Leucodysplasia, microcephaly, cerebral malformation (LMC): a novel recessive disorder linked to 2p16

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We report three related and one unrelated child with an apparently novel neurodevelopmental disorder. The clinical course was very similar in all the four patients: congenital microcephaly with severe failure of post-natal brain growth, neonatal onset of intractable seizures associated with lack of developmental progression and death within the first 3 years of life. The appearance on cerebral neuroimaging was almost identical, with simplified gyration associated with a non-thickened cortex, severe hypoplasia of the corpus callosum, a small flattened brain stem, and specific cystic lesions in the white matter around the temporal and occipital horns. To our knowledge these patients represent a previously unreported, autosomal recessive syndrome. Homozygosity mapping in the consanguineous family has identified a candidate region on the chromosome 2p16.

Keywords: leucodysplasia; microcephaly; cerebral malformation; autosomal recessive; neonatal seizures

Abbreviations: SNP = single nucleotide polymorphism


Introduction

Congenital microcephaly exhibits aetiological heterogeneity with numerous sporadic, environmental and Mendelian causes. When the brain is small, but structurally normal, the condition is known as ‘microcephaly vera’ or ‘primary microcephaly’. This is commonly associated with mild to moderate mental retardation and autosomal recessive inheritance. Genetic studies in affected families have enabled the identification of novel genes important for normal brain growth and development (Bond et al., 2002; Jackson et al., 2002). More profound congenital microcephaly is often associated with other cerebral malformations. The cortical abnormalities vary from a simplified gyral pattern to the thick agyric cortex of lissencephaly, or the small convolutions of polymicrogyria (Barkovich et al., 1998; Dobyns and Barkovich, 1999). Clinical features include neonatal seizures and severe developmental delay. In recent years, several distinct entities have emerged from within this heterogeneous group of disorders, for instance ‘lissencephaly with cerebellar hypoplasia and congenital microcephaly’ (Barkovich et al., 2001; Ross et al., 2001). The syndrome we describe here appears unique, and is not encompassed within the original classification of severe congenital microcephaly and cerebro-cortical disorders (Dobyns and Barkovich, 1999).

We report four children (three related and one unrelated) who presented with severe congenital microcephaly and a unique cerebral malformation, associated with profound mental handicap and seizures. We propose that our patients represent a previously undescribed autosomal recessive disorder of neurodevelopment. The phenotype was very similar between the affected individuals, males and females were...
affected, no parent was affected and there were unaffected siblings of both sexes. In the three related patients, there was a high degree of consanguinity within the family. Given this, we attempted to find the location of the causative gene using autozygosity mapping (Houwen et al., 1994; Mueller et al., 1993) and identified a candidate region on the chromosome 2p16.

**Patient case reports**

Patients 1–3 were born to multiply consanguineous Asian parents (Fig. 1). Patient 4 was the son of unrelated Caucasian parents. All four patients shared a very similar clinical phenotype and appearances on cerebral MRI scan. They died within the first 3 years of life; post-mortems were not performed. Growth parameters are shown as standard deviations (SDs) relative to the mean for age and sex.

**Patient 1**

Patient 1 was the first child of first cousin parents. She was born at term by breech presentation following a normal pregnancy. Microcephaly was noted at birth, with an occipital-frontal circumference (OFC) of 30 cm (−3 SD). She presented with seizures on the second day of life. She made minimal...
developmental progress and by the age of 1 year made only single syllable sounds, no purposeful movements, and had cortical visual impairment with lack of fixation and following. She was dependent on gastrostomy feeds because of lack of co-ordination for swallowing.

Clinical examination at 21 months confirmed marked microcephaly, with an OFC of 38 cm (−7 SD). In comparison, her other growth parameters were between −1 SD and −2 SD for age. She was facially distinct with a low anterior hairline and a short forehead, arched and thick eyebrows with synophrys, long eye-lashes, long palpebral fissures and hypertelorism. She had a hypotonic facial expression, with a tented mouth, a long philtrum, and micro-retrognathia. She had marked truncal hypotonia, but was hypertonic in all the four limbs, with elbow contractures. Deep tendon reflexes were variably present with normal muscle bulk. She was fitting continuously, but made no other spontaneous movements. She had a kyphoscoliosis and a small umbilical hernia, but no other physical malformations. She died suddenly and unexpectedly at the age of 2 years.

The following investigations revealed no abnormality: renal, liver and bone biochemistry; creatinine kinase; viral studies; venous bicarbonate and chloride; serum lactate and glucose; CSF glucose, lactate and white cell count; urine organic and amino acids; and plasma very long chain fatty acids, white cell enzymes and plasma lysosomal enzymes. Chromosome analysis and fluorescent in situ hybridization (FISH) studies for the LIS1 specific deletion at 17p13.3 were both normal.

Her electroencephalogram (EEG) showed a high amplitude background with high amplitude and multifocal epileptic discharges. Ophthalmic assessment identified optic atrophy with macular hypoplasia and unresponsive VEPs. Cerebral ultrasound scans in the newborn period showed dilated lateral ventricles and a subependymal cyst. She subsequently had cranial CT and MRI scans, the findings of which are discussed in detail below.

Her mother had learning difficulties of unknown aetiology, in common with several of her siblings. The mother’s head circumference was normal (between the mean and −0.65 SD), as was a cranial MRI scan.

Patients 2 and 3

Patients 2 and 3 were the male cousins of Patient 1. They too had first cousin parents, each of whom was a sibling of the parents of Patient 1 (Fig. 1). Their clinical course followed a pattern similar to that of Patient 1. Both presented with congenital microcephaly and seizures during the first week of life. Both children had severe failure of post-natal brain growth: at birth, Patient 2 had a head circumference measuring 30.5 cm (−3 SD to −4 SD) and Patient 3 had a head circumference of 31 cm (−3 SD). By the age of 10 months, these measurements had fallen to 35.6 cm (−8 SD) and 36.5 cm (−7 SD), respectively. The boys also shared the same facial features as Patient 1. Of note, Patient 2 was found to have a small penis and cryptorchidism, whereas Patient 3 had normal external genitalia.

In contrast to their female cousin, Patients 2 and 3 were able to successfully bottle feed throughout the first 18 months of life. They gained weight rapidly, increasing to greater than +3 SD, despite an active dietetic management. From the age of 2 years their feeding skills deteriorated and they had prolonged episodes of hypersomnolence. In the last few months of life, their seizures became less frequent and they displayed no spontaneous movements while awake, but showed reflex extensor posturing of upper and lower limbs on handling. Both boys died at the age of 2 years and 9 months from aspiration pneumonia.

The following investigations were performed and were normal: chromosome analysis, creatinine kinase, viral studies, urine amino and organic acids, and blood lactate and ammonia levels. Of interest, Patient 3 was found to have congenital hypothyroidism, when examined by the newborn screening programme, with a grossly raised TSH. A thyroid isotope scan displayed a normally sized and placed thyroid gland. Anti-thyroid peroxidase and anti-g antibodies were low in the mother and the child. T4 and TSH in the mother were normal. These results suggested a defect of thyroxine synthesis. The boy’s hearing assessment was normal. CT scan of the inner ear was not performed. EEGs for Patients 2 and 3 were highly abnormal, with similar findings to Patient 1. The cranial MRI scans for Patients 2 and 3 were also abnormal and are discussed in detail below.

Patient 4

Patient 4 was the second child of unrelated Caucasian parents. Intrauterine growth retardation (IUGR) was noted at 37 weeks of gestation, but otherwise the pregnancy was uneventful. Labour was induced at 38 weeks in view of the IUGR, and delivery was normal. His birth weight was 2183g (around −2 SD). Microcephaly was noted at birth, with an OFC of 30 cm (−3 SD to −4 SD). He developed seizures within the first few weeks of life and also had feeding problems.

When assessed at the age of 17 months, he had intractable fits and profound global developmental delay. Swallowing had become difficult, and he was fed through a gastrostomy tube. His OFC was 40 cm (−6 SD). He was generally hypotonic, but he kept his hands tightly clenched. His ears appeared large even in relation to his head size. He had two upper incisors and a high palate. He had brisk reflexes throughout, upgoing plantars and bilateral ankle clonus. Otherwise the examination was unremarkable. He had normal male genitalia.

The following investigations were normal: chromosome analysis; including FISH for the LIS1 specific deletion at 17p13.3; intrauterine infection screen; urinary amino and organic acids; and auditory brainstem responses. Ophthalmological assessment was normal, although it was noted that he could fixate, but not follow a bright light. Electrodiagnostic testing was not undertaken. His EEGs were very abnormal,
showing both multifocal epileptiform and background activity. His cranial MRI scan findings are discussed below.

**MRI findings**

**Patient 1**
The cranial MRI scan was performed at 5.5 months of age (Fig. 2). It showed simplified gyration, more pronounced posteriorly, with a non-thickened cortex (2–3 mm). The lateral ventricles were dilated, especially the posterior horns, with lack of surrounding white matter. There was no convincing supratentorial myelin. Within the white matter of the temporal and occipital regions there were cystic areas. The corpus callosum was markedly hypoplastic, and the brain stem was small and flat. The cerebellum appeared normal.

**Patient 2**
The cranial MRI scan was performed at 2 years of age. Gyration was simplified, particularly in the frontal regions. There was lack of opercularization. The lateral ventricles were dilated, especially the posterior and temporal horns, with a reduction in the surrounding white matter. The corpus callosum was extremely hypoplastic. The white matter signal on the T2-weighted images was increased, particularly around the posterior horns. Cystic lesions were seen in the white matter of the temporal horns. The brain stem was hypoplastic. The cerebellum appeared normal.

**Patient 3**
The cranial MRI scan was performed at 16 days of age (Fig. 3). The gyration was simplified with an irregular non-thickened cortex (2–3 mm). The lateral ventricles were enlarged, especially posteriorly, with a significant reduction in surrounding white matter. There was increased white matter signal on the T2-weighted images around the posterior horns and cystic lesions were identified. The corpus callosum was severely hypoplastic. The cerebellar hemispheres and the brain stem were hypoplastic, and there was a megacisterna magna.

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![Fig. 2](image1.png)

**Fig. 2** Patient 1. Sagittal T1-weighted image (A) showing the simplified gyration, reduction in white matter (WM) and thin flat brainstem (white arrow). Axial T2-weighted image (B) showing supratentorial absence of myelination (white arrow) and dilatation of the lateral ventricles, particularly the posterior horns (black arrow). Axial T2-weighted image (C) showing the cystic areas in the white matter of the temporal regions (black arrows).

![Fig. 3](image2.png)

**Fig. 3** Patient 3. Sagittal T1-weighted image (A) showing simplified gyration and a small brainstem (white arrow). Axial T2-weighted image (B) indicating the dilation of the lateral ventricles, particularly the posterior horns (white arrows). Axial T1-weighted image (C) showing the cystic areas found in the temporal/occipital regions (white arrows).
Patient 4

The cranial MRI scan was performed at 2.5 months of age (Fig. 4). Gyration was slightly simplified, and the cortex looked irregular, but not thickened (2–3 mm). The supratentorial white matter was under-myelinated, and there was an increased signal on the T2-weighted images throughout, especially in the temporal and occipital regions. In these areas the white matter was also reduced and contained cystic areas. The corpus callosum was extremely hypoplastic, and the pons was flat. The cerebellum appeared normal.

Homozygosity mapping of the LMC locus

Homozygosity mapping was undertaken in the multiply consanguineous family with three affected children (Fig. 1). We made use of the Affymetrix 10K SNP chip, bearing oligonucleotides sufficient to analyse over 10,000 human autosomal single nucleotide polymorphisms (SNPs). DNA from the three affected children was hybridized onto the chip using the manufacturers’ protocol. The data was analysed by the ExcludeAR3 program (Woods et al., 2004). This showed only one significant homozygous region that is shared between the three patients: a 10.3 cM region on chromosome 2 made up of 68 consecutive SNPs. In order to confirm this finding, the affected individuals and their parents were genotyped using polymorphic microsatellite markers, some of which were novel. Such novel markers were generated by use of the human genome browser ‘repeat marker’ and ‘simple repeats’ tracks to select possible polymorphic microsatellite repeats, Primer3 to design primers for their amplification and BLASTn to determine that the primers were unique. They are named by the BAC in which they lie followed by the repeat, i.e. AC069550GT22 is a marker that contains only a run of 22 GT repeats that lies on the bacterial artificial chromosome AC069550. The markers were as follows: D2S2739 (SNP_A-1514906, the pter most homozygous SNP), AC069550GT22, D2S2156, D2S123, D2S2251, D2S2153, D2S393, D2S357, D2S2397, AC007365AC23, AC007386TG19, AC012370AC/CT, AC092669AC27 (SNP_A-1516900, the qter most homozygous SNP) and D2S285. This allowed us to both confirm the SNP finding of a concordant, homozygous segment in all three affected individuals and further refine the region to be flanked pter by D2S2739 and qter by AC012370AC/CT, a physical region of 16 MB containing at least 44 genes. The results are shown in Fig. 1 and allowed the generation of a maximum multipoint LOD score of 3.3 for D2S2397 (using Genehunter, a disease allele frequency of 0.001, known marker allele frequencies were obtained from the GDB Human Genome Database, and for novel markers allele frequencies were derived from the number of alleles observed in the family and a Caucasian control).

Discussion

We describe four children presenting with significant congenital microcephaly and onset of seizures in the neonatal period. They subsequently made little if any developmental progress, were profoundly handicapped and had both primary and secondary microcephaly with a longitudinal reduction in head circumference centile. Brain imaging revealed consistent cerebral abnormalities: a simplified gyral pattern, an irregular cortex in Patients 3 and 4, generalized deficiency of supratentorial white matter with cystic lesions in the area of the temporal and occipital horns, a lack of myelination, severe hypoplasia of the corpus callosum and brain stem hypoplasia with a flat pons. In all but Patient 3 the cerebellum appeared normal. One patient (Patient 2) had microgenitalia, and another (Patient 3) had congenital hypothyroidism, which may have been due to an unrelated autosomal recessive disorder.

The findings in these patients suggest a severe neurodevelopmental disorder affecting both cortical grey and white matter neurogenesis, with post-natal cerebral growth failure. The nature of the cystic areas in the white matter of the temporal and occipital horns is unclear. They were seen on the cerebral MRI of all four patients. The earliest scan undertaken was at the age of 16 days (Patient 3). As no follow-up scans were available, we do not know whether they were part of a
progressive, possibly destructive, process, or they represented a prenatal malformation. There was no conclusive neuroimaging evidence of a primary neuronal migration disorder.

Homozygosity mapping has identified a physical region of 16 MB containing at least 44 genes. Within this region \textit{OTX1} presented as an interesting candidate gene because of the phenotype of the knock-out \textit{Otx1} mouse and \textit{oceilless} (synonyms orthodenticle, OTD and OTX) fly mutant (Acampora et al., 1996). \textit{Otx1}\textsuperscript{-/-} mice have epilepsy and multiple cerebral malformations. However, bidirectional sequencing of all exons and splice sites revealed no abnormalities (data not shown). Analysis of other genes in the area is ongoing.

The early onset of an intractable seizure disorder in these patients could suggest an imbalance of excitatory and inhibitory neural circuit formation. Abnormal migration of GABAergic (\(\gamma\)-aminobutyric-acid-releasing) interneurons has for instance been shown in \textit{Arx} deficient mice (Kitamura et al., 2002), and certain mutations in the human homologue are the cause of X-linked lissencephaly and abnormal genitalia. These patients also present with neonatal, intractable seizures (Dobyns et al., 1999).

The unique features we describe in these four children suggest that they had the same novel disorder, of which we can find no previous description. Pedigree analysis strongly suggests autosomal recessive inheritance, which the results of homozgyosity mapping supported by the finding of a single shared locus on 2p16. The causative gene can be expected to play a vital role in both neurogenesis and normal central nervous system function. We have tentatively named the condition LMC syndrome (leucodysplasia, microcephaly and cerebral malformation), after the principal findings.

Electronic resources

Flybase for Drosophila genomic and phenomic details of \textit{oceilless} at http://flybase.bio.indiana.edu/bin/fbidq.html?
FBgn0004102&resultlist=fbgn28246.data
GDB Human genome database at http://gdbwww.gdb.org/
GLUE interface for Genehunter at http://www.hgmp.mrc.ac.uk/Registered/Webapp/glue/

Human genome browser, May 2004 assembly at http://genome.ucsc.edu/ 
Primer3 at http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi

Acknowledgements

The authors would like to thank the families of the patients for their help.

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