Deleterious Variation in *BRSK2* Associates with a Neurodevelopmental Disorder

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Developmental delay and intellectual disability (DD and ID) are heterogeneous phenotypes that arise in many rare monogenic disorders. Because of this rarity, developing cohorts with enough individuals to robustly identify disease-associated genes is challenging. Social-media platforms that facilitate data sharing among sequencing labs can help to address this challenge. Through one such tool, GeneMatcher, we identified nine DD- and/or ID-affected probands with a rare, heterozygous variant in the gene encoding the serine/threonine-protein kinase BRSK2. All probands have a speech delay, and most present with intellectual disability, motor delay, behavioral issues, and autism. Six of the nine variants are predicted to result in loss of function, and computational modeling predicts that the remaining three missense variants are damaging to BRSK2 structure and function. All nine variants are absent from large variant databases, and *BRSK2* is, in general, relatively intolerant to protein-altering variation among humans. In all six probands for whom parents were available, the mutations were found to have arisen *de novo*. Five of these *de novo* variants were from cohorts with at least 400 sequenced probands; collectively, the cohorts span 3,429 probands, and the observed rate of *de novo* variation in these cohorts is significantly higher than the estimated background-mutation rate ($p = 2.46 \times 10^{-6}$). We also find that exome sequencing provides lower coverage and appears less sensitive to rare variation in *BRSK2* than does genome sequencing; this fact most likely reduces *BRSK2*'s visibility in many clinical and research sequencing efforts. Altogether, our results implicate damaging variation in *BRSK2* as a source of neurodevelopmental disease.

Developmental delay and intellectual disability (DD and ID), attention-deficient/hyperactivity disorder (ADHD), schizophrenia, language communication disorders, autism spectrum disorders (ASDs), and motor and tic disorders lie under a more general umbrella of neurodevelopmental disorders (NDDs).[1,2] Although these are traditionally categorized into discrete disease entities, many symptoms are not unique to a single NDD. Furthermore, many genes have been associated with multiple NDDs,[3] and new genetic associations continue to be discovered. This is particularly true given the recent acceleration in large-scale sequencing and cross-site genotype-phenotype "matchmaking" efforts.[4,5]

Through a Clinical Sequencing Exploratory Research (CSER) project focused on sequence-driven diagnoses for probands with unexplained DD and/or ID,[6] we identified variation likely to be deleterious in *BRSK2* (MIM: 609236) in four unrelated probands. *BRSK2* encodes a serine/threonine-protein kinase, which is involved in axonogenesis and the polarization of cortical neurons.[7] *BRSK2* is predicted to be relatively intolerant to protein-altering variation in the general population (%ExAC v2 residual variation intolerance score [RVIS] = 4.9462%,[8] pLI score (probability that a gene is intolerant to a loss of function mutation) = 0.78[9]). In each proband, the *BRSK2* variant was prioritized, after filtering and manual curation, as the most compelling disease-candidate variant of interest (see details by Bowling and colleagues for additional information about the cohort and analytical methods[6]). Although these observations suggest *BRKS2* as a strong candidate NDD-associated gene, we sought additional cases via GeneMatcher[5] to support pathogenicity. GeneMatcher is a database developed as part of the MatchMaker Exchange and has been shown to facilitate rare-disease-gene discovery.[10] Information about five additional affected probands who were found by research or diagnostic sequencing (Table 1) and who had variants likely to be deleterious in *BRSK2* was independently submitted to GeneMatcher. Informed consent to publish de-identified data was obtained from all affected individuals and/or families (see Supplemental Material and Methods). Altogether, the affected probands ranged in age from 3 years and

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Table 1. Variant Details of Individuals with *BRSK2* Variation

Proband[a]	1	2	3	4	5	6	7	8	9
Sequencing/ analysis site[b]	A	B	C	C	A	D	E	C	C
HGVS nomenclature[c]	g.1459543G>A, c.194G>A (p.Arg65Gln)	g.1462017G>A, c.273–1G>A	g.1463837G>A, c.530+1G>A	g.1464720G>A, c.635G>A (p. Gly212Glu)	g.1464815C>T, c.730C>T (p. Gln244*)	g.1471060_ 1471071del, c.1281_ 1287+5del	g.1471924_ 1471925del, c.1395_1396del (p.Ser466Glnfs*83)	g.1472635_ 1472636del, c.1532_1533del (p.Glu511Valfs*38)	g.1477839C>T, c.1861C>T (p.Arg621Cys)
Predicted effect	missense	splice alteration	splice alteration	missense	premature stop	splice alteration	frameshift	frameshift	missense
Inheritance	*de novo*	*de novo*	*de novo*	*de novo*	*de novo*	unknown	*de novo*	unknown	unknown
CADD_v1.4	25.3	24.8	26.5	26.9	38	32	34	34	29.2
GERP	2.57	2.65	3	3.26	3.27	4.11	3.91	3.81	3.8
Sequencing method	exome	exome	genome	genome	exome	exome	exome	genome	genome

Abbreviations are as follows: HGVS = Human Genome Variation Society, CADD = Combined Annotation Dependent Depletion, and GERP = genomic evolutionary rate profiling.
[a]Probands are numbered on the basis of a 5′ to 3′ ordering of variation.
[b]Sequencing and analysis sites are further defined in the Supplemental Note.
[c]HGVS nomenclature applies to GenBank: NC_000011.9, GenBank: NM_001256627.1, and GenBank: NP_001243556.1.

9 months old to 19 years old and had a mean age of just under 8 years (Table 2). All probands described here display ID except for one. All probands present with developmental delays, including speech delay (9/9) and motor delay (7/9). Eight of nine probands, one of whom (proband 9) was considered borderline, present with autism, and seven were reported to have behavioral abnormalities, including stereotypies (4/7), temper tantrums (3/7), and/or ADHD (3/7). Two probands reported sleep issues that were treatable with melatonin. Although most probands were reported to have facial dysmorphism, we did not observe a consistent set of features (see Supplemental Note). Additional details of each case are provided in the supplement (See Table S1 and Supplemental Note).

Parents were available for testing for six of the nine probands, and in all six of these probands, the variants were found to be *de novo* (Table 1 and Figure 1). Six of the nine described variants, including two frameshift variants, one nonsense variant, and three variants affecting canonical splice sites, are predicted to result in loss of function. The remaining three variants were missense. All nine variants are absent from gnomAD[9] and the Bravo TOPMed database. Although gnomAD does contain variant data aggregated from several disease cohorts, there has been an effort to remove any variants found in individuals with severe pediatric disease.[9] All variants were computationally predicted to be deleterious and had Combined Annotation Dependent Depletion (CADD) scores[11] ranging from 24.8 to 38; these scores indicate that they rank among the most highly deleterious variants possible in the human genome reference assembly, similar to most variants previously reported to cause Mendelian diseases.[11]

The canonical protein encoded by the *BRSK2* locus (GenBank: NP_001243556.1, UniProt: Q8IWQ3, 736 aa) contains several domains, including a protein kinase domain (aa 19–270), a ubiquitin-associated domain (UBA; aa 297–339), a proline-rich domain (aa 424–468), and a kinase-associated domain (KA1; aa 530–653) that contains a KEN box (a degradation signal, aa 603–605) (Figure 1B). An analysis of conservation along the protein identified several regions with elevated measures of conservation (Figure 1C). Two missense variants (c.194G>A [p.Arg65Gln] and c.635G>A [p.Gly212Glu]) are located within the protein kinase domain, and one (c.1861C>T [p.Arg621Cys]) is within the KA1 domain.

We assessed the potential structural effects of the three missense variants by performing computational modeling.[12] All three missense variants lie within conserved linear motifs (Figure 1C) and affect residues that are conserved across many species (Figures 2 and S1). Arg65 lies within the protein kinase domain and has been found to coordinate intramolecularly with Glu330 to form a salt bridge.[13] Arg65 also lies within a mitogen-activated protein kinase (MAPK) docking motif[14] (Figure 2). Thus, p.Arg65Gln is predicted to disrupt both the structure and functional activity of BRSK2. Gly212 lies in the C-lobe of the protein kinase domain,

Table 2. Clinical Characteristics of Individuals with *BRSK2* Variation

Proband	1	2	3	4	5	6	7	8	9
Gender	Male	Male	Female	Male	Male	Male	Male	Male	Male
Age at last examination	5 years	14 years	5 years, 7 months	3 years, 9 months	19 years	4 years	6 years, 10 months	10 years, 11 months	4 years, 7 months
Intellectual disability	moderate	severe	mild	moderate	mild progressed to severe	none reported	mild	moderate	moderate
Developmental delay(s)	speech and motor	speech, including speech regression, and motor	speech and fine motor	speech and social interaction; speech regression	speech and fine motor; regression	speech and gross motor	speech	speech and gross motor	speech and gross motor
Autism	no	yes	yes	yes	yes	yes	yes	yes	borderline
Behavior	none reported	ADD and/or ADHD, stereotypic, temper tantrums, sleep issues	none reported	temper tantrums, easily cries, fidgets, hits others, prefers to play alone	ADHD, hyperactive as toddler, psychoses, schizophrenia, anxiety, hallucinations, impulsive, self-mutilation, aggression, concentration issues	stereotypies, pinching, tantrums and rigidity, sleep issues	stereotypic (mild)	ADD and/or ADHD, impulsive	stereotypic

Abbreviations are as follows: ADD = attention deficit disorder and ADHD = attention-deficient/hyperactivity disorder.

just at the C terminus of a helix, within a flexible linker;[13] thus, p.Gly212Glu might disrupt secondary protein structure. Arg620 and Arg621 comprise a di-arginine endoplasmic-reticulum (ER) retrieval-and-retention motif, and a recent publication found that Arg620 forms a salt bridge with Asp305 when the KA1 domain associates with the UBA domain.[13] The authors of this paper also found that disruption of several key polar residues, including Arg620 and Arg621, in the C terminus of the protein abolishes phospholipid binding. On the basis of these observations, it is plausible that p.Arg621Cys disrupts the localization of this protein to the ER and possibly to other membranes. Given that the p.Arg621Cys variant is of unknown inheritance, it remains a variant of uncertain significance (VUS),[15] and further experimental or computational analyses are needed if we are to better understand its potential molecular and disease effects.

We assessed the degree of enrichment of observed *de novo* variation in *BRSK2* in the sequenced DD- and/or ID-affected cohorts that underlie this study. Two probands (1 and 5) were a part of a cohort of 2,418 DD- and/or ID-affected probands sequenced as trios. An additional proband (2) was sequenced as a trio among a cohort of 550 affected probands, and two others (probands 3 and 4) were among a cohort of 461. In aggregate, these cohorts include five *de novo* variants in 3,429 affected, sequenced individuals. We compared this observed rate to the expected rate estimated by Samocha et al.[16] (2.97×10^{-5} variants per chromosome) of *de novo* missense, nonsense, splice, and frameshift variation in *BRSK2*. The observed rate of *de novo* variation in the DD- and/or ID-affected cohorts considered here is significantly greater than the background mutation rate (five *de novo* variants observed versus 0.20 expected, $p = 2.46 \times 10^{-6}$), and this observation remained significant even after a Bonferroni correction for 20,000 genes ($p = 0.0492$). We note that one proband (7) with a *de novo* variant was sequenced clinically as a trio, but a cohort size was not available for this proband; furthermore, one or both parents were unavailable for testing for three of the nine observed variants. Thus, although these four additional variants add to the evidence supporting a disease role for *BRKS2*, they are excluded from the preceding enrichment calculations.

BRSK2 and its homolog *BRSK1* (MIM: 609235) encode kinases required for neuronal polarization.[7] These two kinases, along with 11 other kinases, form the AMPK-related family of protein kinases.[17] Although knockouts of either *Brsk1* or *Brsk2* alone in mice were healthy and fertile, double knockouts of *Brsk1* and *Brsk2* resulted in pups that exhibited reduced spontaneous movement and little response to tactile stimulation and that died within 2 h of birth.[7] Expression patterns of *BRSK2* also support its role in neurodevelopment. *BRSK2* is most highly expressed in the brain in humans,[18] and *Brsk1* and *Brsk2* are restricted to the nervous system in mice.[7]

BRSK2 interacts with several genes that are associated with NDDs, including autism, tuberous sclerosis, and DD

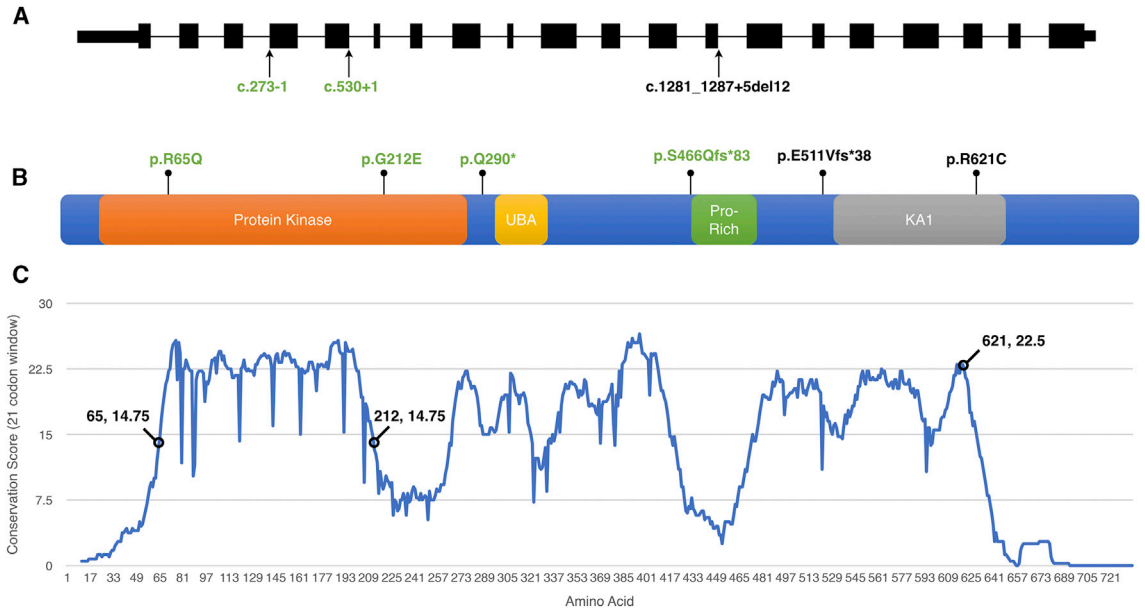

Figure 1. Exon and Domain Structure, Conservation, and Locations of Observed Variation in *BRSK2*

(A and B) Variation observed in *BRSK2* is shown for (A) the canonical, 20-exon transcript, GenBank: NM_001256627.1 and (B) the 736 aa protein, GenBank: NP_001243556.1. Protein domains include protein kinase, ubiquitin-associated (UBA), proline-rich (Pro-Rich), and kinase-associated 1 (KA1) domains. Splice variants are shown below the schematic representation of the canonical transcript, and protein-altering variants are shown above the schematic representation of BRSK2. *De novo* variants are shown in green text, and those of unknown inheritance are shown in black.

(C) Analysis of conservation throughout BRSK2 was performed with amino acid selection scores as previously published[12] and used a 21-codon sliding window. The most-selected motifs of a protein are identified as peaks. The three residues found to be affected by variation here are labeled, along with their respective conservation scores.

and/or ID. For example, BRSK2 has been shown to phosphorylate TSC2 and suppress mTORC1 activity.[19] The tuberous sclerosis complex (TSC) signaling pathway is one of the pathways associated with autophagy during early axonal growth,[20] and TSC2, specifically, is a regulator of cellular size and growth.[21,22] BRSK2 has also been shown to interact with PTEN, which has been associated with various developmental disorders (see MIM: 601728[23,24]) including autism. PTEN-deficient mice display malformation of neuronal structures and autistic features that result from aberrant TSC-mTORC1 signaling.[25] WDR45, also known as WIPI4, is a scaffold protein that controls autophagy and has recently been shown to be dependent on BRSK2 activity.[26] Variation in *WDR45* is associated with an X-linked dominant disorder: neurodegeneration with brain iron accumulation (MIM: 300894). The numerous genetic and biochemical interactions between *BRSK2* and well-established NDD genes further strengthen the conclusion that damaging variation in *BRSK2* underlies an NDD.

Across six recent publications reporting on *de novo* variation in large cohorts with DD and/or ID or autism,[27–32] two protein-altering *BRSK2* variants were reported: GenBank: NM_001256627.1 (c.992_994del, [p.Lys331del]) was found in a cohort of 2,500 probands with autism,[30] and GenBank: NM_001256627.1 (c.770G>A, [p.Arg257His]) was found in a cohort of 4,293 DD- and/or ID-affected probands.[28] Interestingly, this second variant has been observed as a heterozygote seven times in gnomAD, suggesting it is not a highly penetrant allele contributing to DD and/or ID. These data raise an interesting question, namely as to why the frequency of observed *BRSK2* variation in this study is markedly higher than that found in previous studies. This is particularly true for the HudsonAlpha CSER study,[6] in which four variants were found among 581 affected probands (461 of whom were sequenced as trios). Some of the discrepancy is probably due to stochastic variability in observing a small number of rare events. However, one potential systematic explanation is that *BRSK2* is less deeply covered in exomes, and the observed enrichment, in part, reflects the effects of the genome sequencing that was used for the HudsonAlpha probands described here. It has been shown previously that genome sequencing provides better coverage, in general, over coding exons than exome sequencing does,[27,31,33–36] and that some exons, including among clinically relevant genes, tend to be more poorly covered by exomes.[36]

We find that *BRSK2* is less well covered by exomes than by genomes in gnomAD (Figure 3). For example, when requiring a minimum depth of 20× among exonic bases (plus 10 bp on either side of each exon), we found that 76% of gnomAD exome samples, compared to 93% of genome samples, have half of all *BRSK2* bases covered (Figure 3A). Furthermore, we assessed rare-variant detection rates, in particular the rate at which singletons (i.e., variants for which only one alternative allele is observed across the combined set of exomes and genomes) are

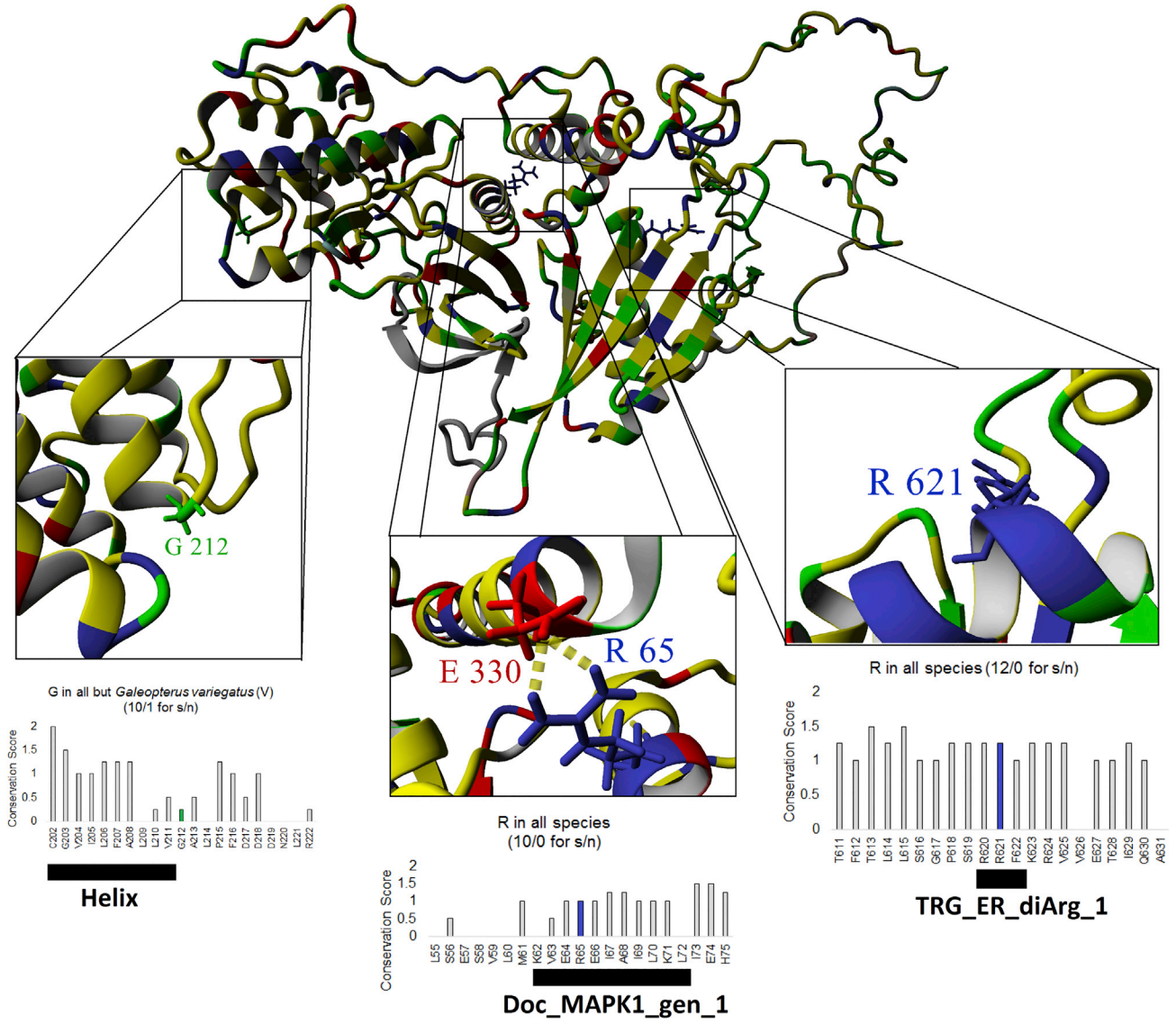

Figure 2. Computational Modeling of BRSK2 Missense Variants

A full model of BRSK2 was created with I-TASSER modeling using PDB: 4YOM, 4YNZ, and 4IW0. This model of BRSK2 was combined via ConSurf mapping with sequences for BRSK2 from 99 species. Amino acid coloring is as follows: gray = not conserved, yellow = conserved hydrophobic, green = conserved hydrophilic, red = conserved polar acidic, blue = conserved polar basic, and magenta = conserved human variants of interest. Zoomed-in views of the three locations are shown, along with codon usage throughout evolution. The conservation score is defined as an additive metric of amino acid conservation and codon selection as previously defined.[12] For example, a conservation score of 2 indicates 100% conservation with >2 standard deviations above the mean for codon selection.[12] s/n indicates synonymous mutations versus non-synonymous mutations observed at the same position in other species; differences are indicated. All three sites are under high selection and have multiple synonymous (s) amino acids in 99 open reading frames (ORFs) of BRSK2 and only a single nonsynonymous (n) change observed at G212. Linear motifs mapped with the Eukaryotic Linear Motif (ELM) tool are shown below each site.

observed. There are 46 singletons detected among 15,708 genomes (0.29%) in gnomAD and 189 singletons detected among 125,748 exomes (0.15%); this difference is significant ($p = 1.5 \times 10^{-4}$, Fisher's exact test) and suggests increased rare-variant sensitivity in genomes relative to in exomes. Additionally, considering only exomes, we compared coverage of *BRSK2* exons to exons in other disease-associated genes that are annotated in the Development Disorder Genotype-Phenotype Database (DDG2P). Although, again, only 76% of samples have at least half of *BRSK2* bases covered at 20× in gnomAD exomes, 99% of samples have half or more of the bases in previously reported DDG2P genes covered in gnomAD exomes (Figure 3B). Thus, we find it likely that the lower rates of *BRSK2* variation found in other DD and/or ID studies reflects, at least in part, reduced variant sensitivity of exome sequencing in *BRSK2*.

We have identified nine individuals harboring rare, heterozygous *BRSK2* variants that are likely to be deleterious, and we provide detailed clinical descriptions of the

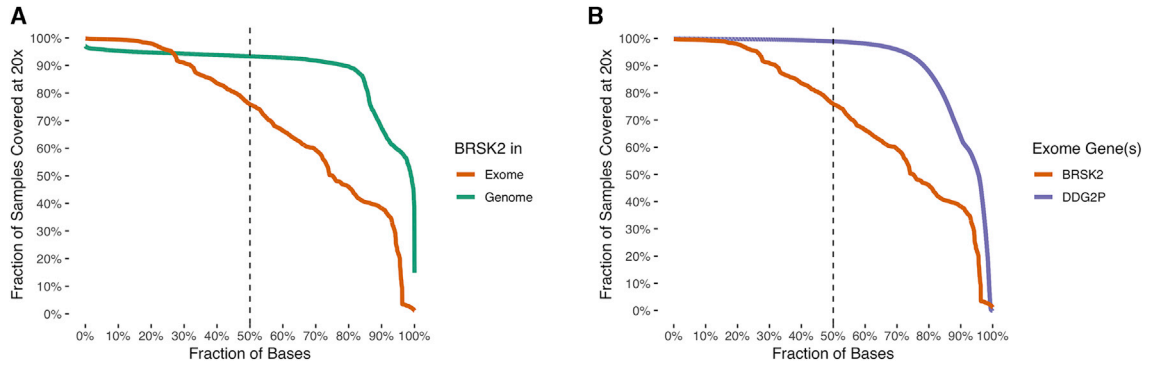

Figure 3. Comparisons of *BRSK2* Sequencing Depth across gnomAD Datasets
Fractions of gnomAD samples that attain a per-base sequencing depth of ≥20× are plotted as a function of the percentage of bases examined, ordered by a decreasing fraction of exonic-base coverage. Only autosomal positions are included. The dashed line shows the fraction of samples covered at the median-depth base.
(A) Using only BRSK2 exonic bases (exons plus 10 bp on either side), coverage is compared in gnomAD exomes (orange; 125,748 individuals) and gnomAD genomes (green; 15,708 individuals).
(B) Using only gnomAD exomes (125,748 individuals), exonic bases (exons plus 10 bp on either side) in BRSK2 (orange) are compared to exonic bases in 1,012 confirmed developmental-delay genes identified by the Developmental Disorders Genotype-Phenotype Database (DDG2P; purple).

phenotypes observed in these individuals, who all present with varying degrees and manifestations of developmental disorders. We believe these observations strongly support the conclusion that damaging variation in *BRSK2* is causally related to an NDD. The key points of evidence are as follows: (1) we observe a statistically significant enrichment of *de novo* variants in affected individuals relative to the estimated background mutation rate ($p = 2.46 \times 10^{-6}$); (2) although one or both parents were unavailable in three cases, none of the variants described here were found to be inherited, and all observed variants are absent from gnomAD and TopMed; (3) *BRSK2* is relatively intolerant to protein-altering variation in the general population;[8,9] (4) all variants in affected probands are either predicted to result in loss of function or are missense variants at highly conserved residues; (5) all variants are computationally predicted to be evolutionarily deleterious and have, for example, CADD[11] scores that are typical for mutations previously reported to underlie Mendelian disease; (6) model organism evidence suggests a role for *BRSK2* in neurodevelopment; and (7) *BRSK2* is known to genetically and/or biochemically interact with several genes that are robustly associated with developmental disease. In summary, these data collectively implicate *BRSK2* as an NDD-related gene.

Accession Numbers

All relevant variant data are supplied within the paper or in supporting files. Complete genome data for probands 3, 4, 8, and 9 are available via dbGAP (accession number phs001089.v3.p1). Complete exome data for other probands is not available for privacy and institutional review board (IRB) reasons.

Supplemental Data

Supplemental Data can be found with this article online at https://doi.org/10.1016/j.ajhg.2019.02.002.

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Declaration of Interests

The authors declare no competing interests.

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Web Resources

Bedtools, https://github.com/arq5x/bedtools2
Bravo Variant Browser, https://bravo.sph.umich.edu/freeze3a/hg19/
Combined Annotation Dependent Depletion (CADD), https://cadd.gs.washington.edu/
Developmental Disorder Genotype-Phenotype Database (DDG2P), https://decipher.sanger.ac.uk/ddd#ddgenes
gnomAD Genome Aggregation Database, https://gnomad.broadinstitute.org/
Online Mendelian Inheritance in Man, http://www.omim.org/
The R Project for Statistical Computing, http://www.r-project.org
UCSC Genome Browser, http://genome.ucsc.edu

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Supplemental Data

Deleterious Variation in *BRSK2* Associates with a Neurodevelopmental Disorder

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Supplemental Note: Case Reports

Proband 1 is a 5-year-old male born at 42 weeks gestational age. Birth weight was in the 68th percentile. He walked independently at 18 months. There is no known specific timing of first words but they were delayed, especially pronunciation. At the age of 3 years, he was diagnosed with global developmental delay in visual, motor cognition and speech abilities. While no formal cognitive evaluation has performed, he was noted by clinicians as having moderate intellectual disability. Visually, he has spasm nutans with hypermetropia (+5.5D), intermittent horizontal nystagmus, and torticollis. He presents with flat nasal bridge, short philtrum, pouting lower lip, widely spaced nipples, thin skin, and phimosis. Through trio exome sequencing, we identified a *de novo* missense (p.Arg65Gln) in *BRSK2*. The proband is also hemizygous for a *de novo* missense (p.Asp256Asn) in *OTUD5*, considered a variant of uncertain significance. *OTUD5* is not currently described as a disease-associated gene.

Proband 2 is a 14-year-old male and the second child of healthy unrelated parents. He was born full-term with mild overgrowth (birth weight 4560 g (98th percentile), birth length 56 cm (99th percentile), OFC 39 cm (97th percentile)) and normal Apgar score. He was a very quiet baby, walked at 17 months and acquired his first words at 2 years. At the age of 3 years, he experienced an autistic regression characterized by isolation, loss of communication skills, repetitive and poor play activities and abundant stereotypies. He developed sleep disturbances that are improved by melatonin. He attends a special school for intellectually disabled children. He has epilepsy, which started when he was 9 years old. Absence seizures are the main seizure type but some tonic-clonic seizures have been reported. Seizures were resistant to sodium valproate and carbamazepine but are partially controlled by levetiracetam and clobazam. Now aged 14 years, he is unable to speak but can feed himself and is toilet trained. He still has numerous stereotypies, plays with water and various objects and has temper tantrums. His behavior met the criteria for autism spectrum disorder according to the Autism Diagnostic Interview. His weight is 66 kg (87th percentile), height 174 cm (91st percentile), occipitalfrontal head circumference (OFC) 57.5 cm (97th percentile). Clinical examination does not reveal altered morphological features nor signs of neurological problems. Through trio exome sequencing, we identified a *de novo* splice variant (c.273-1G>A) in *BRSK2*.

Proband 3 is a 5-year-old female born after an uneventful pregnancy. Birth weight was 3458 g (53rd percentile). Birth length and head circumference were not available. Gross motor development occurred within the normal range, as she crawled at 10 months and walked independently at 15 months. However, she is noted to have dyspraxia and mild gait ataxia. Skeletal evaluation indicates femoral anteversion. She also exhibits tremors. Her first words were at 18 months and first combination of words at three years. She was diagnosed with autism at 5 years and 6 months. At her last examination, she was in the 98th percentile for height and 95th percentile for weight. She is considered to have mild intellectual disability, although no formal cognitive evaluation has performed. She has distinctive upslanting palpebral fissures, large eyes and a beaked shaped nose. She has had normal hearing, vision and echocardiogram evaluations. No brain MRI was performed. Her family history is unremarkable. Through trio genome sequencing, we identified a *de novo* splice alteration (c.530+1G>A) in *BRSK2*.

Proband 4 is a 3-year-old male with unknown birth history. His motor development proceeded within normal range, as he walked at 13 months of age. He exhibits delays in speech and social interaction. He has behavioral abnormalities including temper tantrums and he easily cries, fidgets, and is anxious with loud sounds. He prefers to play alone, has difficulty following directions, and throws toys and hits others. He runs and climbs excessively. He has been diagnosed with autism. The only dysmorphic feature noted is downslanting palpebral fissures. He also exhibits undescended testes. No formal

evaluations for vision or hearing were conducted but there are no obvious problems. At last evaluation, he was within the 63rd percentile of height and 86th percentile for weight. His family history is unremarkable. Through trio genome sequencing, we identified a *de novo* missense variant (p.Gly212Glu) in *BRSK2*.

Proband 5 is a 19-year-old male, who was born by 38+4 weeks gestation with a weight of 4080 g. His APGAR scores were 8-9. There were no congenital anomalies and the neonatal period was normal. He started to walk around 12 months of age, but he did show delay in fine motor development. Speech was unclear as a toddler. From the age of 1 year and 6 months he showed hyperactive behavior. At 5 years an IQ of 77 was measured and signs of autism spectrum disorder and ADHD were noted. He started at regular education but soon went to a special school due to concentration problems. At 8 years of age, attendance at school became more and more stressful due to behavioral difficulties. A disintegration disorder, delusions and hallucinations were noted, and cognitive functioning regressed. From 9 years of age it became more difficult to make contact with him. His developmental level and functioning had shifted to a severe intellectual disability. At 18 years of age he was diagnosed with schizophrenia, autism spectrum disorder, and ADHD. In addition, he shows self-mutilation and aggression towards others. He sometimes speaks in short sentences. There are no reports of seizures. Vision and hearing are normal. He gained weight after antipsychotic medication was changed. Family history consists of several first and second-degree individuals with a psychiatric diagnosis such as bipolar disorder, psychoses, and depression. At physical examination he had a height of 180 cm (-0.5 SD) and head circumference of 57 cm (-0.4 SD). In addition, he had deep-set eyes, mild upslanting of palpebral fissures, synophrys and a short first digits of feet. Previous testing included a normal 250k SNP array, metabolic screen, MRI of the brain, and EEG. Through trio exome sequencing, we identified a *de novo* nonsense variant (p.Gln244Ter) in *BRSK2*.

Proband 6 is a 4-year-old male, born by C-section due to failure to progress at 40 weeks to a 43-year-old mother following a pregnancy notable for maternal gestational diabetes treated with insulin and maternal Zoloft use. Birth weight was 3260 g (31st percentile). Concerns about repetitive behaviors emerged by 9 months of age. He walked at 14 months of age but did not speak any words until 24 months. He was diagnosed with autism spectrum disorder at 21 months. He continued to have repetitive and perseverative behaviors with intense interest in letters and numbers. He was able to read words by 3.5 years of age though language for communication remained limited. Behavioral issues were noted, including tantrums and rigidity. He had difficulty falling asleep, a problem managed successfully with melatonin. When last evaluated at 4.5 years of age, his weight and height were at 99th percentile and 88th percentile, respectively, with head circumference at the 64th percentile. His eyes appeared close set; he had a single transverse palmar crease on the left hand and a supernumerary nipple on the right side. He had mild hypotonia with lordotic posture and mild proximal weakness with difficulty jumping. Genetic testing including microarray, Fragile X, and Prader Willi methylation, each of which were normal. He had a normal creatine phosphokinase (CPK) levels. Through exome sequencing, a deletion (c.1281_1287+5del12) was detected in *BRSK2*. The variant is predicted to destroy the canonical splice donor site in intron 13 and cause abnormal gene splicing. Inheritance status is unknown.

Proband 7 is a 6-year-old male, who was born full-term (40 weeks gestation) after an uneventful pregnancy. Birth weight was 3200 g (28th percentile) and birth length was 52 cm (76th percentile). Birth OFC is not available. Subsequent growth was within normal to high percentiles, including height (90th percentile), weight (50th percentile) and OFC (90-97th percentile). Gross motor development proceeded within the normal range; he was walking independently at 15 months of age. However, mild fine motor delays were progressively observed and speech development was significantly delayed, with first words

after the age of 2 years. In addition, his behavior fell within the range of autism-spectrum disorders, with positive ADOS-testing. He had some stereotypies and initially showed little interest in social interactions, which subsequently improved with psychiatric follow-up and special education support in school. Treatment with methylphenidate since age 5 years appeared to improve his social awareness. No formal developmental or intelligence testing was performed. At age 6 years-10 months, he spoke in simple sentences. A formal hearing test was normal. He presented with two generalized seizures within days of each other at age 11 months, in the setting of an afebrile gastro-enteritis episode. EEG was normal at the time and no further seizures occurred without medication. Neurological examination was unremarkable and no brain MRI was performed. He showed no regression but continuous progress in all developmental aspects. Morphologically, he was slightly brachycephalic with a broad and prominent forehead, triangular face with pointed chin, slight upslanting palpebral fissures, narrow nose, broad mouth with thick lower lip, and cupid's bow of upper lip. His family history was unremarkable, except for an aborted pregnancy with Trisomy 21. Previous investigations were normal for Fragile-X and SNP array. Through exome sequencing, we identified a *de novo* frameshift (p.Ser466Glnfs*83) in *BRSK2*.

Proband 8 is a 10-year-old male with unknown birth history. Gross motor development was delayed with sitting at almost 2 years of age, and crawling and walking occurred after 2 years of age. He is mostly non-verbal and often babbles. Although no IQ was recorded, he is noted to have moderate intellectual disability, ADD/ADHD, and impulsivity. He has also been diagnosed with autism. There is no report of seizures. He has dysmorphic features, including a heart-shaped face, narrow nose, down slanting palpebral fissures and a hypoplastic alae nasi. He has had a normal CT. At last evaluation, his height and weight were at the 13th percentile. Seizures, a brain tumor, and special education were reported in the mother. A maternal half-sibling also presents with moderate intellectual disability, developmental delay, autism spectrum disorder, and seizures. His father also has history of mental illness. Through genome sequencing of the proband and his mother, we identified a frameshift (p.Glu511Vfs*38) in *BRSK2* that was not inherited from his mother; his father was not available for testing.

Proband 9 is a 4-year-old male with unknown birth history. Gross motor development was delayed with crawling starting at 14-15 months and walking at 18-20 months. His speech was also delayed. He presents with stereotypic behaviors and was diagnosed with borderline autism at an unknown age. No IQ was noted but he is considered to exhibit moderate intellectual disability. No seizures were noted. Dysmorphic features consist of oval-shaped face, epicanthal folds, retrognathia, telecanthus, and upturned ear lobe (more prominent ear on the right side). He shows astigmatism and wears glasses. He has mild laryngomalacia and subglottic stenosis. He also has sleep apnea. He has had two episodes of tachycardia. The only significant family history is that the mother had special education as a child. Through genome sequencing, we identified a missense variant (p.Arg621Cys) in *BRSK2*. Inheritance status is unknown. Due to remaining uncertainties, we would classify this BRSK2 variant as a variant of uncertain significance according to the ACMG scoring guidelines[1].

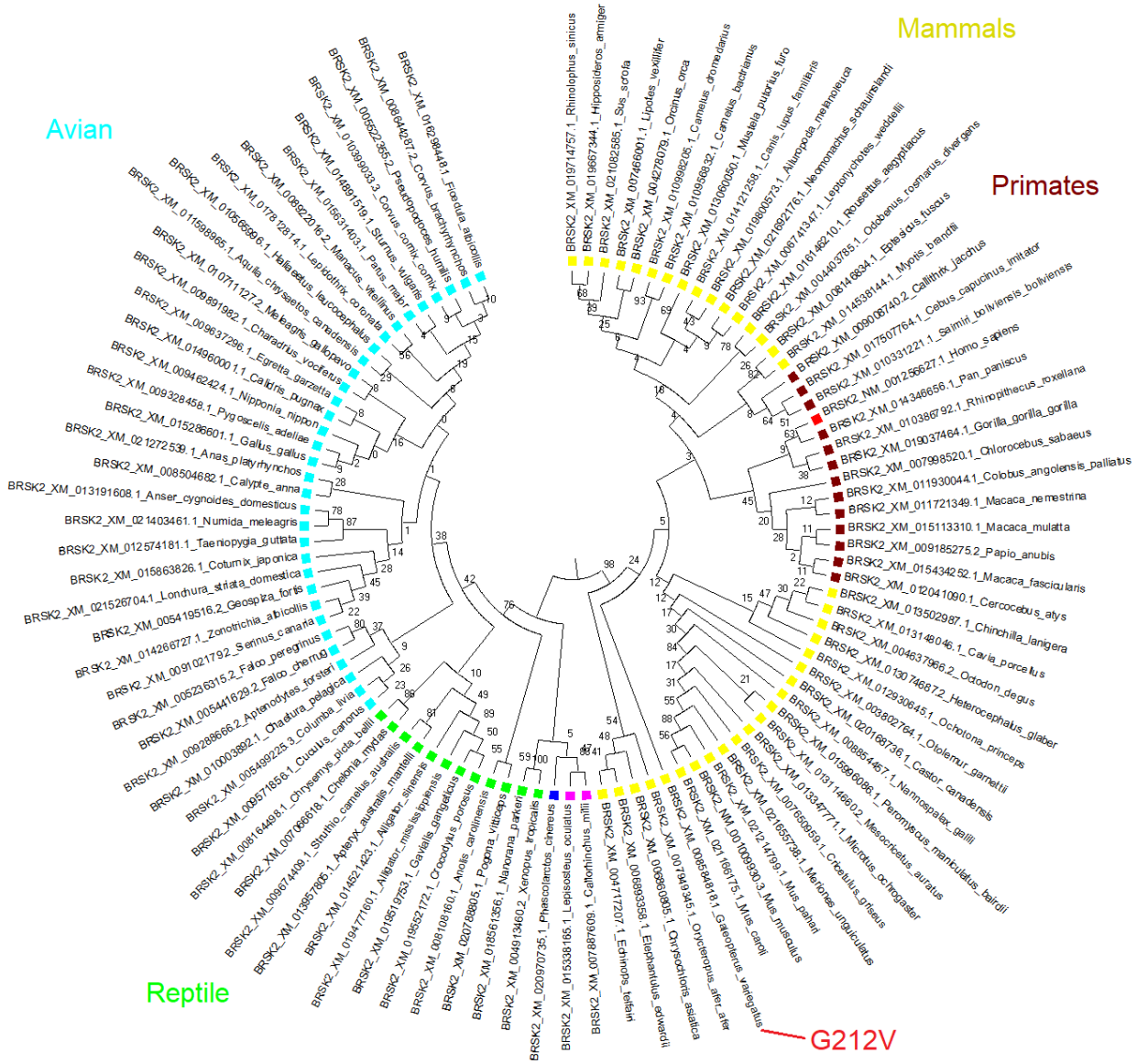

Figure S1. Phylogenetic tree for BRSK2 sequences used. Tree was generated using 1000 bootstrap replicates, showing the values for clustering of trees at each node. Sequences include an array of avian (cyan), reptile (green), marsupial (blue), fish/shark (magenta), mammals (yellow), and primates (brown). At the residues where missense variants were identified (R65, G212, and R621), the amino acid in human is conserved in all other species, with one exception: G212V is found in the Sunda flying lemur (*Galeopterus variegatus*).

Table S1. Additional clinical characteristics of individuals with BRSK2 variation.

Proband	1	2	3	4	5	6	7	8	9
Speech delay	Yes	Yes; Regression	Yes	Yes; Regression	Yes	Yes	Yes	Yes	Yes
First words (months)	n/a	24 m	18 m			24 m	After 24 m	Nonverbal, babbles	
Motor delay	Yes	Yes	Yes; Fine; gross	No	Yes; Fine	Yes; Gross	No	Yes; Gross	Yes; Gross
Age of walking (months)	18 m	17 m	15 m	13 m	12 m	14 m	15 m	After 24 m	18-20 m
Facial dysmorphism	Short philtrum, pouting lower lip, full eyelids, high forehead, flat nasal bridge, wide spaced nipples	None reported	Distinctive upslanting palpebral fissures, large eyes, beaked shaped nose	Downslanting palpebral fissures	Deep set eyes, mild upslanting palpebral fissures, synophrys	Close set eyes	Brachycephaly, broad/prominent forehead, slightly upslanting palpebral fissures, narrow nose, long philtrum, slightly broad mouth with thick lower lip and cupid's bow upper lip, triangular face with pointed chin	Heart-shaped face, down slanting palpebral fissures	Retrognathia, telecanthus, epicanthal folds, upturned ear lobes, oval-shaped face
Other	Nystagmus, phimosis, hyper-	Seizures, first at 9 y	Tall stature, macrocephaly, mild gait	Undescended testes	Short first digits of feet	Supernumerary nipple; Single	Two generalized seizures;	None reported	Mild laryngomalacia and

	metropia, torticollis		ataxia, tremors			transverse palmar crease (L hand), mild hypotonia, mild proximal weakness	possibly linked to gastroenteritis episode at 11 mo; normal EEG, no further seizures		subglottic stenosis; sleep apnea, astigmatism
Family history	No	No	No	No	Yes	Unknown	No	Yes	Yes

Supplemental Methods

Ethics Statement

Informed consent to publish de-identified data was obtained from all patients and/or families. Probands 1, 5 and 6 were consented by established institutional processes. Proband 2 was enrolled in study in France approved by the local ethical committee. Probands 3, 4, 8 and 9 enrolled in a study approved and monitored by review boards at Western (20130675) and the University of Alabama at Birmingham (X130201001). Proband 7 was enrolled in a study approved by the ethical committee of the canton of Zurich under the number EK StV 11/09.

Exome/Genome sequencing

In all cases, the *BRSK2* variant described here was the most compelling, likely causal deleterious variant identified in each proband. Note that a variant of uncertain significance in *OTUD5* was noted for Proband 1, but there is no evidence to support further pathogenicity of this variant.

Site A

For probands 1 and 5, exome sequencing analysis (ES) was performed for the patient and both parents. Exome capture was performed with the Agilent SureSelect Human All Exon enrichment kit version 5 (Agilent Technologies). Whole-exome sequencing was performed on the Illumina HiSeq platform 4000 (BGI, Copenhagen, Denmark) with 2x150bp reads. Sequencing reads were aligned to the GRCh37 reference genome using BWA version 0.7.8 and variants were called with GATK haplotype caller version unified phenotyper 3.3.0 software packages. Variants were annotated using an in-house pipeline version 2.4.1. Prioritization of variants was done by selecting first *de novo* and rare variants (filtering <1% in ExAC database, <1% in-house database, <5% dbSNP) in the coding regions and the splice sites of genes on an institute-defined intellectual disability gene panel (743 genes at the time of analysis for Proband 1, 877 genes at time of analysis for Proband 5). As a second step, *de novo*, X-linked, homozygous and compound heterozygous variants in genes beyond this gene panel were analyzed by using a stringent filtering <5% dbSNP, <1% in-house database, phyloP >3,5 (except when *de novo* or truncating). Variants in genes that had a clear link to the phenotype of the patient (e.g. animal model, pathway, expression pattern) were confirmed by Sanger sequencing and reported.

Site B

For proband 2, ES was performed (QXT Agilent) on proband and both parents in a research setting. Alignment and variant calling were performed using standard software (bwa -0.7.12, samtools-1.1, picard-tools-1.121, GenomeAnalysisTK-2014.3-17g0583018, including Haplotype caller, FastQC 0.10.1). Variants were annotated using SNPEff-4.2 and dbNSFP. Variants were filtered to require 1) minor allele frequency in ExAC <1% and 2) impact on the coding sequence (missense, stop gained, stop loss, start loss, frameshift and inframe indel, splice donor and acceptor variants). Sanger confirmation was performed in all family members using Thermo Fischer Big Dye Terminator V3 and Applied Biosystem 3730 sequencer with POP7. Sequences were analyzed with Applied Biosystem Seqscape v2.5.

Site C

For probands 3, 4, 8, and 9, whole genome sequencing (WGS) was performed on probands and parents, when available. Variant filtering and prioritization were performed as previously described [2]. Sanger validation was performed in a CAP/CLIA-certified laboratory.

Site D

For Proband 6, using genomic DNA from the proband and parents, the exonic regions and flanking splice junctions of the genome were captured using the IDT xGen Exome Research Panel v1.0. Massively parallel (NextGen) sequencing was done on an Illumina system with 100bp or greater paired-end reads. Reads were aligned to human genome build GRCh37/UCSC hg19, and analyzed for sequence variants using a custom-developed analysis tool. Additional sequencing technology and variant interpretation protocol has been previously described[3]. The general assertion criteria for variant classification are publicly available on the GeneDx ClinVar submission page (http://www.ncbi.nlm.nih.gov/clinvar/submitters/26957/).

Site E

For Proband 7, Genomic DNA was extracted from EDTA blood of the patient and his parents. Whole Exome Sequencing (WES) on the patient was performed using the xGen® Exome Research Panel v1.0 (IDT) with paired-end sequencing (HiSeq SBS Kit v4, 125 Fwd-125 Rev, Q30-value: 90.9) on a HiSeq System (Illumina Inc.). Raw fastQ files were aligned to the hg19 reference genome using NextGene (Softgenetics). The average depth of coverage was 270.8x and 98% of the targeted bases were assessed by ≥20 independent sequence reads. By applying filters for known and candidate ID genes (SYSID and In-House) and Minor Allele Frequency ≤ 2% (gnomAD, ExAC) a total of 46 variants were observed in at least 16% of reads with sufficient quality level. Variants were investigated computationally for deleterious effects, by associations of the affected gene with proband's phenotype and by literature search for functional information. The candidate BRSK2 mutation from the WES approach was re-sequenced in the index and his parents after PCR amplification by Sanger sequencing using an ABI Genetic Analyzer 3730 (Applied Biosystems, Foster City, California).

Three-dimensional modeling

Protein modeling was performed as previously described[4].

Statistical enrichment of *BRSK2* variants

We compared the frequency of observed variation to the expected frequency of variation in *BRSK2*[5] using an Exact Binomial Test in R.

Comparison of BRSK2 coverage in exomes and genomes

Bedtools was used to extract base-pair-level coverage data from gnomAD exomes and genomes (release 2.0.2) across the intersection of Consensus CDS exons (CCDS, as of August 2016) +/- 10bp and either 1) *BRSK2* or 2) confirmed genes from the Developmental Disorder Genotype-Phenotype Database (DDG2P, as of November 2018, using coordinates from GRCh37 ENSEMBL build 94). This resulted in three sets of bp-level coverage data: exome-BRSK2, genome-BRSK2, and exome-DDG2P. Using R, sets were sorted by fraction of samples meeting 20x coverage and the percentile rank of each position was calculated and graphed. To assess rates of singleton variants, we used BCFTools to extract and normalize

variants from the CCDS +/- 10bp region. We removed all variants part of a multi-allelic site in either the exome or genome sets and used R and dplyR to full-join the remaining variants and compute the sum of the genome and exome allele counts. Unique variants were counted toward their respective set if their combined allele count was equal to one and the variant had a filter status of PASS in its respective set.

Supplemental References

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