of the presence of 4 β2 subunits in the complexes, were analyzed.

We found the human β2 subunit shifts the voltage-dependence of activation by ~26 mV compared to BK α channels alone (V1/2: BK 36 ± 3.2 mV and BK/β2WT 10 ± 4 mV; Fig. 1C–E). This effect was not different with mutant subunits, as BK/β2G124R currents did not produce a significant difference in the V1/2 compared with BK/β2WT (V1/2: BK/β2G124R 7 ± 5 mV; Fig. 1D–E). Our results agree with prior studies reporting that the BK channel voltage sensitivity (Z) is not affected by β2 (Contreras et al., 2012; Orío et al., 2006). Additionally, we did not observe a significant difference in voltage sensitivity between the BK/β2G124R channels and BK/β2WT (Z = 0.91 ± 0.04 and 0.97 ± 0.06 respectively; Fig. 1D).

β2 subunits also affected BK channel opening and closing. Under these ionic conditions, BK/β2WT currents did not activate differently than BK α channels alone (Fig. 1F). However, the mutation BK/β2G124R increased the activation kinetics (τact), with BK/β2G124R currents activating 2 times faster than BK/β2WT at 0 mV (Fig. 1F). At membrane potentials near the peak of an action potential (+40 mV), the τact for BK/β2G124R currents was 4.2 ± 0.3 ms, while BK/β2WT was 6.6 ± 0.5 ms. In contrast, with channel closing, BK/β2WT currents deactivated up to 4 times slower than the BK channel alone (τinact, Fig. 1G). The presence of the mutation reduced this effect on closing, such that BK/β2G124R was only 2 times slower on average (between –60 and –70 mV; Fig. 1G). At –60 mV, the τinact for BK/β2G124R currents was 4.2 ± 0.9 ms, while BK/β2WT was 5.6 ± 1.1 ms. Taken altogether, the net effect of the G124R mutation is restricted to channel opening (BK/β2G124R channels activated more rapidly than BK/β2WT) and closing kinetics (BK/β2G124R channels deactivated more slowly than BK/β2WT), not affecting the voltage-dependence of activation.

3.4. Effect of G124R mutation on the BK channel inactivation

Although G124R is located extracellularly, away from the N-terminal inactivation particle inside the cell, the mutation could affect inactivation by changing the kinetics for β2 modulation of BK channels. To test this, WT and BK42WT mutant β2 subunits with intact inactivation were used under 3 voltage protocols in physiological ionic conditions to address different aspects of this process. First, to determine how fast inactivation occurs and the basic voltage-dependence, the rate of inactivation (τinact) and the voltage at which voltage the half of the channels are inactivated (V1/2,τinact) were obtained using a protocol stepping the BK/β2WT and BK/β2G124R channels from closed to different activation potentials, and then measuring the current at a post-pulse step to +160 mV (Fig. 2A, and inset in 2B). BK channels co-expressed with α2,β2WT produced macroscopic currents that activated, and then inactivated within 150 ms (Fig. 2A). As a control for αββ subunit stoichiometry, patches were first stepped to +160 mV to confirm they produced τinact values consistent with 4 β2 subunits per BK channel, which was 20 ms in symmetrical K+ and 10 μM Ca2+ from prior studies (Wang et al., 2002). Thus, only patches with currents that produced macroscopic τinact of less than or equal to 20 ± 5 ms (± 2 standard deviations from the average; Fig. 2B) were analyzed.

BK/β2G124R had τinact values that were indistinguishable from BK/β2WT (Fig. 2A–B). Fig. 2C shows the similar distributions from BK/β2WT and BK/β2G124R channels across individual patches for –20 and +60 mV, demonstrating the mutation does not cause a systematic effect. Average τinact values were 52.3 ± 5.7 ms for BK/β2WT and 46.6 ± 4.5 ms for BK/β2G124R at +20 mV, and 18.5 ± 1.3 ms for BK/β2WT and 19.2 ± 1.3 ms for BK/β2G124R at +160 mV. In addition, no difference was observed in the V1/2 for inactivation between BK/β2WT (~141 ± 53 mV) and BK/β2G124R (~148 ± 5.7 mV) currents using the same voltage protocol (Fig. 2D–F).

Next, we assessed recovery from inactivation, previously shown to be between 18 and 25 ms in symmetrical K+ and 10 μM Ca2+ conditions (Li et al., 2007; Wang et al., 2002; Xia et al., 2003). A two-pulse protocol was used, consisting of an activation step to +160 mV to allow activation and inactivation to occur, followed by a second shorter step to +160 mV after a recovery interval at ~150 mV. The second step was 2 ms after the first, with increasing increments of 2 ms to evaluate the time constant of recovery from inactivation (Fig. 3A). The representative trace shows the BK/β2G124R current response, with increasing amount of current obtained from each subsequent recovery step (Fig. 3A). To compare BK/β2WT and BK/β2G124R channel recovery from inactivation, current from second voltage step was normalized to the peak current from the first voltage step (I2nd step/I1st step; Fig. 3A) and plotted as a function of the recovery time interval (Fig. 3B). The recovery time constant (τRecovery) was obtained from fits of normalized current (current normalized to peak current, I2nd step/I1st step) over time curves. In these experimental conditions, no difference was found between the τRecovery for BK channels co-expressed with β2WT (28 ± 2.7 ms) compared to mutant β2G124R (27 ± 3.3 ms) (Fig. 3C).

A final set of conditions using an action potential (AP) waveform stimulus was evaluated, a non-stationary voltage command that integrates the activation and deactivation kinetics during BK/β2 gating. The representative AP waveform was obtained from a central hypothalamic neuron type where the role for BK/β2 channel regulation of neuronal activity is well-characterized and where the alteration of BK current properties due to loss of β2 is detectable in single AP-evoked BK currents (Whitt et al., 2016). As in prior experiments, data was only included from patches with τinact value of 20 ms deviations at +160 mV ± 2 standard deviations (Fig. 4A–B) consistent with channel complexes containing 4 β2 subunits (Wang et al., 2002).

Small currents were evoked from BK/β2WT co-expressed channels (Fig. 4C) using this single AP voltage stimulus in physiological K+ conditions. BK/β2G124R also reliably produced AP-evoked currents. To determine if the faster activation and slower deactivation associated with β2G124R in steady-state experiments could increase AP-evoked BK current, BK current was integrated as area under the curve (AUC). AUC values were normalized to the maximum current evoked by the square voltage step protocol to account for differences in expression between patches. No differences were detected between the β2WT and β2G124R AP-
evoked currents (Fig. 4D). Furthermore, no difference was found in the peak BK current elicited by either β2 subunit (BK/β2WT normalized peak current: 0.03 ± 0.004; n = 16 and BK/β2G124R current: 0.03 ± 0.003, n = 20; P = 0.9, t-test). This experiment further confirmed that the inactivation phenotype conferred by β2 is not affected by the changes in BK channel opening and closing kinetics induced by the β2G124R mutation.

4. Discussion

Here we report a new KCNMB2 mutation, c.370G > A; p.G124R,
from a heterozygous individual with autism that affects BK/β2 channel opening and closing kinetics without obvious effect on inactivation. The present investigation was not designed to establish causality for the G124R variant in ASD, due to the de novo nature of the mutation. As additional patient variants are reported for KCNMB2, it remains to be determined whether the association between this gene or variant and ASD will be substantiated in a larger ASD cohort. Nevertheless, because ASD, learning disability, and other neurodevelopmental dysfunctions are prominent phenotypes in the ASD, learning disability, and other neurodevelopmental dysfunctions will be substantiated in a larger ASD cohort. Nevertheless, because ASD, learning disability, and other neurodevelopmental dysfunctions are prominent phenotypes in the ASD, learning disability, and other neurodevelopmental dysfunctions will be substantiated in a larger ASD cohort. 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extracellular loop on activation kinetics (Huang et al., 2017), other studies did not find differences in kinetics in the context of larger extracellular loop region chimeric swaps between β1 and β2IR (Orio et al., 2006). Interestingly, G124 lies in a stretch of residues with high homology between β1, β2, and β3, but not β4 (Gruslova et al., 2012). This raises the possibility that the similarities between β1 and β2 could have precluded the ability to detect a change in the chimeric experiments (Orio et al., 2006), leaving open the role of the extracellular loop in BK gating kinetics. Consistent with the data in this study, β2 has been shown to interact with the Bκa voltage sensor, which would support a role in gating kinetics (Savalli et al., 2007).

Lastly, although the extracellular location of G124R does not predict a direct role, inactivation is the most obvious effect β2 confers on BK channel gating. An indirect effect on inactivation could be produced if BK/β2G124R channels opened faster, and therefore inactivated earlier, reducing AP-evoked BK current amplitude. However, no differences in the inactivation or recovery from inactivation were found using either activation paradigm: steady-state (square step commands) or dynamic (action potential commands) voltage stimuli. The lack of difference suggests that any differences between the β2WT and β2G124R extracellular loop conformations that produced the kinetic effects in steady-state protocols are not enough to reveal an effect on the inactivation phenotype. This lack of effect was not due to a low number of β2 subunits present in the channel complex, since patches in this study were selected to contain the full 4 β2 subunits by τinact values (Li et al., 2007; Wang et al., 2002; Xia et al., 1999, 2003). Nevertheless, only one set of basic conditions was tested here. Additional deficits with β2G124R may be uncovered with higher intracellular calcium concentrations or by using repetitive firing protocols which produce faster activation and limited recovery between action potentials.

5. Conclusions

Intellectual disability is one of the core phenotypes in KCNMA1-linked channelopathy. This study reports a KCNMB2 variant found in an autistic individual and prompts continued investigation of an association between KCNMB2 and ASD in a larger cohort. Future studies will be required to determine the biophysical mechanism by which the G124R mutation affects BK/β2 gating and whether this alters neuronal activity in ASD-related circuitry. However, the data presented here raise the possibility that KCNMB2 variants could alter BK currents by similar kinetics means as some KCNMA1-linked channelopathy α subunit variants, without affecting inactivation.

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CRediT authorship contribution statement

Hans J. Moldenhauer: Conceptualization, research conception and data collection, statistical analysis design and execution, manuscript writing. Ria L. Dinsdale: data collection. Sara Alvarez: patient case description and sequencing. Alberto Fernandez-Jaen: patient case description and sequencing, All authors approved the final version of the manuscript. Andrea L. Meredith: Conceptualization, Formal analysis, research conception and data analysis, statistical analysis design, manuscript writing, and review and critique.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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