

Early Infantile Epileptic Encephalopathy Associated With the Disrupted Gene Encoding Slit-Robo Rho GTPase Activating Protein 2 (*SRGAP2*)

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We report on a female patient with early infantile epileptic encephalopathy and severe psychomotor disability possessing a *de novo* balanced translocation t(1;9)(q32;q13). The patient showed clonic convulsions of extremities 2 days after birth. Electroencephalogram (EEG) transiently showed atypical suppression-burst pattern. The seizures evolved to brief tonic spasms, and hypsarrhythmia on EEG was noticed at age of 5 months, indicating the transition to West syndrome. By using fluorescent in situ hybridization (FISH), southern hybridization, and inverse PCR, the translocation breakpoints were successfully determined at the nucleotide level. The 1q32.1 breakpoint was located within a segmental duplication and disrupted the gene encoding Slit-Robo Rho GTPase activating protein 2 (*SRGAP2*). The 9q13 breakpoint was suggested to reside in the heterochromatin region. *Srgap2* has been shown to be specifically expressed in developing brain of rodents, negatively regulate neuronal migration and induce neurite outgrowth and branching. Thus, *SRGAP2* is very likely to play a role in the developing human brain. This is a first report of the *SRGAP2* abnormality associated with early infantile epileptic encephalopathy.

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Key words: early infantile epileptic encephalopathy; West syndrome; chromosomal translocation; *SRGAP2*

INTRODUCTION

Many infantile epileptic syndromes show a unique combination of seizure types and electroencephalogram (EEG) findings depending on the patients' age [Kato et al., 2008]. Ohtahara syndrome (OS) and early myoclonic encephalopathy (EME) are characterized by early onset seizures mainly in neonatal period, and suppression-burst pattern on EEG, though their initial seizure type is different

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[Djukic et al., 2006; Ohtahara and Yamatogi, 2006]. Both OS and EME can progress to the West syndrome phenotype age-dependently, which is characterized by brief tonic spasms, a specific EEG pattern called hypsarrhythmia [Kato, 2006], in 75% and 41% of cases, respectively [Djukic et al., 2006; Ohtahara and Yamatogi, 2006]. The three epileptic syndromes (OS, EME, and West syndrome) are generally intractable and show the arrest of psychomotor development [Djukic et al., 2006; Kato, 2006; Ohtahara and Yamatogi, 2006]. Brain malformations and metabolic disorders were found as underlying causes of the three syndromes, but

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many idiopathic or cryptogenic cases remain etiologically unexplained. Recently, several causative genes have been reported: *ARX* in OS and West syndrome, *CDKL5* in West syndrome, *STXBPI* in OS, *SLC25A22* in EME [Stromme et al., 2002; Kalscheuer et al., 2003; Weaving et al., 2004; Molinari et al., 2005; Kato et al., 2007; Saitsu et al., 2008]. Of note, mutations in *ARX* have been found in both OS and West syndrome phenotypes, suggesting a common pathological seizure mechanism between them. However, there are still large numbers of cases remaining to be elucidated. Identification of new causative genes is absolutely necessary for further understanding of infantile epileptic syndromes.

The Slit-Robo signaling controls the neuronal migration and axonal guidance [Brose et al., 1999; Li et al., 1999; Wu et al., 1999], both of which are dependent on cytoskeletal reorganization. The family of Rho-GTPases, including Rac, Cdc42, and Rho, plays important roles in regulating cytoskeletal dynamics [Hall, 1998]. Rho-GTPases alternate between active (GTP-bound) and inactive (GDP-bound) conformation. The activities of Rho GTPases are tightly and antagonistically regulated by Guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs): GEFs catalyze nucleotide exchange and mediate activation, while GAPs increase the intrinsic GTPase activities to promote GTP hydrolysis, leading to inactivation [Lamarche and Hall, 1994]. Slit-Robo Rho GTPase activating proteins (SRGAPs) were identified as a family of GAP proteins which bind to the intracellular domain of Robo [Wong et al., 2001]. Three family members (SRGAP1-3) specifically expressed in developing brain of rodents [Wong et al., 2001; Yao et al., 2008; Bacon et al., 2009]. Recent studies suggested that SRGAPs are involved in neuronal development. SRGAP1 protein is required for Slit-mediated repulsion of migratory cells from the anterior subventricular zone of the fore-brain by blocking Cdc42 activity [Wong et al., 2001]. Functional disruption of SRGAP3 protein is associated with severe mental retardation in 3p-syndrome [Endris et al., 2002]. Moreover, it has been reported that SRGAP2 negatively regulates neuronal migration and induces neurite outgrowth and branching [Guerrier et al., 2009].

Here, we present a patient with infantile epileptic encephalopathy and profound psychomotor delay with a de novo reciprocal translocation t(1;9)(q32;q13), disrupting the *SRGAP2* gene. Detailed genomic analysis is presented.

CLINICAL REPORT

The 5-year-old girl is a product of unrelated healthy parents. She was born at term without asphyxia after an uneventful pregnancy. She showed apnea twice at day 1. Clonic convulsions of extremities started at day 2. Initial EEG performed at 10-day was reported as normal. Subsequently, myoclonus, which was easily induced by stimulation, was observed. Ictal EEG during myoclonus did not indicate that it was an electrical convulsion. Clonic convulsions were increased at 2 months of age when atypical suppression-burst pattern was transiently observed (Fig. 1A). Her seizures were controlled by combination of vitamin B6, zonisamide, phenobarbital, and KBr, but myoclonus continued. Brain magnetic resonance imaging (MRI) showed cortical atrophy and thin corpus callosum at 2 months of age (Fig. 1C–E). West syndrome was

diagnosed at 5 months of age by intellectual disability without head control, series of tonic-spasms, and hypsarrhythmia on EEG (Fig. 1B).

MATERIALS AND METHODS

Molecular Cytogenetic Analysis

G-banded chromosomes of peripheral lymphocytes were analyzed. Fluorescence in situ hybridization (FISH) was performed using peripheral lymphocytes. Labeling, hybridization, wash, and image acquisition were performed as previously described [Saitsu et al., 2008]. RPCI-11 BAC clones and approximately 10-kb probes amplified by long PCR using LA Taq polymerase (Takara Bio, Otsu, Japan) were used as probes. Primer information is available on request.

GeneChip Human Mapping 250K *NspI* Array

Genomic DNA obtained from peripheral blood leukocytes were used for microarray analysis. Experimental procedures were performed according to the manufacturer's protocol with slight modification (fragmentation time was shortened to 25 min). Call rate was 89.5%. Copy number alterations were analyzed by using CNAG2.0 [Nannya et al., 2005].

Cloning of Translocation Breakpoints

The 1q32.1 translocation breakpoint was analyzed by Southern hybridization using *EcoRI*- and *PstI*-digested patient DNA. Her parental DNAs were also analyzed. Probes were synthesized by PCR DIG probe synthesis kit (Roche, Basel, Switzerland) using RP11-134f21 DNA as a template. Primer information is available on request. Hybridization, washing and detection of probes were done according to the manufacturer's protocol. Images were captured on FluorChem (Alpha Innotech, San Leandro, CA). After identification of aberrant DNA fragments by Southern hybridization, size fractionation of electrophoresed *EcoRI*- and *PstI*-digested DNA of the patient was performed using QIAEXII Gel extraction kit (Qiagen, Valencia, CA) in order to obtain der(1) and der(9) translocation junction fragments, respectively. The collected DNA was self-ligated by Ligation high (Toyobo, Osaka, Japan), ethanol precipitated and dissolved in 20 μ l EB buffer (Qiagen). Inverse PCR was performed in 25 μ l of volume, containing 2 μ l ligated DNA, 1 \times LA PCR bufferII, 2.5 mM MgCl₂, 0.4 mM each dNTP, 0.5 μ M each primer, and 1.25 U LA Taq polymerase (Takara Bio). Primers were listed below: *EcoRI*-forward, 5'-GAAATGGCCTGGCTTGGTTGCTAT-3'; *EcoRI*-reverse, 5'-CACTGAAGCTGCCCTTGAGAA-GTGA-3'; *PstI*-forward, 5'-TTTCCCTCCATGATTCCTCTCTGCT-3'; *PstI*-reverse, 5'-CCAGGACAGCGTCTCACTCTCCATA-3'. Negative controls only used either forward or reverse primer. The PCR product was purified with ExoSAP (USB Co., Cleveland, OH) and sequenced for both forward and reverse strands with BigDye Terminator chemistry ver. 3 according to the standard protocol (Applied Biosystems, Foster city, CA). After breakpoint sequences were determined, breakpoint-specific primers for both der(1) and der(9) translocation junctions were designed: der(1)-forward, 5'-CCAAGGAATTGGGATCTCTGGGTCT-3'; der(1)-reverse, 5'-CATTCCATTCCATTCCCCTGCAC-3' (1,098-bp);

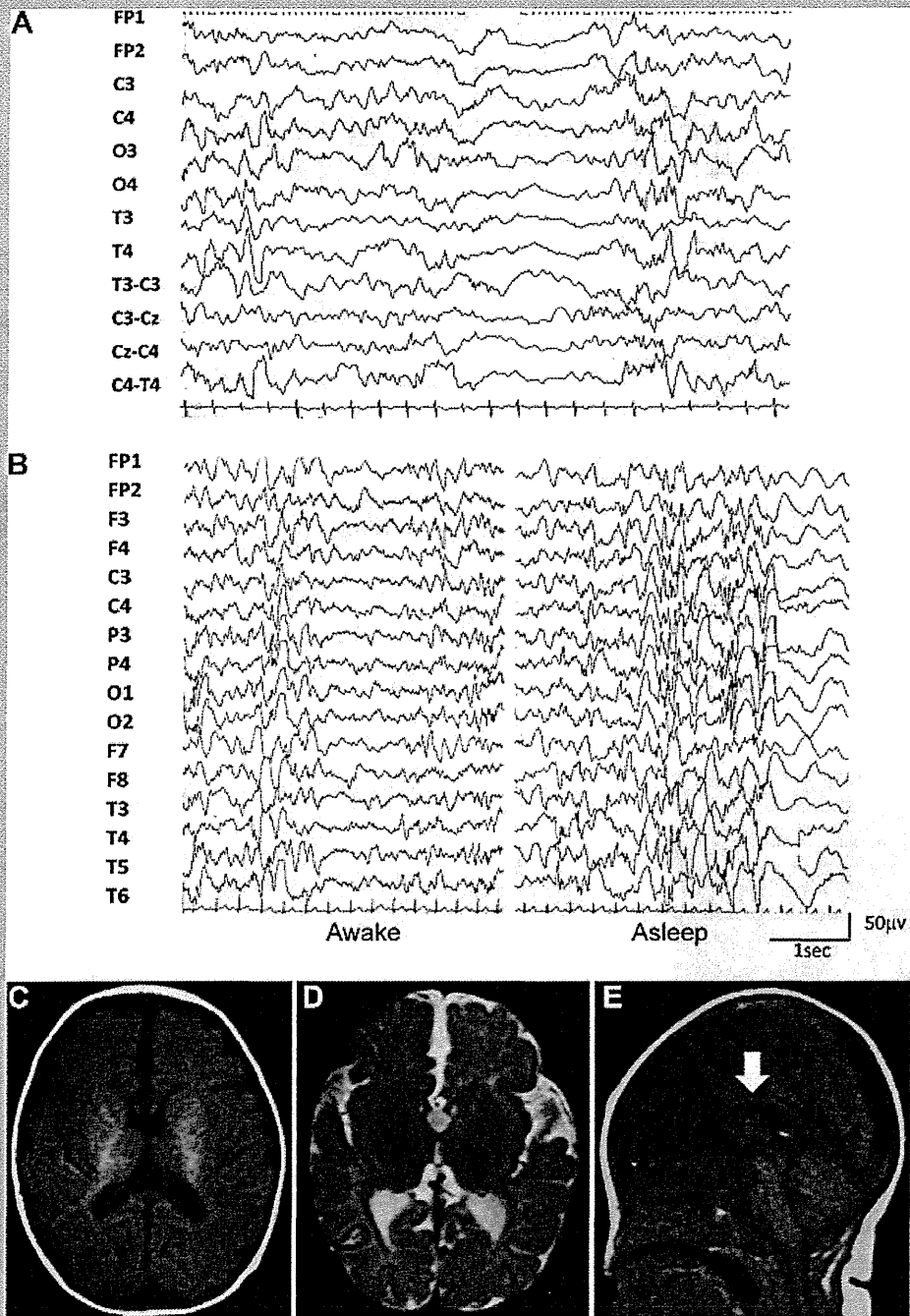


FIG. 1. EEG and brain MRI of the patient. **A:** Interictal high-voltage bursts alternate with low amplitude suppression phases at an approximately regular rate in both awake and asleep states at age of 2 months. Suppression phases do not exhibit "almost flat pattern" as typical suppression-burst pattern. **B:** Interictal EEG at 5 months shows multifocal spikes at awake (left), and high-voltage slow rhythm superimposed with irregular spikes; hypersarhythmia at sleep with some periodicity (right). **C,D:** Brain MRI T1- (C) and T2-weighted (D) axial images show mild cortical atrophy with normal myelination. **E:** Sagittal brain T1-weighted image shows thin corpus callosum (arrow)

der(9)-forward, 5'-GGAAAGGAATGGAATGAAATCAACGCG-3'; der(9)-reverse, 5'-CCAGGACAGCGTCTCACTCTCCATA-3' (495-bp). Junction fragments were amplified by PCR using these primer-sets on DNAs of the patient and her parents.

RESULTS

G-banded chromosomal analysis revealed a balanced translocation t(1;9)(q32;q13). Her parents showed a normal karyotype (data not shown), indicating that the translocation occurred de novo. Subsequent FISH analysis demonstrated that the breakpoint in chromosome 1 was covered by the clones, RP11-1109h22 and 134f21, showing signals all on normal chromosome 1 and derivatives chromosomes 1 and 9 (Fig. 2A,B). The overlapping region of these two clones was localized within the *SRGAP2* locus (Fig. 2A). The 5'-part of *SRGAP2* transcript was not mapped in the Human Genome browser (both in NCBI Build 36.1/hg18 and GRCh37/hg19 assembly) because the genomic contigs covering the immediately upstream regions of *SRGAP2* gene were absent. Thus, we described the putative exon number based on the order of mappable exons to the existing genomic database. The breakpoint was further narrowed down by FISH analysis using long PCR products as probes (Fig. 2A). Probe II showed weak but clear signals all in on chromosome 1, and derivative chromosomes 1 and 9, suggesting that the breakpoint was located within probe II (data not shown). It was of note that the probe II is associated with a segmental duplication (Fig. 2A). Southern hybridization analysis using probes P1 and P3 detected different aberrant bands only in the patient (Fig. 2A,C), indicating that the 1q32 breakpoint was located at the region between the two probes. P2 did not show any aberrant bands in Southern analysis, suggesting that a small deletion may have occurred near the breakpoint (Fig. 2A,C). Inverse PCR [Triglia et al., 1988] on *EcoRI*- and *PstI*-digested DNA was successful in obtaining der(1) and der(9) breakpoint-junction fragments, respectively. Sequence analysis showed that the 1q32 translocation breakpoint was located within the putative intron 5 of *SRGAP2*, and exon 5 was completely deleted (Fig. 2A). Sequences of the 9q13 breakpoint were not uniquely mapped to reference sequences.

However, sequences of 3'-end of the der(1) junction fragment (approximately 6.1-kb apart from the breakpoint) were similar to satellite 3 sequences (GeneBank accession number AF035810.1) (Fig. 2E), suggesting that 9q13 breakpoint was located in the heterochromatin region. Breakpoint-specific PCR analysis of the patient and her parents confirmed that the rearrangements occurred de novo (Fig. 2F). To check genomic copy number alterations accompanied by the rearrangement, GeneChip Human Mapping 250K *NspI* (Affymetrix, Santa Clara, CA) was performed. Besides two known copy number variations, no other imbalances were detected (data not shown).

DISCUSSION

SRGAP2 is a member of Slit-Robo Rho GTPase activating proteins with three domains: an N-terminal F-BAR domain, a RhoGAP domain, and an SH3 domain [Wong et al., 2001; Guerrier et al., 2009]. There are three variants of *SRGAP2* transcripts in humans: variant 1 (GenBank accession number NM_015326.2), variant 2 (GenBank accession number NM_001042758.1), and variant 3 (GenBank accession number NM_001170637.1). In all three variants, the coding proteins commonly possess F-BAR, RhoGAP, and SH3 domains except for an amino acid deletion in F-BAR domain in variant 2. Mouse *Srgap2* is expressed in the entire developing cortex including proliferative zones and postmitotic regions [Bacon et al., 2009; Guerrier et al., 2009]. It has been reported that the *SRGAP2* protein negatively regulates neuronal migration and induce neurite outgrowth and branching through its F-BAR domain [Guerrier et al., 2009]. In addition, GAP activity of the *SRGAP2* protein specifically downregulate Rac1 [Guerrier et al., 2009]. Mutations in *ARHGGEF6*, Rac1/Cdc42 specific GEF, cause X-linked mental retardation [Kutsche et al., 2000]. Moreover, mutation and/or disruption of *OPHN1* and *SRGAP3*, both encoding Rac1-GAPs, are associated with severe mental retardation [Billuart et al., 1998; Endris et al., 2002], indicating the importance of Rac1 regulation in human brain development. Thus, *SRGAP2* is likely to play important roles in developing brain in humans through the ability of the F-BAR and RhoGAP domains. It would be interesting to analyze

FIG. 2. Genomic characterization of t(1;9)(q32;q13). A: Schematic representation of the reciprocal translocation, t(1;9)(q32;q13) [top]. A summarized physical map covering the 1q32.1 translocation breakpoint is indicated [middle]. RP11-1109h22 and 134f21, and PCR probe II span the translocation breakpoint [longitudinal dashed line] in association with the segmental duplication. Four RefSeq genes, including *SRGAP2* spanning the breakpoint, are presented. Note that absence of genomic contigs of the immediately upstream region of the *SRGAP2* gene. More detailed maps are shown [bottom]. A partial restriction map [E, *EcoRI*; P, *PstI*], probes for southern hybridization [P1–P3], and putative exons 5–7 of *SRGAP2* are indicated. Translocation breakpoint [longitudinal dashed line] accompanied with a 1,575-bp deletion encompassing exon 5 of *SRGAP2* [red thick dashed line] are located between P1 and P3. B: FISH analysis using RP11-1109h22 as a probe showed clear signals on chromosome 1, and der(1) [white arrowheads] and der(9) chromosomes [white arrow]. Cross-hybridization was also observed to segmental duplications located at pericentric regions of chromosome 1 and derivative chromosome 1. C: Southern hybridization using probes P1, P2, and P3 on genomic DNAs of the patient and her parents. Arrow shows aberrant bands specific to the patient [not observed in parental DNA]. Pt, patient; Fa, father; Mo, mother. D: Breakpoint junction sequences of der(1) and der(9). In upper part, top and bottom sequence strands show chromosome 1 and derivative chromosome 1 sequences, respectively. In lower part, top and bottom strands show derivative chromosome 9 and normal chromosome 1 sequences, respectively. Breakpoint positions are marked with small longitudinal lines based on the UCSC genome browser coordinate [version Mar. 2006]. Asterisks indicate nucleotides identical to normal chromosomes. E: Sequences of the 3'-end of the der(1) junction fragment. Top and bottom sequence strands show der(1) and satellite 3 sequences, respectively, showing homology between two sequences. F: Breakpoint-specific PCR analysis of the patient's family. Primers specific to der(1) and der(9) breakpoints could successfully amplify 1,098- and 495-bp products, respectively, only from the patient [Pt], indicating the translocation occurred de novo. M, size marker; Fa, father; Mo, mother.

SRGAP2 in a large cohort of patients presenting with early epileptic encephalopathy including West syndrome. Although full-length *SRGAP2* transcripts (functional), which include sequences of putative exons 1–20 at 1q32.1, have been deposited in GeneBank, 5'-part of the *SRGAP2* transcript is not mapped in the Human Genome browser. Furthermore, seven exons of *SRGAP2* were again mapped to two separated segmental duplications at 1q21.1 and 1p11.2 with sequence similarities of 99.29% and 99.30%, respectively (Fig. 2A). This complex genomic structure interfered with full-blown mutation screening especially for the 1,356-bp coding region including the F-BAR domain. A microdeletion within two separate segmental duplications in *SRGAP2* locus has been found in 2 out of 90 Yoruban individuals (presumably with normal phenotype) from the HapMap Project using custom high-density oligonucleotide arrays [Matsuzaki et al., 2009]. However, it is uncertain whether they could confirm the precise locations of the deletions by another method. Thus, there remains a possibility that the deletion actually occurred at highly homologous genomic segments located at 1q21.1 and 1p11.2. Further descriptions about aberrations of the *SRGAP2* gene will be required for establishing in a causative role in early infantile epileptic encephalopathy.

The 9q13 breakpoint is likely to reside within the heterochromatic region. It is possible that some genes adjacent to 1q32.1 breakpoint would suffer from gene silencing by the position effect. *IKBKE* is an IKK (inhibitor of nuclear factor kappaB kinase)-related kinase that is essential for interferon-inducible antiviral transcriptional response [Tenoever et al., 2007]. *Ikbke* knockout mice are protected from high-fat diet-induced obesity, chronic inflammation in liver and fat, hepatic steatosis, and whole-body insulin resistance [Chiang et al., 2009]. However, neurological abnormalities have never been reported. *RASSF5* is a member of the Ras association domain family. A crucial role in the integrin-mediated adhesion and migration of lymphocytes and dendritic cells has been shown in *Rassf5*-deficient mice, but neurological abnormalities have never been mentioned [Katagiri et al., 2004]. Thus, *IKBKE* and *RASSF5*, two adjacent genes to *SRGAP2*, are less likely to be involved in infantile epileptic encephalopathy.

In conclusion, we described a patient with early infantile epileptic encephalopathy, carrying a de novo reciprocal translocation disrupting the *SRGAP2* gene. Clonic convulsions and atypical suppression-burst patterns on EEG at early infantile period did not fit into either OS or EME. However, the seizures became brief tonic spasms, and hypersarrhythmia on EEG was noticed, indicating transition to West syndrome. Disruption of *SRGAP2* may be related to West syndrome which has heterogeneous backgrounds [Kato, 2006].

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REFERENCES

- Bacon C, Endris V, Rappold G. 2009. Dynamic expression of the Slit-Robo GTPase activating protein genes during development of the murine nervous system. *J Comp Neurol* 513:224–236.
- Billuart P, Bienvenu T, Ronce N, des Portes V, Vinet MC, Zemni R, Roest Crolius H, Carrie A, Fauchereau F, Cherry M, Briault S, Hamel B, Fryns JP, Beldjord C, Kahn A, Moraine C, Chelly J. 1998. Oligophrenin-1 encodes a rhoGAP protein involved in X-linked mental retardation. *Nature* 392:923–926.
- Brose K, Bland KS, Wang KH, Arnott D, Henzel W, Goodman CS, Tessier-Lavigne M, Kidd T. 1999. Slit proteins bind Robo receptors and have an evolutionarily conserved role in repulsive axon guidance. *Cell* 96:795–806.
- Chiang SH, Bazuine M, Lumeng CN, Geletka LM, Mowers J, White NM, Ma JT, Zhou J, Qi N, Westcott D, Delproposto JB, Blackwell TS, Yull FE, Saltiel AR. 2009. The protein kinase IKKepsilon regulates energy balance in obese mice. *Cell* 138:961–975.
- Djukic A, Lado FA, Shinnar S, Moshe SL. 2006. Are early myoclonic encephalopathy (EME) and the Ohtahara syndrome (EIEE) independent of each other? *Epilepsy Res* 70:S68–S76.
- Endris V, Wogatzky B, Leimer U, Bartsch D, Zatyka M, Latif F, Maher ER, Tariverdian G, Kirsch S, Karch D, Rappold GA. 2002. The novel Rho-GTPase activating gene MEGAP/ srGAP3 has a putative role in severe mental retardation. *Proc Natl Acad Sci USA* 99:11754–11759.
- Guerrier S, Coutinho-Budd J, Sassa T, Gresset A, Jordan NV, Chen K, Jin WL, Frost A, Polleux F. 2009. The F-BAR domain of srGAP2 induces membrane protrusions required for neuronal migration and morphogenesis. *Cell* 138:990–1004.
- Hall A. 1998. Rho GTPases and the actin cytoskeleton. *Science* 279:509–514.
- Kalscheuer VM, Tao J, Donnelly A, Hollway G, Schwinger E, Kubart S, Menzel C, Hoeltzenbein M, Tommerup N, Eyre H, Harbord M, Haan E, Sutherland GR, Ropers HH, Geck J. 2003. Disruption of the serine/threonine kinase 9 gene causes severe X-linked infantile spasms and mental retardation. *Am J Hum Genet* 72:1401–1411.
- Katagiri K, Ohnishi N, Kabashima K, Iyoda T, Takeda N, Shinkai Y, Inaba K, Kinashi T. 2004. Crucial functions of the Rap1 effector molecule RAPL in lymphocyte and dendritic cell trafficking. *Nat Immunol* 5:1045–1051.
- Kato M. 2006. A new paradigm for West syndrome based on molecular and cell biology. *Epilepsy Res* 70:S87–S95.
- Kato M, Saitoh S, Kamei A, Shiraishi H, Ueda Y, Akasaka M, Tohyama J, Akasaka N, Hayasaka K. 2007. A longer polyalanine expansion mutation in the ARX gene causes early infantile epileptic encephalopathy with suppression-burst pattern (Ohtahara syndrome). *Am J Hum Genet* 81:361–366.
- Kato M, Saitoh S, Kamei A, Shiraishi H, Ueda Y, Akasaka M, Tohyama J, Akasaka N, Hayasaka K. 2008. Genetic etiology of age-dependent epileptic encephalopathy in infancy: Longer polyalanine expansion in ARX causes earlier onset and more severe phenotype. In: Takahashi T, Fukuyama Y, editors. *Biology of seizure susceptibility in developing brain*. Montrouge, Paris: John Libbey Eurotext. pp. 75–86.
- Kutsche KYntema H Brandt A Jantke I Nothwang HG Orth U Boavida MG David D Chelly J Fryns JP Moraine C Ropers HH Hamel BC van Bokhoven H Gal A 2000 Mutations in ARHGAP6 encoding a guanine nucleotide exchange factor for Rho GTPases in patients with X-linked mental retardation *Nat Genet* 26:247–250.

- Lamarche N, Hall A. 1994. GAPs for rho-related GTPases. *Trends Genet* 10:436–440.
- Li HS, Chen JH, Wu W, Fagaly T, Zhou L, Yuan W, Dupuis S, Jiang ZH, Nash W, Gick C, Ornitz DM, Wu JY, Rao Y. 1999. Vertebrate slit, a secreted ligand for the transmembrane protein roundabout, is a repellent for olfactory bulb axons. *Cell* 96:807–818.
- Matsuzaki H, Wang PH, Hu J, Rava R, Fu GK. 2009. High resolution discovery and confirmation of copy number variants in 90 Yoruba Nigerians. *Genome Biol* 10:R125.
- Molinari F, Raas-Rothschild A, Rio M, Fiermonte G, Encha-Razavi F, Palmieri L, Palmieri F, Ben-Neriah Z, Kadhom N, Vekemans M, Attie-Bitach T, Munnich A, Rustin P, Colleaux L. 2005. Impaired mitochondrial glutamate transport in autosomal recessive neonatal myoclonic epilepsy. *Am J Hum Genet* 76:334–339.
- Nannya Y, Sanada M, Nakazaki K, Hosoya N, Wang L, Hangaishi A, Kurokawa M, Chiba S, Bailey DK, Kennedy GC, Ogawa S. 2005. A robust algorithm for copy number detection using high-density oligonucleotide single nucleotide polymorphism genotyping arrays. *Cancer Res* 65:6071–6079.
- Ohtahara S, Yamatogi Y. 2006. Ohtahara syndrome: With special reference to its developmental aspects for differentiating from early myoclonic encephalopathy. *Epilepsy Res* 70:S58–S67.
- Saitu H, Kato M, Mizuguchi T, Hamada K, Osaka H, Tohyama J, Uruno K, Kumada S, Nishiyama K, Nishimura A, Okada I, Yoshimura Y, Hirai S, Kumada T, Hayasaka K, Fukuda A, Ogata K, Matsumoto N. 2008. De novo mutations in the gene encoding STXBP1 (MUNC18-1) cause early infantile epileptic encephalopathy. *Nat Genet* 40:782–788.
- Stromme P, Mangelsdorf ME, Shaw MA, Lower KM, Lewis SM, Bruyere H, Lutchera V, Gedeon AK, Wallace RH, Scheffer IE, Turner G, Partington M, Frints SG, Fryns JP, Sutherland GR, Mulley JC, Gez J. 2002. Mutations in the human ortholog of *Aristaless* cause X-linked mental retardation and epilepsy. *Nat Genet* 30:441–445.
- Tenoever BR, Ng SL, Chua MA, McWhirter SM, Garcia-Sastre A, Maniatis T. 2007. Multiple functions of the IKK-related kinase IKKepsilon in interferon-mediated antiviral immunity. *Science* 315:1274–1278.
- Triglia T, Peterson MG, Kemp DJ. 1988. A procedure for in vitro amplification of DNA segments that lie outside the boundaries of known sequences. *Nucleic Acids Res* 16:8186.
- Weaving LS, Christodoulou J, Williamson SL, Friend KL, McKenzie OL, Archer H, Evans J, Clarke A, Pelka GJ, Tam PP, Watson C, Lahooti H, Ellaway CJ, Bennetts B, Leonard H, Gez J. 2004. Mutations of *CDKL5* cause a severe neurodevelopmental disorder with infantile spasms and mental retardation. *Am J Hum Genet* 75:1079–1093.
- Wong K, Ren XR, Huang YZ, Xie Y, Liu G, Saito H, Tang H, Wen L, Brady-Kalnay SM, Mei L, Wu JY, Xiong WC, Rao Y. 2001. Signal transduction in neuronal migration: Roles of GTPase activating proteins and the small GTPase Cdc42 in the Slit-Robo pathway. *Cell* 107:209–221.
- Wu W, Wong K, Chen J, Jiang Z, Dupuis S, Wu JY, Rao Y. 1999. Directional guidance of neuronal migration in the olfactory system by the protein Slit. *Nature* 400:331–336.
- Yao Q, Jin W-L, Wang Y, Ju G. 2008. Regulated shuttling of Slit-Robo-GTPase activating proteins between nucleus and cytoplasm during brain development. *Cell Mol Neurobiol* 28:205–221.

