Recurrent Distal 7q11.23 Deletion Including *HIP1* and *YWHAG* Identified in Patients with Intellectual Disabilities, Epilepsy, and Neurobehavioral Problems

Melissa B. Ramocki,[1,2,16] Magdalena Bartnik,[3,16] Przemyslaw Szafranski,[4,16] Katarzyna E. Kołodziejska,[4] Zhilian Xia,[4] Jaclyn Bravo,[4] G. Steve Miller,[5,6] Diana L. Rodriguez,[1,2] Charles A. Williams,[7] Patricia I. Bader,[8] Elżbieta Szczepanik,[9] Tomasz Mazurczak,[9] Dorota Antczak-Marach,[9] James G. Coldwell,[5] Cigdem I. Akman,[1,2] Karen McAlmon,[10,11,12] Melinda P. Cohen,[13] James McGrath,[14] Elizabeth Roeder,[15] Jennifer Mueller,[7] Sung-Hae L. Kang,[4] Carlos A. Bacino,[4] Ankita Patel,[4] Ewa Bocian,[3] Chad A. Shaw,[4] Sau Wai Cheung,[4] Tadeusz Mazurczak,[3] and Paweł Stankiewicz[3,4,*]

We report 26 individuals from ten unrelated families who exhibit variable expression and/or incomplete penetrance of epilepsy, learning difficulties, intellectual disabilities, and/or neurobehavioral abnormalities as a result of a heterozygous microdeletion distally adjacent to the Williams-Beuren syndrome region on chromosome 7q11.23. In six families with a common recurrent ~1.2 Mb deletion that includes the Huntingtin-interacting protein 1 (*HIP1*) and tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein gamma (*YWHAG*) genes and that is flanked by large complex low-copy repeats, we identified sites for nonallelic homologous recombination in two patients. There were no cases of this ~1.2 Mb distal 7q11.23 deletion copy number variant identified in over 20,000 control samples surveyed. Three individuals with smaller, nonrecurrent deletions (~180–500 kb) that include *HIP1* but not *YWHAG* suggest that deletion of *HIP1* is sufficient to cause neurological disease. Mice with targeted mutation in the *Hip1* gene ($Hip1^{-/-}$) develop a neurological phenotype characterized by failure to thrive, tremor, and gait ataxia. Overall, our data characterize a neurodevelopmental and epilepsy syndrome that is likely caused by recurrent and nonrecurrent deletions, including *HIP1*. These data do not exclude the possibility that *YWHAG* loss of function is also sufficient to cause neurological phenotypes. Based on the current knowledge of Hip1 protein function and its proposed role in AMPA and NMDA ionotropic glutamate receptor trafficking, we believe that *HIP1* haploinsufficiency in humans will be amenable to rational drug design for improved seizure control and cognitive and behavioral function.

The vast majority (~95%) of patients with Williams-Beuren syndrome (WBS [MIM 194050]), a common multisystem developmental disorder characterized by supravalvular aortic stenosis, multiple peripheral pulmonary arterial stenoses, elfin face, infantile hypercalcemia, and cognitive and behavioral abnormalities, have a recurrent ~1.6 Mb hemizygous deletion in 7q11.23.[1] In a few patients with an unusual association of WBS with infantile spasms (IS) and more severe developmental delay or intellectual disability (DD or ID), larger deletions extending distally have been reported.[2–7] Marshall et al.[7] proposed that deletion of the membrane-associated guanylate kinase inverted-2 (*MAGI2* [MIM 606382]) gene, mapping ~3.5 Mb distal to the WBS common deletion (Figure 1), causes IS, because 15 of 16 individuals with 7q11.23-q21.1 deletions involving *MAGI2* developed IS, whereas 11 of 12 subjects with 7q11.23-q21.1 deletions leaving *MAGI2* intact did not. Supporting this notion, Marshall et al. found that mouse Magi2 interacts with Stargazin and that mutations in *Stargazin* cause epilepsy in mice.[7,8]

However, to date, no point mutations have been identified in *MAGI2*, and although no epilepsy was documented for the 12 subjects with 7q11.23-q21.1 deletions proximal to *MAGI2*, 11 of these subjects had severe DD or ID.[5,7,9,10] In addition, at least three patients with WBS, DD or ID, and IS with 7q11.23 deletions proximal to *MAGI2* have been reported.[4,11,12] Kamoike et al.[12] suggested that in addition to deletion of *MAGI2*, deletion of the tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, gamma (*YWHAG* [MIM 605356]) or Huntingtin-interacting protein 1 (*HIP1* [MIM 601767]) genes, which are both normally expressed in the brain, may contribute to epilepsy and ID in these patients. To further investigate this hypothesis, they studied morpholino antisense

[1]Department of Pediatrics, Section of Pediatric Neurology and Developmental Neuroscience, Baylor College of Medicine, Houston, TX 77030, USA; [2]Texas Children's Hospital, Houston, TX 77030, USA; [3]Department of Medical Genetics, Institute of Mother and Child, Warsaw 01-211, Poland; [4]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA; [5]Children's Medical Center at Hillcrest, Tulsa, OK 74104, USA; [6]University of Oklahoma, Tulsa, OK 74135, USA; [7]Raymond C. Philips Research and Education Unit, Division of Genetics and Metabolism, Department of Pediatrics, University of Florida College of Medicine, Gainesville, FL 32611, USA; [8]Northeast Indiana Genetic Counseling Center, Fort Wayne, IN 46845, USA; [9]Clinic of Neurology of Children and Adolescents, Institute of Mother and Child, Warsaw 01-211, Poland; [10]Children's Hospital Boston, Boston, MA 02115, USA; [11]Harvard Medical School, Boston, MA 02115, USA; [12]Special Care Nursery, Winchester Hospital, Winchester, MA 01890, USA; [13]Division of Medical Genetics and Genomic Medicine, Vanderbilt University, Nashville, TN 37232, USA; [14]Departments of Comparative Medicine and Genetics, Yale University School of Medicine, New Haven, CT 06520, USA; [15]Department of Pediatrics, Division of Genetics and Metabolic Disorders, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, USA
[16]These authors contributed equally to this work
*Correspondence: pawels@bcm.edu
DOI 10.1016/j.ajhg.2010.10.019.

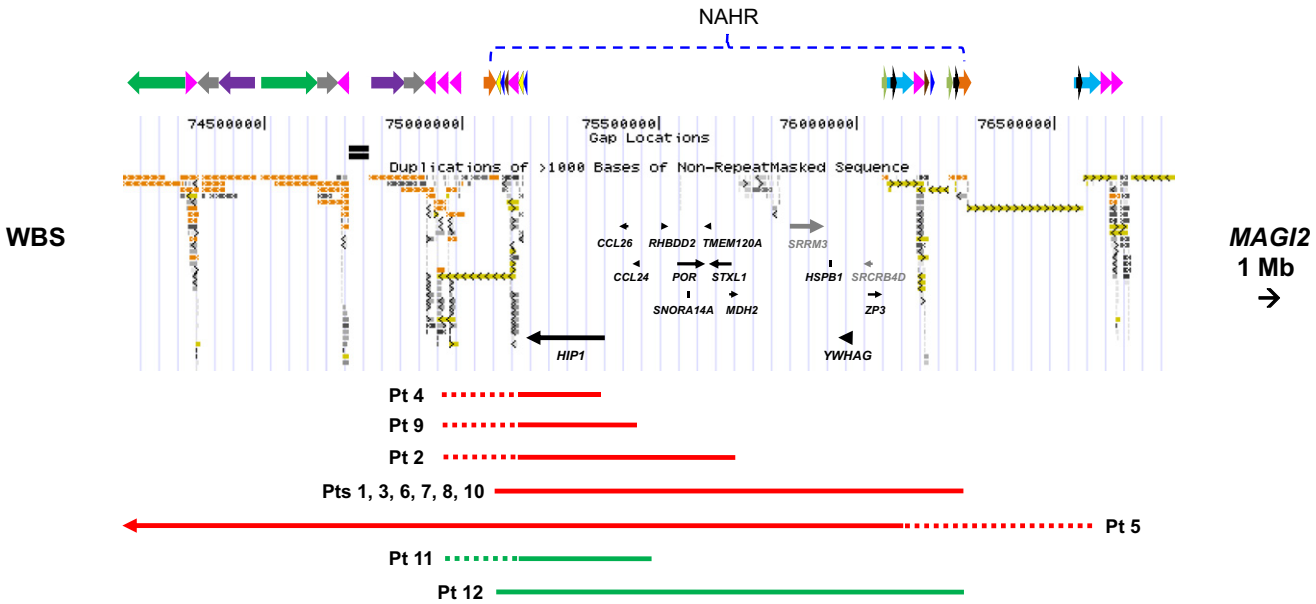

Figure 1. Schematic Representation of the Genomic Architecture in Distal 7q11.23
The extent of the identified deletions is shown in red, and duplications are shown in green. The NAHR sites identified in patients 6 and 8 were mapped to the directly-oriented ~25 kb subunits (orange) with crossovers within the subunits identified in two patients (6 and 8) with recurrent deletions.

knockdowns of *ywhag* and *hip1* in zebrafish. *hip1*$^{-/-}$ zebrafish had reduced or complete absence of yolk extension, narrowing along the dorsoventral axis, and severe mandibular aplasia, but there was no obvious effect on the developing brain. In *ywhag*$^{-/-}$ zebrafish, however, increased diameter of the heart tube and reduced brain size were appreciated, leading Kamoike et al. to postulate that haploinsufficiency of *YWHAG* causes the IS and cardiomegaly phenotypes observed in their patients.[12]

We queried our chromosome microarray database for individuals with distal 7q11.23 deletions or duplications that include the *HIP1* and/or *YWHAG* genes. Three small nonrecurrent distal 7q11.23 deletions, six common recurrent distal 7q11.23 deletions, and one larger-sized deletion harboring *HIP1* were found in ~29,510 individuals referred for chromosomal microarray analysis (CMA) at the Baylor College of Medicine Medical Genetics Laboratories (BCM MGL), yielding a frequency of 1 in 3279, comparable with the 1 in 2960 frequency of the 680 kb *CHRNA7* (MIM 118511) deletion.[13] Our collection of subjects referred for CMA includes mostly children with various combinations of DD, ID, autism, and birth defects; a small percentage has epilepsy, but rarely as an isolated finding.

Initial array CGH analyses were performed with microarrays designed by BCM MGL and manufactured by Agilent Technology as previously described.[14,15] We received DNA samples from all probands and their family members after obtaining informed consent via protocols approved by the Institutional Review Board for Human Subject Research at Baylor College of Medicine (except patient 5, who is deceased, and patient 6, for whom no forwarding contact information was available). Patients' genomic DNA was extracted from peripheral blood via the Puregene DNA isolation kit (Gentra System). To fine map the size and the extent of the genomic deletions and duplications, we performed oligonucleotide microarray CGH analyses with custom-designed 7q11.23 region-specific high-resolution oligonucleotide microarrays: 135K (NimbleGen Systems) according to the manufacturers' instructions. Microarrays were scanned on the NimbleGen MS 200 scanner. Scans were processed with NimbleScan version 2.5 and analyzed with SignalMap version 1.9. Confirmatory and parental fluorescence in situ hybridization analyses in patients and their parents were performed with chromosome 7q11.23-specific bacterial artificial chromosome clones CTB-139P11, RP11-229D13, RP11-622P13, and RP11-845K6 via standard procedures. Clinical histories were obtained from parents and from physicians. For the patients with epilepsy, medical records including EEG reports were reviewed, and physicians and families were interviewed about clinical seizure semiology, thereby allowing us to classify the epilepsy in most cases as generalized, localization related, or mixed.

Overall, we identified 26 individuals from ten unrelated families who exhibit variable expression and/or incomplete penetrance of epilepsy, learning difficulties, intellectual disabilities, and/or neurobehavioral abnormalities as a result of hemizygous genomic deletions, including *HIP1*. The deletions mapped between the WBS region and *MAGI2* ranged in size from ~4 Mb to ~180 kb and included apparent recurrent, as well as nonrecurrent, rearrangements (Figure 2). Our subjects presented with a range of neurological and neurodevelopmental phenotypes (Table 1). Epilepsy was common among probands with distal 7q11.23 deletion with 8 of 10 (80%) affected;

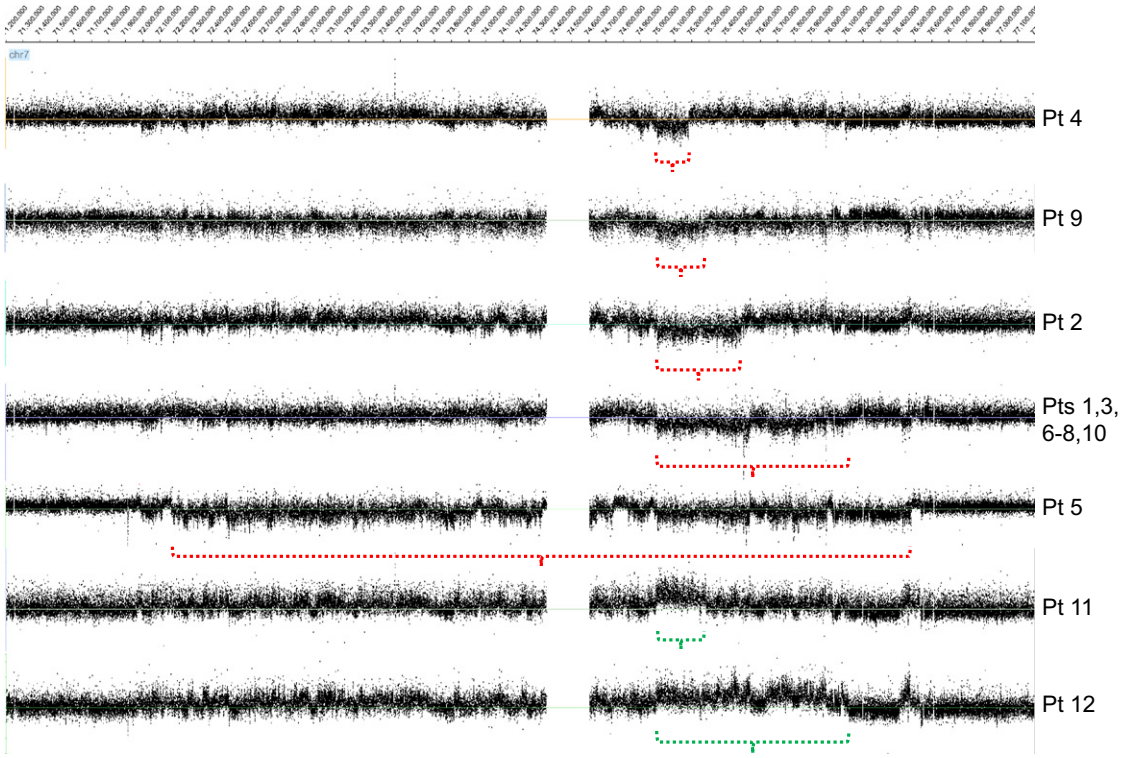

Figure 2. Results of Array CGH Analysis with a 7q11.23 Region-Specific High-Resolution Oligonucleotide Microarray, Including the WBS Common Deletion Region
The extents of the identified deletions (red) and duplications (green) (135K, NimbleGen). Patients 1, 3, 6–8, and 10 had a common ~1.2 Mb deletion.

however, among all family members with the deletion, epilepsy was present in 13 of 26 (50%). Generalized epilepsy was most common, occurring in 6 of 13 (46%); localization-related epilepsy occurred in 4 of 13 (31%), mixed epilepsy in 1 of 13 (8%), epilepsy not otherwise specified in 1 of 13 (8%), and neonatal epilepsy in 1 of 13 (8%). Routine clinical brain MRI exams were typically interpreted as normal, but one subject (patient 4) had a dysembryoplastic neuroectodermal tumor and a temporal lobe cortical dysplasia that were identified on pathological examination status post epilepsy surgery (temporal lobectomy) for medically refractory symptomatic localization-related epilepsy, and a second subject (patient 3) had a mild Chiari 1 malformation.

The intellectual spectrum associated with distal 7q11.23 deletion is broad. Of the 12 surviving children, (eight surviving probands, including patient 10, and their four siblings; patients 1 and 5 are deceased), 7 of 12 (58%) had moderate to severe global DD or ID and/or were on the autistic spectrum, 2 of 12 (17%) had mild ID, 2 of 12 (17%) were diagnosed with learning disabilities only, and 1 of 12 (8%) was developmentally on target. Of the 12 adults, 3 of 12 (25%) had mild ID and had required special education, 3 of 12 (25%) expressed concern that they had really struggled in school or disproportionately with certain subjects, and 6 of 12 (50%) reported no cognitive difficulties, no special needs in the educational system, and no history of neuropsychiatric disease. Behavioral problems were common among children with distal 7q11.23 deletion and include inattention, hyperactivity, impulsivity, and aggression. Depression and self-abusive behaviors were reported in one patient. Autism was diagnosed in 2 of 12 children (siblings, 17%).

We have also identified two reciprocal microduplications inclusive of *HIP1* in three children from two families (Figure 2; Table 1). Although none of these children have developed epilepsy, 1 of 3 (33%) has an expressive language disorder, 2 of 3 (67%) have attention deficit hyperactivity disorder and problems with aggression, 1 of 3 (33%) was formally diagnosed with bipolar disorder, and 1 of 3 (33%) had a large posterior encephalocele (Chiari III malformation).

In contrast to other disease-associated recurrent deletion and duplication CNVs that typically arise de novo (e.g., 16p11.2 and 1q21.1),[16–19] we found that our patients inherited the common or small distal 7q11.23 deletion or duplication in all cases for which parental origin could be determined. Deletions of distal 7q11.23 are therefore reminiscent of the typically inherited 15q13.3 microdeletions that cause epilepsy and other neurodevelopmental phenotypes but exhibit incomplete penetrance and variable expressivity.[20–24] The incomplete penetrance of epilepsy and ID in apparently clinically unaffected transmitting parents can potentially be explained by a two-hit

Table 1. Phenotypic Features of 26 Individuals from Ten Unrelated Families with an *HIP1* Deletion and Four Individuals from Two Unrelated Families with an *HIP1* Duplication

	Age at Diagnosis	Sex	Ancestry[a]	CNV	Cognitive Function, Other Problems	Epilepsy	Inheritance	Method	Other CMA Abnormalities V8
Pt 1	1 mo.	Female	E	Com del	34 wga, cystic encephalomalacia, death in neonatal period	Neonatal seizures	Pat	V5.0, V8.0	None
Pt 1's father	34 yr.	Male	E	Com del	Normal	Generalized epilepsy	Unknown	FISH	Unknown
Pt 2	4 yr.	Male	E/H	Small del	Global DD, microcephaly, PDA	None	Mat	V6.1, V8.0	Xp22.31dup[b]
Pt 2's mother	30 yr.	Female	H	Small del	Normal	None	Unknown	FISH	Unknown
Pt 3	4 yr.	Male	E	Com del	Global DD, ID, ASD, Chiari 1	Febrile seizures, generalized epilepsy	Mat	V6.1, V8.0	None
Pt 3's mother	45 yr.	Female	E	Com del	Struggled in school, miscarriages (singleton and twin)	None	Unknown	FISH	Unknown
Pt 3's sister	7 yr.	Female	E	Com del	ASD	None	Mat	V5.0	None
Pt 4	13 yr.	Female	E	Small del	Severe ID	Medically refractory generalized epilepsy s/p VNS placement	Mat	V6.2, V8.0	5q31.2del[c]
Pt 4's mother	38 yr.	Female	E	Small del	Difficulty with reading	None	Unknown	FISH	Unknown
Pt 4's brother	14 yr.	Male	E	Small del	Severe ID, aggressive, hyperactive	Generalized epilepsy	Mat	V6.2	None
Pt 5	3 mo.	Female	H	Large del	WBS, congenital heart disease	None	De novo	V6.2, V8.0	None
Pt 6	4 yr.	Female	E	Com del	Moderate ID, hyperactivity	Mixed epilepsy	Pat	V6.3, V8.0	None
Pt 6's father	27 yr.	Male	E	Com del	Learning disabilities, special education	Epilepsy	Unknown	FISH	Unknown
Pt 6's brother	1.5 yr.	Male	E	Com del	Mild ID, hyperactivity	None	Pat	V6.4	None
Pt 7	9 yr.	Female	AA	Com del	Learning disabilities	Medically intractable localization-related epilepsy	Pat	V8.0	Xp11.22dup[d]
Pt 7's sister	11 yr.	Female	AA	Com del	Learning disabilities	Localization-related epilepsy	Pat	V8.1	Xp11.22dup[d]
Pt 7's father	38 yr.	Male	AA	Com del	Struggled in math	Localization-related epilepsy	Unknown	FISH	Unknown
Pt 8	13 yr.	Female	E	Com del	Mild ID, ADHD, depression, mood disorder, aggressive	Generalized epilepsy	Unknown/ adopted	V8.0	None
Pt 9	3 yr.	Male	AA	Small del	Normal	Localization-related epilepsy	Mat	V8.1	None
Pt 9's mother	18 yr.	Female	AA	Small del	Normal	None	Pat	FISH	Unknown
Pt 9's MGF	38 yr.	Male	AA	Small del	Normal	Single seizure event as adult	Unknown	FISH	Unknown

Table 1. *Continued*

	Age at Diagnosis	Sex	Ancestry[a]	CNV	Cognitive Function, Other Problems	Epilepsy	Inheritance	Method	Other CMA Abnormalities V8
Pt 10	19 yr.	Male	E	Com del	Severe/ moderate ID	Generalized epilepsy	Mat	V8.0	None
Pt 10's mother	40 yr.	Female	E	Com del	Mild ID, special education	None	Mat	FISH	Unknown
Pt 10's MGM	70 yr.	Female	E	Com del	Normal	None	Unknown	FISH	Unknown
Pt 10's uncle	30 yr.	Male	E	Com del	Mild ID, special education	None	Mat	FISH	Unknown
Pt 10's aunt	41 yr.	Female	E	Com del	Mild ID	None	Mat	FISH	Unknown
Pt 11	3 mo.	Male	E	Small dup	Large posterior encephalocele/ Chiari III malformation, hydrocephalus VPS	None	Pat	V8.0	None
Pt 11's father	34 yr.	Male	E	Small dup	Spinal cord schwannoma	None	Unknown	FISH	Unknown
Pt 12	16 yr.	Male	E	Com dup	Behavior/mood disorder (diagnosed with bipolar disorder), ADHD, aggressive	None	Not mat[e]	V8.0	None
Pt 12's sister	5 yr.	Female	E	Com dup	Speech delay, ADHD, aggressive	None	Not mat[e]	FISH, V8.1	11p14.3p14.2del[f]

The following abbreviations are used: ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; com, common; del, deletion; dup, duplication; FISH, fluorescence in situ hybridization; mat, maternal; MGF, maternal grandfather; MGM, maternal grandmother; mo., months old; pat, paternal; PDA, patent ductus arteriosus; pt, patient; s/p, status post; V, version; VNS, vagus nerve stimulator; yr., years old; VPS, ventriculoperitoneal shunt; wga, weeks of gestational age.

[a] AA, African American; E, European; H, Hispanic.

[b] arrXp22.31(6876449-8075153)x2.

[c] arr5q31.2(138439046- 138591992)x1.

[d] arrXp11.22(53081560-53332267)x3.

[e] Father in prison.

[f] arr11p14.3p14.2(25518340-26613145)x1. (Coordinates according to NCBI build 36, March 2006.)

model,[25,26] single nucleotide variants of the second allele,[27] or a combination thereof. The observed increase in severity of phenotypes among probands is likely explained by ascertainment bias.

We did not study a matched control set of healthy children, but we performed two analyses to consider the frequency of the common recurrent ~1.2 Mb distal 7q11.23 deletion in normal controls from the studies that surveyed CNV incidence. We did not find this deletion in approximately 20,000 individuals from different world populations[28–34] (dbGaP; M. Hurles, personal communication). To further refine our analysis, we calculated a p value for the *HIP1* deletion event among patients with an indication of epilepsy on their submitting diagnosis. Of the ten distinct families with the *HIP1* deletion, eight had epilepsy as an indication and two did not. In our CMA database as a whole, ~5,000 patients have epilepsy as an indication, whereas ~24,500 do not. Together, these values suggest that the incidence of epilepsy as an indication is significantly higher in the *HIP1* deletion subjects, with a Fisher's exact p value of 2.224×10^{-5} and a 95% confidence interval of between 3.91 and 189.12.

Three smaller-sized overlapping deletions (~180–500 kb) in patients 2, 4, and 9 (Figure 2) enabled us to narrow the critical region to a single gene, *HIP1* encoding Huntingtin-interacting protein 1,[35] that has a role in clathrin-mediated endocytosis.[36,37] *HIP1* has been implicated in the pathogenesis of Huntington disease (MIM 143100)[38] and prostate and colon cancers.[39] A balanced reciprocal translocation t(5;7)(q33;q11.2) resulting in a HIP1 and platelet-derived growth factor β receptor (*PDGFβR* [MIM 173410]) fusion protein was identified in a patient with chronic myelomonocytic leukemia.[40]

Hip1$^{-/-}$ mice developed premature testicular degeneration, severe spinal deformities, abnormal hematopoiesis, and cataracts.[41,42] Reportedly, these animals had no gross structural abnormalities of the central nervous system; however, both *Hip1*$^{-/-}$ and *Hip1*$^{+/-}$ mice develop epilepsy (T. Ross, personal communication). In a second loss-of-function model, Metzler et al.[43] showed that by 3 months of age, *Hip1*$^{-/-}$ mice develop neurological problems characterized by failure to thrive, tremor, and gait ataxia (likely secondary to a rigid thoracolumbar kyphosis). *Hip1*$^{-/-}$ mice have defects in presynaptic function, as

demonstrated by a reduction in paired-pulse facilitation in hippocampal brain slices. In addition, *Hip1*$^{-/-}$ mice demonstrate delayed recovery from chemically induced long-term depression, as well as altered α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptor function.[43,44]

Hip1 colocalizes in hippocampal and cortical neurons with NMDA ionotropic glutamate receptors (iGluRs), and *Hip1*$^{-/-}$ mice have a profound decrease in NMDA-induced AMPA-type iGluR clathrin-mediated internalization.[38,43] Endocytic trafficking and recycling maintains a pool of mobile surface AMPA receptors required for synaptic potentiation.[45] Interestingly, the increased internalization of ionotropic receptors for glutamate has been demonstrated to be pathogenic in mouse and *Drosophila* models of Fragile X syndrome (MIM 300624).[46] Mutations in the ionotropic glutamate receptor 3 gene (*GRIA3* [MIM 305915]) have been found in patients with X-linked mental retardation, seizures, and autistic behavior (MRX94 [MIM 300699]).[47] Moreover, AMPA receptors have also been implicated in patients with clinical diagnoses of schizophrenia (MIM 181500), Alzheimer disease (MIM 104300), amyotrophic lateral sclerosis (MIM 105400), stroke, Parkinson disease (MIM 168600), depression, epilepsy, and Rasmussen encephalitis.[48]

Given that in six families the common recurrent ~1.2 Mb deletions are flanked by large complex low-copy repeats (HIP1-LCRs), we hypothesized that they have likely arisen via nonallelic homologous recombination (NAHR). To address this hypothesis, we performed computational analyses of the proximal and distal HIP1-LCR copies. We found that they are organized as clusters of subunits, two of which are in direct orientation (Figure 1). Long-range PCR primers were designed to be specific to regions directly flanking each of these two paralogous subunit pairs in both HIP1-LCR copies and to each subunit (when possible) in order to amplify the predicted junction between the proximal and distal subunits (orange) of HIP1-LCRs. Amplification of the 4–17 kb fragments was performed with Takara *LA Taq* polymerase (TaKaRa Bio USA) following the manufacturer's protocol. Amplification of the GC-rich region in HIP1-LCRs was done in the presence of 4% dimethyl sulfoxide, with the following conditions: 94°C for 1 min 30 s, 61°C for 30 s, 72°C for 6 min. PCR products were treated with ExoSAP-IT (USB) to remove unconsumed dNTPs and primers and were directly sequenced by Sanger method with the initial primers and primers specific for both paralogous subunits in the proximal and distal copies of the HIP1-LCRs (Lone Star Labs). The genomic sequences based on the oligonucleotide coordinates from the array CGH experiments were downloaded from the UCSC Genome Browser (NCBI build 37, February 2009) and assembled with Sequencher version 4.2 software (Gene Codes). Interspersed repeat sequences were identified with RepeatMasker). DNA GC content was analyzed with CPGPlot. Using long-range PCR primers F: 5′-CTCTATGGAGCACAGATTCCATGCTAGACC, specific to the region centromeric and adjacent to the ~25 kb subunit in the proximal HIP1-LCR copy, and R: 5′-AGATCA GAGATCCTCTATTTTCCCCCTTAATC, specific to both subunits, we identified NAHR sites in patients 6 and 8 between the *cis*-morphic nucleotides or paralogous sequence variants in a 368 bp GC-rich interval (hg19; chr. 7:75,071,539-75,071,906; chr. 7:76,256,141-76,256,508 in the proximal and distal HIP1-LCRs, respectively; Figure 3).[49]

These collective data suggest that haploinsufficiency of *HIP1* is sufficient to alter neuronal homeostasis in a manner that predisposes the human brain to both focal and generalized epilepsies, as well as to a broad range of cognitive dysfunction and neurobehavioral abnormalities, including mild to severe intellectual disabilities and inattention, hyperactivity, impulsivity, and aggression through a mechanism involving abnormal AMPA and NMDA iGluR trafficking. However, these data do not exclude haploinsufficiency of *YWHAG* as a contributing factor to the neurological phenotypes in patients with deletions of both *HIP1* and *YWHAG,* nor do they exclude *YWHAG* as a candidate gene that, when altered, is also sufficient to cause neurological dysfunction.

Future studies should include sequence analysis of both the *HIP1* and *YWHAG* genes in patients with unexplained epilepsy and neurodevelopmental or neurobehavioral syndromes, natural history protocols to document the full spectrum of human phenotypes associated with *HIP1* and/or *YWHAG* loss or gain of function, complete neurological and neurobehavioral characterization of *Hip1* and *Ywhag* function in mice, and preclinical drug studies in these mouse models.

Acknowledgments

The authors thank all of the families who participated in this study. We thank B.A. Bejjani, J. Belmont, J.R. Lupski, D.L. Nelson, and S.K. Prakash for helpful discussion, as well as P. Hixson, J. Bratic, and M. Bedell for technical assistance. P.S. was supported in part by grant R13-0005-04/2008 from the Polish Ministry of Science and Higher Education. M.B.R. is grateful for the support of grant 5K08NS062711-03 from the National Institutes of Health/National Institute of Neurological Disorders and Stroke. J.B. was supported by grant T32GM008307 from the National Institute of General Medical Sciences. Some authors are based in the Department of Molecular and Human Genetics at Baylor College of Medicine, which offers extensive genetic laboratory testing, including use of arrays for genomic copy number analysis, and which derives revenue from this activity. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Received: September 5, 2010
Revised: October 12, 2010
Accepted: October 22, 2010
Published online: November 24, 2010

Web Resources

The URLs for data presented herein are as follows:

Baylor Medical Genetics Laboratories, http://www.bcm.edu/geneticlabs/?pmid=16207

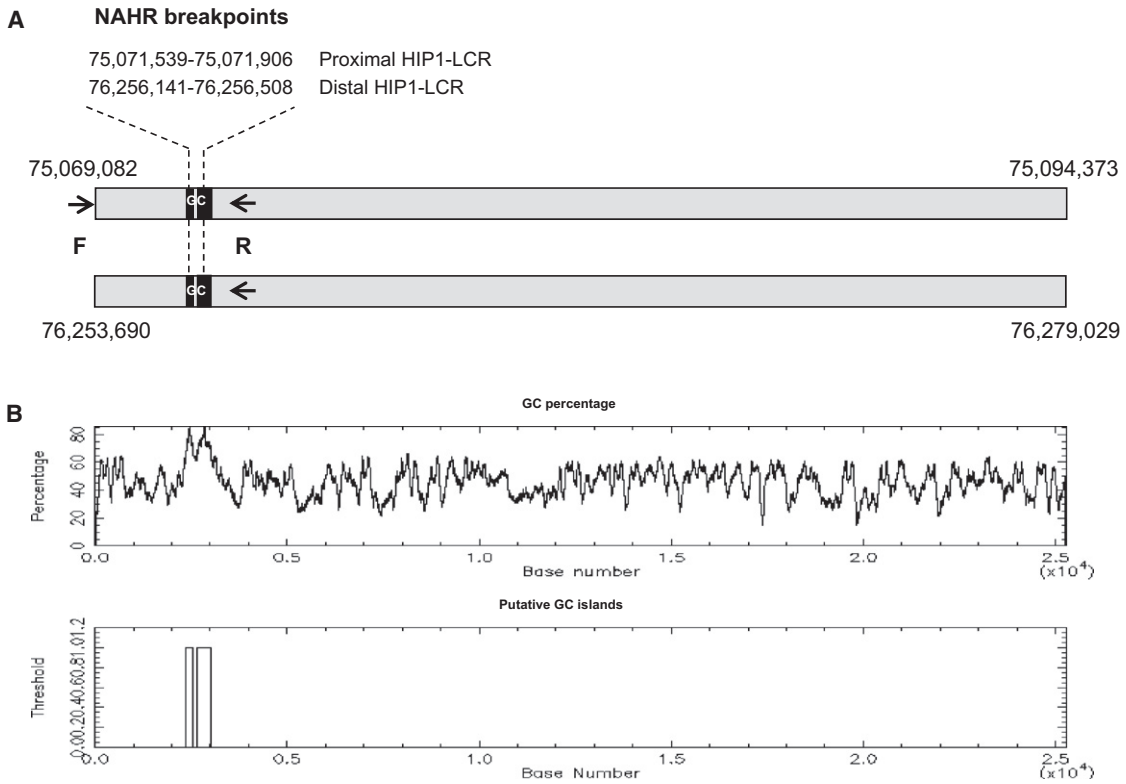

Figure 3. Genomic Structure of the ~25 kb Subunits of 99.2% DNA Sequence Identity from the Proximal and Distal HIP1-LCRs, in which the NAHR Sites Were Mapped in Patients 6 and 8

(A) The NAHR site regions were narrowed in patients 6 and 8 to a 0.4 kb interval by sequencing the long-range PCR products obtained with primer F, specific to the region adjacent to the proximal LCR, and the reverse primer R, specific for both proximal and distal LCRs (arrows depicting primers are not shown to scale).

(B) Schematic representation of GC content of the HIP1-LCRs. The unique feature of the NAHR sites reveals remarkably high GC content (black boxes in A mark regions with GC content of at least 70% within a 100 bp window). Polypurine and pyrimidine sequences with GC content of at least 50% have been associated with human recombination hot spots.[49]

CPGPlot, http://www.ebi.ac.uk/Tools/emboss/cpgplot

Database of Genotypes and Phenotypes (dbGaP), http://www.ncbi.nlm.nih.gov/gap

Online Mendelian Inheritance in Man, http://www.ncbi.nlm.nih.gov/Omim/

RepeatMasker, http://www.repeatmasker.org

UCSC Genome Browser, http://genome.ucsc.edu/

Accession Numbers

Array data have been deposited in Gene Expression Omnibus database under accession number GSE23834. The NAHR site sequences have been deposited in the GenBank database with the accession number HQ148673.

References

1. Ewart, A.K., Morris, C.A., Atkinson, D., Jin, W., Sternes, K., Spallone, P., Stock, A.D., Leppert, M., and Keating, M.T. (1993). Hemizygosity at the elastin locus in a developmental disorder, Williams syndrome. Nat. Genet. *5*, 11–16.
2. Mizugishi, K., Yamanaka, K., Kuwajima, K., and Kondo, I. (1998). Interstitial deletion of chromosome 7q in a patient with Williams syndrome and infantile spasms. J. Hum. Genet. *43*, 178–181.
3. Wu, Y.Q., Nickerson, E., Shaffer, L.G., Keppler-Noreuil, K., and Muilenburg, A. (1999). A case of Williams syndrome with a large, visible cytogenetic deletion. J. Med. Genet. *36*, 928–932.
4. Morimoto, M., An, B., Ogami, A., Shin, N., Sugino, Y., Sawai, Y., Usuku, T., Tanaka, M., Hirai, K., Nishimura, A., et al. (2003). Infantile spasms in a patient with Williams syndrome and craniosynostosis. Epilepsia *44*, 1459–1462.
5. Stock, A.D., Spallone, P.A., Dennis, T.R., Netski, D., Morris, C.A., Mervis, C.B., and Hobart, H.H. (2003). Heat shock protein 27 gene: Chromosomal and molecular location and relationship to Williams syndrome. Am. J. Med. Genet. A. *120A*, 320–325.
6. Tercero, M.F., Cabrera López, J.C., Herrero, M.M., and Rodríguez-Quiñones, F. (2005). Williams-Beuren syndrome and West "syndrome:" Causal association or contiguous gene deletion syndrome? Am. J. Med. Genet. A. *133A*, 213–215.
7. Marshall, C.R., Young, E.J., Pani, A.M., Freckmann, M.L., Lacassie, Y., Howald, C., Fitzgerald, K.K., Peippo, M., Morris, C.A., Shane, K., et al. (2008). Infantile spasms is associated with deletion of the *MAGI2* gene on chromosome 7q11.23-q21.11. Am. J. Hum. Genet. *83*, 106–111.
8. Deng, F., Price, M.G., Davis, C.F., Mori, M., and Burgess, D.L. (2006). Stargazin and other transmembrane AMPA receptor regulating proteins interact with synaptic scaffolding protein MAGI-2 in brain. J. Neurosci. *26*, 7875–7884.

9. Ferland, R.J., Gaitanis, J.N., Apse, K., Tantravahi, U., Walsh, C.A., and Sheen, V.L. (2006). Periventricular nodular heterotopia and Williams syndrome. Am. J. Med. Genet. A. *140*, 1305–1311.
10. Edelmann, L., Prosnitz, A., Pardo, S., Bhatt, J., Cohen, N., Lauriat, T., Ouchanov, L., González, P.J., Manghi, E.R., Bondy, P., et al. (2007). An atypical deletion of the Williams-Beuren syndrome interval implicates genes associated with defective visuospatial processing and autism. J. Med. Genet. *44*, 136–143.
11. Röthlisberger, B., Hoigné, I., Huber, A.R., Brunschwiler, W., and Capone Mori, A. (2010). Deletion of 7q11.21-q11.23 and infantile spasms without deletion of *MAGI2*. Am. J. Med. Genet. A. *152A*, 434–437.
12. Komoike, Y., Fujii, K., Nishimura, A., Hiraki, Y., Hayashidani, M., Shimojima, K., Nishizawa, T., Higashi, K., Yasukawa, K., Saitsu, H., et al. (2010). Zebrafish gene knockdowns imply roles for human *YWHAG* in infantile spasms and cardiomegaly. Genesis *48*, 233–243.
13. Shinawi, M., Schaaf, C.P., Bhatt, S.S., Xia, Z., Patel, A., Cheung, S.W., Lanpher, B., Nagl, S., Herding, H.S., Nevinny-Stickel, C., et al. (2009). A small recurrent deletion within 15q13.3 is associated with a range of neurodevelopmental phenotypes. Nat. Genet. *41*, 1269–1271.
14. Lu, X., Shaw, C.A., Patel, A., Li, J., Cooper, M.L., Wells, W.R., Sullivan, C.M., Sahoo, T., Yatsenko, S.A., Bacino, C.A., et al. (2007). Clinical implementation of chromosomal microarray analysis: Summary of 2513 postnatal cases. PLoS ONE *2*, e327.
15. Ou, Z., Kang, S.H., Shaw, C.A., Carmack, C.E., White, L.D., Patel, A., Beaudet, A.L., Cheung, S.W., and Chinault, A.C. (2008). Bacterial artificial chromosome-emulation oligonucleotide arrays for targeted clinical array-comparative genomic hybridization analyses. Genet. Med. *10*, 278–289.
16. Weiss, L.A., Shen, Y., Korn, J.M., Arking, D.E., Miller, D.T., Fossdal, R., Saemundsen, E., Stefansson, H., Ferreira, M.A., Green, T., et al; Autism Consortium. (2008). Association between microdeletion and microduplication at 16p11.2 and autism. N. Engl. J. Med. *358*, 667–675.
17. Shinawi, M., Liu, P., Kang, S.H., Shen, J., Belmont, J.W., Scott, D.A., Probst, F.J., Craigen, W.J., Graham, B.H., Pursley, A., et al. (2010). Recurrent reciprocal 16p11.2 rearrangements associated with global developmental delay, behavioural problems, dysmorphism, epilepsy, and abnormal head size. J. Med. Genet. *47*, 332–341.
18. Mefford, H.C., Sharp, A.J., Baker, C., Itsara, A., Jiang, Z., Buysse, K., Huang, S., Maloney, V.K., Crolla, J.A., Baralle, D., et al. (2008). Recurrent rearrangements of chromosome 1q21.1 and variable pediatric phenotypes. N. Engl. J. Med. *359*, 1685–1699.
19. Brunetti-Pierri, N., Berg, J.S., Scaglia, F., Belmont, J., Bacino, C.A., Sahoo, T., Lalani, S.R., Graham, B., Lee, B., Shinawi, M., et al. (2008). Recurrent reciprocal 1q21.1 deletions and duplications associated with microcephaly or macrocephaly and developmental and behavioral abnormalities. Nat. Genet. *40*, 1466–1471.
20. Sharp, A.J., Mefford, H.C., Li, K., Baker, C., Skinner, C., Stevenson, R.E., Schroer, R.J., Novara, F., De Gregori, M., Ciccone, R., et al. (2008). A recurrent 15q13.3 microdeletion syndrome associated with mental retardation and seizures. Nat. Genet. *40*, 322–328.
21. Ben-Shachar, S., Lanpher, B., German, J.R., Qasaymeh, M., Potocki, L., Nagamani, S.C., Franco, L.M., Malphrus, A., Bottenfield, G.W., Spence, J.E., et al. (2009). Microdeletion 15q13.3: A locus with incomplete penetrance for autism, mental retardation, and psychiatric disorders. J. Med. Genet. *46*, 382–388.
22. Miller, D.T., Shen, Y., Weiss, L.A., Korn, J., Anselm, I., Bridgemohan, C., Cox, G.F., Dickinson, H., Gentile, J., Harris, D.J., et al. (2009). Microdeletion/duplication at 15q13.2q13.3 among individuals with features of autism and other neuropsychiatric disorders. J. Med. Genet. *46*, 242–248.
23. van Bon, B.W., Mefford, H.C., Menten, B., Koolen, D.A., Sharp, A.J., Nillesen, W.M., Innis, J.W., de Ravel, T.J., Mercer, C.L., Fichera, M., et al. (2009). Further delineation of the 15q13 microdeletion and duplication syndromes: A clinical spectrum varying from non-pathogenic to a severe outcome. J. Med. Genet. *46*, 511–523.
24. Helbig, I., Mefford, H.C., Sharp, A.J., Guipponi, M., Fichera, M., Franke, A., Muhle, H., de Kovel, C., Baker, C., von Spiczak, S., et al. (2009). 15q13.3 microdeletions increase risk of idiopathic generalized epilepsy. Nat. Genet. *41*, 160–162.
25. Lupski, J.R. (2007). Structural variation in the human genome. N. Engl. J. Med. *356*, 1169–1171.
26. Girirajan, S., Rosenfeld, J.A., Cooper, G.M., Antonacci, F., Siswara, P., Itsara, A., Vives, L., Walsh, T., McCarthy, S.E., Baker, C., et al. (2010). A recurrent 16p12.1 microdeletion supports a two-hit model for severe developmental delay. Nat. Genet. *42*, 203–209.
27. Kurotaki, N., Shen, J.J., Touyama, M., Kondoh, T., Visser, R., Ozaki, T., Nishimoto, J., Shiihara, T., Uetake, K., Makita, Y., et al. (2005). Phenotypic consequences of genetic variation at hemizygous alleles: Sotos syndrome is a contiguous gene syndrome incorporating coagulation factor twelve (FXII) deficiency. Genet. Med. *7*, 479–483.
28. Redon, R., Ishikawa, S., Fitch, K.R., Feuk, L., Perry, G.H., Andrews, T.D., Fiegler, H., Shapero, M.H., Carson, A.R., Chen, W., et al. (2006). Global variation in copy number in the human genome. Nature *444*, 444–454.
29. Pinto, D., Marshall, C., Feuk, L., and Scherer, S.W. (2007). Copy-number variation in control population cohorts. Hum. Mol. Genet. *16 Spec No. 2*, R168–R173.
30. Marshall, C.R., Noor, A., Vincent, J.B., Lionel, A.C., Feuk, L., Skaug, J., Shago, M., Moessner, R., Pinto, D., Ren, Y., et al. (2008). Structural variation of chromosomes in autism spectrum disorder. Am. J. Hum. Genet. *82*, 477–488.
31. Shaikh, T.H., Gai, X., Perin, J.C., Glessner, J.T., Xie, H., Murphy, K., O'Hara, R., Casalunovo, T., Conlin, L.K., D'Arcy, M., et al. (2009). High-resolution mapping and analysis of copy number variations in the human genome: A data resource for clinical and research applications. Genome Res. *19*, 1682–1690.
32. Itsara, A., Cooper, G.M., Baker, C., Girirajan, S., Li, J., Absher, D., Krauss, R.M., Myers, R.M., Ridker, P.M., Chasman, D.I., et al. (2009). Population analysis of large copy number variants and hotspots of human genetic disease. Am. J. Hum. Genet. *84*, 148–161.
33. Kirov, G., Grozeva, D., Norton, N., Ivanov, D., Mantripragada, K.K., Holmans, P., Craddock, N., Owen, M.J., and O'Donovan, M.C.; International Schizophrenia Consortium; Wellcome Trust Case Control Consortium. (2009). Support for the involvement of large copy number variants in the pathogenesis of schizophrenia. Hum. Mol. Genet. *18*, 1497–1503.
34. Conrad, D.F., Pinto, D., Redon, R., Feuk, L., Gokcumen, O., Zhang, Y., Aerts, J., Andrews, T.D., Barnes, C., Campbell, P., et al; Wellcome Trust Case Control Consortium. (2010). Origins and functional impact of copy number variation in the human genome. Nature *464*, 704–712.

35. Kalchman, M.A., Koide, H.B., McCutcheon, K., Graham, R.K., Nichol, K., Nishiyama, K., Kazemi-Esfarjani, P., Lynn, F.C., Wellington, C., Metzler, M., et al. (1997). *HIP1*, a human homologue of *S. cerevisiae Sla2p*, interacts with membrane-associated huntingtin in the brain. Nat. Genet. *16*, 44–53.
36. Metzler, M., Legendre-Guillemin, V., Gan, L., Chopra, V., Kwok, A., McPherson, P.S., and Hayden, M.R. (2001). HIP1 functions in clathrin-mediated endocytosis through binding to clathrin and adaptor protein 2. J. Biol. Chem. *276*, 39271–39276.
37. Waelter, S., Scherzinger, E., Hasenbank, R., Nordhoff, E., Lurz, R., Goehler, H., Gauss, C., Sathasivam, K., Bates, G.P., Lehrach, H., and Wanker, E.E. (2001). The huntingtin interacting protein HIP1 is a clathrin and alpha-adaptin-binding protein involved in receptor-mediated endocytosis. Hum. Mol. Genet. *10*, 1807–1817.
38. Metzler, M., Gan, L., Wong, T.P., Liu, L., Helm, J., Liu, L., Georgiou, J., Wang, Y., Bissada, N., Cheng, K., et al. (2007). NMDA receptor function and NMDA receptor-dependent phosphorylation of huntingtin is altered by the endocytic protein HIP1. J. Neurosci. *27*, 2298–2308.
39. Rao, D.S., Hyun, T.S., Kumar, P.D., Mizukami, I.F., Rubin, M.A., Lucas, P.C., Sanda, M.G., and Ross, T.S. (2002). Huntingtin-interacting protein 1 is overexpressed in prostate and colon cancer and is critical for cellular survival. J. Clin. Invest. *110*, 351–360.
40. Ross, T.S., Bernard, O.A., Berger, R., and Gilliland, D.G. (1998). Fusion of Huntingtin interacting protein 1 to platelet-derived growth factor β receptor (PDGFbetaR) in chronic myelomonocytic leukemia with t(5;7)(q33;q11.2). Blood *91*, 4419–4426.
41. Rao, D.S., Chang, J.C., Kumar, P.D., Mizukami, I., Smithson, G.M., Bradley, S.V., Parlow, A.F., and Ross, T.S. (2001). Huntingtin interacting protein 1 is a clathrin coat binding protein required for differentiation of late spermatogenic progenitors. Mol. Cell. Biol. *21*, 7796–7806.
42. Oravecz-Wilson, K.I., Kiel, M.J., Li, L., Rao, D.S., Saint-Dic, D., Kumar, P.D., Provot, M.M., Hankenson, K.D., Reddy, V.N., Lieberman, A.P., et al. (2004). Huntingtin Interacting Protein 1 mutations lead to abnormal hematopoiesis, spinal defects and cataracts. Hum. Mol. Genet. *13*, 851–867.
43. Metzler, M., Li, B., Gan, L., Georgiou, J., Gutekunst, C.A., Wang, Y., Torre, E., Devon, R.S., Oh, R., Legendre-Guillemin, V., et al. (2003). Disruption of the endocytic protein HIP1 results in neurological deficits and decreased AMPA receptor trafficking. EMBO J. *22*, 3254–3266.
44. Parker, J.A., Metzler, M., Georgiou, J., Mage, M., Roder, J.C., Rose, A.M., Hayden, M.R., and Néri, C. (2007). Huntingtin-interacting protein 1 influences worm and mouse presynaptic function and protects Caenorhabditis elegans neurons against mutant polyglutamine toxicity. J. Neurosci. *27*, 11056–11064.
45. Petrini, E.M., Lu, J., Cognet, L., Lounis, B., Ehlers, M.D., and Choquet, D. (2009). Endocytic trafficking and recycling maintain a pool of mobile surface AMPA receptors required for synaptic potentiation. Neuron *63*, 92–105.
46. Nakamoto, M., Nalavadi, V., Epstein, M.P., Narayanan, U., Bassell, G.J., and Warren, S.T. (2007). Fragile X mental retardation protein deficiency leads to excessive mGluR5-dependent internalization of AMPA receptors. Proc. Natl. Acad. Sci. USA *104*, 15537–15542.
47. Wu, Y., Arai, A.C., Rumbaugh, G., Srivastava, A.K., Turner, G., Hayashi, T., Suzuki, E., Jiang, Y., Zhang, L., Rodriguez, J., et al. (2007). Mutations in ionotropic AMPA receptor 3 alter channel properties and are associated with moderate cognitive impairment in humans. Proc. Natl. Acad. Sci. USA *104*, 18163–18168.
48. Bowie, D. (2008). Ionotropic glutamate receptors & CNS disorders. CNS Neurol. Disord. Drug Targets *7*, 129–143.
49. Bagshaw, A.T.M., Pitt, J.P.W., and Gemmell, N.J. (2006). Association of poly-purine/poly-pyrimidine sequences with meiotic recombination hot spots. BMC Genomics *7*, 179.