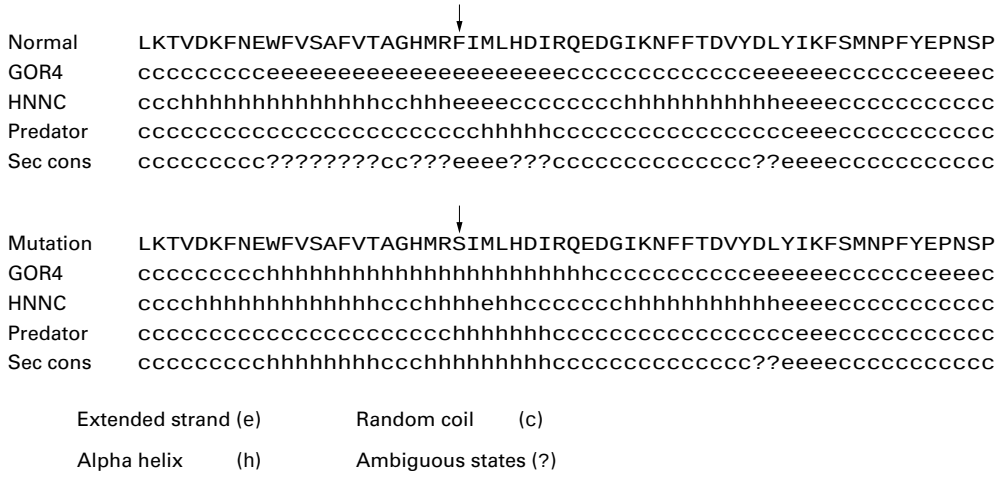

Figure 2 SEDL secondary structure. SEDL secondary structure was predicted to change from an extended strand (e) and random coil (c) in normal controls (normal) to an alpha helix (h) in SED patients (Mutation) with F→S mutation at position 83 (marked by arrow).

phenotype as a mutation that results in truncation of the protein. The affected patients in the family described here had very mild clinical symptoms. Contrary to other pedigrees with X linked SED, in which females heterozygous for the gene defect suffered from subtle skeletal abnormalities and arthritis by middle age,[10 11] the female carriers in the family described here had no objective evidence of SED. This may reflect the subtle impact of the C→T mutation on SEDL protein function.

In the family described here we were able to confirm that a male of uncertain status (IV.8) had no mutation in SED. Thus, the ability clearly to diagnose SED by molecular methods in children with joint or spine abnormalities is of extreme importance, as it may allow appropriate and early intervention.

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Molecular characterisation of a new case of microphthalmia with linear skin defects (MLS)

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EDITOR—Microphthalmia with linear skin defects (MLS) is a clinically complex and highly variable phenotype in XX subjects and has been considered to be at least partially determined by three features: the pattern of X chromosome inactivation; the extent of the Xp22.3 segmental monosomy; and the nature of the chromosomal anomaly (deletion or translocation).The recurring features of microphthalmia with linear skin defects, generally restricted to the face and neck in all the early reported cases, led to its designation as the MLS syndrome. The consistent association of these two manifestations with Xp22.3 segmental monosomy suggests that MLS is a contiguous gene syndrome.[1 2] However, the phenotype may be complicated by additional abnormalities which include sclerocornea, chorioretinal abnormalities, agenesis of the corpus callosum, hydrocephalus, infantile seizures, mental retardation, and congenital heart defects. To date, around two dozen cases of MLS syndrome

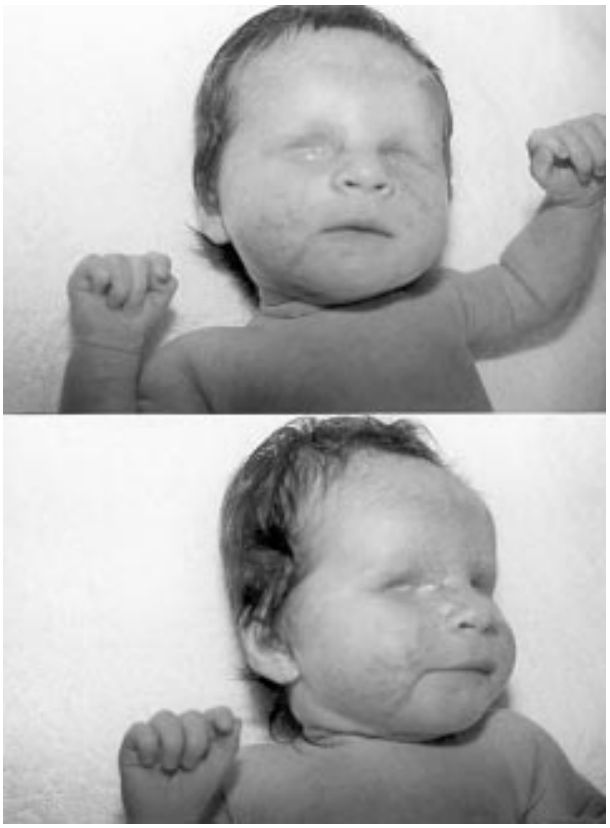

Figure 1 The proband at 34 days of age showing red, reticulolinear skin lesions on the cheek and nose and bilateral microphthalmia.

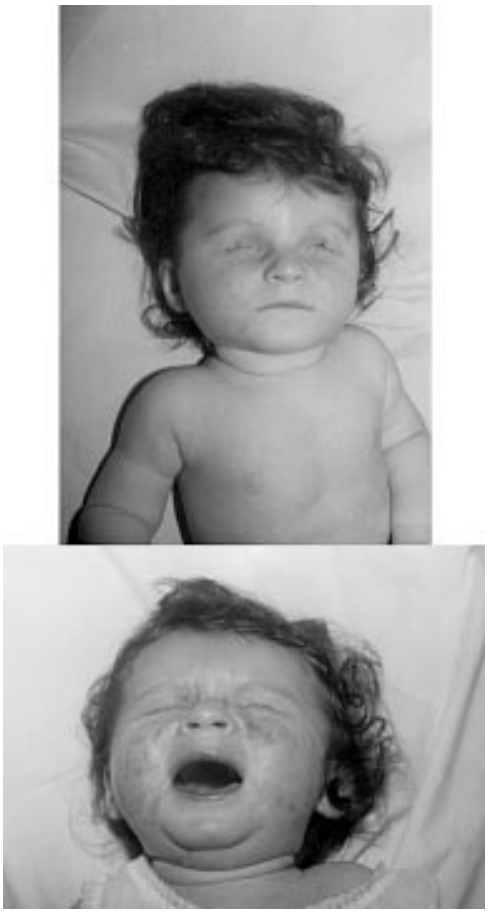

Figure 2 The patient at the age of 6 months. Note bilateral microphthalmia and irregular linear areas of skin hypoplasia involving the face and neck.

J Med Genet
2001;**38**:411–417

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(including our case) have been published and in approximately half (including our case), the Xp22.3 disruption has resulted from a terminal deletion.[3–10] In the remaining cases, Xp22.3 segmental monosomy is a consequence of X;Y[8 11–13] or X;autosome translocations.[14–18]

In all cases, there is monosomy for over 10 megabase pairs and comparison with patients harbouring smaller deletions has defined a ~570 kb interval that must contain the gene(s) giving rise to the diagnostic clinical features of the disorder, including microphthalmia, sclerocornea, and linear skin defects. Subsequent and ongoing investigations have led to the identification and characterisation of three genes from this minimal region as well as the precise mapping of a number of expressed sequence tags (ESTs). However, it is still currently unknown which gene(s) are responsible for this unique combination of features. Wapenaar *et al*[19 20] used cell lines from 10 MLS cases with deletions and translocations involving the Xp22 region to investigate the minimal region of monosomy leading to all the diagnostic features of the syndrome.

The entire region spanning the defining breakpoints has been cloned into overlapping cosmids, establishing the critical region to be approximately 570 kb in size and located just distal to the *AMG* locus.[19–21] Recent efforts directed at identifying the causative gene(s) for MLS have resulted in the mapping and preliminary characterisation of three genes from the critical interval: the X linked Opitz syndrome gene, *MID1*,[22 23] a gene encoding a holocytochrome c synthase (*HCSS*),[24] and a gene encoding a GTPase activating protein, *ARHGAP6* (this paper).[25] None of these genes has yet been implicated in the diagnostic features of the MLS phenotype.

Case report

Here we present a female patient with MLS, who had unusual, red, reticulolinear, non-vesicular, erythematous skin lesions on her face and neck and bilateral microphthalmia at birth. Relative microcephaly and linear streaks of erythematous skin on the face starting medial to the inner canthi (3-4 mm in width) and

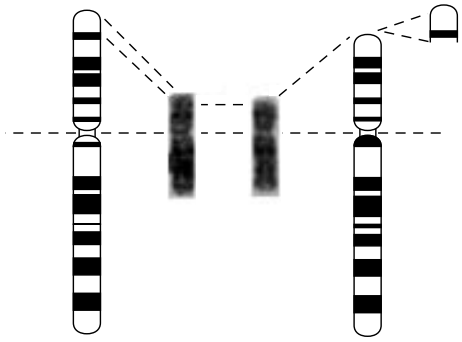

Figure 3 Partial karyotype of the X chromosome. The deleted X chromosome with a breakpoint at p22.3 is shown on the right.

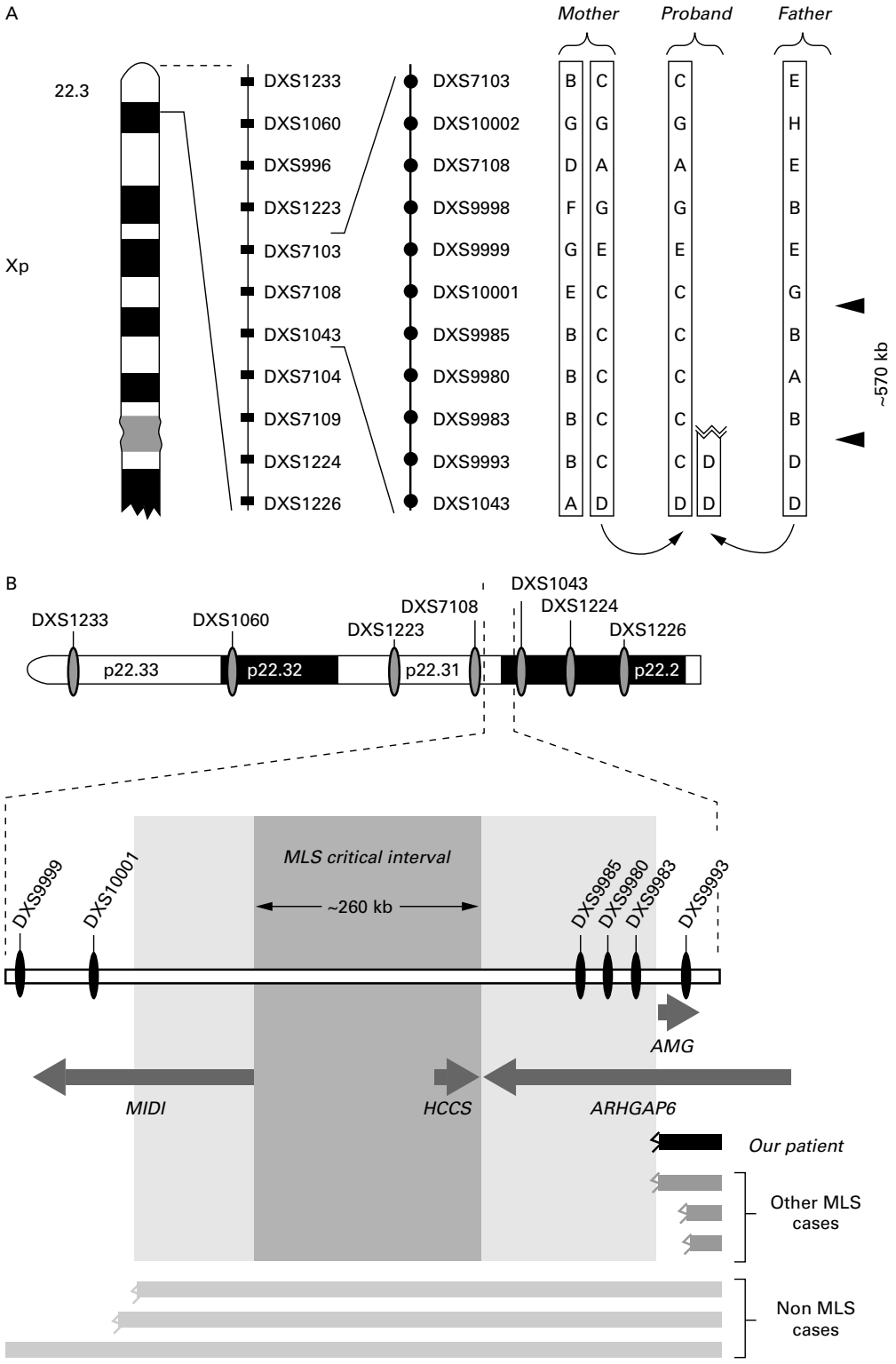

Figure 4 (A) Molecular analysis of the extent of the Xp deletion in the proband using microsatellite markers within and surrounding the MLS critical region. The distances between markers are not proportionately represented. Only informative markers are shown. Allele sizes from the parental and proband's chromosomes are shown as letters. The previous boundaries of the MLS critical region are indicated by arrowheads. (B) Location of genes and breakpoints from selected patients with MLS and without MLS features. Light shading=previously designated MLS critical region. Darker shading=proposed location of the gene(s) causing diagnostic features of MLS. The approximate size of the new MLS critical region (260 kb) corresponds to the interval between the 5' end of the MID1 gene and the 3' end of the ARHGAP6 gene.

continuing down along the side of the nose and onto the cheeks were noted. The streak (medial to the nasolabial sulcus) on the right continued down the jaw line. The skin lesions on the neck were less linear and more reticular in pattern. The skin on the remainder of the body was normal (fig 1). Apart from small ears, a high arched palate, and hypoplastic genitalia, other systems were normal. A CT scan showed small bulbus oculi (5 mm in diameter) bilaterally,

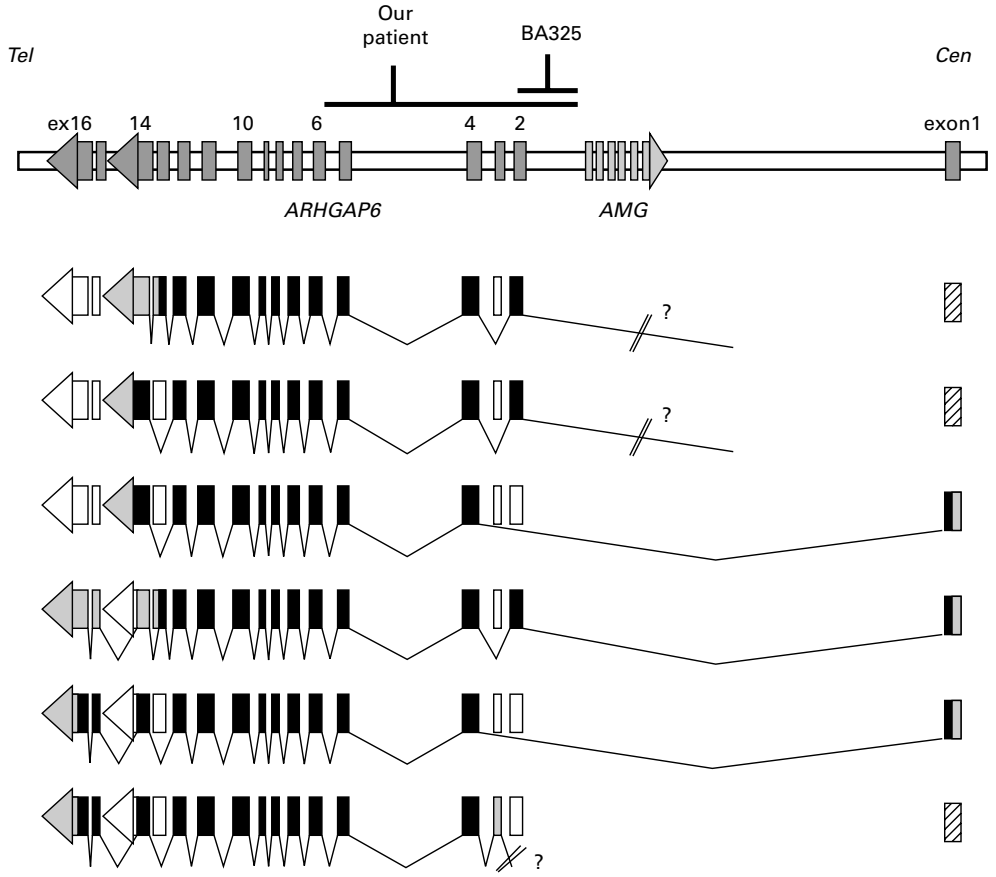

Figure 5 Exonic structure and transcriptional orientation of the ARHGAP6 and AMG genes. Six different mRNAs are formed as a result of complex alternate splicing at both the 3' and 5' ends of the ARHGAP6 gene. The size of the ARHGAP6 gene is ~350 kb. Also indicated are the locations of the breakpoints in our patient and case BA325. Light shading=untranslated regions. Black boxes=translated regions. White boxes=exons not used in particular transcripts. The cDNA clones for three of the mRNA isoforms do not represent complete sequences (indicated by question marks at their 5' ends). However, it is likely that exon 1 (hatched) is also used in these transcripts. The exon and intron sizes are not drawn to scale.

intact extraocular muscles and optic nerves, and agenesis of the corpus callosum. On re-evaluation at 6 months of age microcephaly (OFC of 38 cm) was prominent with a normal height and weight. The skin lesions were milder in appearance than at the previous examination but became more prominent with crying (fig 2). Her developmental milestones were moderately delayed. High resolution chromosome studies showed a 46,X,del(X) (p22.3→pter) karyotype (fig 3). Both parental karyotypes were normal.

To investigate the extent of the deletion further, we typed 15 polymorphic markers that map to Xp22 on DNA prepared from the proband and her parents. In fact, most of these markers were known to map within or immediately around the MLS critical region.[21] The results clearly and precisely showed the localisation of the patient's breakpoint (fig 4A). All informative microsatellites that were tested from Xpter to DXS9983 (within the MLS critical region) showed absence of the paternally derived X chromosome in the patient. DXS9993, and all markers further centromeric to it, clearly showed the presence of both paternal and maternal alleles in the proband (fig 4A). Unfortunately, other recently developed markers (DXS9982, DXS9984, and DXS9986) located between DXS9983 and DXS9993[21] were uninformative and thus the position of the breakpoint could not be further refined. Verification of the microsatellite findings was, however, obtained by both fluorescence in situ hybridisation analysis of metaphase chromosomes and quantitative Southern blot analysis of DNA from the parents and the proband using three different cosmids and subcloned cosmid fragments, respectively, as probes (data not shown). Trapped exons and selected cDNA fragments were used as complex probes on cDNA libraries derived from various tissues. Multiple cDNA clones were isolated from an Adult Retina library that each derived from the same gene. Sequencing and compilation of these cDNAs indicated that the gene probably encodes a GTPase activating protein (data not shown). The gene was subsequently reported by others as the *ARHGAP6* gene.[25]

We have determined the relative position and exonic organisation of the *ARHGAP6* and *AMG* genes, which are known to map in the vicinity of the position of the breakpoint in our patient. These analyses showed the *ARHGAP6* gene to be composed of 16 exons which are transcribed in the centromere to telomere direction (fig 4B, fig 5). These new data have led to the identification of an additional 5' untranslated exon approximately 160 kb further centromeric to the previously designated first exon, extending the *ARHGAP6* gene size to approximately 350 kb. This finding thus

Table 1 Clinical and laboratory findings of patients with Xp22.2/Xp22.3 disruption owing to deletions

	Reference												
Trait	*3*	*4*	*5*	*6 Case 1*	*6 Case 1 mother*	*7 Case 1*	*7 Case 2*	*8 Case 1*	*8 Case 2*	*8 Case 2 mother*	*9*	*10*	*Our case*
Sex	F	F	F	F	F	F	F	F	F	F	F	F	F
Eye anomalies			Iridoschisis		–						Red lid fissures	Strabismus	+
Sclerocornea/corneal opacity	+	+		+		+	+	+		+	+	–	–
Microphthalmia	+	+	+ Unilateral	+		+	+	+			+	–	+
Lacunae													
Pigmentary chorioretinopathy						+				Hypopigment		+	
Linear skin defects (face, neck)	+	+		+	+	+	+	+		+	+	–	+
CNS anomalies		–			–				Exencephaly	–	+	Hydrocephalus +	
Callosal agenesis	+			Displaced CC		+	+				+		+
Infantile seizures												+	
Other				Sept pellicidum		Colpocephaly	–	Hyperextensibility		–	Colpocephaly	Prof retardation	
Skeletal anomalies		–			–		–				Elbow hypoextension	–	–
Rib/vertebral						+							–
Growth retardation						+						–	+
Short stature					+			<3rd centile		+			+
Mental retardation		–			–			+		–			+
Other	Nail dysplasia	–	Goitre, hip joint dysplasia, labium synechia, craniotabes	Diaphragmatic hernia, dysmorphism	Tooth anomalies	Dysmorphism, high arched palate, macular depig	A-V block, dysmorphism, nail dysplasia	Dysmorphism		Short limbs	Patent ductus arteriosus, dysmorphism, hypotonia, anterior anus	Inguinal hernia, hermaphroditism, hyperpigmentn	Microtia, high arched palate, hypoplastic genitalia
Karyotype	del	del	del	del	del	del	del	del	del	45,X/del	del	del	del
Deletion Xp-pter	p22.3	p22.2	p22	p22.2	p22.2	p22.31	p22.3	p22.2	p22.2	p22.2	p22.1	p21.1	p22.3
Origin of disrupted X	ND	ND	pat	mat	ND	pat	ND	ND	mat	ND	ND	ND	pat
Inactivation pattern	ND	ND	der(X)	ND	ND	der(X)	ND	der(X)	ND	ND	ND	der(X)	ND

ND: not done, mat: maternal, pat: paternal, del: deletion, der: derivative.

places the small *AMG* gene within the first intron of *ARHGAP6* and transcribed in the opposite direction. Strikingly, our cDNA data and genomic sequence analysis suggest that complex alternate splicing is occurring at both the 3' and 5' ends of the *ARHGAP6* gene. Consequently, multiple protein isoforms are predicted to be encoded by this gene. Our microsatellite data therefore position the breakpoint in our patient between the *AMG* gene and exon 6 of the *ARHGAP6* gene (fig 4B). Interestingly, the breakpoint in the patient (BA325) previously defining the proximal boundary of the MLS critical interval is located between *AMG* and exon 2 of *ARHGAP6* (fig 5).[26]

Discussion

The cytogenetic and molecular analysis of the present case have clearly identified one of the smallest reported Xp deletions in a patient who expresses the full diagnostic phenotype of MLS. In fact, the breakpoint occurs within the same region as seen in a case (BA325) previously defining the proximal boundary of the critical interval. Our new data on the structure of genes around these breakpoints have indicated that both occur distal to the *AMG* gene (in intron 1 of *ARHGAP6*) and proximal to exon 6 (our case) or exon 2 (BA325) of the *ARHGAP6* gene. Consequently, no functional product is expected to be produced from the severely truncated *ARHGAP6* allele in these two patients. Our recent finding that the 5' end of *MID1* is located approximately 120 kb proximal to the telomeric boundary of the MLS critical interval[23] implies that the minimal interval harbouring the gene(s) causing the diagnostic features of MLS must lie between the end of the *MID1* gene and the breakpoint defined in this report, a region of only 450 kb. Notably, however, Prakash *et al*[26] have shown that even male mice harbouring a targeted disruption of the *Arhgap6* gene show no detectable phenotypic or behavioural abnormalities. As approximately 190 kb of the 450 kb MLS region is taken up by the remainder of the *ARHGAP6* gene, these findings suggest that the causative MLS gene(s) may reside within the 260 kb interval between the 5' end of *MID1* and the 3' end of *ARHGAP6*. To date, only one full length gene (*HCCS*) has been described in this interval.[24] Targeting of this gene in mice is currently being performed to address its contribution to the MLS phenotype.

Both our patient and BA325 show considerable similarity in their clinical features over and above those necessary for diagnosis (that is, microphthalmia and linear skin defects). However, there are some differences. For example, case BA325 was documented as having sagittal clefting of the vertebrae, a small, "punched out" skull defect, and macular depigmented skin lesions on the trunk,[7] features not present in our case. As both cases have been shown to be terminal deletions of paternal origin, it follows that any differences in the clinical presentation of both cases is most likely attributable to differences in the pattern of X chromosome inactivation. However, differences in severity of particular features may also conceivably be the

Table 2 Clinical and laboratory findings of patients with Xp22.2/Xp22.3 disruption owing to translocations

Trait	Reference 8 Case 3	11 Case 1	11 Case 2	12 Case 1	12 Case 1 mother	13	14	15	16	17	18
Sex	M	F	F	F	F	M	F	F	F	F	F
Eye anomalies			Orbital cyst	Glaucoma	Blindness	Lens dysp + glaucoma					
Sclerocornea/corneal opacity		+	+	+	–	+	+	+	+	+	–
Microphthalmia	Right>left	+	+	+	+	+	+	+ Unilateral	–	+	–
Lacunae							+			–	
Pigmentary chorioretinopathy								Teratoma/lipoma?		–	
Linear skin defects (face, neck)	+	+	+	+	+	+ Sm muscle hamartoma	–	–	+	+	–
CNS anomalies		Hydrocephalus				+				–	
Callosal agenesis				+		+	+	–			–
Infantile seizures							Hydrocephalus	+			
Other		–		Forebrain atrophy	Mild retardation	Colpocephaly	Dandy-Walker	–	Cerebral cysts		
Skeletal anomalies	–	–		–	–	–				Proximal thumbs	Right bifid thumb
Rib/vertebral							+	–	–		
Growth retardation	+ (3–10 p)	+	+			+	+	+		+	Normal stature
Short stature							+	+ (3–25 p)		+ (5 p)	–
Mental retardation	–		–				+	–			+
Other	Hypospadias	2nd/3rd toe syndactyly	Midline sacral dimple, anterior anus	Tetraplegia, hermaphroditism		Hearing loss, anterior/ imperforate anus, ASD, cryptorchidism, hypospadias		Dysmorphism, hypotonia		ASD, VSD, dysmorphism, hearing loss	Dysmorphism
Karyotype	t(X;Y)	t(X;Y)	t(X;Y)	t(X;Y)	t(X;Y)	t(X;Y)	t(X;3)	t(X;3)	t(X;2)	+der(X)	t(X;3)
Deletion Xp-pter	p22.2	p22.3	p22.3	p22.3?	p22.3?	p22.3?	p22.2	p22.2	p22.1	p22.3	p22.3
Origin of disrupted X	pat	pat	pat	mat	pat	pat	ND	mat	mat	ND	ND
Inactivation pattern	ND	der(X)	ND	ND	ND	ND	der(X)	ND	ND	der(X)	Mosaic

ND: not done, mat: maternal, pat: paternal, del: deletion, der: derivative.

result of differences in the genetic background, a phenomenon that has generally been ignored as a possible explanation in previous publications. In reviewing the clinical findings of the two dozen reported cases of MLS, it is likely that many of the frequently associated but non-diagnostic features observed in MLS females can be accounted for by monosomy of the X linked Opitz syndrome gene, *MID1*, which partially overlaps the MLS critical interval at its distal end (tables 1 and 2). Although Opitz syndrome is more severe in males, many females have been reported with some features of the disorder. In fact, clinical variability is also seen between affected males of the same family, indicating that the variability in females (including MLS females) is likely to be determined by factors other than the status of X chromosome inactivation, such as genetic background or environmental influences. Among the clinical features shared by MLS females and cases of Opitz syndrome are facial dysmorphism, deformed ears, high arched palate, structural heart defects, mild craniosynostosis, anteriorly displaced or imperforate anus, and various genital defects. However, microphthalmia, sclerocornea, and linear skin defects have not been reported in Opitz syndrome.

This work was supported in part by the Italian Telethon Foundation. During this work, TCC was supported by a C J Martin Fellowship and an R Douglas Wright Fellowship both from the National Health and Medical Research Council of Australia. TCC also acknowledges the support provided by an AMRAD Post-Doctoral Award.

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J Med Genet
2001;**38**:417–420

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Cryptic telomeric rearrangements in subjects with mental retardation associated with dysmorphism and congenital malformations

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EDITOR—Cryptic telomeric rearrangements are a significant cause of idiopathic mental retardation. Knight *et al*[1] found 7.4% of these rearrangements in children with moderate to severe mental retardation. Clinical selection criteria for testing patients with subtelomeric chromosome specific probes are still not clear cut and the importance of other surveys to define this point has been stressed.[2] With this aim, we examined 200 patients with idiopathic mental retardation, either isolated or associated with dysmorphism and/or congenital anomalies, using FISH analysis with subtelomeric chromosome specific probes.

Material and methods

The sample was collected in four Italian Genetic Centres. Patients were selected on the basis of the following criteria: (1) the presence of mental retardation that was classified as mild, moderate, or severe according to DSM IV[3]; (2) patients under 1 year of age, too young for psychological assessment, were selected for the presence of developmental delay; (3) exclusion of pre- or perinatal distress through an accurate evaluation of the pre-, peri-, and postnatal patient history; and (4) exclusion of Mendelian syndromes and of genomic disorders[4 5] for which a specific diagnostic test is available.

The essential elements of evaluation also included family history, a complete physical and neurological examination of the patients with particular attention to the presence of mental retardation and multiple congenital anomalies, and assessment of the behavioural phenotype. Electroencephalograms, brain CT scan, and MRI were performed in specific situations.

Abnormal methylation and expansion at FRAXA and FRAXE[6] were excluded in 52 and 50 males and in 37 and 32 females, respectively.

Routine cytogenetic analysis at the 400-550 band level was performed in all the patients. In those patients in whom a cryptic subtelomeric rearrangement was identified by FISH, prometaphase chromosomes were also analysed to determine if the rearrangement could be detected in retrospect by cytogenetic analysis. Chromosome preparations from peripheral blood or from lymphoblastoid cell lines were used for FISH analysis. The Chromoprobe-T kit with telomere specific clones[7] was used according to the supplier's instructions (Cytocell, UK) with minor modifications. To establish the origin of each rearrangement, FISH and microsatellite analysis with subtelomeric probes were performed in the parents of the patients. FISH experiments with different YACs from each rearranged chromosomal region were performed to define its size.

Results

Among our 200 patients (table 1), 44 had mild mental retardation (IQ 50-70), 62 were moderately retarded (IQ 50-35), and 55 were severely retarded (IQ 35-20). A total of 39 patients had mental retardation not otherwise specified.