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ARTICLE

A revision of the lissencephaly and Miller–Dieker syndrome critical regions in chromosome 17p13.3

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Miller–Dieker syndrome (MDS) is a multiple malformation syndrome characterized by classical lissencephaly and a characteristic facies. It is associated with visible or submicroscopic deletions within chromosome band 17p13.3. Lissencephaly without facial dysmorphism has also been observed and is referred to as isolated lissencephaly sequence (ILS). Apparently partial and non-overlapping deletions of the 5′ or 3′ end of a candidate gene *LIS1* in one ILS and one MDS patient had suggested that MDS was a single gene disorder, and that *LIS1* spans in excess of 400 kb. However, the originally presumed 5′ end of *LIS1* was found to belong to the *14-3-3*ε gene residing more distally on 17p13.3. We have now isolated the correct 5′ end of *LIS1*, constructed a ~500 kb genomic contig encompassing *LIS1*, and estimated its gene extent to be ~80 kb. Fluorescence *in situ* hybridization analysis of an ILS patient with a *de novo* balanced translocation, as well as analysis of several other key MDS and ILS deletion patients, localizes the lissencephaly critical region within the *LIS1* gene. Therefore, *LIS1* remains the strongest candidate gene for the lissencephaly phenotype in ILS and MDS. Our analyses also suggest that additional genes distal to *LIS1* may be responsible for the facial dysmorphology and other abnormalities seen in MDS but not in ILS patients, supporting our original concept of MDS as a contiguous gene deletion syndrome.

INTRODUCTION

Classical lissencephaly or ‘smooth brain’ is a severe malformation of the brain manifest by a smooth cerebral surface with lesser involvement of the cerebellum and other rhombic lip derivatives. It results from incomplete neuronal migration to the cerebral cortex and rhombic lip derivatives at 9–13 weeks of embryonic development (1). Affected children have severe or profound mental retardation, epilepsy, and subtle facial abnormalities especially bitemporal hollowing and small jaw (2).

Classical lissencephaly occurs in several malformation syndromes. Miller–Dieker syndrome (MDS) consists of classical lissencephaly, characteristic facial abnormalities and sometimes other birth defects (3). The facial changes consist of prominent forehead, bitemporal hollowing, short nose with upturned nares, flat midface, protuberant upper lip with thin vermillion border and small jaw. It is associated with visible or submicroscopic rearrangements within chromosome band 17p13.3 in almost all patients (4). Isolated lissencephaly sequence (ILS) consists of classical lissencephaly and its direct sequela with no other major anomalies (2). Submicroscopic deletions of chromosome 17p13.3 have been detected in almost 40% of patients (4). Classical lissencephaly also occurs in X-linked lissencephaly and subcortical band heterotopia, which has been mapped to chromosome Xq22.3–q23 (5).

We previously isolated a candidate gene for MDS (*LIS1*; 6) which was subsequently shown to be the human homologue of the 45K subunit of the brain isoform of platelet activating factor acetylhydrolase isolated from bovine cerebral cortex (7). One of the four LIS1 cDNA clones (8-1) was initially thought to contain the 5′ end of the gene, such that apparently non-overlapping deletions in the 5′ or 3′ end of the gene were detected in one ILS and one MDS patient (although reported as two MDS patients). This suggested that partial or complete deletion of *LIS1* was necessary and sufficient for expression of the complete MDS phenotype (6). We recently discovered that clone 8-1 does not contain the 5′ end of *LIS1*, but instead is a chimera of downstream

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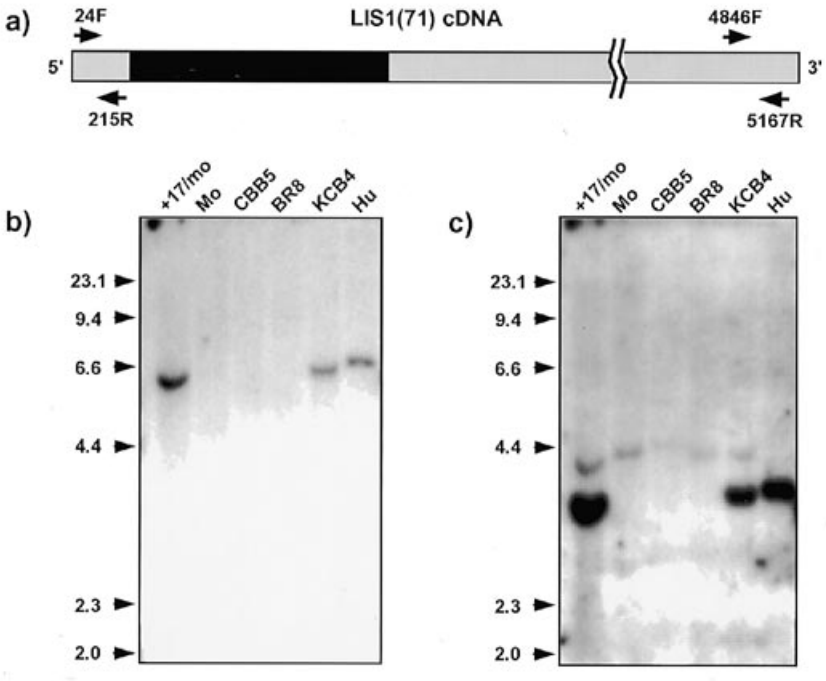

Figure 1. Southern blot analysis of LIS1 cDNA clone 71 on a panel of MDS/ILS chromosome 17 somatic cell deletion hybrids. (**a**) Schematic of LIS1(71) cDNA showing positions of primer pairs used to generate hybridization probes. Primer sequences and amplicon lengths are found in Materials and Methods. (**b**) Results of hybridization with probe 24F/215R. Lanes beginning from the left are mouse hybrid containing only human chromosome 17 (+17/mo), total mouse genomic (Mo), mouse hybrids of ILS-024 (CBB5), MDS-009 (BR8), and MDS-019 (KCB4), and total human genomic (Hu). DNAs were digested with *Pst*I. This probe, which detects a ~6.5 kb fragment from human chromosome 17, is deleted in patients ILS-024 and MDS-009 but is not deleted in patient MDS-019. Size markers are in kb (λ*Hin*dIII digest). (**c**) The same blot after removal of probe 24F/215R and rehybridization with probe 4846F/5167R, showing its deletion in patients ILS-024 and MDS-009 and presence again in MDS-019.

LIS1 sequences and another more distally located gene *14-3-3ε* (8). Since clone 8-1 was used in initial characterization studies of *LIS1* deletions in MDS patients (6), we reassessed the candidacy of *LIS1* as the MDS causative gene. For this purpose, we have isolated the true 5′ end of *LIS1* including the promoter region, and generated a genomic contig encompassing *LIS1*.

Using these reagents, we performed fluorescence *in situ* hybridization analysis (FISH) on an ILS patient with a *de novo* 17;19 balanced translocation. As a result, we have localized his chromosome 17 breakpoint to a large intron within the 5′ untranslated region (UTR) of *LIS1*. Additionally, our FISH analyses of patients with interstitial deletions have revealed one MDS and three ILS patients whose proximal or distal breakpoints also occur in the same interval within *LIS1*. Based on these findings, we have redefined the lissencephaly minimal critical region to a <100 kb region centromeric to *D17S379* and telomeric to *D17S1566*, coinciding with the *LIS1* gene. The ILS translocation breakpoint within *LIS1*, and the partial deletions of *LIS1* in ILS and MDS deletion patients, strongly suggest that *LIS1* is the lissencephaly causative gene. The data, however, do not support the hypothesis that *LIS1* is also responsible for the facial dysmorphism and other anomalies of MDS patients.

RESULTS

LIS1 clone 71 is not deleted in patient MDS-019

Patient MDS-019 was previously shown to be deleted for the 5′ end of the *LIS1* gene by analysis of somatic cell hybrids using cDNA clone 8-1 as probe, as well as by fluorescence *in situ* hybridization (FISH) analysis of patient lymphoblasts using cosmids isolated with clone 8-1 (6). LIS1 clone 8-1 has since been determined to be chimeric and does not contain the 5′ end of the *LIS1* gene (8). We therefore reassessed patient MDS-019 by Southern blot analysis of a somatic cell hybrid containing her abnormal chromosome 17, using the largest non-chimeric LIS1 cDNA we had as probe (clone 71, Fig. 1a). Using probe 24F/215R representing the 5′ end of clone 71, a ~6.5 kb human chromosome 17 specific *Pst*I fragment is present for the hybrid of MDS-019 and absent for the hybrids from patients MDS-009 and ILS-024 (Fig. 1b). When the same blot was rehybridized with probe 4846F/5167R representing the 3′ end of clone 71, a ~4 kb human 17-specific *Pst*I fragment is again detected for patient MDS-019 but not for patients MDS-009 and ILS-024 (Fig. 1c). These data indicate that MDS-019 is not deleted for the *LIS1* gene portion represented by the 5.2 kb cDNA clone 71.

Isolation of the *LIS1* promoter

Since deletion of the promoter of any gene can effectively extinguish its expression, we wished to determine if the promoter and 5′ UTR sequences of *LIS1* could be deleted in MDS-019. The high homology of the 5′ UTR between human LIS1(47) and mouse Lis1 cDNAs (9) strongly suggests that the entire 5′ UTR of LIS1(47) is *LIS1*-specific. Nonetheless, based on the ~7.5 kb size of the largest LIS1 transcript (9), the lack of a polyadenylation signal close to the 3′ end of the 5.2 kb LIS1(71) cDNA (6), and the presence of a long 5′ UTR in the bovine homolog (7), we

Figure 2. Sequence of 1481 bp genomic fragment containing the 5′ end of *LIS1*, beginning from a *Dra*I restriction site (*). CCAAT and TATAA boxes are double underlined. Sequence overlap with 5′ UTR of LIS1(47) cDNA is indicated in bold. A complete Alu sequence and a partial Alu fragment are indicated by dotted lines. The *Ssp*I site and *Dra*I half-site are italicized. Positions and directions of extension of primers used in genomic PCR and RT-PCR analyses are indicated (sequences of 'R' primers are the complement of those underlined).

deduced that among other things a portion of the 5′ UTR of LIS1 could still be unaccounted for.

Attempts to isolate additional 5′ UTR sequences of LIS1 cDNA by reverse-transcription-PCR (RT-PCR) did not yield any distinct fragments (data not shown). We therefore chose a genomic PCR approach and isolated a 1.5 kb fragment extending upstream of the LIS1(47) 5′ UTR. Within this fragment resides a TATAA box, as well as a CCAAT box ~10 nucleotides upstream of it (Fig. 2). Northern blot analysis using this 1.5 kb genomic fragment detects the characteristic double LIS1 transcripts of ~7.5 and ~5.5 kb (Fig. 3), confirming that it represents the 5′ end of *LIS1*. RT-PCR analysis of human brain poly(A)$^+$ RNA using primer pair CP2F and CP6R did not yield any product, nor was product obtained when either CP9F or CP7F was paired with LIS1(47)73R or LIS1(47)86R, whereas a ~200 bp fragment was obtained when CP8F was used as the forward primer against LIS1(47)73R or LIS1(47)86R (data not shown). These results suggest that LIS1 transcription initiates between the CP7F and CP8F primer sites (Fig. 2).

The *LIS1* promoter is deleted in MDS-019

We retested patient MDS-019 and the two other patients described above for the presence of the *LIS1* promoter by Southern blot analysis, and the results now show all three patients to be deleted for a ~3 kb chromosome 17-specific *Hin*dIII fragment containing the *LIS1* promoter (Fig. 4). Thus, while patients MDS-009 and ILS-024 are deleted for all of *LIS1* on the affected chromosome 17, patient MDS-019's deletion involves only part of the 5′ UTR and sequences upstream. Based on the results using LIS1(71) cDNA and the *LIS1* promoter fragment, we conclude that the proximal deletion breakpoint of MDS-019 must lie downstream of the promoter but upstream of the LIS1(71) 5′ UTR sequence. Significantly, no altered fragment size is detected in MDS-019 using the LIS1(71) 24F/215R probe (Fig. 1b) and no fragment of any size is detected with the *LIS1* promoter probe (Fig. 4). These observations imply the presence of a large intron within the 5′ UTR of LIS1, which is supported by our Southern analysis results and confirmed by exon–intron

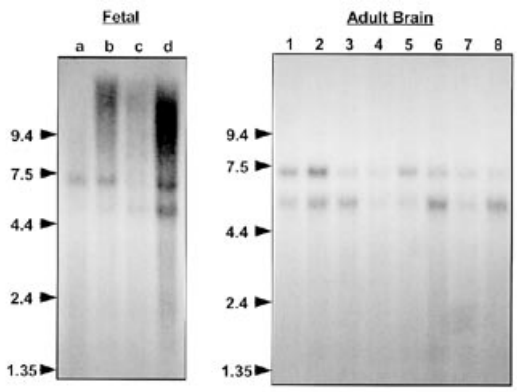

Figure 3. Northern blot analysis of *LIS1* 5′ end probe on fetal tissues and adult brain tissues. The fetal blot was hybridized with the entire 1.5 kb fragment, while the adult brain blot was hybridized with a truncated 0.9 kb fragment starting from the *Ssp*I site and extending 3′ (see Fig. 2). a, brain; b, lung; c, liver; d, kidney; 1, amygdala; 2, caudate nucleus; 3, corpus callosum; 4, hippocampus; 5, whole brain; 6, substantia nigra; 7, subthalamic nucleus; 8, thalamus. LIS1-specific transcripts of ~7.5 and ~5.5 kb are detected in varying intensities within and between tissues. Size markers are in kb.

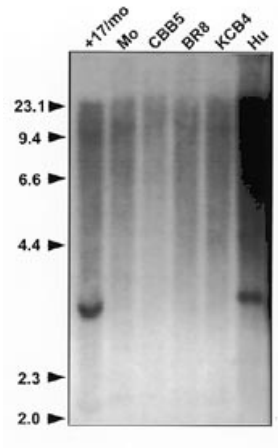

Figure 4. Southern blot analysis of *LIS1* 5′ end probe on MDS/ILS chromosome 17 somatic cell hybrid panel. DNAs were digested with *Hin*dIII. This probe detects a ~3 kb fragment from human chromosome 17, and is deleted in all three patients (ILS-024, MDS-009 and MDS-019). Size markers are in kb (λ*Hin*dIII digest).

sequence analysis of *LIS1*-containing genomic clones (see below, 10).

A cosmid/P1/PAC contig spanning the *LIS1* gene

To determine the extent of the *LIS1* gene and its relationship to the lissencephaly and Miller–Dieker syndrome critical regions, we isolated cosmids, P1s and PACs and generated a contig encompassing *LIS1* that extends centromeric for ~150–200 kb and telomeric by the same distance to the L132 (*D17S379*) locus (Fig. 5a). While several cosmids, P1s and PACs contain portions of *LIS1*, only one PAC (95H6) contains the entire gene. Further screening of the LA17NC01 and L4/FS17 chromosome 17-specific libraries did not yield additional cosmids spanning the gap between cosmids 120A7 and 37E9. Based on restriction digestion analyses, we calculated the insert sizes of c120A7, c37E9, PAC-95H6 and PAC-308F9 to be ~35, ~35, ~110 and ~120 kb, respectively (data not shown). Hybridization analyses of *Not*I/*Eco*RI double-digests of PACs 95H6 and 308F9 were also performed using LIS1(71) cDNA, *LIS1* promoter fragment, c154B4, c120A7, c37E9 and c135A6 as probes separately. Examination of these separate hybridization results revealed several internal fragments common to both PAC inserts that did not hybridize with any of the above probes, and that by deduction are derived from a large intron downstream of the 1.5 kb promoter fragment but upstream of the genomic region represented by LIS1(71) cDNA, i.e. within the 5′ UTR (data not shown). We estimate the size of this intron to be ~50 kb from the combined sizes of all the non-hybridizing fragments. The presence of this intron within the 5′ UTR has been confirmed by exon–intron sequencing of *LIS1*-containing genomic clones, as shown in the accompanying paper (10). Additionally, LIS1(71) cDNA probe detected fragments of ~13 and ~7.5 kb from PACs 95H6 and 308F9 as well a ~6 kb fragment from PAC-95H6 (data not shown). The portions of *LIS1* containing the open reading frame (ORF) and 3′ UTR thus constitute ~26.5 kb, and we therefore estimate the entire *LIS1* gene to be ~80 kb in size. Two simple sequence repeats (SSRs), *D17S1566* and *D17S379*, were recently developed and assigned to the Miller–Dieker syndrome chromosome region (11; A. Tanigami *et al.*, in preparation), and we have mapped these markers within the contig to positions flanking the *LIS1* gene (Fig. 5a).

FISH analysis of ILS and MDS patient lymphoblasts

To further define the lissencephaly minimal critical region, we used these genomic clones as probes for FISH analysis on several MDS and ILS deletion patients as well as on a previously unreported ILS patient (ILS-096) who carries a 17p;19q balanced translocation. From these studies we have identified three ILS patients (ILS-035, ILS-103 and ILS-136), apart from MDS-019, who show partial deletions of *LIS1*, and have determined the chromosome 17 translocation breakpoint of ILS-096 (Fig. 5b).

Figure 6 illustrates the FISH results for patients MDS-019 and ILS-103 using two cosmids, c37E9 which contains the promoter and partial 5′ UTR of *LIS1*, and c120A7, containing the ORF and 3′ UTR exons of *LIS1*. The presence of hybridization signal with c120A7 and lack of signal with c37E9 in the affected chromosome 17 from patient MDS-019 confirms our Southern blot data on the position of her proximal deletion breakpoint (Fig. 6a and b). FISH results of patient ILS-035 showed the same results using c120A7 and c37E9 (data not shown). In contrast, no signal is detected on the affected chromosome from patient ILS-103 with c120A7 probe while a signal is present with c37E9, thus defining her distal deletion breakpoint (Fig. 6c and d). FISH analysis of patient ILS-136 using these two probes yielded similar results (data not shown).

Initial cytogenetic analysis of ILS-096 revealed a balanced translocation between chromosome 17 at sub-band p13.3 and chromosome 19 at sub-band q13.13. Using c120A7, signals were detected on the normal 17 and der(17) chromosomes, whereas with c37E9 signals were present on the normal 17 and der(19) chromosomes (Fig. 7). Using a PAC (308F9) that overlaps both cosmids, signals are now detected on both the der(17) and der(19) chromosomes as well as the normal 17. FISH analysis with a second PAC clone (95H6) confirms the findings using PAC-308F9 (data not shown), thus localizing the chromosome 17 translocation breakpoint of ILS-096 to the region of the 5′ UTR and intervening intron of *LIS1* (Fig. 5b).

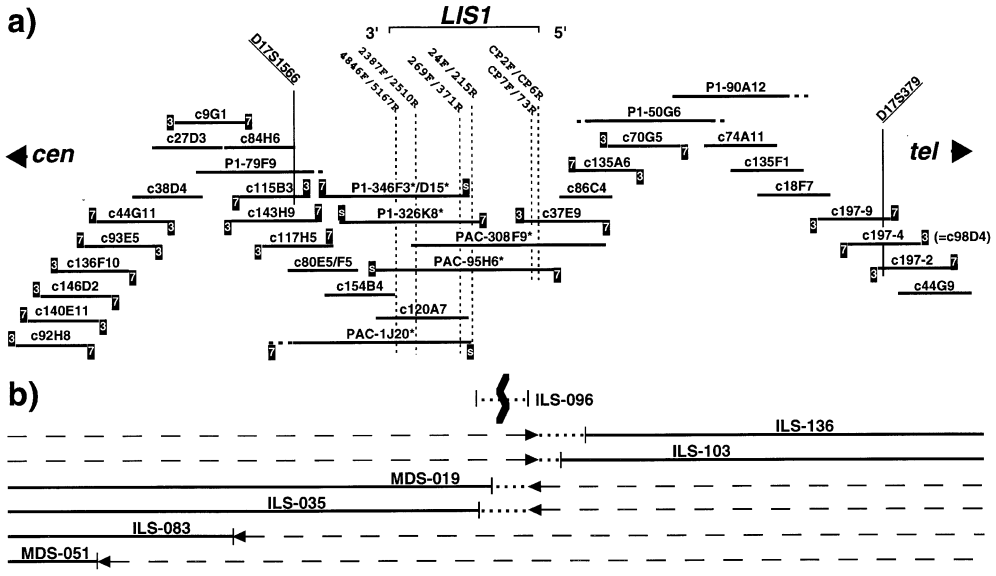

Figure 5. (**a**) Contig of cosmid, P1, and PAC clones spanning the Miller–Dieker lissencephaly critical region (not drawn to scale). Where determined, T3, T7 and Sp6 ends of clones are indicated by 3, 7 and S respectively. Position and orientation of *LIS1* within the contig is indicated. Positions of some primer pairs used in alignment of clones and determination of the *LIS1* gene extent are shown by dotted vertical lines. Positions of the flanking SSR $(CA)_n$ markers *D17S1566* and *D17S379* are indicated by solid vertical lines. Clones isolated from commercial libraries are indicated by asterisks. (**b**) Schematic representation of approximate chromosome 17p breakpoints in balanced translocation patient ILS-096 and six other interstitial deletion patients. Intact regions are shown by solid horizontal lines, broken lines signify deletions, and dotted lines indicate approximate breakpoint ranges.

DISCUSSION

We have identified the correct 5′ end of *LIS1* and estimated the physical extent of this gene to be ~80 kb, oriented 5′ to 3′ from telomere to centromere. Collectively, the FISH analyses of several key ILS and MDS patients have enabled localization of the lissencephaly minimal critical region to the *LIS1* gene locus, which lies between the SSR markers *D17S1566* centromerically and *D17S379* telomerically. These data thus strongly implicate *LIS1* as the gene responsible for the lissencephaly phenotype in ILS and MDS. Curiously, there is an overlap in the deletions of ILS-136 and ILS-103 with those of MDS-019 and ILS-035, occurring where the ~50 kb 5′ UTR intron resides (Fig. 5b). There thus exists the possibility that a small gene resides within this intron, and that it may be deleted or disrupted in these deletion patients and in the translocation patient. However, as shown in the accompanying paper (10), we have recently identified point mutations in two coding exons as well as a small exon–intron deletion within the *LIS1* gene in patients with ILS, thus ruling out that possibility.

Cytogenetic deletions of 17p13.3 have been observed in many MDS patients, while more recent FISH analyses using cosmid probes representing the *D17S379* locus have revealed submicroscopic deletions within 17p13.3 in a significant percentage of ILS patients (4). These findings led us to originally hypothesize that MDS was caused by disruption of more than one gene. Our subsequent identification of apparently non-overlapping deletions of *LIS1* in patients MDS-019 and MDS-021 had suggested otherwise, and instead implicated *LIS1* in the etiology of the complete MDS phenotype (6). Patient MDS-021 had actually been reclassified as patient ILS-084 (by WBD) prior to detection of this abnormality.

We have since discovered that one of the cDNA clones used in that study and presumed to contain the 5′ end of *LIS1* (8-1) actually contains a fragment of another more distal gene *14-3-3ε* (8). With the true 5′ end of *LIS1* now identified, we reassessed these two patients and have shown that patient MDS-019 is deleted for part of the 5′ UTR and promoter region. Our FISH analysis of patient ILS-084 was inconclusive and suggestive of a complex combination of deletion and mosaicism in a lymphoblast cell line. For these reasons, we have not been able to confirm nor revise our previous findings of her deletions. Notwithstanding this patient, our analyses of all other key ILS and MDS deletion patients have enabled the definition of a small region of overlap representing the lissencephaly minimal critical region. Additionally, the co-incident localization of the distal breakpoint of ILS-103 and the proximal breakpoint of MDS-019 (Fig. 5b) to the same region of *LIS1* co-localizes the lissencephaly minimal critical region with the proximal boundary of the Miller–Dieker syndrome chromosome region (MDCR).

Furthermore, our results are consistent with the notion that a gene or genes in addition to *LIS1* are necessary for expression of the full Miller–Dieker syndrome phenotype. For example, patients ILS-035 and MDS-019 have similar proximal deletion breakpoints. Also, complete deletions of *LIS1* occur in ILS and MDS patients alike, for example in patients ILS-083 and MDS-051 (Fig. 5b). Additionally, deletions that start from *LIS1* and extend towards the centromere have only been documented in ILS patients (ILS-103 and ILS-136, Fig. 5b). In contrast,

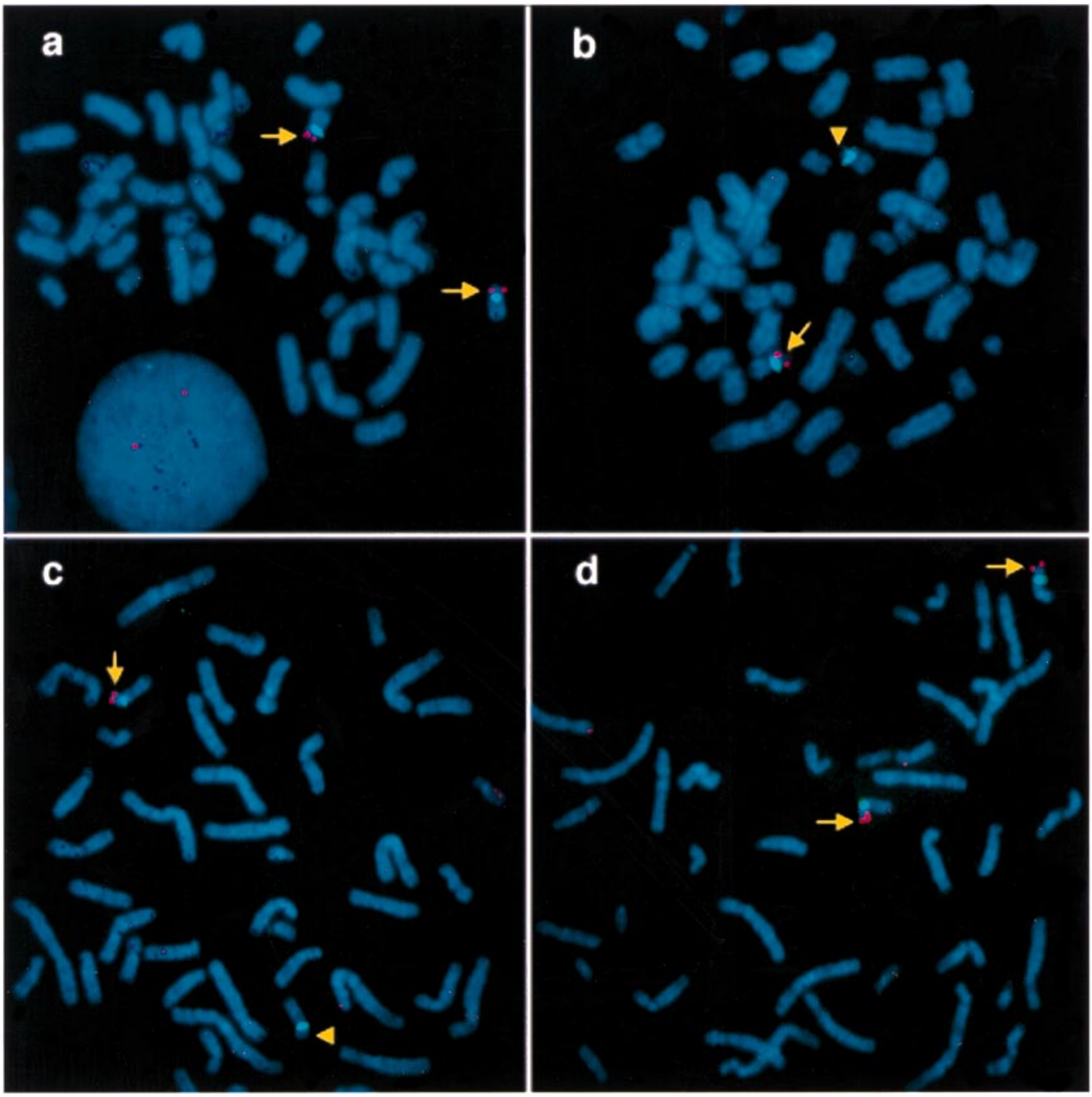

Figure 6. FISH analysis of patients MDS-019 (**a, b**) and ILS-103 (**c, d**). When cosmid 120A7 is used as probe, a signal is present on both chromosomes 17 of MDS-019 (a) but only one chromosome 17 of ILS-103 (c). With cosmid 37E9, only one signal is now detected for MDS-019 (b) and both chromosomes 17 of ILS-103 are now positive (d). Cosmid signals are red, and chromosome 17 α-satellite signals are green. Arrows indicate presence of probe signal and arrowheads indicate deletion of probe.

deletions of MDS patients may or may not involve loci centromeric to *LIS1*, but always involve *LIS1* and other telomeric loci in excess of 250 kb. Although deletions of ILS patients may also include more distal loci (e.g. ILS-024), FISH analyses using newly isolated cosmids within the MDCR have shown that the distal breakpoints of MDS patients are always more telomeric than those of ILS patients (our unpublished data). These observations are thus consistent with the concept of an additional gene or genes telomeric to *LIS1* contributing to the facial dysmorphology and other abnormalities seen in MDS but not in ILS patients, supporting our original hypothesis of MDS as a contiguous gene deletion syndrome. The possibility of modifier loci outside of 17p13 being responsible for the MDS-specific phenotype is inconsistent with previous observations in familial cases of MDS cases involving balanced translocations or inversions, in which all unbalanced children with 17p monosomy have the full MDS phenotype.

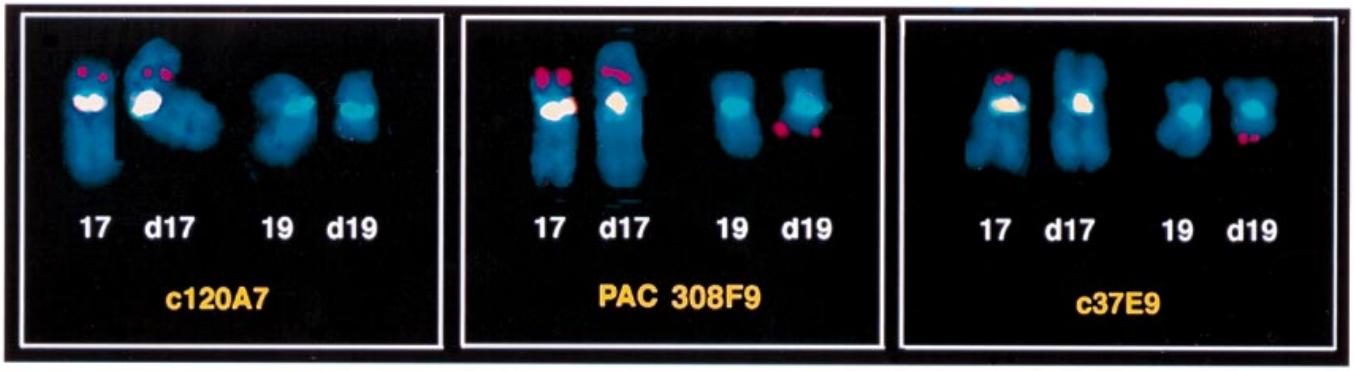

Figure 7. FISH analysis of balanced translocation patient ILS-096, showing a partial karyotype including the normal and derivative chromosomes 17 and 19 only. Hybridization with c120A7 reveals signals on the normal and derivative chromosomes 17 whereas c37E9 detects signals on the normal 17 and der(19). Hybridization with PAC-308F9, which overlaps both cosmids, results in signals on both the der(17) and der(19), indicating that this PAC spans the translocation breakpoint. Cosmid and PAC signals are red, chromosome 17 α-satellite signals are yellow, and chromosome 19 α-satellite signals are green.

Table 1. ILS and MDS patients and somatic cell hybrids described in this study

Diagnosis number	Log number	Hybrid cell line
ILS-024	LP89-035	CBB5
ILS-035	LP90-023	–
ILS-083	LP93-003	–
ILS-084[a]	LP88-009	–
ILS-096	LP93-008	–
ILS-103	LP94-013	–
ILS-136	LP95-125	–
MDS-009	LP87-001	BR8
MDS-019	LP84-001	KCB4
MDS-051	LP95-059	–

[a]Previously listed as patient MDS-021.

MATERIALS AND METHODS

Patient data

We have evaluated 56 patients with MDS and 151 patients with ILS over the past 14 years. The presence of classical lissencephaly was confirmed by review of brain imaging studies or autopsy, and syndrome diagnosis was established prior to special laboratory studies in all but a few patients by either direct physical examination or review of medical records and photographs (by WBD). Those patients referred to in this study are listed in Table 1.

cDNA and genomic clones and somatic cell hybrids

LIS1 cDNA clones 71, 47, and 8-1 and the full-length human 14-3-3ε cDNA clone have been described (6,8) and sequences are available in GenBank through accession numbers L13385, L13386, L13388, and U54778, respectively. The sequence of the 1.5 kb genomic fragment containing the promoter (Fig. 2) has been deposited in GenBank under the accession number U58678.

Somatic cell hybrids KCB4 and BR8 constructed from patients MDS-019 and MDS-009 have been described elsewhere (12), while CBB5 is derived from patient ILS-024 who has an interstitial 17p13 deletion occurring from but not including *D17S5* (YNZ22) distally and extending proximally towards but not including TP53 (unpublished data).

Isolation of *LIS1* promoter fragment

The 1.5 kb *LIS1* promoter-containing fragment was isolated using the Human PromoterFinder™ kit (Clontech, Palo Alto, CA) with modifications. Briefly, a primer specific to the 5′ UTR of the LIS1 cDNA, LIS1(47)86R (5′-AGCCGTTCCGGGGCTCGAGTC-3′) and an adaptor-specific primer AP1 (5′-GTAATACGACTCAC-TATAGGGC-3′) were used in a first round amplification from a 'library' of total human genomic DNA digested with *Dra*I and ligated to adaptors. An aliquot from this reaction was subjected to a second round (nested) amplification using an overlapping LIS1-specific primer LIS1(47)73R (5′-CTCGAGTCCAAGCT-CGGCGCTC-3′) and an overlapping adaptor-specific primer AP2 (5′-ACTATAGGGCACGCGTGGT-3′). For both rounds of amplification, 1 µl of template was used in 50 µl reaction volumes, with final reagent concentrations of 20 mM Tris–HCl (pH 8.75), 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.1% Trition® X-100, 0.1 mg/ml BSA, 10% DMSO, 0.25 mM of each dNTP, 0.2 µM of each primer, and 0.025 U/µl exo(–) *Pfu* polymerase (Stratagene, La Jolla, CA). Amplifications for both rounds were performed in a PTC-200 thermal cycler (MJ Research, Watertown, MA), beginning with 7 cycles of 94°C for 2 s and 72°C for 3 min, followed by 32 cycles of 94°C for 2 s and 67°C for 3 min, and ending with a final extension at 67°C for 4 min. The amplified product was separated across a 1% agarose, 0.5× TBE gel, then gel purified and subcloned, or used as probe in Southern or northern analyses. Complete sequencing of both strands of this fragment was performed commercially (Seq-Wright, Houston, TX). Sequence alignments and analyses were accomplished using the DNASTAR program (DNASTAR, Madison, WI).

Probes

Probes 24F/215R (192 bp) and 4846F/5167R (322 bp) were PCR-amplified from LIS1 cDNA clone 71 using the primer pairs 5′-TCCGGTGGAATGAATCTTAC-3′/5′-TGGCTGTAATGTCA-AGCTTATC-3′ and 5′-GTCACGGCTGGACTGAATG-3′/5′-GCAACAAATCTTACTGTAAAACACG-3′, respectively. Standard *Taq* polymerase-mediated PCR reactions were performed in

a PE480 thermal cycler (Perkin Elmer-ABI), with an initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 45 s, 57°C for 1 min and 72°C for 1 min. Amplified products were separated across a 1.5% agarose gel in 0.5× TBE at 10 V/cm, gel-purified using the Qiaquick-spin kit (Qiagen, Chatsworth, CA), and labelled to high specific activity with [α-^{32}P]dCTP using the DECAprime II random-decamer labelling kit (Ambion, Austin, TX). The 1.5 kb genomic fragment containing the *LIS1* promoter was similarly labelled, and preassociated with excess human CoT1 DNA (Gibco-BRL, Gaithersburg, MD) prior to blot hybridization.

Southern and northern analyses

DNA from somatic cell hybrids was isolated by routine methods and digested with restriction endonucleases according to supplier instructions. Digested DNA was separated across a 0.7% agarose, 1× TBE gel, followed by alkaline capillary transfer to Zeta-Probe™ GT membrane (Bio-Rad, Hercules, CA) according to supplier instructions. Prehybridizations and hybridizations were performed in Rapid-hyb buffer (Amersham, Arlington Heights, IL) according to supplier instructions. Final wash was in 0.2× SSC, 0.1% SDS at 65°C for 30 min.

Multiple-tissue adult and fetal northern blots were obtained from a commercial source and probe hybridizations and blot washes were performed according to manufacturer instructions (Clontech, Palo Alto, CA).

Genomic clones and contig assembly

Cosmids spanning the lissencephaly critical region were isolated by hybridization screening of a flow-sorted human chromosome 17 gridded library (LA17NC01) constructed at the Los Alamos National Laboratory, Los Alamos, NM and available from the Physical Mapping Core (PMC), National Center for Human Genome Research, NIH. Additional screenings were performed on a chromosome 17-specific cosmid library (L4/FS17) obtained from the Reference Library Database, Max-Planck-Institute for Molecular Genetics, Berlin, Germany. Several P1 clones were obtained by PCR screening of a P1 library available from the PMC. Other P1 and PAC clones were obtained by hybridization screening of gridded PAC and P1 libraries (Genome Systems, St. Louis, MO).

The genomic contig was assembled using a combination of STS/SSR PCR analysis and Southern blot analysis of cosmid, P1 and PAC digests using cDNAs, whole cosmids or partial cosmid fragments. cDNAs and cosmid DNA were isolated using the Qiagen plasmid kit, while P1 and PAC DNA was obtained from Genome Systems. Cosmids, cosmid fragments, and cDNAs were radio-labelled as above and preassociated with excess human CoT1 DNA prior to hybridization. Primers 24F, 215R, 4846F and 5167R are as described under 'Probes', while LIS1(47)73R, CP2F, CP6R, CP7F, CP8F, and CP9F are indicated in Figure 2. Other primers used were 269F (5′-TATCTTCGTTCAAATGGC-TATGAAG-3′), 371R (5′-CCAAAAGACCAGCATACTTTTT-ATC-3′), 2387F (5′-GTGTGCCCATTTGAAAGGAGTG-3′), and 2510R (5′-GCACGCTCCATTAAACCCTG-3′). Amplification conditions were as described under 'Probes' with the exception that annealing of the CP and LIS1(47) primers were at 55°C, and 60°C annealing was used for the remaining primers.

Fluorescence *in situ* hybridization

Chromosome preparations were made from lymphoblastoid cell lines by conventional methods and aged slightly (1–2 weeks) to maintain morphology during denaturation. Slides were washed in 2× SSC at 37°C for 30 min, serially dehydrated in 70%, 80%, and 95% ethanol at room temperature, denatured in 70% formamide/0.65× SSC at 72°C for 2 min, then serially dehydrated at –20°C in 70%, 80%, 90% and 100% ethanol.

Cosmid, P1 or PAC DNA was labeled with digoxigenin-11-dUTP by nick translation (Boehringer Mannheim, Indianapolis, IN), precipitated in ethanol with 50× excess of human CoT-1 and herring testis DNA (Gibco-BRL), and resuspended to a final concentration of 25 ng/μl in hybridization solution (50% formamide, 2× SSC, 10% dextran sulfate). Probes were denatured at 76°C for 10 min and preassociated at 37°C for 30 min prior to hybridization. Chromosome-specific α-satellite probes were selectively amplified from human–rodent monochromosomal hybrids as described (13). Chromosome 19 α-satellite was amplified in the presence of biotin-16-dUTP (Boehringer Mannheim) while chromosome 17 α-satellite was amplified separately using either biotin- or digoxigenin-dUTP, then mixed in a 1:1 ratio.

Probe-hybridization mix (10 μl) was applied under a 22 mm × 24 mm coverslip and slides were incubated in a moist chamber for 16 h at 37°C, then washed as previously described (14). Probes were detected in 50 μl of rhodamine anti-digoxigenin (Boehringer Mannheim) at 1 μg/μl and FITC-avidin D (Vector Laboratories, Burlingame, CA) at 5 μg/μl. Slides were washed three times in 4× SSC, 0.1% Tween®-20 at 45°C and mounted in antifade solution (Vector) containing DAPI. Analysis was performed using a Zeiss Axiophot microscope equipped with filters to detect DAPI, FITC and rhodamine separately, as well as dual and triple band pass filter sets to detect signals simultaneously. Images were collected and merged using a cooled CCD camera (KAF 1400, Photometrics) and IP Lab Spectrum software.

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