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

D T Pilz, A Dalton, A Long, T Jaspan, E L Maltby, O W J Quarrell

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.

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 each type.12 During life the two types are determined by typical features on neuroimaging.13-6 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. 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.16 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 callosum16 (fig 1). Hydrocephalus and cere-
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,¹⁸ whereas in ILS the appearance of the cerebral surface ranges between agyria and complete pachygyria and dysmorphic features are less obvious.¹⁸

MDS was originally thought to be autosomal recessive.⁷ In 1983 Dobyns et al.⁹ 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.³ In 1991 the first case of ILS with a microdeletion in 17p13.3 was
Deletions in the critical region for lissencephaly on 17p13.3

shown using Southern blotting.\textsuperscript{11} In a study of
45 patients with ILS by Ledbetter et al\textsuperscript{2} a
deletion was found in six (13.3\%) patients; fluorescent
in situ hybridisation (FISH) proved the most sensitive method. In 1993 Carozzo
and Ledbetter\textsuperscript{13} 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\textsuperscript{14} 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 be-
tween the sequence of the 45S 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.\textsuperscript{15} PAF acetylhydrolase in-
activates PAF and their role in neuro-
development 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 \mu{}g/ml RNase A
for one hour at 37°C, followed by 0.05% pepsin
in 0.1 NHCL. Slide denaturation was in 70%
formamide/2 \times 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 \times SSC at 43°C, and the probe
was detected by incubating with fluorescein
isothiocyanate (FITC) conjugated avidin (4 \mu{}g/
ml) (Vector Laboratories) at room temperature
for 20 minutes. The signal was amplified using
the method of Pinkel et al.\textsuperscript{16} Slides were
mounted in “Vectashield” (Vector Laboratories) and
counterstained with propidium iodide (0.5 \mu{}g/
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\textsuperscript{13} which amplify a CA
repeat polymorphism within the lissencephaly
critical region on 17p13.3 resulting in frag-
ments 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 \mu{}l containing 1 \mu{}l DNA, 1.5 mmol/l MgCl\textsubscript{2},
200 \mu{}mol/l of each dNTP, 1–2.5 units Taq Poly-
merase, 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), electro-
phoresed for five hours at 200V and silver
stained.

Results

On clinical and radiological criteria we iden-
tified 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.\textsuperscript{17} 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 de-
letions, one being the result of an unbalanced
rearrangement of a pericentric inversion of a
maternal chromosome 17 (H Kingston, per-
sonal communication). Of the 11 patients with
ILS who were studied, two (18\%) had a de-
letion. None of the five patients with focal or
diffuse cortical dysplasia showed a deletion. Both deletions detected with the PCR assay were paternal (table).

**Discussion**

Identification of microscopic and submicroscopic 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. 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, FISH studies should be performed on the parental chromosomes when a deletion is detected in the proband.

Of 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 useful, would be particularly helpful as a clinical tool to assist 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 al12 in a review of the causal heterogeneity in patients with 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. There were no recurrences in our study and Dobyns et al12 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 Bar- kovic and Kjos.17 Three of them met the clinical criteria of ILS and clinical similarities between ILS and NLCD have been described.17 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.

We would like to thank the families and their clinicians for their support, and Action Research for funding this study. The consumables were obtained courtesy of the Chris Fund.

References


7erry M, Rogers AW. The migration of neuroblasts in the developing cerebral cortex. J Anat 1965;99:691.


