

# Detecting deletions in the critical region for lissencephaly on 17p13.3 using fluorescent in situ hybridisation and a PCR assay identifying a dinucleotide repeat polymorphism

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## Abstract

**During a study of lissencephaly in England and Wales, 23 children were identified with this diagnosis. They were classified as follows: three children had Miller-Dieker syndrome (MDS), 13 had isolated lissencephaly sequence (ILS), two had type II lissencephaly, and five children were reclassified as focal or diffuse cortical dysplasia.**

**Microdeletions of chromosome 17p13.3, also known as the Miller-Dieker critical region, have been associated with both MDS and ILS. We used the commercially available Oncor probe for fluorescent in situ hybridisation (FISH) studies on 14 patients and a further four were studied elsewhere. Deletions were identified in all three MDS patients and two of the ILS patients. These results are consistent with previously reported data. No deletions were found in those patients with focal or diffuse cortical dysplasia.**

**In addition, a CA repeat polymorphism which maps to the Miller-Dieker critical region was studied in 12 families and was informative in nine; the results were consistent with the FISH data.**

**We conclude that FISH is a reliable method to detect deletions in patients with MDS and ILS and also useful to identify chromosome rearrangements in their parents which are not detected by conventional cytogenetic analysis. The PCR assay, if informative, is also reliable and a useful alternative if only DNA is available.**

**None of the five children with atypical radiological features had a deletion. We therefore suggest that as well as looking for other aetiologies a careful review of the diagnosis should be made of the MDS or ILS cases in whom a deletion is not found.**

each type.<sup>1,2</sup> During life the two types are determined by typical features on neuroimaging.<sup>1,3-6</sup> As deletions in the lissencephaly critical region on 17p13.3 have so far only been recognised in MDS and ILS which are associated with type I lissencephaly, further discussion will be limited to this type.

Neuronal migration occurs between 2 and 4 months' gestation with "waves" of migrating cells continuing until the 25th week.<sup>7</sup> The characteristic histological pattern of type I lissencephaly is the four layered neocortex as a result of a neural migration defect at around 3 to 4 months.<sup>1,6</sup> Macroscopic features seen on neuroimaging include: incomplete opercularisation of the sylvian fissure with an exposed insula which gives the brain a figure of eight appearance, an agyric or pachygyric cerebral surface with a thick cortex, enlarged posterior horns of the lateral ventricles (colpocephaly), and dysplasia of the corpus callosum<sup>1,6</sup> (fig 1). Hydrocephalus and cere-

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Lissencephaly is a heterogeneous neuronal migration disorder characterised by the complete or partial absence of cerebral gyri which gives the surface of the brain a smooth appearance, hence the name lissencephaly, derived from the Greek words "lissos" = smooth and "encephalos" = brain.

Neuropathologically two major types, type I and type II lissencephaly, are distinguished and clinically several syndromes are recognised with

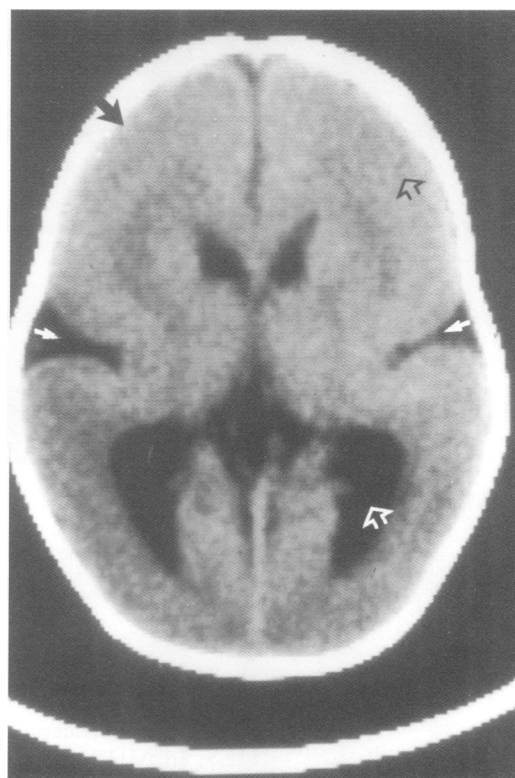
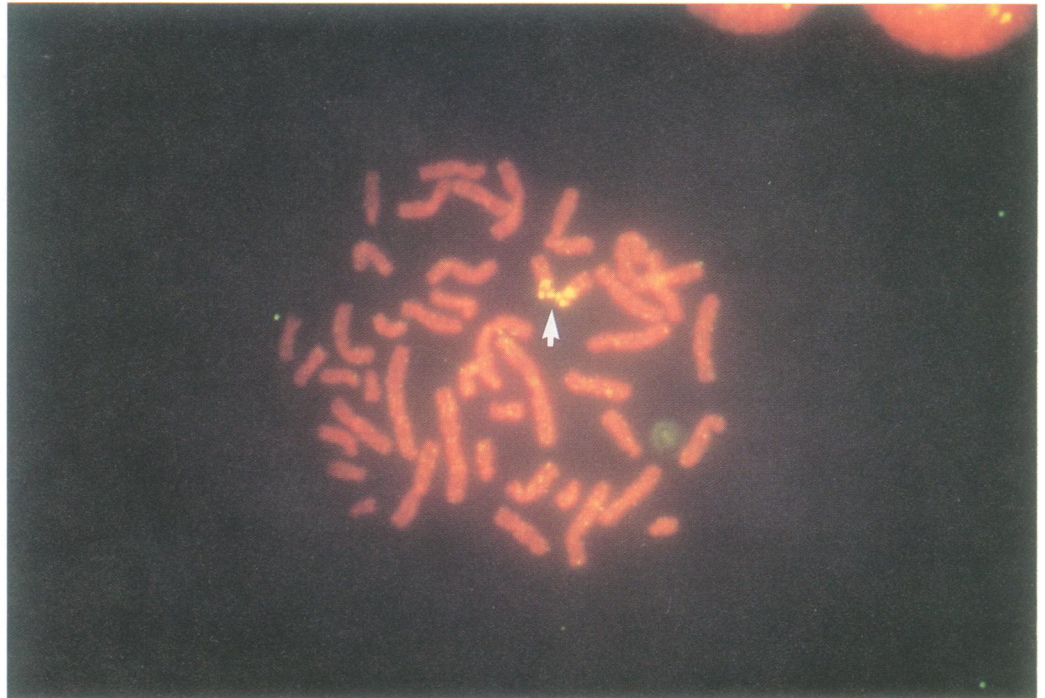
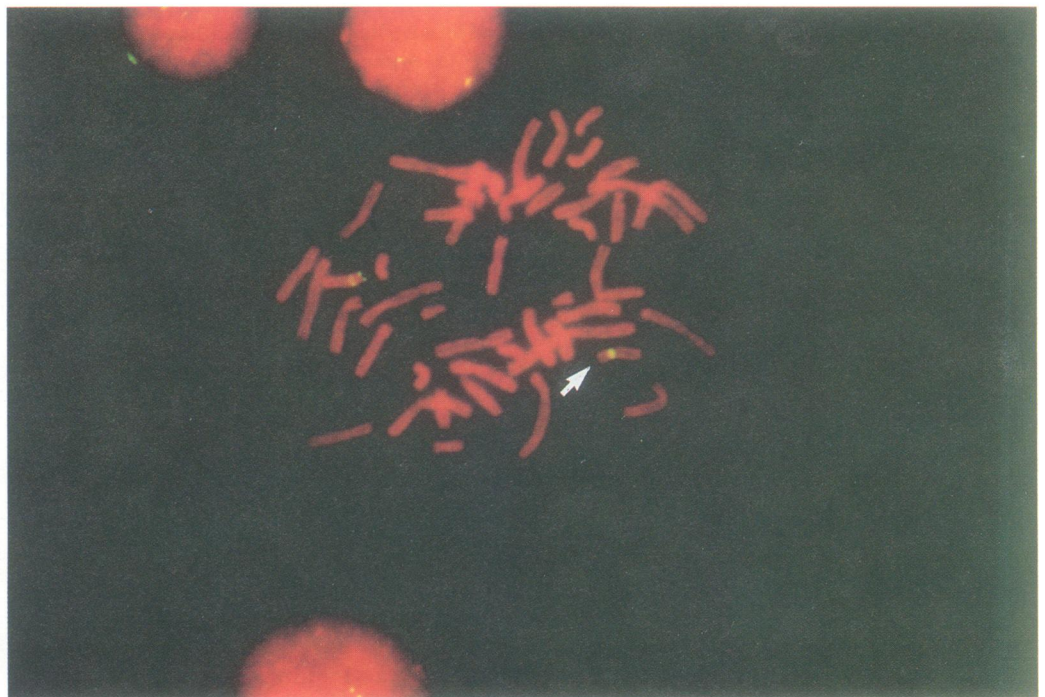


Figure 1 Cranial CT scan showing lissencephaly type I with an agyric cerebral surface (filled black arrow), thick cerebral cortex (open black arrow), incomplete/absent opercularisation (filled white arrows), and colpocephaly (open white arrow).



A



B

Figure 2 In situ hybridisation studies using the *Oncor* biotin labelled probe for the Miller-Dieker region on chromosome 17p13.3. (A) Hybridisation (arrow) to both chromosomes 17 in a non-deleted patient. (B) Absence of hybridisation (arrow) to one of the chromosomes 17 in a patient with a deletion.

bellar abnormalities point towards type II lissencephaly.

Type I lissencephaly is found in MDS and ILS. In MDS an agyric or almost completely agyric brain is associated with typical dysmorphic features,<sup>38</sup> whereas in ILS the appearance of the cerebral surface ranges between agyria and complete pachygyria and dysmorphic features are less obvious.<sup>48</sup>

MDS was originally thought to be autosomal recessive.<sup>9</sup> In 1983 Dobyns *et al*<sup>10</sup> first reported an association between MDS and a deletion of 17p13 in two families. In a recent study microscopic and submicroscopic deletions in the region of 17p13.3 were identified in about 90% of MDS patients; 12% were the result of familial rearrangements.<sup>3</sup> In 1991 the first case of ILS with a microdeletion in 17p13.3 was

shown using Southern blotting.<sup>11</sup> In a study of 45 patients with ILS by Ledbetter *et al.*<sup>12</sup> a deletion was found in six (13.3%) patients; fluorescent in situ hybridisation (FISH) proved the most sensitive method. In 1993 Carozzo and Ledbetter<sup>13</sup> reported a CA repeat polymorphism contained in the cosmid c197-9 (assigned to 17p13.3), which showed only one parental allele in patients with MDS and ILS in whom deletions had already been shown by other methods. Subsequently Reiner *et al.*<sup>14</sup> reported the cloning of a candidate gene they called "LIS-1" (lissencephaly 1), deleted in two patients with MDS. The deduced amino acid sequence of LIS-1 indicated a relationship with the beta subunits of G proteins, which are known to be involved in the signal transduction of neuronal pathways. Recently, homology between the sequence of the 45K subunit of platelet activating factor (PAF) acetylhydrolase present in the bovine cerebral cortex and the protein encoded by the LIS-1 gene was found by Hattori *et al.*<sup>15</sup> PAF acetylhydrolase inactivates PAF and their role in neurodevelopment has yet to be determined.

In this paper we report the results of the FISH studies or PCR assays identifying the CA repeat polymorphism in three patients with MDS, 13 with ILS, and five with focal or diffuse cortical dysplasia.

## Methods

### PATIENTS

Twenty-three patients were identified through clinicians and the national support group for families with lissencephaly as part of a clinical, radiological, and genetic study of lissencephaly in England and Wales. They were all examined clinically by one of us (DTP). The CT scans were evaluated by a neuroradiologist (TJ); MRI scans were only available in two patients.

### FISH

In situ hybridisation studies were performed using the Oncor biotin labelled probe for the Miller-Dieker region on chromosome 17. The probe mixture also contained the D17Z1 17 alpha satellite region for use as a reference. Slide pretreatment was with 10 µg/ml RNAase for one hour at 37°C, followed by 0.05% pepsin in 0.01 NHCL. Slide denaturation was in 70% formamide/2 × SSC at 70°C for two minutes; the probe was applied and hybridisation was at 37°C in a humidified chamber for 16 hours. The post-hybridisation wash was with 50% formamide in 2 × SSC at 43°C, and the probe was detected by incubating with fluorescein

isothiocyanate (FITC) conjugated avidin (4 µg/ml) (Vector Laboratories) at room temperature for 20 minutes. The signal was amplified using the method of Pinkel *et al.*<sup>16</sup> Slides were mounted in "Vectashield" (Vector Laboratories) and counterstained with propidium iodide (0.5 µg/ml) before examination on a Leitz Laborlux microscope equipped for FITC fluorescence.

### PCR ASSAY

We used a set of PCR primers as published by Carozzo and Ledbetter<sup>13</sup> which amplify a CA repeat polymorphism within the lissencephaly critical region on 17p13.3 resulting in fragments of 342 to 362 bp. We modified the method by using a *HaeIII* restriction digest on the PCR product to reduce the size of the variable product to approximately 180 bp. This separates well on a polyacrylamide gel. The PCR assay was performed in a total volume of 50 µl containing 1 µl DNA, 1.5 mmol/l MgCl<sub>2</sub>, 200 µmol/l of each dNTP, 1–2.5 units *Taq* Polymerase, and approximately 4 pmol of each primer. Initial denaturation was at 95°C for four minutes followed by 25 cycles of 94°C/one minute, 55°C/one minute, 72°C/two minutes, and a final extension of seven minutes at 72°C (Perkin-Elmer).

The digest was run on an 8% polyacrylamide gel (19:1 acrylamide:bisacrylamide), electrophoresed for five hours at 200 V and silver stained.

## Results

On clinical and radiological criteria we identified three patients as having MDS and 13 as having ILS. Five patients, of whom three met the clinical criteria of ILS and two who had unrelated dysmorphic features, did not show the typical radiological appearances of type I lissencephaly and we classified these as having focal or diffuse cortical dysplasia as described by Barkovich and Kjos.<sup>17</sup> Two patients had type II lissencephaly.

FISH studies were undertaken on all three patients with MDS, 11/13 patients with ILS, and 4/5 patients with focal or diffuse cortical dysplasia. The analysis was performed in our laboratory in 14 of the cases; four patients had been studied elsewhere by the time of the survey (fig 2A+B). A PCR analysis was possible in 12 families and informative in nine.

All three patients with MDS showed deletions, one being the result of an unbalanced rearrangement of a pericentric inversion of a maternal chromosome 17 (H Kingston, personal communication). Of the 11 patients with ILS who were studied, two (18%) had a deletion. None of the five patients with focal or

Detection of deletions in the lissencephaly critical region 17p13.3 with FISH and a CA repeat polymorphism

Diagnosis	1 MDS	2 MDS	3 MDS	4 ILS	5 ILS	6 ILS	7 ILS	8 ILS	9 ILS	10 ILS	11 ILS	12 ILS	13 ILS	14 ILS	15 BFCD	16 DCD	17 BFCD	18 BFCD	19 FCD
FISH	DEL	DEL	DEL	DEL	DEL	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	—	ND
PCR	NI	DEL	—	DEL	NI	ND	ND	—	—	—	—	ND	ND	—	—	NI	ND	ND	ND

DEL=deleted; ND=not deleted; NI=not informative; MDS=Miller-Dieker syndrome; ILS=isolated lissencephaly sequence; BFCD=bilateral focal cortical dysplasia; DCD=diffuse cortical dysplasia; FCD=focal cortical dysplasia.

diffuse cortical dysplasia showed a deletion. Both deletions detected with the PCR assay were paternal (table).

### Discussion

Identification of microscopic and sub-microscopic deletions within 17p13.3 in most patients with MDS and some with ILS has allowed more precise counselling for affected families and the offer of prenatal diagnosis in some. In keeping with previous studies we found that FISH is a reliable method to detect deletions in patients with MDS and ILS and that the percentage of deletions found in our patients coincided with those previously reported.<sup>3,12</sup> The PCR assay we used also produced reliable results when informative, and is a useful alternative when only DNA is available. In both cases in which a deletion was identified with this method the paternal allele was absent. However, both maternal and paternal deletions have been reported.<sup>3</sup>

All our three patients with MDS had deletions and in a series of 25 patients with MDS, Dobyns *et al*<sup>3</sup> and Ledbetter *et al*<sup>12</sup> reported deletions in 22 (88%). They concluded that with appropriate methods, deletions would eventually be detectable in all patients with MDS. As subtle balanced parental chromosomal rearrangements<sup>3,18</sup> can be the cause of the deletion, FISH studies should be performed on the parental chromosomes when a deletion is detected in the proband.

In our study the remaining 18 patients who did not have MDS had usually remained unclassified under the general diagnosis of lissencephaly. However, identification of those with ILS, where deletions on 17p13.3 have been reported, and those with atypical lissencephaly or a non-lissencephalic cortical dysplasia, where a deletion in this area may not be expected, is important for genetic counselling purposes. Only 13 of our remaining 18 patients fulfilled both the current clinical and radiological criteria on the neuroimaging available for the diagnosis of ILS. Of these 11 had FISH studies and two (18%) had a deletion. This result compares with deletions found in six out of 44 patients (13.6%) discussed by Dobyns *et al*<sup>4</sup> in a review of the causal heterogeneity in ILS. Recurrence risks in cases of de novo deletions are likely to be low. Other causes of ILS include intrauterine infection, possibly intrauterine hypoperfusion, some cases of autosomal recessive inheritance and other loci.<sup>4</sup> There were no recurrences in our study and Dobyns *et al*<sup>4</sup> suggested an empirical risk of 7%, when known causes are excluded. This figure is likely to be refined as further rearrangements become detectable in non-deleted cases of ILS.

Five of the 21 children included in this study did not fit the diagnosis of either MDS or ILS. On radiological assessment primarily on the basis of cranial CT scans, they appeared to fall into the group of non-lissencephalic cortical

dysplasias (NLCD) recently reviewed by Barkovic and Kjos.<sup>17</sup> Three of them met the clinical criteria of ILS and clinical similarities between ILS and NLCD have been described.<sup>17</sup> The other two had unrelated dysmorphic features. None of these five children had a deletion on 17p13.3 using FISH or PCR or both. Recurrence risks in patients with focal or diffuse cortical dysplasias are undefined; there were none in our small series with a total of 10 sibs.

We conclude that a clinical and radiological diagnosis of MDS or ILS should be attempted in each patient with lissencephaly, and in those who do not show the deletions other aetiologies should be considered and the diagnosis reviewed. Neuroimaging would be very important in such a review and where not previously undertaken an MRI scan should be recommended, as the extent of the cortical changes are more readily assessed, as are any concomitant white matter changes, commonly associated grey matter heterotopias, the presence of polymicrogyria, and callosal dysgenesis, which can provide further clues as to the aetiology.

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