RBFOX1 and RBFOX3 Mutations in Rolandic Epilepsy

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Abstract

Partial deletions of the gene encoding the neuronal splicing regulator RBFOX1 have been reported in a range of neurodevelopmental diseases, including idiopathic generalized epilepsy. The RBFOX1 protein and its homologues (RBFOX2 and RBFOX3) regulate alternative splicing of many neuronal transcripts involved in the homeostatic control of neuronal excitability. In this study, we explored if structural microdeletions and exonic sequence variations in RBFOX1, RBFOX2, RBFOX3 confer susceptibility to rolandic epilepsy (RE), a common idiopathic focal childhood epilepsy. By high-density SNP array screening of 289 unrelated RE patients, we identified two hemizygous deletions, a 365 kb deletion affecting two untranslated 5’-terminal exons of RBFOX1 and a 43 kb deletion spanning exon 3 of RBFOX3. Exome sequencing of 242 RE patients revealed two novel probably deleterious variants in RBFOX1, a frameshift mutation (p.A233Vfs*74) and a hexanucleotide deletion (p.A299_A300del), and a novel nonsense mutation in RBFOX3 (p.Y287*). Although the three variants were inherited from unaffected parents, they were present in all family members exhibiting the RE trait clinically or electroencephalographically with only one exception. In contrast, no deleterious mutations of RBFOX1 and RBFOX3 were found in the exomes of 6503 non-RE subjects deposited in the Exome Variant Server database. The observed RBFOX3 exon 3 deletion and nonsense mutation suggest that RBFOX3 represents a novel risk factor for RE, indicating that exon deletions and truncating mutations of RBFOX1 and RBFOX3 contribute to the genetic variance of partial and generalized idiopathic epilepsy syndromes.

Introduction

Rolandic epilepsy (RE), or benign epilepsy with centrotemporal spikes (BECTS), is one of the most common epilepsy syndromes of childhood, comprising about 15% of epilepsies in children under the age of 16 years [1]. Age of onset ranges from years 3 to 13 and peaks between 8–9. Characteristic features are 1.) a somatosensory onset with unilateral parasthesias involving the tongue, lips, gums, and inner cheeks 2.) unilateral, tonic or tonic-clonic convulsions involving the face, lips, tongue as well as the pharyngeal and laryngeal muscles, causing 3.) speech arrest and drooling due to sidorrhoea and saliva pooling. At this stage the seizure may end, or it may develop into a generalized tonic clonic seizure. Nocturnal seizures, the most frequent variant of this syndrome, frequently become generalized. The electroencephalographic hallmark, a prerequisite of diagnosis, is blunted high-voltage characteristically shaped centrotemporal spikes (CTS), often followed by slow waves [2,3]. Based on a number of family studies it is generally assumed that, like in all other common idiopathic epilepsies a multifactorial mode of inheritance appears most likely [2,4,5]. The most recent family study on RE reported an increased rate of RE, febrile seizures, and an “epilepsy aphasia spectrum disorder” in relatives of children with RE [6]. To date, a number of loci or genes have been linked to various forms of idiopathic focal epilepsies or the EEG endophenotype of centrotemporal spikes (CTS). Linkage was reported to markers on 15q13.2 and to 16p12-11.2 [7,8]. But no causative gene has been reported at either loci yet. In a small number of cases variants in KCNQ2 and KCNQ3 were found to be associated with RE and the respective EEG trait [9]. In addition, mutations in SRPX2 in two families with mental retardation, severe language dysfunction and rolandic seizures have been reported [10]. By genome-wide linkage analysis RE and the CTS trait have been associated with the elongator protein complex 4 [11]. Furthermore, in a girl with early-onset epileptic encephalopathy and CTS a de novo GRIN2A mutation was identified [12]. However, all these findings in single or few patients or small families still lack replication.

Rare copy number abnormalities of RBFOX1 have also been associated with mental retardation in comorbidity with and without seizures, attention deficit disorder and autism [13,14,15,16]. Recently, we have shown in 1400 idiopathic...
generalized epilepsy (IGE) patients and 2256 population controls that
deletions affecting 5'- located exons of RBFOX1 are
significantly enriched in IGE cases compared to population
matched controls [17]. The RBFOX genes (RBFOX1: chr16:6069132-7763340, NM_001142333; RBFOX2: chr22:36134783-36424583, NM_001082578, and RBFOX3: chr17:77065427-77512230; NM_001082575) encode neuron-specific
splicing factors predicted to regulate neuronal splicing
networks. Several epilepsy candidate genes are downstream targets of
Rhox proteins (FLNA, SLC1A3, DCX, GABBR3, GAD2, KCNQ2, SCN8A, SLC12A5, SYN1, RBFOX3, SCN1A, SLC12A3) and their regulation of expression
and splicing has been demonstrated [18,19]. FOX family members regulate splicing of the other FOX members and autoregulate themselves [20,21]. The present candidate gene analysis tested whether (i) deletions in RBFOX1, RBFOX2 and RBFOX3 might increase risk of RE and (ii) exonic mutations affecting the protein structure occur more
frequently in RE compared to control subjects.

Methods
The institutional review board of the University Giessen, Germany specifically approved this study. Registration number: No 03/11. Written informed consent was obtained from all subjects or their legal guardians according to study protocols approved by the institutional ethical review board of the University Giessen, Germany under the title “genomic variation in patients with idiopathic epilepsy”.

Diagnostic Criteria
Diagnosis of RE was performed according to the International Classification of Seizures and Epilepsies as described [3]. Sleep activation, characteristic shape, and classification by two independent individuals were required for classification of the EEG trait. Electrical Status Epilepticus in slow sleep (ESEs) was diagnosed if prolonged generalized discharges of CTS dominated sleep EEG recordings [22,23,24]. Atypical benign partial epilepsy of childhood (ARE) was diagnosed employing the following criteria: Characteristic EEG trait of CTS, however, with trains of continuous generalized nocturnal discharges as a prerequisite of diagnosis in all ABPE cases. In addition at least one of the following two features needed to be present: (1) seizures compatible with BECTS plus one or more additive seizure types like atonic seizures, atypical absences (“dreamy states”) or myoclonic seizures as reported. (2) seizures compatible with BECTS plus a significant mental handicap, and/or severe developmental speech disorder [25,26,27]. If a child had a seizure symptomatology compatible with BECTS but had prolonged generalized discharges of CTS during sleep EEG, without any additional seizure types and a normal global and speech development it was diagnosed as BECTS.

Patient Cohort
Families. The investigated cohort consisted of 98 index cases
selected from 98 multiplex families with at least two affected
siblings. In 96 families at least one of the affected probands
suffered from RE or ARE, the second affected sibling presented either with RE, ARE or the EEG trait only. In two families all
affected children did not suffer from seizures, but displayed the
EEG trait only. Of all 98 index patients tested, 78 presented with
RE, 15 with ARE, 3 with ESES, and 2 with CTS only.

Sporadic cases. 191 non-familial cases were included into the
study cohort. Of these patients 133 suffered from classic RE, 11 from ESES, 26 from ARE and 1 with CTS only.

Genotyping and Copy Number Variation Detection
Whole blood DNA from the patients was genotyped for using the Infinium OmniExpressExome BeadChip (Illumina Inc., San
Diego, CA) according to the manufacturer’s protocol. Briefly, 200 ng of DNA were amplified, biotin labeled, and hybridized to the microarray. CNV calls were generated with the PennCNV software [28], using the log R ratio (LRR) and B allele frequency (BAF) for 730.525 probes designed for the genotyping array. CNV analysis was restricted to microdeletions covered by at least 20 probes and spanning 40 kb or more in size. To exclude technical artifacts, all potential microdeletions were manually inspected for regional SNP heterozygosity state and log2 ratios of the signal intensities using the Illumina Genome Viewer (Illumina Inc., San
Diego, CA). Only one RBFOX1 deletion and one RBFOX3 deletion could be confirmed by manual variant evaluation. Segregation in the families of the microdeletions was examined by real-time quantitative PCR using TaqMan CNV probes (RBFOX1: Hs04461212_cn; RBFOX3: Hs03975574_cn) (Life Technologies, Darmstadt, Germany).

Exonic Sequence Analysis
Sequence analysis was performed using next generation sequencing techniques. In brief, DNA was fragmented using sonification technology (Covaris, Woburn, MA, USA) and fragments were end repaired and adaptor ligated. SeqCap EZ Human Exome Library v2.0 (Roche NimbleGen, Madison, WI, USA) was used for enrichment and samples were analyzed on the Illumina HiSeq 2000® sequencer. For 242 patients exome data were generated which featured an average coverage >30x for 77% of the target sequences. Data were filtered using Illumina Realtime Analysis® (RTA) software v1.8 and mapped to the human genome reference build hg19 via the ELANDv2 alignment algorithm on a multi-node compute cluster. PCR duplicates were excluded using CASAVA v1.8. Variant calling was performed by SAMtools (version 0.1.7) for Indel detection. Scripts developed in-house at the Cologne Center for Genomics (Cologne, Germany) were applied to detect protein changes, affected splice sites, and overlaps with known variants. In particular, variants were filtered for high-quality unknown variants in RBFOX1, RBFOX2, and RBFOX3 by comparison to an in-house variation database, dbSNP build 137 (www.ncbi.nlm.nih.gov/projects/SNP/), 1000 Genomes database (www.1000genomes.org/), and the Exome Variant Server (http://eva.gs.washington.edu/EVS/). Variant validation and segregation analyses were performed by Sanger sequencing following standard protocols.

Results
Detection of RBFOX1 and RBFOX3 Microdeletions in RE Patients
In total, 289 RE patients were screened for copy number variations in RBFOX1, RBFOX2, and RBFOX3 using the Infinium OmniExpressExome BeadChip® (Illumina Inc., San Diego, CA). We identified one RE patient among 289 (0.34%) with a hemizygous deletion in both RBFOX1 and RBFOX3. A deletion of 365 kb was found to be located in the genomic region of RBFOX1 affecting the untranslated 5'-terminal exons 3 and 1B (Fig. 1A, S1, exon annotation according to [17]) whereas a smaller deletion of 43 kb affected RBFOX3 by removing exon 3 of the known isoform NM_001082575 along with flanking intronic sequences (Fig.1B, S1). The RBFOX1 gene deletion affects the largest transcript variants 4–5 and 6 (NM_018723, NM_001142333, NM_001142334). No additional mutations were
identified in the remaining undeleted RBFOX1 and RBFOX3 exonic sequences.

Detection of Rare Exonic Variants in RBFOX1 and RBFOX3

Mutational screening of RBFOX1, RBFOX2, RBFOX3 in 242 RE patients did not reveal any mutation in RBFOX2, while a total of three rare mutations (1.2%) were identified in RBFOX1 and RBFOX3 (Fig. 1A,1B; Table 1). In contrast to the identified RBFOX1 and RBFOX3 microdeletions, all exonic variants were located near the 3'-terminal region of both genes. The identified exonic variants included two RBFOX1 variants in exon 11 (c.690_696delGTATCCAins(GTATCCA)2; p.A233Vfs*74, NM_001142333) and exon 13 (c.893_898delCTGCCG, p.A299_A300del, NM_001142333), as well as a nonsense mutation in exon 13 of the RBFOX3 gene (c.861C>A, p.Y287*, NM_001082575). The p.A299_A300del variant of patient E699 (Table 1, S1) deletes two out of three consecutive alanine residues which are conserved among mammals but not vertebrates (Fig. S2, S3).

To our knowledge nonsense mutations in FOX genes have not been described in the literature and are not found in any of the available databanks. They are absent from 6503 individuals whose exomes were deposited in the EVS database. Furthermore, no deleterious mutations have been identified in >450 exomes of our in-house database (various non epilepsy projects; about 80% Caucasian ancestry). In the truncated proteins the C-terminal fragments of RBFOX1 and RBFOX3 are affected which are critical for cassette-exon activation and repression [29] and the nuclear localization of the FOX proteins [21,30,31].

Familial Segregation and Comorbidity Analysis

The segregation of RBFOX1 and RBFOX3 variants identified in the RE index-patients were tracked in four families (Fig. 2). Where testing was possible (n = 3), all variants identified were inherited, one maternally and two paternally. Five out of ten variant carriers were affected by RE, one by an encephalopathy with status epilepticus during sleep (ESES) and one by the RE-characteristic CTS EEG-trait only. The transmitting parents were all unaffected at the date of evaluation. Notably, we cannot rule out that the parents might have expressed the RE-characteristic CTS EEG-trait at younger age, considering its age-related expression with maximum manifestation during childhood. Six out of eight family members affected by either RE, CTS or ESES carried one exonic variant of RBFOX1 or RBFOX3 and one RE patient had a deletion in both genes (Fig. 2). The index patient in Family 1 had RE and exon-removing microdeletions in both RBFOX1 and RBFOX3 whereas her sister with the CTS EEG-trait alone carried only the RBFOX1 microdeletion. In Families 1, 2 and 4, all affected family members carried a RBFOX1 deletion or one of the truncating mutations. In family 3, the RBFOX1 p.A299_A300del deletion identified in the RE-index patient was not present in his RE-affected sister. The phenotypic RE features of the four variant carrying index cases did not differ from those RE patients lacking RBFOX1 and RBFOX3 variants (Table 1). The ESES phenotype variant is generally assumed to represent the most severe expression of the RE CTS EEG-trait. A mild to moderate developmental speech delay, that frequently resolves later, is a known feature in many RE patients [32].

Discussion

A previous study of 1408 unrelated individuals with idiopathic generalized epilepsy revealed exon-removing RBFOX1 microdeletions in five patients, whereas none was found in 2256 ethnically matched controls [17]. Furthermore, rare copy number abnormalities of RBFOX1 have been reported for patients with neurological diseases like epilepsy, mental retardation and autism [13,14,15,16] strongly indicating that partial RBFOX1 deletions are a recurrent risk factor of neurodevelopmental defects in human. The heterozygous Rbfox1 knockout mouse model shows deregulated splicing which impacts genes involved in synaptic transmission and membrane excitability, leading to an increased susceptibility for seizure events. Notably, homo- and heterozygous Rbfox1 knockouts display normal brain morphology [33]. Consistent with the splicing alterations in mice, RNA interference-
Table 1. RBFOX1 and RBFOX3 variants and phenotype of index-patients.

| Family | Index-patient | Patient | Variant | Epilepsy Syndrome | Diagnosis/seizure types | Comorbidity |
|--------|---------------|---------|---------|-------------------|-------------------------|-------------|
| 1      |               | E103    | RBFOX1:365kb Deletion, RBFOX3:43 kb Deletion | RE | Nocturnal generalized tonic clonic seizures, Postictal speech arrest | None (normal global development, normal speech acquisition) |
| 1      |               | E103b   | RBFOX1:365 kb Deletion | CTS only | No seizures, EEG trait only | None (normal global development, normal speech acquisition) |
| 2      |               | EG1208  | RBFOX1: p.A233Vfs*74 | RE | Nocturnal rolandic seizures with postictal speech arrest | Initially delayed language development, later normal |
| 2      |               | EG1209  | RBFOX1: p.A233Vfs*74 | RE | Nocturnal and diurnal rolandic seizures with postictal speech arrest | Initially delayed language development, later normal |
| 3      |               | E699    | RBFOX1: p.A299_A300del | RE | Nocturnal rolandic seizures with postictal speech arrest | None (normal global development, normal speech acquisition) |
| 3      |               | E699b   | -        | RE | Nocturnal rolandic seizures with postictal speech arrest | None (normal global development, normal speech acquisition) |
| 4      |               | E136    | RBFOX3: p.Y287* | RE | Nocturnal generalized tonic clonic seizures with postictal speech arrest | Initially delayed language development, later normal |
| 4      |               | E679c   | RBFOX3: p.Y287* | ESES | ESES without seizures | Moderate developmental delay, delayed speech development, mild oral dyspraxia |

Survey on RBFOX1 and RBFOX3 variants in patients. Seizure type and comorbidity overview of variant carrier. Abbreviations: RE = rolandic epilepsy; CTS = centrotemporal spikes; ESES = epileptic encephalopathy with status epilepticus during sleep.

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Figure 2. Segregation of RBFOX1 and RBFOX3 affecting variants. For three mutations for which DNA samples of family members were available, segregation analyses could be performed. The respective RBFOX1 and RBFOX3 truncating mutations co-segregated with a variable phenotype of either seizures or pathologic EEG patterns in most family members. Only a few individuals carried the respective familial mutation but did not present any clinical features, indicating incomplete penetrance of the mutations. However, subclinical phenotypes (e.g. EEG patterns) have not been investigated in these individuals (indicated by question mark). In family 3 the variant (deletion of two consecutive alanine residues at position 299–300 of RBFOX1) did not segregate with the epilepsy phenotype. Abbreviations: n.a = DNA was not available for testing; RE = rolandic epilepsy; CTS = centrotemporal spikes; ESES = encephalopathy with status epilepticus during sleep.

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mediated 50% knockdown of RBFOX1 transcripts in human neurons changes the alternative splicing pattern and expression of primarily neuronal genes involved in synapse formation and function [19].

Our identification of a RE patient carrying a rare microdeletion affecting the 5′ part of RBFOX1 replicates the association found in idiopathic epilepsy, but now in an entirely different, i.e. focal, epilepsy syndrome. Her sister, who also carried the deletion, exhibited the RE-specific EEG trait but did not suffer from overt seizures. In agreement with our previous IGE study, this indicates that RBFOX1 microdeletions act as a susceptibility factor but not as a highly penetrant variant. The frequency of the RBFOX1 deletion in the RE patient cohort (0.34%) is in the range of that observed for IGE (0.35%; [17]). The RBFOX1 microdeletion presented here is located in the untranslated 5′-terminal region of the gene, like the previously reported microdeletions and translocations [13–17]. A female with autism carrying a 5′-terminal microdeletion of RBFOX1 due to a de novo translocation t(15p;16p) displayed a significantly reduced RBFOX1 mRNA expression in lymphocytes [14]. A second FOX microdeletion also detected in patient E103 is affecting the paralogous RBFOX3 gene which also encodes the highly conserved RNA recognition motif (RRM) [20]. Interestingly, the sister of E103, who also carries the RBFOX1 deletion but lacks the RBFOX3 deletion, only expressed CTS but without having epileptic seizures. Due to the lack of further statistical and functional evidence, we can only hypothesize that both deletions affect neuronal splicing in Family 1 synergistically. Microdeletions in RBFOX1 are extremely rare. Only two other exon-removing microdeletions have been noted in the Database of Genomic Variants (DGV, accessed 2/2013) in the general population. Being present in control individuals might indicate a variable expressivity of RBFOX1 microdeletions or a lack of careful assessment of neuropsychiatric phenotypes in these probands. Both microdeletions do not delete exon 3 (NM_001142333) of RBFOX3 as in our RE patient. It has been demonstrated that Rbfox3 regulates alternative splicing and nonsense mediated decay of Rbfox2 mRNA [21]. This complex interplay of Fox family members has been further reported in the Rbfox1 knockout mouse model where the loss of Rbfox1 inhibits an upregulation of Rbfox2 [33]. Mutational screening did not reveal any exonic mutation in RBFOX2, while three rare mutations have been identified (1.2%) in RBFOX1 and RBFOX3 together. The C-terminal fragment is critical for cassette-exon activation and repression [29] in RBFOX1 as well as for nuclear localization for RBFOX1 [30,31] and RBFOX3 [21]. These mechanisms are likely to be affected by the observed mutations. Furthermore, no frameshift nor nonsense mutations have been found in the exomes of 6503 control subjects reported in the ESV database. All individuals with an epileptic phenotype were carriers of the truncating mutations in family 1, 2 and 4. Only in family 3 the variant (deletion of two consecutive alanine residues at position 299–300 of RBFOX1) did not segregate with the epilepsy phenotype. This deleted sequence is conserved among mammals only, suggesting that most likely only truncating variants may be risk-conferring for RE. In vitro studies demonstrated that the C-terminal domain is critical for cellular localization in both FOX genes and also for targeted splicing in RBFOX1 [21,30,31]. As a model for haploinsufficiency in vitro knock down of RBFOX1 in primary human neuronal stem cells resulted in an altered expression and splicing of several epilepsy candidate genes (FLNA, SLC1A3, DCX, GABRB3, GAD2, KCNQ2, SLC1A5, SV2B, SVN) [19]. Interestingly, variants and mutations in KCNQ2 were recently found associated with RE [9].

In summary, our results strengthen the association of partial RBFOX1 deletions in neurodevelopmental diseases and extend the RBFOX1-related phenotypic spectrum by RE. The present RBFOX3 mutations highlight this neuronal gene as a plausible novel genetic risk factor of RE, and suggest that besides genomic microdeletions affecting the 5′-terminal exons, truncating mutations of RBFOX1 and RBFOX3 increase the risk of RE.

Supporting Information

Figure S1 Raw SNP intensity data of all samples carrying exon-disrupting microdeletions affecting the RBFOX1 and RBFOX3 genes. Red frames represent the area of the observed microdeletions. Signal intensities of a SNP probe are represented by dots, one dot per each probe (Log R ratio track). A decline of neighboring probe signal intensities and B allele frequencies (B Allele Freq track) near 1 and 0 indicate a genomic deletion. The deletions have been visualized using the Illumina Genome Studio Software. (DOC)

Figure S2 UCSC Genome Browser RBFOX1 transcript, common SNP and GERP conservation annotation tracks. Top track: Highlighted in red, deleted nucleotides of patient E699. Middle track: The deleted sequence is abundant all six known RBFOX1 transcripts. Lower track: No common SNP (≤1%) is annotated in dbSNP137 for the shown sequence interval. Bottom track: RBFOX1 Genomic Evolutionary Rate Profiling (GERP) scores. The rejected substitutions score (RS) is based on an alignment of 35 mammal scores. A RS score threshold of 2 provides high sensitivity while still strongly enriching sequence conservation sites (http://www.genome.ucsc.edu). For the deleted sequence of RBFOX1, high and low RS scores are shown. (DOC)

Figure S3 Multiple Sequence Alignment RBFOX1 variant A299_A300del (c.893_898delCTGCGG, p.A299_A300del, NM_001142333). Multiple sequence alignments: The top line indicates the human amino acid sequence according to genome build hg19. Amino acids highlighted in red are hemizygously deleted in patient E699. Three alanine residues are conserved among mammals but only one alanine residue in none mammalian vertebrates. Sequence annotations were taken from the UCSC Genome Browser (http://www.genome.ucsc.edu) and for multiple sequence alignments we used ClustalW (http://www.ebi.ac.uk/Tools/services/web_clustalw2/). (DOC)

Table S1. RBFOX1 and RBFOX3 exonic sequence variants.

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Author Contributions

Conceived and designed the experiments: DL BN. Performed the experiments: DL JA MT EMR. Analyzed the data: DL HT. Contributed reagents/materials/analysis tools: RSM AH TS PN FZ BN HM. Wrote the paper: DL EMR HL FZ TS PN BN.
References

1. Sidenvall R, Forsgren L, Blomquist HK, Heijbel J (1993) A community-based prospective incidence study of epileptic seizures in children. Acta Paediatr 82: 69–65.

2. Doose H, Brügger-Heuer B, Neuhauser B (1997) Children with focal sharp waves: clinical and genetic aspects. Epilepsia 38: 788–796.

3. Berg AT, Berkovic SF, Brodie MJ, Buchhalter J, Cross JH, et al. (2010) Revised terminology and concepts for organization of seizures and epilepsy: report of the ILAE Commission on Classification and Terminology, 2005–2009. Epilepsia 51: 676–685. doi:10.1111/j.1522-1165.2010.02522.x.

4. Ottman R (1989) Genetics of the partial epilepsies: a review. Epilepsia 30: 107–111.

5. Bari B, Bull LL, Strug LJ, Clarke T, Murphy PL, et al. (2007) Autosomal dominant inheritance of centrotemporal sharp waves in rolaniic epilepsy families. Epilepsia 48: 2266–2272. doi:10.1111/j.1522-1165.2007.01221.x.

6. Veara DF, Tsai M-H, Sadleir LG, Grimson KE, Liddle LM, et al. (2012) Clinical genetic studies in benign childhood epilepsy with centrotemporal spikes. Epilepsia 53: 319–324. doi:10.1111/j.1522-1565.2011.03668.x.

7. Neuhauser BA, Fredler B, Himmelebn B, Kämpfer F, Lasser U, et al. (1998) Centrotemporal spikes in families with rolandic epilepsy: linkage to chromosome 13q14. Neurology 51: 1608–1612.

8. Guerrini R, Bonanni P, Nardocci N, Parmeggiani L, Piccirilli M, et al. (1999) Autosomal recessive rolandiic epilepsy with paroxysmal exercise-induced dystonia and writer’s cramp: delineation of the syndrome and gene mapping to chromosome 16p12–11.2. Ann Neurol 45: 344–352.

9. Neubauer BA, Waldgeger S, Heinzinger J, Hahn A, Kurlemann G, et al. (2008) KCNQ2 and KCNQ3 mutations contribute to different idiopathic epilepsy syndromes. Neurology 71: 17–103. doi:10.1212/01.wnl.0000317090.92183.cc.

10. Roll P, Rudolf G, Pereira S, Röher B, Scheffer IE, et al. (2006) SRPX2 mutations in disorders of language cortex and cognition. Hum Mol Genet 15: 1103–1107. doi:10.1038/hmg/dd0835.

11. Roll P, Rudolf G, Pereira S, Röher B, Scheffer IE, et al. (2006) SRPX2 mutations in disorders of language cortex and cognition. Hum Mol Genet 15: 1103–1107. doi:10.1038/hmg/dd0835.

12. Strug LJ, Clarke-T, Chiang T, Chien M, Baskurt Z, et al. (2009) Centrotemporal sharp wave EEG trait in rolandic epilepsy maps to Elongator Protein Complex 4 (ELP4). Eur J Hum Genet 17: 1171–1181. doi:10.1038/ejhg.2008.267.

13. Endele S, Rosenberger G, Geider K, Popp B, Tamer C, et al. (2010) Mutations in GRIN2A and GRIN2B encoding regulatory subunits of NMDA receptors cause variable neurodevelopmental phenotypes. Nat Genet 42: 1021–1026. doi:10.1038/ng.1013.

14. Martin CL, Duvall JA, Ilkin Y, Simon JS, Arreaza MG, et al. (2007) Cytogenetic syndromes. Neurology 71: 177–183. doi:10.1212/01.wnl.0000317090.92185.ec.

15. Elia J, Glessner JT, Wang K, Takahashi N, Shtir CJ, et al. (2012) Genome-wide copy number variation study associates metabotropic glutamate receptor gene networks with attention deficit hyperactivity disorder. Nat Genet 44: 78–84. doi:10.1038/ng.841.

16. Davis LK, Malott N, Mosconi MW, Macmillan C, Schmitt L, et al. (2012) Rare inherited A2BP1 deletion in a proband with autism and developmental hemiparesis. Am J Med Genet A 158A: 1654–1661. doi:10.1002/ajmg.a.35396.

17. Lal D, Trucks H, Moller RS, Hjølgrimm H, Koellemann BPC, et al. (2013) Rare exonic deletions of the RBFOX1 gene increase risk of idiopathic generalized epilepsy. Epilepsia 54: 265–271. doi:10.1111/j.1528-1167.2012.03606.x.

18. O’Brien JE, Drews VL, Jones JM, Dugas JC, Barres BA, et al. (2012) Rbfox proteins regulate alternative splicing of neuronal sodium channel SCN1A. Mol Cell Neurosci 49: 120–126. doi:10.1016/j.mcn.2011.10.005.

19. Vogel BL, Weser E, Walmich A, Friedrich T, Vijayardran C, et al. (2012) RBFOX1 regulates both splicing and transcriptional networks in human neuronal development. um Mol Genet 21: 4171–4186. doi:10.1038/ajmg.dld.240.

20. Damianov A, Black DL (2010) Autoregulation of Fox protein expression to produce dominant negative splicing factors. RNA 16: 405–416. doi:10.1261/rna.1838210.

21. Dredge BK, Jensen KB (2011) Neur/NRibo3 nuclear and cytoplasmic isoforms differentially regulate alternative splicing and nonsense-mediated decay of Ribo3. PLoS ONE 6: e21585. doi:10.1371/journal.pone.0021585.

22. Calvet AF, Bancaud J (1976) Electroencephalography of waves associated with eye movements in man during wakefulness. Electroencephalogr Clin Neurophysiol 40: 457–469.

23. Billard C, Autret L, Laffont F, de Giovanni E, Lucas B, et al. (1981) [Acquired aphasias in epileptic children-four cases with electrical infracritical status epilepticus during sleep [author’s transl]. Rev Electroencephalogr Neurophysiol 40: 457–469.

24. Yasahara A, Yoshihara T, Takeda T, Ueki S, Kobayashi Y, et al. (1991) Epilepsy with continuous spike-waves during slow sleep and its treatment. Epilepsia 32: 59–62.

25. Aicardi J, Chevrie J (1982) Atypical benign partial epilepsy of childhood. Dev Med Child Neurol 24: 281–292.

26. O’Brien JE, Drews VL, Jones JM, Dugas JC, Barres BA, et al. (2012) Rbfox proteins regulate alternative splicing of neuronal sodium channel SCN1A. Mol Cell Neurosci 49: 120–126. doi:10.1016/j.mcn.2011.10.005.

27. Neubauer BA, Waldgeger S, Heinzinger J, Hahn A, Kurlemann G, et al. (2008) KCNQ2 and KCNQ3 mutations contribute to different idiopathic epilepsy syndromes. Neurology 71: 17–103. doi:10.1212/01.wnl.0000317090.92183.cc.

28. Wang K, Li M, Hadley D, Liu R, Glessner J, et al. (2007) PennCNV: an integrated hidden Markov model designed for high-resolution copy number variation detection in whole-genome SNP genotyping data. Genome Res 17: 1665–1674. doi:10.1101/gr.601907.

29. Damianov A, Black DL (2010) Autoregulation of Fox protein expression to produce dominant negative splicing factors. RNA 16: 405–416. doi:10.1261/rna.1838210.

30. Nakahata S, Kawamoto S (2005) Tissue-dependent isoforms of mammalian FoxI homologs are associated with tissue-specific splicing activities. Nucleic Acids Res 33: 2078–2089. doi:10.1093/nar/gkj338.

31. Lee J-A, Tse Z-Z, Black DL (2009) An inducible change in Fox-1/A2BP1 splicing modulates the alternative splicing of downstream neuronal target exons. Genes Dev 23: 2294–2293. doi:10.1101/gad.dds240.

32. Pal DK (2011) Epilepsy and neurodevelopmental disorders of language. Curr Opin Neurol 24: 126–131. doi:10.1097/WCO.0b013e328344634a.

33. Gehman LT, Stoilov P, Maguire J, Damianov A, Lin C-H, et al. (2011) The splicing regulator Rbfox1 (A2BP1) controls neuronal excitation in the mammalian brain. Nat Genet 43: 706–711. doi:10.1038/ng.841.