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ARTICLE

Intragenic deletions and duplications of the *LIS1* and *DCX* genes: a major disease-causing mechanism in lissencephaly and subcortical band heterotopia

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Classical lissencephaly, or isolated lissencephaly sequence (ILS), and subcortical band heterotopia (SBH) are neuronal migration disorders associated with severe mental retardation and epilepsy. Abnormalities of the *LIS1* and *DCX* genes are implicated in the majority of patients with these disorders and account for approximately 75% of patients with ILS, whereas mutations of *DCX* account for 85% of patients with SBH. The molecular basis of disease in patients with ILS and SBH, in whom no abnormalities have been identified, has been questioned. We studied a series of 83 patients with ILS, SBH or pachygyria, in whom no abnormalities of the *LIS1* or *DCX* genes had been identified, for intragenic deletions and duplications by multiplex ligation-dependent probe amplification (MLPA). In 52 patients with ILS, we identified 12 deletions and 6 duplications involving the *LIS1* gene (35%), with the majority resulting in grade 3 lissencephaly. Three deletions of the *DCX* gene were identified in the group of nine female patients with SBH (out of 31 patients with *DCX*-suggestive brain anomalies), ie 33%. We estimate an overall mutation detection rate of approximately 85% by *LIS1* and *DCX* sequencing and MLPA in ILS, and 90% by *DCX* sequencing and MLPA in SBH. Our results show that intragenic deletions and duplications of the *LIS1* and *DCX* genes account for a significant number of patients with ILS and SBH, where no molecular defect had previously been identified. Incorporation of deletion/duplication analysis of the *LIS1* and *DCX* genes will be important for the molecular diagnosis of patients with ILS and SBH.
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Introduction

Classic lissencephaly (LIS) and subcortical band heterotopia (SBH) comprise a spectrum of severe cortical malformations resulting from abnormal neuronal migration during brain development. The most severe form of LIS consists of absent gyri (or agyria), while a less severe or intermediate form consists of abnormally wide gyri (or pachygyria).[1–3] The less severe forms of LIS merge with the phenotype of SBH, a less severe cortical malformation characterized by the presence of bilateral smooth bands of gray matter in the central or superficial white matter of the brain.[4] Both LIS and SBH may occur in isolated form as isolated lissencephaly sequence (ILS) or isolated SBH, or as part of malformation syndromes such as Miller–Dieker or Baraitser–Winter syndrome, with the former comprising a contiguous gene deletion disorder resulting from large microdeletions of the 17p13.3 region.[5–8]

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The genetic basis of ILS and SBH are reasonably well understood, with specific patterns of brain malformation emerging that can assist in distinguishing the gene in which a causative mutation is most likely to be found, most often either the *LIS1* (PAFAH1B1) or *DCX* gene. Abnormalities in the *LIS1* gene, located on chromosome 17p13.3, cause a malformation pattern or gradient that is more severe in posterior as compared to anterior brain regions (posterior-to-anterior), while mutations in the *DCX* gene result in brain malformation patterns that are more severe in the frontal regions of the brain becoming less severe posteriorly (anterior-to-posterior).[1,3,9]

Abnormalities of the *LIS1* and *DCX* genes account for approximately 75% of ILS.[2,3,10] *LIS1* abnormalities comprise whole gene deletions that are detectable by fluorescence *in situ* hybridization (FISH) analysis and mutations within the gene that are detectable by DNA sequencing. *DCX* abnormalities that result in ILS consist primarily of mutations within the gene. Mutations in *DCX* have been described in approximately 38% of males with ILS but only very rarely in females with ILS, and in approximately 85% of females and approximately 29% of males with SBH.[2,3,11–13] For both the *LIS1* and *DCX* genes, mutations that abolish function such as entire gene deletions, nonsense, frameshift and splicing mutations generally result in a more severe phenotype, while missense mutations tend to result in the milder phenotypes.[9,10,13,14]

Intragenic deletions of the *LIS1* and *DCX* genes also result in lissencephaly, but the frequency of these types of mutation as well as the associated disease severity has not been well characterized. Intragenic deletion mutations of the *LIS1* gene, detected by Southern blot, have previously been found to account for approximately 8% of disease-causing mutations.[2,10] We set out to determine the incidence of intragenic deletions and duplications in the *LIS1* and *DCX* genes in patients with varying degrees of lissencephaly and SBH.

In this study, we systematically screened a cohort of 83 patients for *LIS1* and *DCX* intragenic deletions and duplications who were previously found to be negative for microdeletions in the 17p13.3 region by FISH and also negative for mutations detected by sequencing of the *DCX* and *LIS1* genes. The cohort of patients studied has been well characterized in terms of brain abnormality patterns and disease severity. This study provides a comprehensive analysis of the *DCX* and *LIS1* genes and allows us to correlate deletions and duplications of these two genes with disease severity. Our results indicate that deletions and duplications of the *LIS1* and *DCX* genes account for up to 35% of patients with lissencephaly and SBH in whom the molecular basis of disease had previously not been identified. With the inclusion of deletion/duplication analysis of the *LIS1* and *DCX* genes, the molecular basis of 85–90% of patients with lissencephaly and SBH can now be determined.

Materials and methods

Patient selection

Blood samples were obtained from patients after informed consent was given. Protocols were approved by the appropriate IRB committees before the initiation of the research.

Patients

For this study, we classified patients as having lissencephaly based on the presence of agyria, pachygyria, SBH or combinations of these malformations. We defined agyria as areas of cortex lacking sulci (convolutions or gyri) larger than 3 cm and pachygyria as areas of cortex lacking sulci between $\sim$1.5 and 3 cm, both with the cortical ribbon typically thicker than 1 cm. We used the same grading system as in our earlier studies, which consists of completely absent gyri or agyria (grade 1), diffuse agyria with a few shallow sulci usually frontally (grade 2), mixed agyria and abnormally broad gyri or pachygyria (grade 3), diffuse pachygyria (grade 4), mixed pachygyria and SBH (grade 5) and SBH only (grade 6).[1,3,9,10]

We searched our large brain malformation database for all patients with ILS or SBH for whom original brain imaging studies were available and reviewed by one of the authors (WBD). All patients were classified with attention to the pattern (severity and gradient), and the presence or absence of associated features. We excluded all patients with syndromic forms of lissencephaly, including lissencephaly with moderate or severe cerebellar hypoplasia (many patients with *DCX* or *LIS1* mutations have mild cerebellar vermis hypoplasia), Baraitser–Winter syndrome, X-linked lissencephaly with abnormal genitalia, and several other novel syndromes observed in single patients. We separated the remaining patients into three groups based on the pattern of the cortical malformation. From this group, we then selected 83 patients who met the following criteria: negative findings by FISH for a microdeletion of the 17p13.3 region and in whom no mutations were identified in either the *LIS1* and/or *DCX* genes by sequence analysis (Supplementary Table 1).

In total, 52 patients exhibiting severe lissencephaly in the parietal and occipital regions of the brain (severity following an posterior-to-anterior gradient – ie, an *LIS1* pattern) were investigated for intragenic deletions and duplications of the *LIS1* gene, while 31 patients (22 females and 9 males) with SBH and lissencephaly encompassing the frontal regions of the brain (severity following an anterior-to-posterior gradient – ie, a *DCX* pattern) were analyzed for intragenic deletions and duplications in the *DCX* gene. The patient cohort consisted of 83 subjects in total.

DNA isolation

DNA was extracted from leukocytes from EDTA-treated blood using either the Puregene kit (Gentra Systems Inc., Minneapolis, MN, USA) or the MagNAPure Total Nucleic

Acid Extraction system (Roche Diagnostics, Indianapolis, IN, USA) following the manufacturer's protocols.

Detection of intragenic deletions and duplications

We used multiplex ligation-dependent probe amplification (MLPA) to screen our series of 83 patients. All 83 patients had been analyzed for mutations in the *LIS1* and/or *DCX* genes and the 17p13.3 microdeletion and found to be negative. Analysis of the *LIS1* and *DCX* genes was performed using the Lissencephaly P061 MLPA kit (MRC-Holland, Amsterdam, the Netherlands) following the manufacturer's protocol. The MLPA kit contains probes for all the exons (coding and upstream non-coding) of the *DCX* gene and all exons (coding and upstream non-coding) of the *LIS1* gene plus the *LIS1*-flanking genes, *YWHAE*, *HIC1*, *MGC3329*, *KIAA0664* and *KIAA1039*. MLPA fluorescently labeled PCR products were size fractionated on the automated fragment analyzer ABI3130 (Applied Biosystems, Foster City, CA, USA) and analyzed using the fragment sizing program GeneMarker (Soft Genetics Inc.). Positive controls (samples with microdeletions of 17p13.3) and normal controls were included with each run. Ratios of 0.4–0.6 or $x > 1.4$ were considered to denote the presence of a deletion or duplication, respectively, while a ratio of 0.8–1.2 was considered to be normal by MLPA. Any MLPA results that indicated the presence of a single or multiple exon deletion or duplication in a particular patient sample were confirmed by real time quantitative-PCR (RT-qPCR). RT-qPCR was performed using an internal control gene along with the regions of the *LIS1* and *DCX* genes being tested. Short amplicons between 60 and 100 bases were amplified utilizing *Power*SYBR Green (Applied Biosystems) following the manufacturer's protocol, and the ratio of the *LIS1/DCX* region of interest to internal control gene amplification was compared to normal controls. Two independent amplicons within the region containing a potential deletion or duplication were analyzed by RT-qPCR and were run concurrently in duplicate with positive and normal controls. A ratio of 0.4–0.6 represented the presence of a deletion, while a value over 1.4 was interpreted as a duplication. A ratio of 0.8–1.2 was indicative of no deletion or duplication.

Results

Intragenic deletion/duplication analysis of the *LIS1* gene

Of the 52 patients studied for *LIS1* deletions/duplications, 18 patients were found to have either a deletion or duplication affecting the *LIS1* gene (35%) (Table 1). Twelve of the 18 patients harbored a deletion, whereas 6 were found to have a duplication (Figure 1). One patient with a *LIS1* deletion also had a duplication involving the upstream *HIC1* gene. The deletions and duplications identified were of varying sizes ranging from the deletion or duplication of a single exon to deletions involving the entire coding region of the *LIS1* gene. Although the deletions and duplications were found scattered within the gene, exons 3–6 appeared to be involved in the majority of cases (11 of 18). Several of the deletions included genes that flank the *LIS1* gene, such as the *MGC3329* and *HIC1* genes upstream and the *KIAA0664* and *KIAA1039* genes downstream (6 of 18). Two of the deletions involved the upstream non-coding region of *LIS1* and did not appear to include any of the coding region. One duplication was found to involve only the downstream *KIAA0664* gene and did not appear to involve any of the coding and non-coding regions of the *LIS1* gene. These results suggest the presence of regulatory elements important for LIS1 expression that are located both upstream and downstream of the gene.

Of the 18 deletions and duplications identified, 16 were found in patients with grade 3 LIS (Table 3). Twenty of the 52 patients studied had grade 3 LIS, resulting in 80% of this group (16 of 20) harboring either a deletion or duplication. All six duplications identified were in patients with grade 3 lissencephaly, while 10 of the 12 deletions were present in this group. Of the six duplications, four involved multiple exons, one was a duplication encompassing a single exon (exon 5) and one duplication was of a flanking gene 3′ of the *LIS1* gene (*KIAA0664*). All five duplications of the *LIS1* exonic region involved the coiled-coil domain as well as one or more of the WD40 repeats, with three including the critical second WD40 repeat. The four multiple exonic duplications are predicted to be out-of-frame, while the exon 5 duplication is predicted to result in a duplication of a portion of the coiled-coil and the first WD40 domains. The sixth duplication does not appear to affect the *LIS1* gene but rather involves a downstream gene, *KIAA0664*. We have not characterized the size of this duplication further, so it remains possible (and most likely) that 3′UTR and regulatory elements of the *LIS1* gene are affected.

All 10 of the deletions seen in the grade 3 lissencephaly patients are predicted to be deleterious. Two of the deletions appeared to involve the entire coding region of the *LIS1* gene as well as additional downstream genes. These deletions were not detectable by FISH analysis indicating that the size is smaller than that traditionally detected by this technology. Of the smaller deletions that involved a part of the *LIS1* gene, three included exon 2, which harbors the ATG translational start site. Three of the intragenic deletions involved multiple or single exons that were all predicted to alter the reading frame and truncate the protein. The remaining two deletions included the non-coding exon 1 of the *LIS1* gene and additional upstream genes. These deletions are predicted to affect promoter and regulatory elements of the *LIS1* gene. A significant number of the intragenic deletion and duplication break points were located within introns 1, 2 and 5 (44.8%).

Table 1 Frequency of intragenic deletions and duplications in the *LIS1* gene

Gene	*Gradient*	*Grade*	*No. of patients*	*Intragenic deletion/duplication positive*	*%*
		Grade 1 or 2	5	0	0
		Grade 3	20	16	80
LIS1	P>A	Grade 4	22	2	9
		SBH	5	0	0
		Total	52	18	35

Cohort of patients with lissencephaly characterized by an posterior-to-anterior gradient of severity, a strong indicator for a mutation present in the *LIS1* gene. The 52 patients are grouped by causative gene and grade of disease with the severity gradient extending from complete agyria (grades 1 and 2) to pachygyria-agyria (grade 3) to pachygyria (grade 4) to SBH (grade 6).

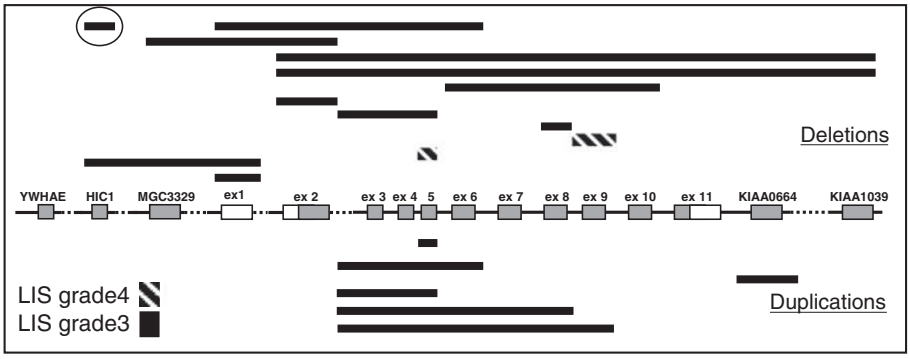

Figure 1 Schematic of intragenic deletions and duplications detected in the *LIS1* and surrounding genes. Each bar denotes a deletion/duplication identified in a patient with the deletions depicted on top and the duplications depicted below the diagram of the gene. The bars indicate the extent of the deletions/duplications and show the regions of the gene/s involved. The associated lissencephaly grade for each patient in whom a deletion/duplication was identified is also indicated. The 11 exons of the *LIS1* gene are shown to be located between the genes *MGC3329* and *KIAA0064*, with non-coding exons and untranslated portions of exons indicated in white and the coding exons indicated in gray. The circled region in the top left corner represents an individual that was found to have an intragenic deletion encompassing exons 1–6 of the *LIS1* gene as well as a duplication of the *HIC1* gene. This figure is not drawn to scale.

Of 22 patients with grade 4 lissencephaly, 2 were identified as having an intragenic deletion of the *LIS1* gene, ie 9%. Both were single exon deletions (exons 5 and 9) and resulted in in-frame deletions within the *LIS1* gene. The complete deletion of exon 5 or 9 results in disruption of the first WD40 repeat and the fourth and fifth WD40 repeats, respectively.[3] Exon 5 also contains the coiled-coil domain (amino acids 44–78), which may play a role in PAFAH1B1 protein–protein interaction.[10] The disruption of these important functional domains of the *LIS1* gene most likely explains the resulting lissencephaly phenotype observed. These two deletions may represent slightly milder mutations that manifest as grade 4 lissencephaly, as they do not result in a frame shift causing a prematurely truncated PAFAH1B1 protein.

No deletions or duplications of the *LIS1* gene were detected in the small group of patients with grade 1 or 2 lissencephaly (0 out of 5 patients). Our retrospective review of their phenotype showed atypical features in four of five patients, consisting of mild to borderline moderate cerebellar vermis hypoplasia in three patients, which was not quite sufficient for us to exclude them from the study, and congenital microcephaly that was more severe than usual with mutations of *DCX* or *LIS1* (−3 standard deviations) in the other patient. In addition, no deletions or duplications of the *LIS1* gene were found in the group of patients with SBH and a posterior-to-anterior gradient of severity (0 out of 5 patients). Although SBH is generally associated with defects in the *DCX* gene, a posterior-to-anterior grade of severity has been associated with *LIS1* mutations.[11,15] This group of patients was also found not to have deletions or duplications of the *DCX* gene.

Intragenic deletion/duplication analysis of the *DCX* gene

Patients included for *DCX* analysis included those with either SBH, pachygyria-SBH or pachygyria manifesting with an anterior-to-posterior gradient that is suggestive of a *DCX* abnormality.[16] This group included 22 females and 9 males (Table 2). Three deletions of the *DCX* gene were observed, all in female patients (Figure 2). Two of the deletions involved exons 3 and 4 and one involved exons 6 and 7 (Table 3). Exon 4 contains the translation start site and a deletion of this region is predicted to affect protein production. The deletion involving exons 6 and 7 is out-of-frame and therefore either leads to loss of the protein

Table 2 Frequency of intragenic deletions and duplications in the *DCX* gene

Gene	*Gradient*	*Grade*	*Female*	*Intragenic deletion/duplication positive*	*%*	*Male*	*Intragenic deletion/duplication positive*	*%*
		SBH	8	2	25	0	0	0
DCX	A>P	Pachygyria-SBH	1	1	100	2	0	0
		Pachygyria	13	0	0	7	0	0

Cohort of patients with SBH and lissencephaly characterized by an anterior-to-posterior gradient of severity, a strong indicator for a mutation present in the *DCX* gene. The 31 patients are grouped by causative gene, grade of disease severity as well as patient gender with the severity gradient extending from SBH (grade 6) to pachygyria-SBH (grade 5) to pachygyria (grade 4).

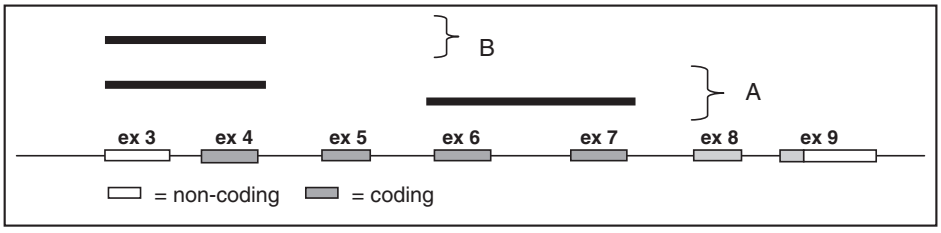

Figure 2 Schematic of intragenic deletions detected in the *DCX* gene in three female patients with subcortical band heterotopia (SBH) or SBH-pachygyria. The A group indicates the *DCX* intragenic deletions identified in two females with SBH, while B represents the deletion identified in one female with SBH-pachygyria. The solid black bars indicate the minimum size of the intragenic deletions and show the region of the gene involved. This figure is not drawn to scale.

Table 3 Features of patients positive for an intragenic deletion/duplication in the *DCX* or *LIS1* gene

LP no.	*Sex*	*Ethnicity*	*Grade*	*Gradient*	*MLPA results*	*Sporadic/Familial*
LR00–085	F	NA	3	P>A	*LIS1*: del ex1–2+*MGC3329*	Sporadic
LR01–088	M	NA	3	P>A	*LIS1*: del ex2	Sporadic
LR01–132	M	Caucasian/Hispanic	3	P>A	*LIS1*: del ex6–10	Sporadic
LR01–137	F	Caucasian	3	P>A	*LIS1*: dup ex5	Sporadic
LR01–155	F	NA	3	P>A	*LIS1*: dup *KIAA0664*	Sporadic
LR02–044	M	NA	3	P>A	*LIS1*: dup IVS2-ex9	Sporadic
LR03–215	M	NA	3	P>A	*LIS1*: del ex2–11+*KIAA0664*+*KIAA1039*	Sporadic
LR03–253	M	NA	3	P>A	*LIS1*: del ex1+*MGC3329*+*HIC1*	Sporadic
LR03–369	M	NA	3	P>A	*LIS1*: del ex1	Sporadic
LR03–414	M	NA	3	P>A	*LIS1*: del IVS2-ex5	Sporadic
LR04–117	F	Caucasian	3	P>A	*LIS1*: del ex1-6+dup *HIC1*	Sporadic
LR04–163	M	Caucasian/Hispanic	3	P>A	*LIS1*: dup IVS2-ex6	Sporadic
LR04–294	M	Caucasian/Hispanic	3	P>A	*LIS1*: dup IVS2-ex5	Sporadic
LR06–010	F	Caucasian	3	P>A	*LIS1*: del ex8	Sporadic
LR06–366	M	Caucasian	3	P>A	*LIS1*: del ex2-11+*KIAA0664*+*KIAA1039*	Sporadic
LR04–219	M	NA	3	P>A	*LIS1*: dup IVS2-ex8	Sporadic
LP93–011	M	Caucasian	4	P>A	*LIS1*: del ex9	Sporadic
LR03–288	M	NA	4	P>A	*LIS1*: del ex5	Sporadic
LP97–115	F	NA	5	A>P	*DCX*: del ex3–4	Sporadic
LP94–056	F	Caucasian	6	SBH	*DCX*: del ex6–7	Sporadic
LR02–330	F	NA	6	SBH	*DCX*: del ex3–4	Sporadic

Features of patients positive for an *LIS1* ($n=18$) or *DCX* ($n=3$) intragenic deletion or duplication. All patients were found to have similar phenotypes consisting of developmental delay, severe mental retardation, seizures and feeding difficulties, typical of children with severe developmental handicaps. Ethnicity represents self-reported information, NA = not known. All 21 patients represented sporadic occurrences of lissencephaly or SBH within their respective families.

through the nonsense-mediate mRNA decay pathway,[17] or truncates the protein leading to loss of the carboxyl end of the protein that contains a CDK5 phosphorylation site at Ser297 and a serine-proline-rich region containing at least four other potential phosphorylation sites near the end of the coding region.[18,19]

All three deletions occurred in the group of nine female patients with either SBH or SBH-pachygyria, ie 33%. None of the 13 female patients with the more severe pachygyria phenotype (lissencephaly grade 4) had *DCX* abnormalities. In addition, none of the male patients included in this group with either SBH-pachygyria or pachygyria

(lissencephaly grades 4 and 5) were found to have *DCX* abnormalities. These results further increase the percentage of female patients with SBH and *DCX* abnormalities.

Discussion

To further characterize the molecular basis of the neuronal migration disorder lissencephaly and the phenotypically milder SBH, we undertook a study to examine the frequency of intragenic deletions and duplications in the *LIS1* and *DCX* genes. Our results showed that deletions and/or duplications in both the *LIS1* and *DCX* genes account for a significant proportion of patients with ILS and SBH.

Through systematic phenotypic classification of brain malformation gradients and severity in ILS and SBH patients negative for a 17p13.3 microdeletion detected by FISH, or a point mutation in *LIS1* or *DCX* detected by sequencing, we were able to identify 18 intragenic deletions and duplications of the *LIS1* gene in our group of 52 patients. Deletions and duplications of the *LIS1* gene therefore appear to account for approximately 35% of this group. When divided according to the severity of LIS, we found that a large majority of deletions and duplications occurred in patients with the classical grade 3 form of LIS. In fact, 80% (16 of 20) of our patients in this group were found to harbor a deletion or duplication of *LIS1*. The milder grade 4 form of LIS (which consists of complete pachygyria) had a lower incidence of deletions and duplications with only 9% (2 of 22) of patients in this group having an intragenic deletion. No deletions or duplications were identified in patients with even milder grade 6 form of LIS (posterior SBH). These results suggest that the milder forms of LIS are genetically heterogeneous and that perhaps other genetic factors may be involved in their etiology. In addition, intragenic deletions and duplications were not found in the more severe group (grades 1 and 2 that consist of complete or nearly complete agyria). These results appear to differ somewhat from our previous findings where *LIS1* deletions were identified in some patients with grade 2 LIS.[10] This may be due to the small number of patients with grade 2 LIS included in this study. This group of patients often has severe *DCX* mutations in boys or microdeletions of the 17p13.3 region, but our experience suggests that additional causative genes are most likely to be found, such as the new *TUBA1A* gene.[20]

Intragenic deletions of the *DCX* gene were found in 33% of female patients with SBH in whom no mutations of *DCX* had previously been identified. The SBH phenotype in the patients with *DCX* deletions was similar to those with *DCX* mutations detected by sequencing. Two of the deletion patients had typical diffuse SBH, while another had a slightly more severe phenotype with a combination of pachygyria and SBH. The pachygyria-SBH phenotype showed an anterior greater than posterior severity that has been associated with *DCX* abnormalities.[3] A series of 20 patients (13 females and 7 males) with the more severe pachygyria-only phenotype showing an anterior-to-posterior gradient were also analyzed for *DCX* with no deletions or duplications identified. On reviewing the phenotype of these patients, we found that most have somewhat less extensive pachygyria and less marked thickening of the cortex than typical for *DCX* mutations. Our entire research database shows *DCX* mutations in only 36 of 138 (26%) patients with frontal predominant pachygyria. We take these observations together to mean that other gene(s) are responsible for this phenotype. Our results suggest that intragenic deletions and duplications of *DCX* result in a more classic SBH phenotype.

The intragenic deletions and duplications identified were of varying sizes and encompassed different regions of the *LIS1* and *DCX* genes. While there were no obvious hot spots for deletions and duplications, break points within introns 1, 2 and 5 appeared to be more common in the *LIS1* gene. The intragenic deletions and duplications observed are all predicted to be loss-of-function mutations that either result in an unstable protein or the lack of protein. Interestingly, the two small in-frame deletions in the *LIS1* gene were found in patients with less severe lissencephaly (grade 4) compared to the remaining out-of-frame deletions and duplications (all found in grade 3 patients). It is possible that the in-frame deletions result in protein that retain some function and manifest as a milder phenotype, as we have observed for some missense mutations of *LIS1*.[14]

The smallest deletions/duplications observed in the *LIS1* gene involved only a single exon, whereas the largest appeared to involve the entire coding region plus additional flanking genes. The exact size of the deletions and duplications however was not determined. Two of the largest deletions that affected the entire *LIS1* coding region have a minimum size of $\sim$158 kb (*LIS1* exon 2 to *KIAA1039* exon 1). These deletions were not detected by previous FISH analysis. Smaller deletions of the 17p13.3 region that partially affect the *LIS1* gene have previously been described that were detected by *LIS1*-specific FISH probes but missed by the commercially available FISH probe for *LIS1*.[21]

We identified an impressive number of intragenic *LIS1* duplications in our cohort of ILS patients. Of the 18 abnormalities identified, one-third were duplications that involve one or multiple exons. Three of the multiple exonic duplications included the critical second WD40 repeat, whereas the other two intragenic *LIS1* gene duplications encompassed the first WD40 repeat as well as the coiled-coil domain. As the WD40 domains and protein interaction domains are considered to be extremely important for the correct folding and function of the PAFAHB1 protein, intragenic duplications could represent a severe type of mutation that causes significant disruption

of the *LIS1* gene product. The sixth duplication occurred downstream of the *LIS1* gene and most likely affects important regulatory elements, although this has not been proven conclusively. Intragenic duplications, in general, tend to be less described as a disease-causing mechanism compared to intragenic deletions. This could be due to duplications occurring less frequently, not resulting in a recognizable phenotype, or not being detected. As the mechanisms that give rise to intragenic deletions are also predicted to result in intragenic duplications, the incidence of both should be similar and it is possible that duplications are being under-detected. Our results showed that intragenic duplications of the *LIS1* gene are a significant cause of disease in ILS.

Microdeletions of 17p13.3 are found in about 40% of patients, while mutations within the *LIS1* and *DCX* genes are seen in approximately 35% of patients.[2,3] Our results indicate that intragenic deletions and duplications of the *LIS1* gene account for an additional 10% of this group (35% of patients without microdeletions or mutations). These results are similar to Mei *et al* (2008) who described the presence of *LIS1* intragenic deletions and duplications in 42% of their ILS patients negative for a 17p13.3 microdeletion detected by FISH,[22] however we identified a larger percent of intragenic duplications in our set of patients. A combination of DNA sequencing and deletion/duplication analysis of the *LIS1* and/or *DCX* genes will therefore detect abnormalities in approximately 85% of patients with ILS, considering 17p13.3 microdeletions, *LIS1* and *DCX* mutations detected by sequencing (which includes *DCX* deletions in males) and *LIS1* intragenic deletions/duplications. Mutations in the *TUBA1A* gene have also been implicated in a small percentage of patients with LIS or SBH,[20,23] and result in a malformation pattern very similar to that caused by defects in the *LIS1* gene, although additional features have been recognized that may distinguish a subset of these patients. Mutations in the other three known LIS genes – *ARX*, *RELN* and *VLDLR* – have been associated with additional abnormalities that exclude them from the ILS category, and none of these genes are associated with SBH.

Similarly, the molecular basis of approximately 90% of SBH is now understood. Mutations of the *DCX* gene are present in approximately 85% of SBH patients[24] and we have found intragenic deletions of the *DCX* gene in an additional 4% (27% of patients without *DCX* mutations). Our results are similar to Mei *et al* (2007) who described the presence of *DCX* intragenic deletions in 27% of their SBH patients in whom no *DCX* mutations had been identified by sequencing.[25] A combination of DNA sequencing and deletion/duplication analysis of the *DCX* gene will therefore detect abnormalities in approximately 90% of patients with SBH. The remaining 10% of SBH are most likely due to abnormalities in other genes, such as *TUBA1A*.

Intragenic deletions and duplications are emerging as contributing to a significant percentage of the molecular basis of disease in multiple different diseases. As these abnormalities have been well documented to contribute significantly to genetic disorders, such as Duchenne and Becker muscular dystrophies, their presence has remained under-detected. This has largely been due to the more common technologies used such as FISH and BAC comparative genomic hybridization that detect larger genomic deletions and duplications (>80 kb) and DNA sequencing that detects smaller deletions and duplications (ranging from a few base pairs to couple of hundred base pairs), all of which will miss exonic deletions and duplications. Southern blot analysis can detect this intermediate size deletion/duplication, but this technique is not routinely or widely used for mutation analysis. Technologies for intragenic deletion/duplication analysis that are more amenable to routine use such as MLPA or RT-qPCR and more recently oligo array CGH will be useful for better characterization of this group of mutations. Intragenic deletions and duplications are likely to account for a sizeable fraction of mutations in genetic disease, and comprehensive mutation analysis should therefore include DNA sequencing as well as intragenic deletion/duplication analysis.

Our findings show that the lissencephaly phenotype associated with mutations and deletions/duplications (both intragenic and large microdeletions) in the *LIS1* and *DCX* genes are very similar. On the basis of these findings, we recommend intragenic deletion/duplication testing by MLPA, or other intragenic deletion/duplication method as the initial assessment (this will detect both intragenic deletions/duplications and large microdeletions), followed by sequencing of *LIS1* and then *DCX* for a classic LIS phenotype. For SBH, we recommend *DCX* sequencing followed by MLPA. In a subset of male patients with grade 1 or frontal lissencephaly, we suggest *DCX* sequencing before *LIS1* sequencing or deletion/duplication analysis. To perform the most appropriate range of testing, a thorough review of patient information by an experienced physician will most likely increase accuracy and mutation yield from genetic testing.

In conclusion, our study of a large group of well-characterized patients with ILS and SBH shows that intragenic deletions and duplications of the *LIS1* and *DCX* genes play a significant role in these disorders and our findings highlight the importance of developing systematic approaches and methods for detection of such mutations. These results are important for diagnostic and genetic counseling purposes. Genetic testing for ILS and SBH should include both mutation and deletion/duplication analysis for the *LIS1* and *DCX* genes.

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