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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].

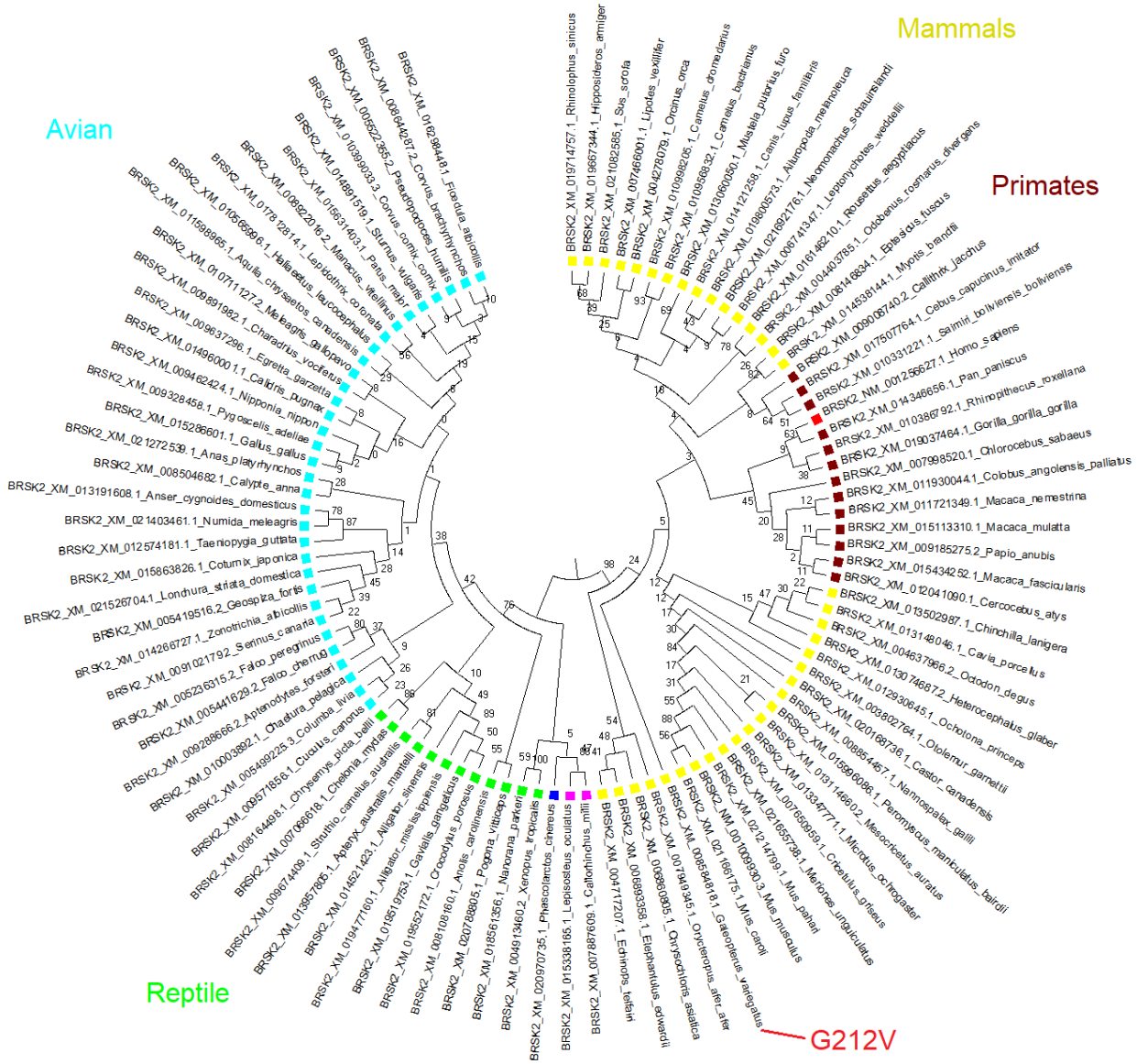

Figure S1. Phylogenetic tree for BRSK2 sequences used. Tree was generated using 1000 bootstrap replicates, showing the values for clustering of trees at each node. Sequences include an array of avian (cyan), reptile (green), marsupial (blue), fish/shark (magenta), mammals (yellow), and primates (brown). 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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