MUTATION UPDATE

Clinical and Molecular Basis of Classical Lissencephaly: Mutations in the *LIS1* Gene (*PAFAH1B1*)

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Classical lissencephaly (LIS) and subcortical band heterotopia (SBH) are related cortical malformations secondary to abnormal migration of neurons during early brain development. Approximately 60% of patients with classical LIS, and one patient with atypical SBH have been found to have deletions or mutations of the *LIS1* gene, located on 17p13.3. This gene encodes the LIS1 or PAFAH1B1 protein with a coiled-coil domain at the N-terminus and seven WD40 repeats at the C-terminus. It is highly conserved between species and has been shown to interact with multiple proteins involved with cytoskeletal dynamics, playing a role in both cellular division and motility, as well as the regulation of brain levels of platelet activating factor. Here we report 65 large deletions of the *LIS1* gene detected by FISH and 41 intragenic mutations, including four not previously reported, the majority of which have been found as a consequence of the investigation of 220 children with LIS or SBH by our group. All intragenic mutations are de novo, and there have been no familial recurrences. Eight-eight percent (36/41) of the mutations result in a truncated or internally deleted protein—with missense mutations found in only 12% (5/41) thus far. Mutations occurred throughout the gene except for exon 7, with clustering of three of the five missense mutations in exon 6. Only five intragenic mutations were recurrent. In general, the most severe LIS phenotype was seen in patients with large deletions of 17p13.3, with milder phenotypes seen with intragenic mutations. Of these, the mildest phenotypes were seen in patients with missense mutations. Hum Mutat 19:4–15, 2002.

KEY WORDS: lissencephaly; LIS1; subcortical band heterotopia; SBH; cortical malformation; Miller-Dieker syndrome; MDS; PAFAH1B1; platelet activating factor; PAF; neuronal migration.

DATABASES:
PAFAH1B1 – OMIM: 601545, 247200 (MDS); GDB: 120525, 677430; GenBank: NM_000430, U72342, L13386, L13385; HGMD: PAFAH1B1; http://www.lissencephaly.org (The Lissencephaly Network); http://www.genestar.org (Gene Clinics)

CLASSICAL LISSENCEPHALY

Classical lissencephaly (LIS) or agyria/pachygyria is a brain malformation characterized by absent or reduced gyration and increased cortical width. Although thick, the cortex is simplified and contains only four of the usual six layers [Crome, 1956]. Associated brain abnor-

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malities include hypogenesis of the rostrum of the corpus callosum, ventricular dilatation, abnormal positioning of the olivary nuclei in the midbrain, and sometimes, mild cerebellar vermis hypoplasia. Subcortical band heterotopia (SBH) is a related disorder in which there are bilateral bands of gray matter found interposed in the white matter between the cortex and the lateral ventricles [Barkovich et al., 1994]. The overlying cortex is usually normal with the exception of shallow sulci. Both LIS and SBH have been presumed to be secondary to an abnormality in the process of migration of immature neurons from the periventricular zone to the developing cortical plate during the first half of pregnancy [Dobyns et al., 1993]. According to this hypothesis, a percentage of neurons will fail to reach their correct position in the cortex. In LIS, these neurons form the neuron-rich deepest cortical layer and in SBH these neurons form the heterotopic subcortical band.

LIS is associated with severe mental retardation, epilepsy, and cerebral palsy, while SBH generally has milder clinical sequelae including seizures and mild-to-moderate intellectual disability [Dobyns et al., 1993; Barkovich et al., 1994]. Less severe phenotypes of both of these disorders have also been reported [Gleeson et al., 2000; Leventer et al., 2001]. LIS was first characterized in pathological studies, and later described as one component of the Miller-Dieker syndrome (MDS; MIM# 247200) [Dobyns et al., 1983]. MDS is a contiguous gene deletion syndrome involving chromosome 17p13.3. It includes severe lissencephaly, characteristic dysmorphic facial features, and occasionally, other congenital malformations such as omphalocele and heart defects. LIS in the absence of other significant malformations has been termed the isolated lissencephaly sequence (ILS) [Dobyns et al., 1993].

Although mutations in either gene can result in either LIS or SBH, the majority of cases of classical LIS are due to deletions or mutations of the LIS1 gene (approved symbol, PAFAH1B1; MIM# 601545) on 17p13.3 [Dobyns et al., 1993; Lo Nigro et al., 1997; Pilz et al., 1998a], whereas the majority of cases of SBH are due to mutations of the DCX gene on Xq22.3 (see MIM# 300121) [Gleeson et al., 1998; Des Portes et al., 1998a,b]. Differences on imaging between LIS caused by LIS1 mutations, and LIS caused by DCX mutations, have been recognized. The former results in LIS more severe in posterior brain regions (the p>a gradient), whereas the latter results in LIS more severe in anterior brain regions (the a>p gradient) [Pilz et al., 1998a; Dobyns et al., 1999]. The overall spectrum of severity of malformations seen in LIS has been divided into six grades. For patients with LIS1 deletions or mutations they are: complete agyria (grade 1), posterior agyria with a few shallow sulci at the frontal pole (grade 2), posterior agyria with anterior pachygyria (grade 3), generalized pachygyria worse posteriorly (grade 4a1), posterior pachygyria with normal anterior gyri (grade 4a2), frontal SBH and posterior pachygyria (grade 5, not shown), and posterior SBH only (grade 6) (Fig. 1).

THE LIS1 GENE AND LIS1/PAFAH1B1 PROTEIN

The LIS1 gene was cloned in 1993 following recognition of an association between LIS and deletions of chromosome 17p13.3 [Reiner et al., 1993]. The LIS1 gene (Genbank accession number: NM_000430) spans ~92 kb at the genomic level and is comprised of 11 exons with a coding region of 1,233 base pairs from exons 2 to 11 (Fig. 2). There are two alternative transcripts (5.5 kb and 7.5 kb) that differ in the length of their respective 3′ UTR regions [Lo Nigro et al., 1997]. Both transcripts are expressed ubiquitously and encode a protein of 410 amino acids, known as LIS1 or PAFAH1B1 [Hattori et al., 1994]. LIS1/PAFAH1B1 is highly conserved between species with only a single amino acid difference between mouse and human [Peterfy et al., 1994], three amino acid differences between human and bovine [Hattori et al., 1995], 70% identity with Drosophila LIS1 [Liu et al., 1999], and 42% homology with the nuclear migration protein NudF in Aspergillus nidulans [Morris et al., 1998]. The LIS1/PAFAH1B1 protein contains a coiled-coil domain at the N-terminus and seven WD40 repeats at the C-terminus [Reiner et al., 1993; Cardoso et al., 2000].

In vitro and in vivo studies of the LIS1/PAFAH1B1 protein suggest two functions. First, LIS1/PAFAH1B1 forms the noncatalytic subunit of the cytosolic G protein-like heterotrimeric cytosolic platelet activating factor (PAF) acetylhydrolase brain isoform Ib (PAF-AH) [Hattori et al., 1994]. In complex with PAFAH1B2 (MIM# 602508; the 29 kDa subunit) and

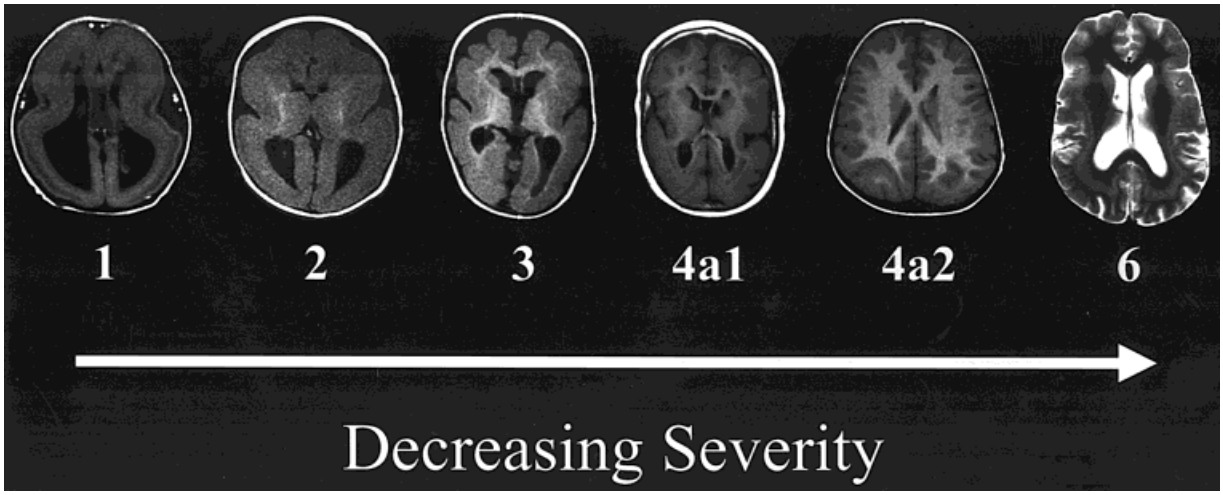

FIGURE 1. **Lissencephaly severity grading system. Magnetic resonance images demonstrating the spectrum of malformations seen in patients with LIS1 deletions or mutations. Grade 1 = complete agyria, grade 2 = posterior agyria and anterior shallow sulci, grade 3 = posterior agyria and anterior pachygyria, grade 4a1 = generalized pachygyria most severe posteriorly, grade 4a2 = posterior pachygyria transitioning to normal gyri anteriorly, and grade 6 = posterior band heterotopia transitioning to normal gyri anteriorly. All scans are axial T1-weighted images, with the exception of the example of grade 6, which is T2-weighted.**

PAFAH1B3 (MIM# 603074; the 30 kDa subunit), LIS1/PAFAH1B1 regulates the level of platelet activating factor in the brain [Hattori et al., 1996]. The regulation of optimal concentrations of brain PAF by this trimeric complex may be critical for correct neuronal migration [Albrecht et al., 1996; Adachi et al., 1997; Bix and Clark, 1998]. Second, it has been shown that LIS1/PAFAH1B1 regulates the microtubule cytoskeleton. The first evidence for this role

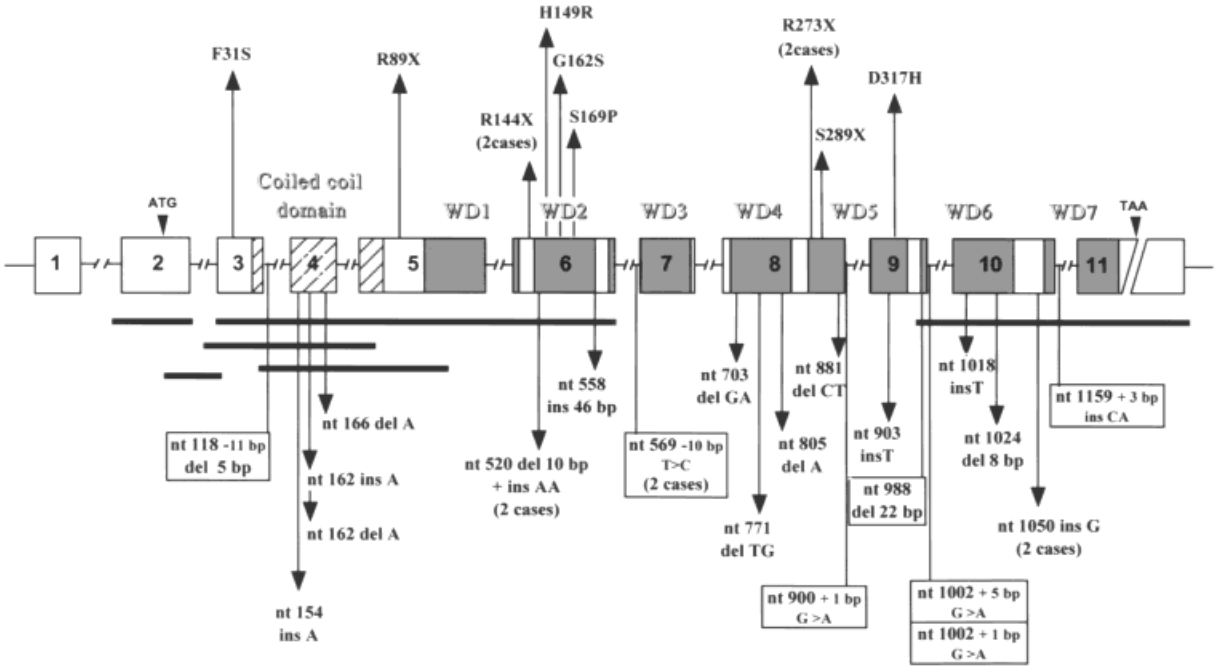

FIGURE 2. **Schematic representation of the LIS1 gene showing genomic structure, known protein domains, and mutations in 41 patients with intragenic mutations of LIS1. Missense and nonsense mutations are denoted by arrows above the gene and frameshift mutations by arrows, splicing defects by boxes and partial deletions by solid black lines (indicating the deleted region) below the gene. Black hatches of the gene indicate the coiled-coil domain and gray shading indicates the seven WD40 repeats.**

came from studies that demonstrate that LIS1/PAFAH1B1 may interact with tubulin, the major component of microtubules, to reduce microtubule events [Sapir et al., 1997]. More recently, it has been showed that LIS1/PAFAH1B1 forms a cytoskeletal complex by interacting with proteins associated with the centrosome and involved in microtubule dynamics such as cytoplasmic dynein, dynactin, NUDE, and NUDEL (two mammalian homologues of the Aspergillus gene NudE) [Efimov and Morris, 2000; Faulkner et al., 2000; Feng et al., 2000; Kitagawa et al., 2000; Niethammer et al., 2000; Sasaki et al., 2000; Smith et al., 2000]. In cooperation with these proteins, LIS1/PAFAH1B1 plays a central role in the organization of the cytoskeleton, which in turn affects neuronal proliferation and migration.

ANIMAL MODELS

Creation of mutants of the Lis1 homologue in Drosophila, has shown that DLis1, similar to the dynein heavy chain gene (Dh64c), is essential for germline cell division [Liu et al., 1999], nuclear positioning [Swan et al., 1999], and oocyte differentiation [Liu et al., 1999; Swan et al., 1999]. Functional analysis of the Lis1 mutant revealed a physical interaction between DLis1 and cytoplasmic dynein and dynactin components, supporting the idea that DLis1 plays a role in microtubule motor based motility [Lei and Warrior, 2000]. More recently using a genetic mosaic analysis of DLis1 in the developing Drosophila brain, Liu and colleagues have analyzed the function in vivo of DLis1 in neurons. These studies showed that underexpression of Lis1 caused a reduction in the proliferation of neurons and this effect could be rescued by a wild type Lis1 transgene [Liu et al., 2000]. The fact that Lis1 is required for both neuroblast proliferation [Liu et al., 2000] and cell division in oogenesis [Liu et al., 1999] supports a role for Lis1 in cell division.

Two knockout mice have been generated by gene targeting [Hirotsune et al., 1998; Cahana et al., 2001]. Both of them confirmed the importance of Lis1, with homozygous null mice dying early in embryogenesis. Heterozygous mice survive, showing evidence of delayed neuronal migration (cortical, hippocampal, cerebellar, and olfactory bulb disorganization) confirmed by in vitro and in vivo cell migration assays [Hirotsune et al., 1998; Cahana et al., 2001] and aberrant morphology of cortical neurons and radial glia in the developing cortex [Cahana et al., 2001]. Interestingly, the heterozygote Lis1 mutant mice created by Cahana et al. showed the production of a shorter mutant protein (sLIS1) which mimics a mutation identified in a lissencephaly patient with a relatively mild phenotype (Table 1 (ILSI07)) [Fogli et al., 1999]. sLIS1was not capable of forming homodimers [Ahn and Morris, 2001] or interacting with PAFAH1B2 and PAFAH1B3. In contrast, these authors found elevated PAFAH enzymatic activity in the brain from heterozygote embryos supporting the demonstration of an in vivo role of LIS1/PAFAH1B1 as a subunit of PAFAH. At present it is uncertain whether the involvement of LIS1/PAFAH1B1 as a component of PAFAH and as a microtubule-related protein are distinct or have related roles in the developing nervous system.

MUTATIONS AND POLYMORPHISMS

Mutations and Large Deletions of the LIS1 Gene

Deletions involving LIS1 are more common than mutations [Pilz et al., 1998b]. In our study of 220 children with LIS (including both ILS and MDS) we found a deletion or mutation of LIS1 in 98 patients. Of these 98 patients, 65 had a deletion detected by fluorescence in situ hybridization (FISH) using the PAC 95H6 clone [Chong et al., 1997] containing the entire LIS1 gene, and 33 had a mutation within the LIS1 gene, or an intragenic deletion. Most were identified by direct sequencing (27/33), while 6/33 were identified by Southern blot analyses [Lo Nigro et al., 1997; Pilz et al., 1998a; Pilz et al., 1999; Cardoso et al., 2000]. A smaller study by Sakamoto et al. [1998] confirmed the high deletion to mutation ratio, showing six of 12 LIS patients having a deletion, and only one a mutation (detected by SSCP) [Sakamoto et al., 1998]. However another study of 24 patients with LIS by Fogli et al. [1999], found three with large deletions of the LIS1 gene, and seven with an intragenic mutation set.

To date, 41 intragenic LIS1 mutations have been found in 41 unrelated individuals (Table 1 and Fig. 1). Of these, five are missense mutations, six are nonsense mutations, 16 are small deletions or insertions causing a frameshift of the open reading frame. Of the remainder, eight are

TABLE 1. *LIS1* Mutations Found in Patients With Isolated Lissencephaly Sequence

Patient	Sex	Exon/ intro	Nucleotide change	Predicted protein	Lissencephaly severity grade	Mutation origin	Reference
Missense mutations							
LP 97-077	F	3	92T>C	F31S	4	De novo	Cardoso et al. [2000]
LP 93-012	M	6	446A>G	H149R	3	De novo	Lo Nigro et al. [1997]
LP 99-127	M	6	484G>A[a]	G162S	4	De novo	Cardoso et al. [2000]
LP 94-051	M	6	499T>C	S169P	6	De novo	Pilz et al. [1999]
LP 98-063	M	9	949G>C	D317H	4	De novo	Cardoso et al. [2000]
Nonsense mutations							
LP 99-058	M	5	265C>T	R89X	3	De novo	Cardoso et al. [2000]
ILS-I12	M	6	430C>T	R144X	2	De novo	Fogli et al. [1999]
LP 97-078	M	6	430C>T	R144X	3	De novo	Pilz et al. [1998a]
LP 89-005	M	8	817C>T	R273X	3	De novo	Lo Nigro et al. [1997]
DP-0201	M	8	817C>T	R273X	4	De novo	Cardoso et al. [2000]
LP 97-086	F	8	866C>G	S289X	3	De novo	Cardoso et al. [2000]
Small deletions or insertions							
ILS-E03	M	4	154-155 ins A	Frameshift	2	De novo	Fogli et al. [1999]
LP 97-068	F	4	162-163 ins A	Frameshift	2	De novo	Pilz et al. [1998a]
Case 4	—	4	162 del A	Frameshift	–	De novo	Sakamoto et al. [1998]
LP 99-053	M	4	166 del A	Frameshift	4	De novo	Cardoso et al. [2000]
ILS-I02	F	6	0-529 del 10bp+ins A	Frameshift	2	De novo	Fogli et al. [1999]
ILS-I05	M	6	0-529 del 10bp+ins A	Frameshift	2	De novo	Fogli et al. [1999]
ILS-S03	M	6	558-559 ins 46bp	Frameshift	2	De novo	Fogli et al. [1999]
LP 97-071	F	8	703-704 del GA	Frameshift	3	De novo	Pilz et al. [1998a]
LR 01-130	**M**	**8**	**771-772 del TG**	**Frameshift**	**3**	**De novo**	**This report**
LP 91-019	F	8	805 del A	Frameshift	3	De novo	Pilz et al. [1998a]
LP 99-039	M	8	881-882 del CT	Frameshift	3	De novo	Cardoso et al. [2000]
LP 97-021	M	9	903-904 ins T	Frameshift	3	De novo	Pilz et al. [1998a]
LP 90-014	M	10	1018-1019 ins T	Frameshift	4	De novo	Pilz et al. [1998a]
LP 01-097	**M**	**10**	**1024-1031 del 8bp**	**Frameshift**	**—**	**De novo**	**This report**
LP 98-100	M	10	1050-1051 ins G[b]	Frameshift	3	De novo	Cardoso et al. [2000]
LR 00-159	**F**	**10**	**1050-1051 ins G**	**Frameshift**	**3**	**De novo**	**This report**
Splice site mutations							
ILS-I07	F	Intron 3	c.118-11 bp del 5bp	Acceptor splice site	3	De novo	Fogli et al. [1999]
LP 99-014	M	Intron 6	c.569-10bp T>C	Acceptor splice site	3	De novo	Cardoso et al. [2000]
LP 99-088	F	Intron 6	c.569-10bp T>C	Acceptor splice site	3	De novo	Cardoso et al. [2000]
LR 00-083	**M**	**Intron 8**	**c.900+1bp G>A**	**Donor splice site**	**3**	**De novo**	**This report**
LP 93-005	M	Intron 9	c.988 del 22bp	Missing exon 9	4	De novo	Lo Nigro et al. [1997]
LP 91-012	M	Intron 9	c.1002+1bp G>A	Donor splice site	3	De novo	Cardoso et al. [2000]
LP 97-069[c]	F	Intron 9	c.1002+5bp G>A	Donor splice site	3	De novo	Pilz et al. [1998]
LP 90-020	M	Intron 10	c.1159+3bp ins CA	Donor splice site	3	De novo	Pilz et al. [1998]
Partial deletions							
LP 85-002	F	2	Rearrangement Southern	Deletion	2	De novo	Cardoso et al. [2000]
LP 91-022	M	2 to 7	Rearrangement Southern	Deletion	3	De novo	Cardoso et al. [2000]
LP 88-009	F	2, 3	Rearrangement Southern	Deletion	2	De novo	Cardoso et al. [2000]
LP 90-012	F	3, 4, 5	Rearrangement Southern	Deletion	2	De novo	Pilz et al. [1998a]
LP 97-064	F	3, 4 ,5	Rearrangement Southern	Deletion	2	De novo	Pilz et al. [1998a]
ILS-F01	F	3′end	Rearrangement Southern	Deletion	2	De novo	Fogli et al. [1999]

All novel reported mutations (in bold face type) have been confirmed as causative by showing their absence in 100 controls and have been verified by at least two independent detection methods.
[a]In Cardoso et al. [2000], G>T at 484 was incorrectly reported as G>T at 486.
[b]In Cardoso et al. [2000], 1050 ins G was incorrectly reported as 1049 ins G.
[c]In Pilz et al. [1998a], LP97-069 was incorrectly numbered as LP97-067.

splicing defects, which were confirmed by RT-PCR analysis where cell lines were available, and six are intragenic or partial deletions of the LIS1 gene. The mutations are spread throughout the gene, with clustering (17/41) in exons 8, 9, and 10, which code for WD40 repeats 4, 5, and 6 of the LIS1/PAFAH1B1 protein. Interestingly, no mutations have been found so far in exon 7, which codes for WD40 repeat 3. Another hot spot for mutations is in exon 4, between nucleotides 154 and 162, corresponding to a stretch of adenines. In this region, three mutations with an insertion or deletion of one adenine, resulting in a frameshift, have been identified. This region codes for the coiled-coil domain, which is the domain of interaction of LIS1/PAFAH1B1 with several cytoskeletal proteins [Feng et al., 2000; Kitagawa et al., 2000; Sasaki et al., 2000].

Only five missense mutations have been identified so far, found in exons 3, 6, and 9, which code for the coiled-coil domain, and the second and fifth WD40 repeats, respectively (Table 1). Of these, 4/5 have been identified in one of the WD40 repeat domains, with 3/5 located in the second WD40 repeat. This repeat has been shown to be critical for the binding of the LIS1/PAFAH1B1 protein to the PAFAH1B2 and PAFAH1B3 subunits, to form the trimeric complex of PAF-AH [Sweeney et al., 2000]. In four missense mutations, the amino acids removed are highly conserved between species and within species. The first (F31S) is located in the coiled-coil domain, and results in a conserved aromatic and hydrophobic amino acid (phenylalanine) being changed to a hydrophilic short polar side chain amino acid (serine). For the remaining three mutations, the histidine at position 149, the serine at position 169, and the aspartate at position 317 cannot be replaced, as they are highly conserved in all proteins with WD40 repeats, including those from other species [Smith et al., 1999]. Even if a change to a closely related amino acid occurs, such as histidine to arginine (H149R) or aspartate to histidine (D317H), it seems that these substitutions have significant consequences on the integrity of the LIS1/PAFAH1B1 protein.

The fifth missense mutation identified results in a change to a functionally related amino acid (a hydrophobic glycine to hydrophilic serine (G162S)). This amino acid change has been seen as a variant in the usual amino acid sequence in other members of the WD40 protein family [Smith et al., 1999]. The clinical features of this patient are very interesting as he has the mildest lissencephaly phenotype of any reported patient with lissencephaly secondary to a LIS1 mutation including normal intelligence, infrequent seizures, and mild speech delay. His brain MRI shows grade 4a(2) LIS with pachygyria restricted to the occipital and posterior parietal lobes bilaterally transitioning to a normal cortex anteriorly. The rest of the brain was normal [Leventer et al., 2001]. Consequently, it can be speculated that the amino acid change in this patient may result in a milder conformational change of the protein and thus less deleterious functional consequences. The imaging and clinical features of the five patients with missense mutations of the LIS1 gene are generally milder and more heterogeneous than patients with other mutations. Of these five patients, the most severely affected had a LIS grade of average severity for intragenic LIS1 mutations (grade 3), and the other four patients had milder grades (grades 4 and 6). In addition, three of these patients had features not seen in other patients with LIS1 mutations including one with moderate to severe cerebellar vermis hypoplasia, one with SBH, and another with a completely normal gyral pattern anteriorly [Leventer et al., 2001].

Five mutations were recurrent (Table 1), including a C>T substitution at position 430 in exon 6, and a C>T substitution at position 817 in exon 8, resulting in protein truncations R144X and R273X, respectively. The other three recurrent mutations are a 10 bp deletion with the insertion of two A in exon 6 at position 520 causing a frameshift with a stop codon 16 bp downstream; an insertion of a G at position 1050; and a splice donor site change (T>C) in intron 6. None of the mutations described here were detected in parental DNA or in 100 unrelated controls, indicating that they are de novo events.

Description of Novel Mutations

Four mutations in unrelated patients have not been previously reported, including one recurrent mutation (Table 1). They were identified by direct sequencing of the LIS1 gene from genomic patient DNA, undertaken twice to confirm the change. The mutations were not found in the parental DNA or in 100 normal chromosomes sequenced, confirming that the mutations

were de novo. Among these, three are small deletions or insertions that predict a truncated protein (frameshift), and one affects an essential nucleotide in the splice donor site of intron 8. The recurrent mutation is an insertion of a G at position 1050 of the LIS1 gene leading to a frameshift.

SBH Caused by a LIS1 Mutation

One of the missense mutations results in a serine to proline change at position 169 of the LIS1/PAFAH1B1 protein (see above), and was identified in a male patient with SBH (Grade 6) [Pilz et al., 1999]. This patient's SBH was posterior predominant, as opposed to the anterior predominance or diffuse bands seen in patients with a DCX mutation [Pilz et al., 1998a; Dobyns et al., 1999]. The modeling of this mutation in recent studies has shown that the change of a conserved serine to a proline in the second WD40 repeat of the LIS1/PAFAH1B1 protein abolishes its interaction with the protein NUDE [Feng et al., 2000]. Based on this data, it would be expected that this mutation would lead to a more severe lissencephaly phenotype. The most plausible explanation for the relatively mild nature of this patient's malformation, as we suggested previously, is the presence of somatic mosaicism, which we could neither prove nor exclude by DNA dilution and PCR-RFLP studies [Pilz et al., 1999].

Polymorphisms

The LIS1 gene is highly conserved and only a few polymorphisms have been found so far, none of which affects the amino acid sequence (Table 2). These include an A>T change at nucleotide position 687 in exon 8 (T229) and a C>T change at nucleotide position 1188 in exon 11 (V396), found in two unrelated boys and their unaffected mothers. We also identified a T>C substitution in intron 6 at nucleotide position 568 + 27 bp and two polymorphisms in the 3′UTR of the LIS1 transcript, a C >T substitution at nucleotide position 1250 and a T>G substitution at nucleotide position 1236.

CLINICAL SIGNIFICANCE

Mild Cases, Survival, and Medical Complications

LIS associated with abnormalities of the LIS1 gene usually results in severe clinical sequelae. These include severe or profound mental retardation, early-onset intractable epilepsy, early and persistent hypotonia that may progress to mild spastic quadriparesis, and major feeding problems [Dobyns et al., 1993]. Early data suggested that lifespan is usually shortened with few patients surviving beyond the first decade, the major causes of death being aspiration pneumonia and sepsis [Dobyns et al., 1993]. However, our experience suggests that lifespan has been improved with more aggressive management of nutrition, aspiration, and seizures. In general, the clinical severity correlates with the extent of agyria and cortical thickening [Barkovich et al., 1991], although the presence of intractable epilepsy may worsen clinical severity beyond that predicted by the malformation. Recently a number of patients have been reported with milder malformations and milder clinical phenotypes, including one boy with normal intelligence whose infrequent seizures, poor fine motor coordination, and mild speech problems are his only significant disabilities [Leventer et al., 2001]. These milder phenotypes are rare, and all are associated with missense mutations. We have suggested that the low prevalence of these milder phenotypes may be related to ascertainment bias in that only those with typical severe LIS are generally referred for sequencing [Cardoso et al., 2000; Leventer et al., 2001].

Genotype–Phenotype Correlation and Functional Consequences

A recent analysis of the relationship between genotype and phenotype has revealed a correla-

TABLE 2. *LIS1* Polymorphic Variants

Position in the gene	Nucleotide change	Sequence variation	Frequency (estimation)	Reference
Intron 6	**c.568+27bpT>C**	**Intronic**	**~1.5%**	**This report**
Exon 8	687A>T	T229T	Rare (1 case)	Cardoso et al. [2000]
Exon 11	1188C>T	V396V	Rare (1 case)	Cardoso et al. [2000]
Exon 11	1236T>G	3′UTR	~10%	Cardoso et al. [2000]
Exon 11	1250C>T	3′UTR	~40%	Koch et al. [1996]

Bold face indicates new polymorphisms not previously published.

tion between LIS severity and the location and type of LIS1 mutation [Cardoso et al., 2000]. LIS secondary to deletions on 17p13.3 result in the most severe phenotype, with the largest deletions producing the most severe LIS grade (complete agyria), and often the clinical picture of MDS. This suggests that there may be a second gene for cortical development located on 17p13.3, which is deleted in addition to LIS1 in patients with MDS. Intragenic mutations resulting in a truncated or internally deleted protein will generally result in a more severe phenotype than missense mutations. In addition, mutations other than missense mutations occurring in the N-terminal region of the gene (coding for the coiled-coil domain) will generally produce a more severe phenotype than will mutations occurring towards the C-terminal end (coding for the WD40 repeats).

Several studies of the LIS1/PAFAH1B1 protein show that two domains within the protein are essential for its function. Both domains are involved in protein/protein interaction. The first is a coiled-coil domain at the N-terminal region spanning exon 2 to 4. This domain allows the LIS1/PAFAH1B1 protein to interact with itself to form homodimers [Ahn and Morris, 2001]. In vitro and in vivo studies also showed that LIS1/PAFAH1B1 interacts with NUDE and NUDEL through their respective N-terminal regions [Efimov and Morris, 2000; Kitagawa et al., 2000]. The second important region is the seven WD40 repeats domain forming a seven-bladed propeller-like structure involved in protein-protein interactions [Garcia-Higuera et al., 1996]. The WD40 repeat region appears to have three important domains for protein/protein interaction. The first is the second WD40 repeat encoded by exon 6. This domain appears to be critical for the correct interaction of PAFAH1B1 with the other two subunits of the PAF-AH complex (PAFAH1B2 and PAFAH1B3) [Sweeney et al., 2000]. The second is the seventh WD40 repeat by which LIS1/PAFAH1B1 has been proposed to interact with DCX [Caspi et al., 2000]. The third is a poorly localized domain within the WD repeats that appears to interact with β spectrin trough, its pleckstrin homology domain [Wang et al., 1995].

To understand the functional consequences of specific mutations, four independent studies have been performed which included the modeling of known human lissencephaly-causing mutations of LIS1 [Sapir et al., 1999; Feng et al. 2000; Sasaki et al., 2000; Sweeney et al., 2000]. These studies have all shown that the modeled mutations abrogate the binding of LIS1/PAFAH1B1 with protein partners such as PAFAH1B2, PAFAH1B3, NUDE, or NUDEL, and thus may interfere with functions such as neuroblast proliferation and migration or maintenance of brain levels of PAF. The finding that intragenic mutations generally result in milder LIS phenotypes than complete gene deletion may imply that partial function is maintained by the mutant LIS1/PAFAH1B1 protein. To address this question two studies used western blot experiments to detect the presence of mutant protein in a total of 14 patients with LIS secondary to intragenic LIS1 mutations using lymphoblastoid cell lines [Fogli et al., 1999; Cardoso et al., 2000]. Of these, a mutant protein was only found in one case—a child with a relatively mild LIS phenotype (grade 4) and a splice site mutation leading to the skipping of exon 4 (Table 1; ILSI07) [Fogli et al., 1999]. In the other 13 patients for whom no mutant protein could be detected, it is possible that mutant proteins were not detected due to instability and rapid protein degradation.

DIAGNOSTIC STRATEGIES

Our approach to the evaluation of children with LIS or SBH is summarized in Figure 3. The initial assessment begins with a clinical evaluation, which involves obtaining an accurate family history and performing a careful examination for dysmorphic features or additional congenital anomalies. The presence of a maternal family history of similar problems is suggestive of a DCX mutation, whereas the presence of dysmorphic facial features is more consistent with a deletion of 17p13.3 involving LIS1. To date, there have been no familial recurrences of intragenic LIS1 mutations. Following clinical evaluation, the brain images should be carefully reviewed by an expert familiar with the assessment of cortical malformations. It is usually possible to distinguish between the LIS1 (p>a) and DCX (a>p) malformation patterns with high quality imaging. The initial laboratory investigation should consist of a high quality cytogenetic analysis. If an abnormality is found, then parental chromosomes must be analyzed to exclude an inherited rearrangement. If there are no structural cytogenetic

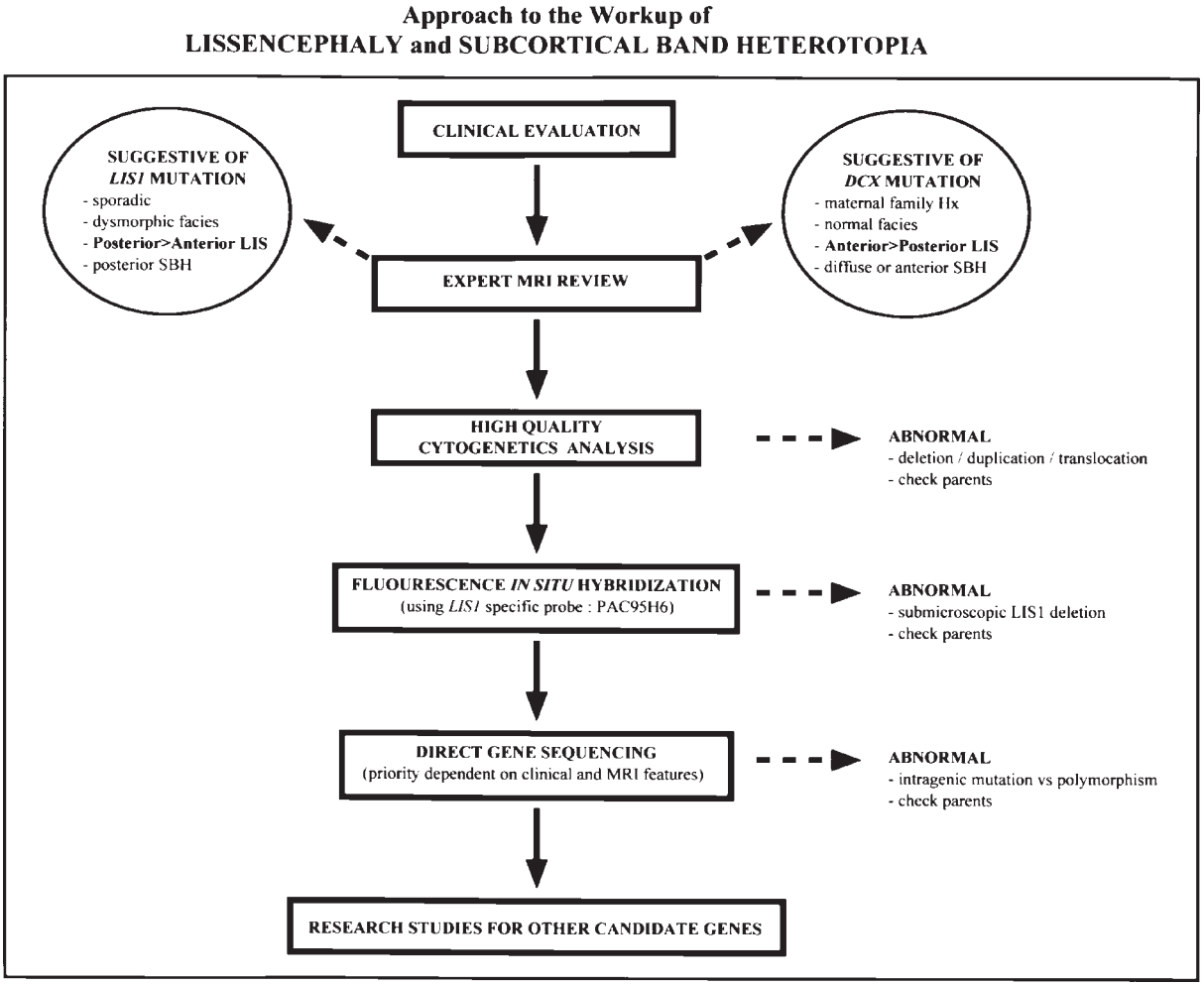

FIGURE 3. **Strategy for diagnostic testing of lissencephaly. Recommended approach to the clinical, imaging, and genetic diagnostic work-up of a patient with lissencephaly or subcortical band heterotopia. (Consultation with a center specialized in the field of cortical malformations will be required for atypical cases and for referral of patients for inclusion in research studies to identify new candidate genes.)**

abnormalities, then further specialized testing, including FISH and direct gene sequencing, should be pursued, guided by the clinical and imaging data. If the gradient is p>a, suggesting an abnormality in LIS1, FISH using PAC 95H6 is indicated, followed by LIS1 sequencing if FISH is normal. An a>p gradient or SBH in females suggests an abnormality in DCX. In these cases, DCX sequencing is indicated. In some cases, where no deletion or mutation can be identified by FISH or direct sequencing, but where the suspicion of a LIS1 abnormality is high, we propose the use of specialized FISH probes or Southern blot analysis. To date, this approach has proven helpful in identifying several cases of children with lissencephaly due to a partial deletion of the LIS1 gene (Table 1).

FUTURE PROSPECTS

Our recent finding of patients with LIS1 missense mutations associated with milder cerebral malformations and clinical sequelae raises the question as to the true phenotypic spectrum associated with mutations within this gene [Cardoso et al., 2000; Leventer et al., 2001]. The few missense mutations found so far may imply that patients with milder phenotypes are not being referred for LIS1 sequencing. To answer this question, LIS1 mutation screening will need to be performed on a wider group of patients with milder clinical features (such as epilepsy or mental retardation), or more limited cortical malformations (such as localized pachygyria or atypical SBH).

Rapid advances in our understanding of the function of the LIS1/PAFAH1B1 protein at the

cellular level will no doubt lead to further functional experiments. These experiments will facilitate a greater understanding of the precise role and importance of LIS1/PAFAH1B1 in the processes of cell division, cell motility, and cortical organization. Through such studies, we may gain a better insight into the pathways and partners with which LIS1/PAFAH1B1 interacts, both within normal brain development and within other organ systems. Concurrent with this work, the modeling of naturally occurring human LIS1 mutations may shed light into the mechanisms by which the maldevelopment of the cortex occurs.

Even after a complete assessment for deletions or mutations of LIS1 or DCX, a significant percentage of patients with LIS or SBH remain in whom no genetic etiology can be identified. In our experience studying 220 children with these rare malformations, an etiology could not be identified in over 40% of patients. The majority of those without an identified cause have been found to have an atypical malformation type, such as LIS and severe cerebellar hypoplasia. For typical cases of classical LIS or SBH, the chances of finding a genetic abnormality in either LIS1 or DCX are closer to 80%. Identification of the pathways in which LIS1/PAFAH1B1 and DCX function, and their cooperating proteins, may help identify candidate genes to be considered for sequencing in those patients without LIS1 or DCX mutations.

CONCLUSIONS

The study of patients with LIS or SBH has enabled the identification of two proteins involved in cortical development, and has accelerated our understanding of the process of neuronal migration. The LIS1 gene appears to be a critical component within this process. In addition, it appears to have other roles in fundamental biological processes conserved across species. Mutations of LIS1 generally result in significant clinical sequelae, yet the full spectrum of disorders related to dysfunction of this and related proteins remains to be elucidated.

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