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LIS1-Related Isolated Lissencephaly

Spectrum of Mutations and Relationships With Malformation Severity

Yoann Saillour, PhD; Nathalie Carion, MS; Chloé Quelin, MD; Pierre-Louis Leger, MD; Nathalie Boddaert, MD, PhD; Caroline Elie, MD; Annick Toutain, MD, PhD; Sandra Mercier, MD; Marie Anne Barthez, MD; Mathieu Milh, MD, PhD; Sylvie Joriot, MD; Vincent des Portes, MD, PhD; Nicole Philip, MD, PhD; Dominique Broglin, MD; Agathe Roubertie, MD, PhD; Gaëlle Pitelet, MD; Marie Laure Moutard, MD; Jean Marc Pinard, MD; Claude Cancès, MD; Anna Kaminska, MD; Jamel Chelly, MD, PhD; Chérif Beldjord, MD, PhD; Nadia Bahi-Buisson, MD, PhD

Objective: With the largest data set of patients with *LIS1*-related lissencephaly, the major cause of posteriorly predominant lissencephaly related to either *LIS1* mutation or intragenic deletion, described so far, we aimed to refine the spectrum of neurological and radiological features and to assess relationships with the genotype.

Design: Retrospective study.

Subjects: A total of 63 patients with posteriorly predominant lissencephaly.

Interventions: Of the 63 patients, 40 were found to carry either *LIS1* point mutations (77.5%) or small genomic deletions (20%), and 1 carried a somatic nonsense mutation. On the basis of the severity of neuromotor impairment, epilepsy, and radiological findings, correlations with the location and type of mutation were examined.

Results: Most patients with *LIS1* mutations demonstrated posterior agyria (grade 3a, 55.3%) with thin cor-

pus callosum (50%) and prominent perivascular spaces (67.4%). By contrast, patients without *LIS1* mutations tended to have less severe lissencephaly (grade 4a, 41.6%) and no additional brain abnormalities. The degree of neuromotor impairment was in accordance with the severity of lissencephaly, with a high incidence of tetraplegia (61.1%). Conversely, the severity of epilepsy was not determined with the same reliability because 82.9% had early onset of seizures and 48.7% had seizures more often than daily. In addition, neither the mutation type nor the location of the mutation were found to predict the severity of *LIS1*-related lissencephaly.

Conclusion: Our results confirm the homogeneity profile of patients with *LIS1*-related lissencephaly who demonstrate in a large proportion Dobyns lissencephaly grade 3a, and the absence of correlation with *LIS1* mutations.

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GENETICALLY INHERITED disorders of neuronal migration in humans represent important causes of epilepsy and mental retardation. Of these, classical lissencephaly is a severe brain malformation caused by an arrest of neuronal migration from 9 to 13 weeks of gestation and characterized by absent or reduced gyration and an abnormally thick, poorly organized cortex with 4 primitive layers.¹ It encompasses a continuous spectrum of malformations from complete agyria to variable degrees of pachygyria to subcortical band heterotopia.² Lissencephaly may occur either as a component of the contiguous gene deletion disorder known as Miller-Dieker syndrome or as an isolated form known as isolated lissencephaly sequence.³⁻⁵ Clinical manifestations range from profound mental retardation, intractable epilepsy, and spasticity with reduced life span⁴ to milder forms with infrequent seizures and intel-

lectual disability.⁵ The clinical severity correlates generally with the degree of agyria and cortical thickness.¹

Approximately 80% of classical lissencephaly (isolated lissencephaly sequence) cases show abnormalities in either the *LIS1* or the *DCX* genes.⁶ Most cases are due to variations (deletions or mutations) in the *LIS1* gene on 17p13.3,⁶⁻⁸ whereas most subcortical band heterotopias are due to mutations in the *DCX* gene on Xq22.3.^{9,10} Both proteins are known to be associated with and to be required for correct neuronal migration.¹¹⁻¹³ The radiological pattern of *LIS1*-related lissencephaly differs from doublecortin-related lissencephaly. One of the main distinctive criteria between the 2 conditions is the gradient of lissencephaly, which is known to be more severe in posterior regions (giving a posterior to anterior gradient) in *LIS1*-related lissencephaly, whereas it is more severe in the anterior brain regions in lissencephaly cases.^{2,6}

Author Affiliations are listed at the end of this article.

The *LIS1* gene comprises 11 exons, 10 of which encode the LIS1 protein, named PFAFH1B1, which contains a LisH homology domain, a coiled coil domain, and 7 WD40 repeats.¹⁴ Large deletions of the *LIS1* gene account for most (40%) isolated lissencephaly sequence cases.⁶ To date, 61 intragenic *LIS1* mutations have been described,^{6,7,15-21} with most (84%) being truncating mutations scattered throughout the entire gene.¹⁸ Missense mutations are less frequent (16%),^{18,21} whereas recent data suggest a high prevalence of small genomic deletions/duplications suspected to account for a large number of 49% of all *LIS1* alterations.²²

Data on genotype phenotype correlation in *LIS1*-related lissencephaly are conflicting. Initially, a putative correlation was suggested, with the less severe lissencephaly associated with missense mutations and late truncating mutations localized at the 3' end of the *LIS1* gene.¹⁵ A recent study did not confirm this relationship.^{15,21} Moreover, recent data on patients with small deletions in *LIS1* suggest that they tend to have the same severity of malformation as those with a frameshift mutation, suggesting that the functional consequences of haploinsufficiency due to either truncating or frameshift mutations or exon(s), deletion(s), or duplication(s) could be comparable. However, owing to the small size of the population (21 in Uyanik et al²¹ and 15 in the Cardoso et al¹⁵) and the lack of major differences between the patients, the statistical analysis might not have been powerful enough to give significant results.

As part of our ongoing lissencephaly research, we have recruited 63 unrelated patients with posteriorly predominant lissencephaly. Of these, 40 patients were found to carry either mutations or small genomic deletions, and 1 patient had a nonsense somatic mutation in the *LIS1* gene. The goal of this study was to evaluate in detail the spectrum of neurological and radiological features of *LIS1*-related lissencephaly. In combination with molecular genetic findings, we have provided additional data concerning the relationship between the severity of the phenotype and the nature of the mutation in the *LIS1* gene and compare this with the phenotypes of patients without mutations in *LIS1* gene. Our data reinforce the concept that neither the mutation type nor location can predict the severity of the clinical and radiological phenotype in the *LIS1*-related lissencephaly gene.

METHODS

Of the 63 patients with classical posteriorly predominant lissencephaly referred to our laboratory for molecular screening, 40 were identified with *LIS1*-related lissencephaly. Clinical data and blood samples were obtained with informed consent from parents and/or patients.

LIS1 MUTATION ANALYSIS

The DNA was extracted using standard protocols. Mutation analysis was performed for *LIS1* for all patients with classical lissencephaly with no deletion detected by fluorescence in situ hybridization (for 17p13.3). Mutation detection was performed by direct sequencing of genomic DNA, as described previously.²³ In all patients, the mutation was con-

firmed to be de novo by direct sequencing of both parents' DNA.

We subsequently performed Quantitative Multiplex PCR (polymerase chain reaction) of Short Fluorescent fragments (QMPSF) in patients who had negative results of mutation screening to detect genomic deletions or duplications in the *LIS1* gene, based on the simultaneous amplification of short genomic fragments using dye-labeled primers under quantitative conditions.²⁴ The PCR products were visualized and quantified as peak areas using an automated DNA sequencer with the gene-scan mode in which both peaks' heights and areas are proportional to the quantity of template present for each target sequence. We designed 2 distinct QMPSF assays that contain the following targeted exons of the *LIS1* gene: assay 1 for exons 3, 4, 5, 6, 8, 9, and 11, and assay 2 for exons 2, 7, and 10. Primer pairs were designed for each of these exons to generate, in both assays, PCR fragments ranging from 75 to 360 base pairs and chosen in such a way that they do not encompass known polymorphisms. The PCRs were run on 100 ng of genomic DNA in 25 μ L of a dilution with 0.2 mmol/L each of deoxynucleotide, 2 mmol/L magnesium chloride, 2.5 U of AmpliTaq Gold DNA polymerase (Applied Biosystems-Roche, Foster City, California), and 0.5 to 2 mmol/L of each primer, 1 primer of each pair carrying a 6-FAM label.

We excluded the case of somatic mosaicism to facilitate the genotype-phenotype analysis and to avoid the phenotype variation caused by such mosaicism.²¹ Patients without mutations and deletion in the *LIS1* gene were also screened for *TUBA1A* mutations, as previously described.²⁵ The investigators were unaware of the mutation type at the time of the initial neuroimaging review.

PATIENTS AND PHENOTYPIC ANALYSIS

All patients were followed up regularly in various departments of pediatric neurology and were known personally to at least 1 of the authors. Detailed information regarding family history, prenatal and perinatal events, age of onset of first seizure, motor development, cognitive function, and neurological examination were collected.

Brain magnetic resonance imaging (MRI) and computed tomographic scans were available for all patients and reviewed independently by 2 authors (N.B. and N.B.-B.). They were graded according to the following lissencephaly patterning scale: grades 1 through 6 denote the overall severity of lissencephaly and refer to the maximum number of abnormalities in the posterior region (ie, posterior to anterior gradient of lissencephaly), as seen on neuroimaging.²⁶ Cerebellar, white matter, and corpus callosum abnormalities were also assessed.

Protocols were approved by the appropriate institutional review board human committee.

RESULTS

RANGE AND DISTRIBUTION OF *LIS1* MUTATIONS AND SMALL GENOMIC DELETIONS

Thirty-one heterozygous *LIS1* mutations and 8 small genomic deletions in the *LIS1* gene were identified. Of the 31 *LIS1* mutations, 12 were nonsense, 8 frameshift, 6 missense, and 5 splicing defect mutations confirmed by reverse transcriptase-PCR. The mutations described here were found scattered throughout the gene (except in exons 3 and 9). All of these mutations were confirmed to be de novo by parental DNA screening. One nonsense

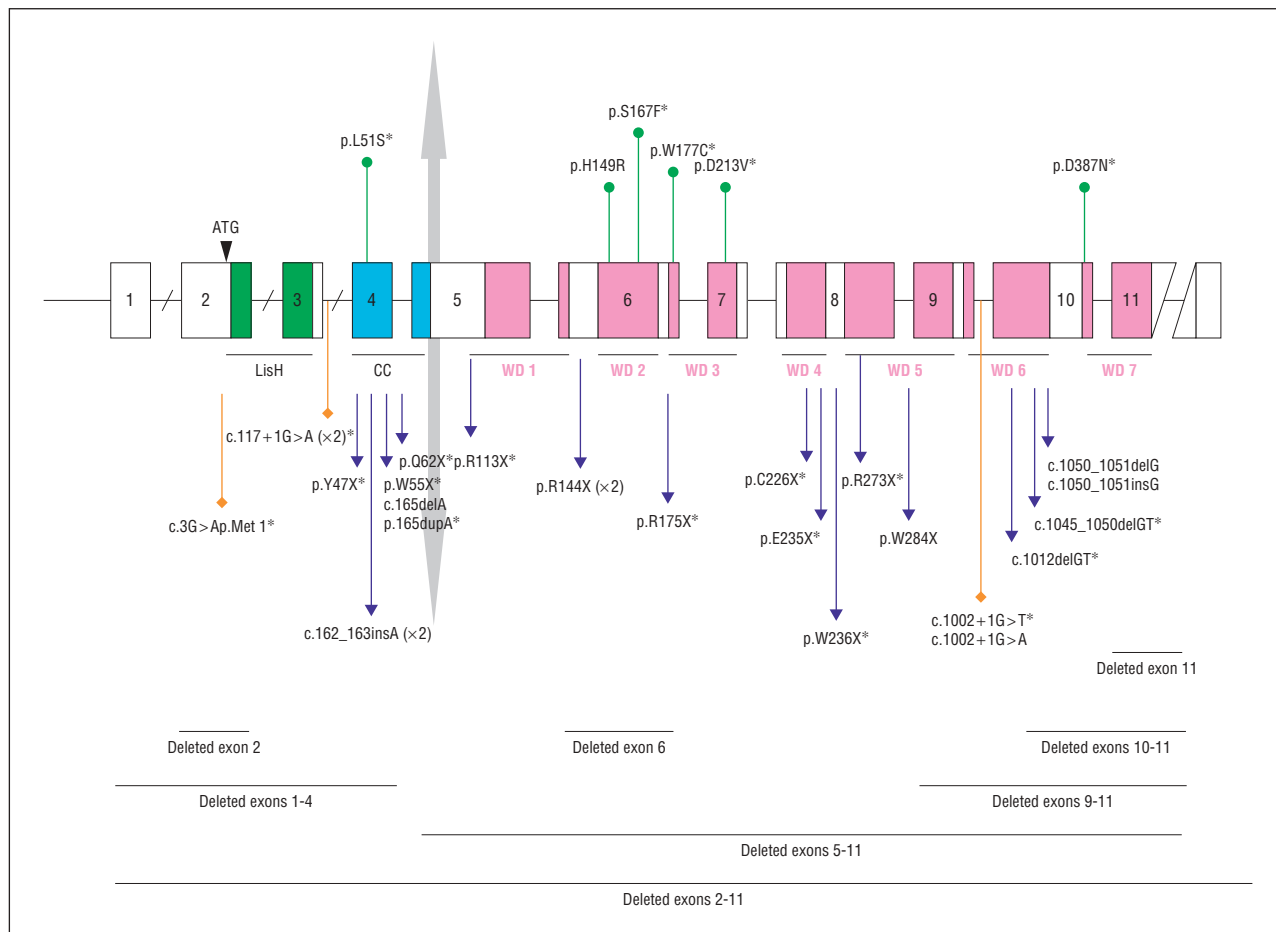


Figure 1. Summary of the *LIS1* mutations identified in the 40 patients described. Missense mutations are denoted by a green circle above the gene; nonsense/frameshift mutations, purple arrows; and splicing defects, orange diamonds below the gene. The encoded LisH homology domains (dark green), coiled-coil (CC) domains (blue), and WD40 repeats (pink) of the *LIS1* protein are displayed. An asterisk denotes the mutations described in the article.

somatic mutation (c.531G>A; p.G177X) present in 30% of the blood was also found but, unfortunately, other tissues were not available for testing.

Eight small intragenic deletions ranging from 1 exon to almost the entire gene (exons 2-11) were found by QMPSF in samples for which DNA sequencing had failed to detect any gene alterations (**Figure 1**).

CLINICAL FINDINGS OF PATIENTS WITH *LIS1*-RELATED LISSENCEPHALY

The age range at the time of the study was 1 to 39 years (median, 6 years). The sex ratio was not evenly distributed (male: female, 18: 22). Clinical features recorded in 38 patients consisted of hypotonia and/or developmental delay in 17 patients (44.7%) and seizures in 21 patients (55.3%).

All patients except for 4 exhibited severe developmental delay (90%) with severe motor impairment including axial hypotonia and spastic quadriparesis in 24 patients (60%), virtually no language development in all cases, and moderate to severe behavioral disturbances including autistic features and sleep disorders in 12 cases (30%). Head circumference was normal in almost half of the patients, and 16 (40%) showed postnatal microcephaly.

All patients experienced seizures within the 3 first years of life, with onset before 7 months in most (30 of 36 patients; 83.3%) and infantile spasms in 28 (77.7%), either isolated or combined with various seizure types including generalized tonic seizures.

At the time of evaluation, all patients had ongoing seizures and refractory epilepsy, with up to 10 seizures daily in 19 patients (47.5%). For 21 other patients, the seizure frequency ranged from 1 per week (50%) to 1 per month (50%), despite polytherapy. Individual data are detailed in **Table 1**.

MRI ABNORMALITIES

The severity of lissencephaly, graded according to Dobyns and Truwit,²⁶ ranged from 1 to 5 (Table 1). Posterior agyria and anterior pachygyria (grade 3a) was the prominent radiological phenotype, affecting 21 patients (55.3%). Grade 2a (diffuse agyria with shallow sulci in anterior regions) was observed in 9 patients (23.7%) and grade 4a (posteriorly predominant pachygyria) in 6 (15.8%). Only 1 patient demonstrated grade 1a lissencephaly (generalized agyria). The 2 remaining patients had grade 5a (pachygyria posteriorly with subcortical band heterotopia); of these, 1 had the nonsense somatic mutation (**Figure 2**).

Table 1. Mutation Analysis, Radiological Findings, and Overview of Neurological Data for 40 Patients With *LIS1*-Related Lissencephaly With Mutations and Intragenic Deletion in the *LIS1* Gene

Patient/Sex/ Age, y ^a	Nucleotide Change (Protein Variation)	Mutation Type	Protein Location	Dobyns Grade ^b	Corpus Callosum	White Matter Abnormalities ^c	Initial Concerns	Head Circumference, Percentile	Gross Motor Function	Language	Behavior and/or Sleep Disorder
Lis001/F/20	c.141T>G (p.Y47X) ^d	Nonsense	N terminal (CC)	NA	NA	NA	Seizures	NA	Spastic quadripareisis	Absent	Moderate autistic features
Lis002/F/3	c.165G>A (p.W55X) ^d	Nonsense	N terminal (CC)	NA	NA	NA	Developmental delay	3rd	Virtually no purposeful movement	Absent	Absent
Lis003/F/10	c.186C>T (p.Q62X) ^d	Nonsense	N terminal (CC)	3a	Thin	Moderate	Hypotonia	3rd	Spastic quadripareisis	Absent	Absent
Lis004/F/8	c.165delA ^d	Frameshift	N terminal (CC)	3a	Thick	Moderate	Hypotonia	10th	Walks independently	Few words in association	Moderate autistic features
Lis005/F/3	c.162_163insA	Frameshift	N terminal (CC)	3a	Normal	Moderate	NA	50th	Walks with aid	Absent	Absent
Lis006/F/15	c.162_163insA	Frameshift	N terminal (CC)	4a	Thick	Moderate	Developmental delay	50th	Walks independently	Absent	Severe autistic features
Lis007/F/7	c.165dupA ^d	Frameshift	N terminal (CC)	2a	Thin	Severe	Hypotonia	<3rd	Walks with aid	Absent	Moderate autistic features
Lis008/M/16	c.152T>C (p.L51S) ^d	Missense	N terminal (CC)	4a	Thin	Moderate	Hypotonia	3rd	Walks with aid	Absent	Absent
Lis009/F/13	c.337C>T (p.R113X) ^d	Nonsense	WD1	3a	NA	NA	NA	3rd	Walks with aid	Absent	Absent
Lis010/F/2	c.430C>T (p.R144X)	Nonsense	WD1/2	2a	Normal	Moderate	Seizures	25th	Walks with aid	Absent	Absent
Lis011/M/27	c.430C>T (p.R144X)	Nonsense	WD1/2	5a	Thick	Moderate	Developmental delay	50th	Walks independently	Few words in association	Absent
Lis012/M/3	c.523A>T (p.K175X) ^a	Nonsense	WD3	3a	Normal	Normal	Hypotonia	3rd	Walks with aid	Absent	Absent
Lis013/F/3	c.678T>A (p.C226X) ^d	Nonsense	WD4	4a	Thin	Moderate	Developmental delay	10th	Walks with aid	Absent	Severe autistic features
Lis014/M/11	c.705G>T (p.E235X) ^d	Nonsense	WD4	3a	Normal	Moderate	Hypotonia	25th	Spastic quadripareisis	Absent	Absent
Lis015/M/4	c.706G>A (p.W236X) ^d	Nonsense	WD4	3a	Thin	Normal	Developmental delay	10th	Walks with aid	Few words in association	Absent
Lis016/M/16	c.817C>T (p.R273X) ^d	Nonsense	WD5	3a	Normal	Normal	Seizures	10th	Walks with aid	Absent	Absent
Lis017/M/4	c.851G>A (p.W284X)	Nonsense	WD5	3a	NA	NA	Hypotonia	<3rd	Virtually no purposeful movement	Absent	Moderate autistic features
Lis018/F/3	c.1012delGT ^d	Frameshift	WD6	3a	Thin	Moderate	Seizures	<3rd	Spastic quadripareisis	Absent	Absent
Lis019/M/14	c.1050_1051delG	Frameshift	WD6	2a	Thin	Moderate	Seizures	<3rd	Spastic quadripareisis	Absent	Moderate autistic features
Lis020/M/5	c.1050_1051insG	Frameshift	WD6	3a	Thin	Severe	Seizures	<3rd	Virtually no purposeful movement	Absent	Absent
Lis021/F/8	c.531G>C (p.W177C) ^d	Missense	WD3	2a	Thin	Moderate	Seizures	50th	Spastic quadripareisis	Absent	Moderate autistic features
Lis022/F/6	c.638A>T (p.D213V) ^d	Missense	WD3	4a	Thin	Moderate	Seizures	3rd	Walks with aid	Absent	Moderate autistic features

(continued)

Additionally, 22 of 34 patients (64.7%) had corpus callosum abnormalities that consisted of mild hypogenesis of the rostrum and splenium, with flattening of the body in 17 and thick and dysmorphic corpus callosum in 5 others. Two patients had cerebellar hypoplasia, with brain stem hypoplasia in 1 case (**Figure 3**, A and C). The white matter was roughly normal, although delayed myelination (3 cases) and prominent perivascular (Virchow-Robin) spaces were observed in 23 cases. Twenty-eight patients (73.7%) had enlarged ventricles, mostly prominent posterior horns of the lateral ventricles. For the 2

remaining patients, both addressed for posteriorly predominant agyria, MRI scans were not available for our reevaluation of Dobyns grade.

GENOTYPE-PHENOTYPE CORRELATIONS

First, we compared patients exhibiting missense mutations (n=6) with those showing nonsense/frameshift mutations (n=18; MRI data of 20 patients) and with small deletions (n=8). It is of interest that in the

Table 1. Mutation Analysis, Radiological Findings, and Overview of Neurological Data for 40 Patients With *LIS1*-Related Lissencephaly With Mutations and Intragenic Deletion in the *LIS1* Gene (continued)

Patient/Sex/ Age, y ^a	Nucleotide Change (Protein Variation)	Mutation Type	Protein Location	Dobyns Grade ^b	Corpus Callosum	White Matter Abnormalities ^c	Initial Concerns	Head Circumference, Percentile	Gross Motor Function	Language	Behavior and/or Sleep Disorder
Lis023/M/4	c.500C>T (p.S167F) ^d	Missense	WD2	2a	Thin	Severe	Developmental delay	50th	Virtually no purposeful movement	Absent	NA
Lis024/F/3	c.446A>G (p.H149R)	Missense	WD2	2a	NA	NA	Seizures	<3rd	Virtually no purposeful movement	Absent	Absent
Lis025/F/7	c.3G>Ap.Met1 ^d	Splicing defect	LisH	3a	Normal	Moderate	Seizures	50th	Walks with aid	Absent	Moderate autistic features
Lis026/M/5	c.1002 + 1G>A	Splicing defect	...	2a	Thin	Moderate	Hypotonia	<3rd	Spastic quadriparesis	Absent	Moderate autistic features
Lis027/M/7	c.117 + 1G>A ^d	Splicing defect	LisH	3a	NA	NA	Hypotonia	3rd	Spastic quadriparesis	Absent	Absent
Lis028/F/3	c.117 + 1G>A ^d	Splicing defect	LisH	3a	Thin	Moderate	Seizures	50th	Walks with aid	Absent	Absent
Lis029/M/39	c.1002 + 1G>T ^d	Splicing defect	...	1a	Thin	Normal	Hypotonia	<3rd	Spastic quadriparesis	Absent	Absent
Lis030/M/12	c.1045-1050delG	Frameshift	WD6	4a	Thin	Normal (cerebellar hypoplasia)	Hypotonia	3rd	Virtually no purposeful movement	Absent	Absent
Lis031/F/3	c.1159G>A (p.D387N)	Missense	WD7	3a	Thin	Moderate	Seizures	50th	Spastic quadriparesis	Absent	Absent
Lis032/F/1	Deleted exon 2	Intragenic deletion	...	3a	Normal	Normal	Seizures	50th	Spastic quadriparesis	Absent	Absent
Lis033/M/3	Deleted exon 1-4	Intragenic deletion	...	3a	Normal	Normal	Seizures	50th	Spastic quadriparesis	Absent	Absent
Lis034/F/6	Deleted exons 5-11	Intragenic deletion	...	3a	Normal	Moderate	Seizures	10th	Virtually no purposeful movement	Absent	Absent
Lis035/F/10	Deleted exons 6	Intragenic deletion	...	3a	Thick	Moderate	Seizures	10th	Spastic quadriparesis	Absent	Severe autistic features
Lis036/F/5	Deleted exons 9-11	Intragenic deletion	...	2-3a	Normal	Moderate	Seizures	50th	Sits independently	Eye contact	Absent
Lis037/M/2	Deleted exon 11	Intragenic deletion	...	2a	Normal	Moderate	Seizures	10th	Spastic quadriparesis	Absent	Absent
Lis038/M/18	Deleted exons 10-11	Intragenic deletion	...	3a	Thick	Moderate	Seizures	10th	Spastic quadriparesis	Absent	Absent
Lis039/M/11	Deleted exons 2-11	Intragenic deletion	...	4a	Thin	Moderate (cerebellar hypoplasia)	Seizures	10th	Spastic quadriparesis	Absent	Absent
Lis040/F/8	c.531G>A (p.G177X) (30% in blood)	Nonsense somatic mosaic	WD3	5a	Normal	Normal	Seizures	50th	Walks independently	Few words in association	Absent

Abbreviations: CC, coiled-coil; ellipses, no data; LisH, *LIS1* homology domain; NA, not available; WD, WD40 repeat.

^aAge at last evaluation.

^bDobyns grade 1 is defined as generalized agyria; grade 2a, diffuse agyria with a few shallow sulci over frontal and temporal; grade 3a, mixed parietooccipital agyria and frontal pachygyria; grade 4a, diffuse pachygyria, which is more severe posteriorly; grade 5a, mixed pachygyria posteriorly and subcortical band heterotopia.^{2,26}

^cRefers to widening of Virchow-Robin spaces.

^dMutations newly described in the article.

nonsense/frameshift and small deletions groups, most patients had Dobyns grade 3, 11 (61.1%), and 6 (75%). When compared with the 6 patients with missense mutations, it appears that half of the patients in this group tend to have more severe lissencephaly (50%; grade 2a). However, owing to the small number of patients in this group, we cannot consider that the repartition of the lissencephaly severity score is significantly different.

Second, we divided the nonsense/frameshift mutations according to their location within the *LIS1* protein, considering the boundary at the level of the end

of the coiled-coil domain (exon 5), as described previously.^{15,27} Mutations up to the 3' region of the end of the coiled-coil domain are termed *early* nonsense/frameshift (n=5; MRI data of 7 patients), while the remaining located in the WD40 repeats are termed *late* nonsense/frameshift mutations (n=13). We found no difference between the 2 groups, most of which also had a grade of 3 (3 [50%] and 8 [61.5%]).

Finally, we compared all patients with either mutations or deletions in the *LIS1* gene (n=37) with patients without abnormalities in the *LIS1* or *TUBA1A* genes (n=24). Patients without mutations tended to

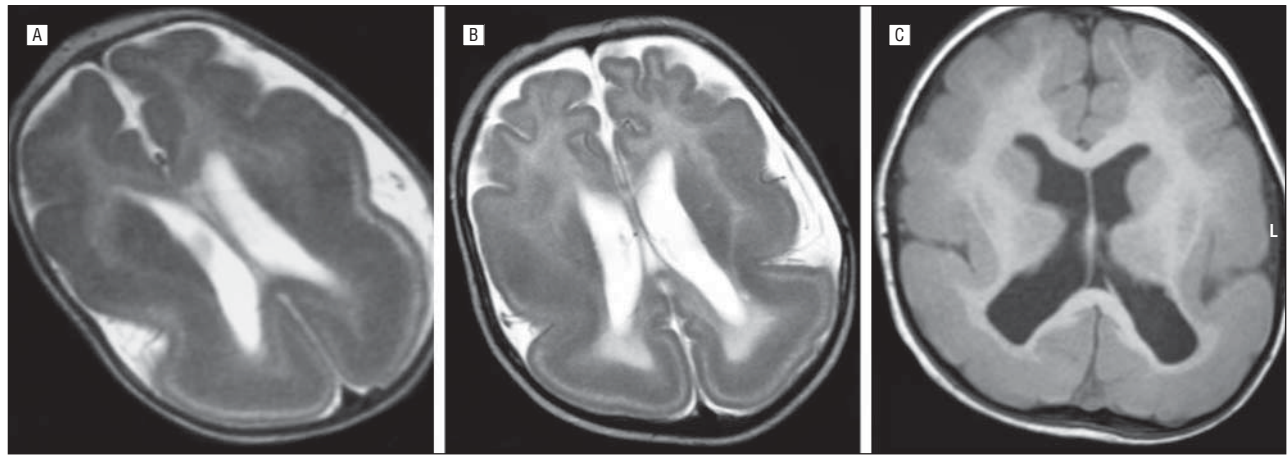


Figure 2. Axial images of patients with *LIS1*-related lissencephaly representing the main lissencephaly grades found in patients in this study. Lissencephaly severity decreases with increasing grade (left to right), illustrated by a case of grade 2a lissencephaly in patient Lis023, aged 5 months (T2-weighted) (A); grade 3a in patient Lis014, aged 20 months (T2-weighted) (B); and grade 4a in patient Lis013, aged 3 years (T1-weighted) (C). Note that the gradients of lissencephaly are more obvious with lower severity.

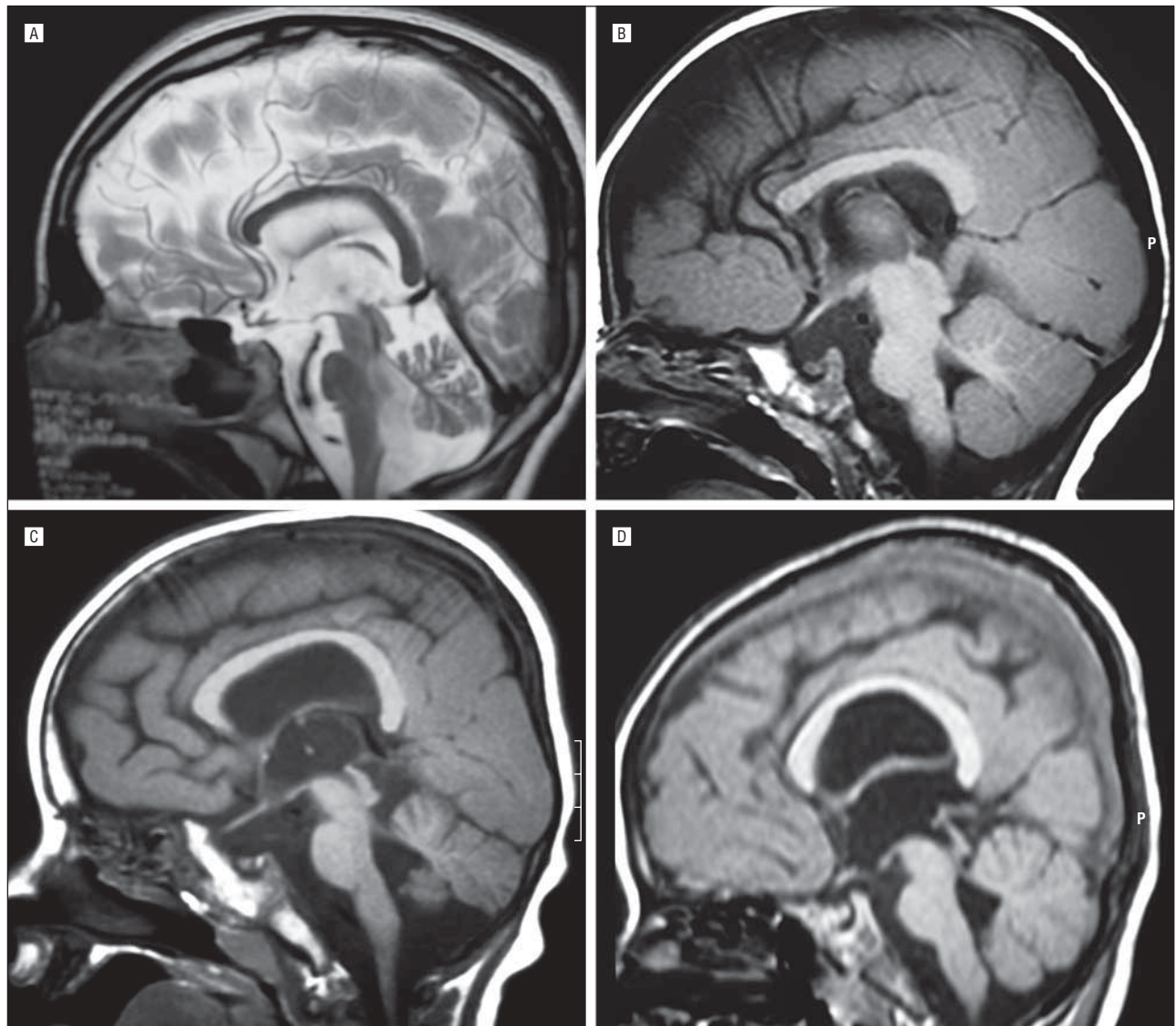


Figure 3. Midline sagittal images showing dysmorphic corpus callosum in all cases, with cerebellar hypoplasia in 2 patients (Lis030 [A] and Lis039 [C]) with brainstem hypoplasia [C] or normal appearance (Lis022 [B] and Lis018 [D]).

Table 2. Comparison of Mutation Type and Location in *LIS1* Mutated Patients

Lissencephaly Grade	Patients With Mutation by Type, No. (%)						
	1 ^a			2 ^b		3 ^c	
	Nonsense and Frameshift (n=18) ^d	Intragenic Deletion (n=8)	Missense (n=6)	Early Nonsense and Frameshift (n=5) ^e	Late Nonsense and Frameshift (n=13)	<i>LIS1</i> (n=37)	None (n=24)
1	0	0	0	0	0	1 (2.7)	0
2	3 (16.6)	1 (12.5)	3 (50.0)	1 (16.6)	2 (15.4)	8 (21.6)	5 (20.8)
3	11 (61.1)	6 (75.0)	1 (16.6)	3 (50.0)	8 (61.5)	21 (56.7)	5 (20.8)
4	3 (16.6)	1 (12.5)	2 (33.3)	1 (16.6)	2 (15.4)	6 (16.2)	10 (41.6)
5	1 (5.6)	0	0	0	1 (7.7)	1 (2.7)	4 (16.6)

^aIn part 1, missense and nonsense/frameshift mutations are compared.

^bIn part 2, nonsense/frameshift mutations were divided according to their location within the *LIS1* protein, considering the boundary at the level of the end of the coiled-coil domain. Mutations up to the 3' region of the end of the coiled-coil domain are termed *early* nonsense/frameshift and the remaining located in the WD40 repeats are termed *late* nonsense/frameshift mutations.

^cIn part 3, all patients with mutations in *LIS1* including those with splicing defects (n=5 of 31) were compared with patients without mutations in *LIS1* and *TUBA1A* genes (n=24).

^dMagnetic resonance images were available for 18 of 20 patients with nonsense and frameshift mutation.

^eA total of 5 of 7 magnetic resonance images were available for the Fisher exact test.

have less severe Dobyns grades, with most patients without mutations having a grade of 4 (of 10; 41.6%), compared with 6 (16.2%) of those with mutations (**Table 2**).

COMMENT

Here, we describe the largest series of 40 patients with *LIS1*-related lissencephaly with detailed clinical and imaging analysis, combined with molecular studies of the *LIS1* gene. Consistent with previous studies, the present findings confirm that the phenotypic variability among patients with the *LIS1* mutation may not be correlated with the *LIS1* mutant genotype.²¹ In addition, we observed that most patients with posteriorly predominant lissencephaly not mutated for *LIS1* tend to exhibit a less severe phenotype.

LIS1-related lissencephaly is responsible for severe neuromotor impairment with moderate microcephaly and a high frequency of early seizures, mainly consisting of infantile spasms, evolving into drug resistance in half, with low interindividual variability. Most patients with *LIS1* mutations have Dobyns grade 3a, consistent with previous data,^{2,6,15,21} often combined with prominent perivascular (Virchow-Robin) spaces that we presume to be secondary to the migrational disorder and corpus callosum hypogenesis. It is of interest that only 1 patient had cerebellar hypoplasia reminiscent of the phenotype lissencephaly with cerebellar hypoplasia type a, suggesting that this feature is rarely associated with *LIS1*-related lissencephaly.²⁸

The current mutation analysis of the *LIS1* gene revealed 31 intragenic mutations distributed over the entire gene, with about a third at the 5' end and the remaining mutations located in the WD40 repeat domains encoded by exons 5 through 11. This repartition differs from the series by Uyanik et al²¹ that reported a high prevalence of mutation in exons 10 and 11, but is consistent with previous studies.^{6,15} Most of the mutations (23 of 31) reported here are newly described, with few recur-

rent mutations, suggesting that no mutational hot spot characterizes mutations in the *LIS1* gene. Six are missense, and 5 of these are newly described, with a total of 15 missense mutations identified in the *LIS1* gene.^{2,6,18,21} Most of them are clustered in WD2 and 3 are encoded by exons 6 and 7. The other newly described mutations (nonsense/frameshift and splicing defect mutations) follow the repartition of mutation types usually reported for the *LIS1* gene. Moreover, we confirm the high prevalence of small deletions because 8 of 40 patients with typical posteriorly predominant isolated lissencephaly sequence with QMPSF gave results comparable with recent data from Mei et al,²⁴ who used a multiplex ligation-dependent probe amplification assay. In our series, of the 2 patients that showed a milder phenotype with grade 5 lissencephaly, 1 patient was found to have a somatic nonsense mutation. Somatic mutations in *LIS1* are uncommon (2.5% of patients in our series and 14% in Uyanik et al²¹) and are supposed to lead to milder phenotype.¹⁹ However, our data suggest that such milder form can also be observed with germline mutation.

To provide further insight into putative genotype phenotype correlations, we examined the relationship between the mutation type and location, but no significant correlation could be observed. Several explanations can be proposed. First, in spite of detailed neurological and radiological analyses, there is little variability among patients with the *LIS1* mutation who exhibit, in most cases, Dobyns grade 3 to 4, tetraplegia and refractory epilepsy. This observation, coupled with the limited size of each group studied, leads to a lack of power in the statistical tests. Second, the limits set between the early and late nonsense/frameshift mutations proposed by Cardoso et al¹⁸ and used here might not reflect the physiological functions of the *LIS1* protein (PAFAH1B), which is known to be involved in multiple protein to protein interactions, playing different roles in the complex process of cortical development through participation in different protein complexes. Biochemical data suggest that all domains of the *LIS1* protein are involved as the noncata-

lytic subunit of a heterodimeric complex that regulates brain levels of the platelet activating factor (PAFAH)²⁹ and as a microtubule-associated protein involved in cell proliferation and neuronal migration.³⁰⁻³³ Moreover, data on the biochemical outcomes of mutations showed they variably affect either the stability of the LIS1 protein, its folding, and/or its interactions with its partners. The full length of the LIS1 protein is required for a correct interaction with most of LIS1 partners, in particular nuclear distribution factor E homolog 1 (NDE1)^{14,34,35} and NDE-like 1 (NDEL1).³⁶ The dimerization properties of LIS1 are mediated via WD3, WD6, and the N-terminal region.³⁷ For the interaction of LIS1 with the PAFAH catalytic subunits $\alpha 1$ and $\alpha 2$, the WD domains WD2, WD3, and WD7 appear to be most important. Finally, WD2, WD5, WD6, WD7 and the N-terminal region are involved in microtubule binding.³⁷ Thus, many regions of the LIS1 protein seem critical for its multiple functions. Finally, the absence of a relationship between phenotype and genotype could be related to the nonsense-mediated mRNA decay that is suspected to play a major role in the processing of the LIS1 transcript, as suggested by Western blot.¹⁵ For this reason, early and late nonsense/frameshift mutations described here would be subjected to nonsense-mediated mRNA decay, leading to haplo insufficiency in each case.^{38,39}

Finally, patients without these mutations tend to have less severe lissencephaly, with most in our series having Dobyns grade 4. Recent data suggest that other genetic causes of lissencephaly could account for posteriorly predominant lissencephaly, including *TUBA1A* mutations. Although accurate analysis of the phenotype of the patients without the mutations allowed us to confirm that they did not demonstrate the distinctive features of *TUBA1A*-related lissencephaly consisting of dysmorphic basal ganglia and cerebellar hypoplasia,^{25,40} *TUBA1A* mutations were excluded in all patients without *LIS1* mutations. We hypothesize that either partner of the functional cascade of *LIS1* or *TUBA1A* genes could explain these posteriorly predominant lissencephalies.

In conclusion, with the largest series of *LIS1*-related lissencephaly, combined with patients found negative for *LIS1* mutation and deletion, our results confirm the homogeneity of patients with *LIS1*-related lissencephaly, a large proportion of whom have Dobyns lissencephaly grade 3a, with no correlation with *LIS1* mutations. On the other hand, patients with posteriorly predominant lissencephaly without the *LIS1* mutation tend to have a less severe phenotype, suggesting either additional molecular basis or unexplored alteration in the *LIS1* gene.

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Author Affiliations: Conseil National de la Recherche Scientifique, Université Paris Descartes (Unités Mixtes de Recherche 8104) and Institut national de la santé et de la recherche médicale (INSERM) U567, Paris (Drs Saillour, Quelin, Chelly, Beldjord, and Bahi-Buisson), Institut Cochin, Paris; Laboratoire de Génétique Moléculaire, Pavillon Cassini, Hôpital Cochin, Assistance publique Hôpitaux de Paris (AP-HP), Université Paris Descartes, Paris (Drs Carion, Chelly, and Beldjord); Service de Neurologie Pédiatrique, Département de Pédiatrie (Drs Leger and Bahi-

Buisson), Service de Radiologie Pédiatrique (Dr Boddaert), Unité de bioinformatique (Dr Elie), and Laboratoire de Neurophysiologie (Dr Kaminska), Hôpital Necker Enfants Malades, AP-HP, Université Paris Descartes, Paris; INSERM U797—Commissariat à l'Énergie Atomique (CEA), Orsay (Dr Boddaert); Service de Génétique (Drs Toutain and Mercier) and Service de Neuropédiatrie (Dr Barthez), Hôpital Clocheville, Centre Hospitalo-Universitaire (CHU) de Tours, Tours; Assistance publique Hôpitaux de Marseille Neurologie Pédiatrique, Marseille (Dr Milh); Service de Neuropédiatrie, Clinique Hôpital, Jeanne de Flandres Centre Hospitalo-Universitaire de Lille, Lille (Dr Joriot); Service de Neuropédiatrie, CHU Lyon, Lyon (Dr des Portes); Service de Génétique, Hôpital La Timone, CHU Marseille, Marseille (Dr Philip); Service de Neurologie, Centre Saint Paul, Marseille (Dr Broglin); Service de Neuropédiatrie, CHU Montpellier, Montpellier (Dr Roubertie); Service de Neuropédiatrie, CHU Nice, Nice (Dr Pitelet); Service de Neurologie Pédiatrique, Hôpital Trousseau, AP-HP, Paris (Dr Moutard); Service de Neurologie Pédiatrique, Hôpital Raymond Poincaré Garches, AP-HP, Université Paris Descartes, Paris (Dr Pinard); Service de Neurologie Pédiatrique, Hôpital des Enfants, CHU Toulouse, Toulouse (Dr Cancès); and INSERM U663, Paris, France (Drs Kaminska and Bahi-Buisson).

Correspondence: Nadia Bahi-Buisson, MD, PhD, Pediatric Neurology, Hôpital Necker Enfants Malades, 149 rue de Sevres, 75015 Paris, France (nadia.bahi-buisson@nck.aphp.fr).

Author Contributions: *Study concept and design:* Saillour, Chelly, and Bahi-Buisson. *Acquisition of data:* Saillour, Carion, Quelin, Leger, Boddaert, Toutain, Mercier, Barthez, Milh, Joriot, des Portes, Philip, Broglin, Roubertie, Pitelet, Moutard, Pinard, Cancès, Kaminska, Chelly, and Bahi-Buisson. *Analysis and interpretation of data:* Saillour, Quelin, Elie, Pinard, Kaminska, Beldjord, and Bahi-Buisson. *Drafting of the manuscript:* Saillour, Leger, and Bahi-Buisson. *Critical revision of the manuscript for important intellectual content:* Carion, Quelin, Boddaert, Elie, Toutain, Mercier, Barthez, Milh, Joriot, des Portes, Philip, Broglin, Roubertie, Pitelet, Moutard, Pinard, Cancès, Kaminska, Beldjord, and Bahi-Buisson. *Administrative, technical, and material support:* Carion. *Study supervision:* Milh, Chelly, Beldjord, and Bahi-Buisson.

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