BRIEF COMMUNICATION

Analysis of *ELP4*, *SRPX2*, and interacting genes in typical and atypical rolandic epilepsy

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Summary

Rolandic epilepsy (RE) and its atypical variants (atypical rolandic epilepsy, ARE) along the spectrum of epilepsy–aphasia disorders are characterized by a strong but largely unknown genetic basis. Two genes with a putative (*ELP4*) or a proven (*SRPX2*) function in neuronal migration were postulated to confer susceptibility to parts of the disease spectrum: the *ELP4* gene to centrotemporal spikes and *SRPX2* to ARE. To reexamine these findings, we investigated a cohort of 280 patients of European ancestry with RE/ARE for the etiological contribution of these genes and their close interaction partners. We performed next-generation sequencing and single-nucleotide polymorphism (SNP)–array based genotyping to screen for sequence and structural variants. In comparison to European controls we could not detect an enrichment of rare deleterious variants of *ELP4*, *SRPX2*, or their interaction partners in affected individuals. The previously described functional p.N327S variant in the X chromosomal *SRPX2* gene was detected in two affected individuals (0.81%) and also in controls (0.26%), with some preponderance of male patients. We did not detect an association of SNPs in the *ELP4* gene with centrotemporal spikes as previously reported. In conclusion our data do not support a major role of *ELP4* and *SRPX2* in the etiology of RE/ARE.

KEY WORDS: **Idiopathic focal childhood epilepsy, Mutation, Gene, CNV, Association, SNP.**

Rolandic epilepsy (RE), also known as benign childhood epilepsy with centrotemporal spikes (BECTS), is the most common childhood epilepsy syndrome. Although RE usually takes a benign course, there is an overlap with more severe forms of idiopathic focal childhood epilepsies such as atypical benign partial epilepsy (ABPE), Landau-Kleffner syndrome (LKS), and the continuous spike-and-waves during slow-wave sleep syndrome (CSWSS). These latter syndromes are often referred to as atypical rolandic epilepsy (ARE), and are considered to form together with RE the spectrum of epilepsy–aphasia disorders (RE/ARE), with a presumably shared strong genetic etiology.[1,2]

In most RE- and ARE-affected individuals, a complex, polygenic inheritance with additional acquired factors emerges as the most likely etiology.[3] Yet, only a few chromosomal loci and an even smaller number of individual genes could be associated with the disease spectrum so far. Pathologic variants of the glutamate receptor *GRIN2A* can be detected in 7.5–20 % of RE/ARE patients, with higher rates in the more severe phenotypes.[4–6] Recently we reported on patients carrying mutations in *RBFOX1/3* and *DEPDC5* genes, although these findings still need to be reproduced in larger cohorts.[7,8]

Two other genes that were considered to be associated with phenotypes of the epilepsy–aphasia spectrum are *ELP4* and *SRPX2*.[9,10] *ELP4* (elongator acetyltransferase complex subunit 4) is part of the multisubunit (ELP1-ELP6) elongator complex which, among other functions, was shown to regulate the maturation of cortical projection neurons.[11] Its role in rolandic epilepsy was suggested by a genome-wide linkage scan for the electroencephalography (EEG) trait of RE–centrotemporal spikes (CTS).[9] This study on 38 families (ascertained through RE index cases) yielded a locus for the CTS trait at chromosome 11p13, with a maximum logarithm of the odds (LOD) score of 4.30. Subsequent fine mapping revealed an association of three intronic single-nucleotide polymorphisms (SNPs) within the *ELP4* gene. However, re-sequencing of the coding region of this gene in a total of 76 patients failed to identify any pathogenic variants.[9,12]

The second candidate gene *SRPX2* (sushi-repeat containing protein X-linked 2) was identified in a French family with rolandic seizures, orofacial dyspraxia, and mental retardation fitting our definition of atypical rolandic epilepsy.[10] Linkage in this family of seven affected members led to a ~20 cM region (containing 82 genes) between Xq21 and Xq22 (LOD score 3.01). In a subsequent mutation search a gain-of-glycosylation variant in the *SRPX2* gene (p.N327S) was identified as the presumed disease-causing mutation. A follow-up screen in a series of individuals with related epilepsy disorders (including 81 RE and 27 ARE patients) revealed a second missense mutation (p.Y72S) in a patient with RE and bilateral perisylvian polymicrogyria.[10] In a different study a splice-site variant (c.961 + 1G>A) was identified in one male patient with autism spectrum disorder.[13] The two *SRPX2* missense mutations were considered to cause a misfolding of the secreted protein, with the p.N327S variant exerting a dominant negative effect and the p.Y72S variant leading to a loss of protein function.[10,12] The relevance of the p.N327S mutation was called into question once a missense mutation in the *GRIN2A* gene was found to cosegregate with the epilepsy phenotype in all and with verbal dyspraxia in most of the affected individuals in this family.[5]

Legitimate doubts were raised for an association of these two genes with CTS in the context of RE (*ELP4*) and respectively with ARE (*SRPX2*), particularly as no positive replications have been reported so far and the proposed pathogenic *SRPX2* variants were also found in control individuals.[5,12,14] The purpose of the present study was to reinvestigate the disease association of these two candidate genes in a cohort of 280 patients with RE/ARE. We also gathered indirect evidence by including close interaction partners of *ELP4* and *SRPX2* in our search for pathogenic variants and genomic rearrangements.

Patients and Methods

In total 290 patients of European ancestry diagnosed with RE (157 male and 98 female) or ARE (19 males and 16 females) according to the international classification were

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recruited in Germany, Austria, and Canada. Two hundred six patients were ascertained, irrespective of their family history, and 84 children through multiplex families (one sibling with RE/ARE or CTS was required). One hundred ninety-six healthy German volunteers (96 male, 100 female) served as population controls for the association analysis of *ELP4* SNPs, and 403 Austrian population controls (192 male, 211 female) were genotyped for the *SRPX2* p.N327S variant. The study was approved by all respective local institutional review boards.

Whole exome sequencing for candidate genes analysis was performed in a subset of 204 patients (RE = 182, ARE = 22) using the Nimblegen-SeqCapEZ-V2 44M enrichment kit on the Illumina HiSeq2000 system (Illumina, Inc., San Diego, CA, U.S.A.). For 280 patients (RE = 248, ARE = 32) SNP-array genotyping and copy number variant (CNV) detection was performed on the Illumina OmniExpress platform and analyzed with the Illumina Genome Viewer, PennCNV and PLINK.1.7 software. Genotyping of the *SRPX2* rs121918363 variant (p.N327S) was done with a TaqMan SNP Genotyping Assay (Life Technologies, Carlsbad, CA, U.S.A.). For the functional prediction of rare variants, we employed the dbNSFPv2.3 database (http://sites.google.com/site/jpopgen/dbNSFP) and for the identification of *SRPX2* and *ELP4* interaction partners the STRING tool (http://string-db.org/ filtered for experimental evidence). Statistical power was calculated with the online Genetic power calculator (http://pngu.mgh.harvard.edu/%7Epurcell/gpc). The exome variant server (EVS; accessed 12/2013; http://evs.gs.washington.edu/EVS/) was queried for the frequency of genetic variants in the respective genes in the European control population. Further details on patients and methods can be found in the supporting information.

Results

Elp4

Analyzing the coding sequences of *ELP4* in 204 patients with RE/ARE revealed four missense variants (three known and one unknown), none of which was predicted as damaging by the dbNSFPv2.3 database (using the LR/RadialSVM ensemble score, which is composed of 10 widely used component scores) (Table S1). The frequency of rare variants did not differ from the one obtained in European controls of the EVS; neither did any of the close interaction partners of *ELP4* (*ELP2-6, IKBKAP, URM1, SIRT1, PUS3, NFYB, MED31, MOCS3*) show an excess of rare or damaging variants (Table 1).

Next we replicated the previously reported association of three intronic SNPs in the *ELP4* gene with the CTS trait[9] in 280 RE/ARE patients and 196 controls. We genotyped one SNP (rs986527) directly and the others via proxy SNPs in high linkage disequilibrium (LD). Although we had a power of 99% to detect the reported allelic odds ratio (OR); e.g., OR 1.88 for rs986527, alpha = 0.05, allelic 1 d.f. test), our association analysis failed to replicate the initial association claim (Table 2). We also performed an extended SNP association analysis covering the whole *ELP4* gene (as done in

Table 1. Frequency of rare variants in *ELP4, SRPX2*, and interacting genes in 204 RE/ARE patients and 4,300 European individuals of the EVS

Gene	RE cases variant[a] alleles/total alleles (%)	EVS controls variant alleles/ total alleles (%)	p- Value[b]	RE cases damaging alleles[c]/total alleles (%)	EVS controls damaging alleles[c]/total alleles (%)	p- Value[b]
ELP4	8/408 (1.96)	161/8,222 (1.96)	1	0/408 (0)	0/8,222 (0)	1
SRPX2	2/285 (0.70)	34/6,728 (0.51)	0.66	0/287 (0)	1/6,728 (0.01)	1
ELP4 interaction partners						
ELP2	12/408 (2.94)	259/8,600 (3.01)	1	0/408 (0)	13/8,600 (0.15)	1
ELP3	1/408 (0.25)	37/8,600 (0.43)	1	0/408 (0)	15/8,600 (0.17)	1
ELP5	1/408 (0.25)	16/8,600 (0.19)	0.55	0/408 (0)	1/8,600 (0.01)	1
ELP6	1/408 (0.25)	32/8,352 (0.38)	1	0/408 (0)	0/8,352 (0)	1
IKBKAP	5/408 (1.23)	174/8,600 (2.02)	0.36	0/408 (0)	2/8,600 (0.02)	1
URM1	1/408 (0.25)	12/8,600 (0.14)	0.45	0/408 (0)	6/8,600 (0.07)	1
SIRT1	2/408 (0.49)	75/8,600 (0.87)	0.59	0/408 (0)	0/8,600 (0)	1
PUS3	1/408 (0.25)	137/8,600 (1.59)	0.02	0/408 (0)	12/8,600 (0.14)	1
NFYB	1/408 (0.25)	0/8,600 (0)	0.05	0/408 (0)	0/8,600 (0)	1
MED31	1/408 (0.25)	6/8,600 (0.07)	0.28	0/408 (0)	0/8,600 (0)	1
MOCS3	12/408 (2.94)	121/8,600 (1.41)	0.02	2/408 (0.49)	8/8,600 (0.09)	0.07
SRPX2 interaction partners						
ADAMTS4	6/408 (1.47)	204/8,600 (2.37)	0.31	0/408 (0)	11/8,600 (0.13)	1
CTSB	6/408 (1.47)	103/8,600 (1.20)	0.64	3/408 (0.74)	69/8,600 (0.80)	1
PLAUR	4/408 (0.98)	110/8,600 (1.28)	0.82	0/408 (0)	0/8,600 (0)	1
FOXP2	1/408 (0.25)	29/8,600 (0.34)	1	0/408 (0)	13/8,600 (0.15)	1

[a]Refers to variant alleles (missense, nonsense, splice-site, frameshift variants) with a minor allele frequency <1%.
[b]Fisher's exact test, gene-specific comparison of variant alleles to all alleles in patients versus controls.
[c]Refers to the subset of the above missense variants predicted as damaging by dbNSFPV2.3 database. This prediction is based on a 10-component score.

Table 2. ELP4–SNP association results, 280 RE cases versus 196 German controls

SNP	Minor allele	Frequency patients	Frequency controls	p-Value
rs1232186	A	0.420	0.424	0.9
rs986527	T	0.354	0.380	0.4

SNPs reported as associated with CTS (rs986527, rs11031434, rs964112)[9] were genotyped directly (rs986527) or via proxy SNPs in high LD (rs986527, rs964112: $r^2 = 0.92$; rs1232186, rs11031434: $r^2 = 0.96$) in 280 patients with RE/ARE and 196 German controls.

the screening stage of the original report[9]), but none of the 10 SNPs tested achieved nominal significance ($p > 0.05$). Finally we performed a screen for structural variations in 280 patients covering the genomic region of *ELP4* or any of the other 11 interacting genes and did not find any CNVs affecting any of the investigated genes.

Srpx2

Next-generation sequencing of *SRPX2* exons in 204 patients identified two boys with the previously described p.N327S mutation in this X chromosomal gene (rs121918363; c.980A>G; NM_014467.2). Other rare variants were not detected. Genotyping a further 43 RE/ARE patients for the p.N327S variant did not reveal additional mutation carriers. In contrast, we detected one female heterozygous p.N327S carrier by genotyping 403 Austrian controls and a search for this mutation in 4,300 European controls (2,428 female, 1,872 male) of the EVS yielded 11 additional individuals (8 heterozygous females, 3 hemizygous males). A statistical comparison between patients and controls (both sexes) did not suggest a specific enrichment of this variant among RE/ARE patients, neither in separate comparison to the Austrian or the EVS controls (2/247 vs. 1/403 or 11/4300) nor to the merged European control set (12/4703; Fisher's exact test $p = 0.15$). A subanalysis in males yielded a borderline significance that was lost after Bonferroni correction (2/146 male patients vs. 3/2064 male controls p-(uncorrected) = 0.04). The two patients carrying the mutation, one diagnosed with typical rolandic epilepsy and the other one with LKS, share a common haplotype between chrX:98482335-100213232. For both patients the magnetic resonance imaging (MRI) scan was normal and the family history for epilepsy or seizures was negative. None of the two boys harbors a *GRIN2A* variation.

We also performed a search for sequence variants in four genes identified as close *SRPX2*-interaction partners (*ADAMTS4, CTSB, PLAUR, FOXP2*) in 204 affected individuals, without finding any excess of rare or deleterious variants in comparison to European controls of the EVS (Table 1). Finally, a SNP-array based screen for structural variations within the genomic region of the *SRPX2* gene and its four interaction partners in 280 RE/ARE patients did not reveal any CNVs.

Discussion

Our elaborate reexamination analysis does not support a major role for *ELP4* and *SRPX2* genes in RE/ARE.

The main evidence in favor of *ELP4* as an underlying gene for CTS was a linkage peak and the subsequent association of three intronic markers within the *ELP4* gene in a study of 38 families with this EEG trait.[9] Our failure to replicate the SNP-association analysis (despite having adequate power) suggests that common *ELP4* alleles do not significantly contribute to CTS in the European population. The lack of deleterious variants and CNVs in *ELP4* or its close interaction partners further questions the role of *ELP4* in CTS. However, our data do not exclude that variations in this gene or its regulatory elements might still be of relevance in odd families.

The claim of *SRPX2* mutations being pathogenic for ARE mainly rests on the cosegregation of the p.N327S variant in a family with ARE, but the later identification of a concomitant co-segregating missense mutation in the *GRIN2A* questioned this hypothesis.[5] In support of its importance are subsequent experimental data demonstrating the functional relevance of the p.N327S variant for cortical development and epileptogenicity. Significantly, expression of the mutant N327S-SRPX2 protein leads to an impairment of neuronal migration resulting in spontaneous epileptiform activity and in reduced vocalizations in mice.[12,15] This last observation is appealing because alterations in language development can be part of the phenotypic spectrum of ARE and because genes interacting with *SRPX2* were associated with language development (*FOXP2*) or cerebral lateralization in dyslexia (*PCSK6*).[16–18]

Our detection of the p.N327S variant in controls with an almost similar frequency to affected individuals, argues against a major pathogenic effect in RE/ARE. We observed only a suggestive excess in affected boys, although this did not remain significant after correction. This later phenomenon would contrast with the dominant negative effect of the variant, which may lead to functional similarity in both genders due to the partial use of the mutant gain-of-glycosylation site.[10]

One limitation of this study is that the controls were not specifically screened for the presence of RE/ARE, let alone milder manifestations such as CTS or minor speech and behavioral problems. Therefore, we cannot exclude a coincidental accumulation of these phenotypes in the control groups, which could have blurred the analysis. As no other sequence or structural variants could be identified for the *SRPX2* gene and no specific enrichment of variants was observed for *SRPX2* interacting partners, our results do not support a major contribution of this gene to rolandic epilepsy and its atypical variants, although a relevance in odd families or for related subtle phenotypes cannot be excluded.

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Disclosure

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Methods.

Table S1. Rare variants found in 204 RE-spectrum epilepsy patients.