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SHORT REPORT

A very high density microsatellite map (1 STR / 41 kb) of 1.7 Mb on Xp22 spanning the microphthalmia with linear skin defects (MLS) syndrome critical region

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Microphthalmia with linear skin defects (MLS) syndrome is an X-linked disorder presenting only in XX individuals. It is characterised by dysmorphic features such as microphthalmia, sclerocornea, and linear streaks of erythematous and hypoplastic skin restricted to the head and neck. Karyotype analyses have so far revealed a terminal deletion or translocation causing monosomy for the distal Xp region (Xp22.3) in all patients. We have used existing cosmid clones from the region to perform a saturation screen for AC-type microsatellites with the goal of facilitating analysis of five novel patients with features of MLS. Three of these cases had an Xp22.3 abnormality, while the other two showed some characteristic features of MLS but had apparently normal karyotypes. Forty-two novel microsatellite markers have now been developed from the 1.7 Mb cloned interval. Ninety-three percent of the novel markers exhibited allelic variation, representing an average of one polymorphic PCR-based marker (STR) every 41 kb.

Keywords: Xp22; microsatellites; MLS; Aicard syndrome; Goltz syndrome; X chromosome; contiguous gene syndrome; microphthalmia; linear skin; focal dermal hypoplasia; paraffin-embedded

Introduction

The high frequency of naturally occurring chromosomal rearrangements involving the distal short arm of the X chromosome has made this region particularly amenable to deletion/phenotype correlation mapping. Recently, a new syndrome called microphthalmia with linear skin defects (MLS; MIM#309801) has been added to this list of contiguous gene syndromes. The salient features of this syndrome include severe microphthalmia, sclerocornea, and linear streaks of erythematous and hypoplastic skin restricted to the head and neck. Clinical presentation in only XX individuals implies embryonic lethality in hemizygous individuals. With one possible exception,[1] all published cases with a 'classical' MLS phenotype have presented with cytogenetically visible abnormalities involving Xp22 and this

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feature is generally accepted as a criterion for diagnosis.[2–7]

Some authors have noted clinical overlap between the MLS syndrome phenotype and that observed in two other X-linked dominant disorders exhibiting embryonic male lethality, Aicardi syndrome and focal dermal hypoplasia (FDH) or Goltz syndrome, and this has led to the hypothesis that the latter two disorders may be due to different patterns of X-inactivation of the same gene or genes involved in expression of the MLS phenotype.[6,8,9] Based on the molecular characterisation of the chromosomal abnormalities in MLS patients, a 0.6 Mb genomic region was reported to contain all or part of the disease gene(s).[10,11]

Here we report the near-complete and fully annotated cosmid contig covering approximately 1.7 Mb distal to Amelogenin and encompassing the critical region for MLS. Over 40 new polymorphic repeat markers have been developed and these, together with all relevant anchor STS markers from the larger linkage and YAC-based physical maps.[12,13] have been precisely positioned on the cosmids to unequivocally correlate genetic and physical distances. The microsatellites have been used to analyse breakpoint positions in three MLS patients with Xp22 cytogenetic abnormalities and to search for submicroscopic deletions in two exceptional karyotypically normal cases with features of MLS.

Materials and Methods

Cosmid Library Screening and Contig Assembly

Some of the cosmids have been previously described.[11] Closure of gaps in this map was achieved by screening both the LLOXNC01 and the Reference Library Database X-chromosome specific cosmid libraries with probes derived from cosmid ends closest to each gap (see Figure 1). Filters were hybridised as previously described.[11]

Isolation of Polymorphic Microsatellite Markers

Digested Cosmid DNAs were immobilised on Hybond N+ membrane and hybridised with a probe containing an uninterrupted dinucleotide stretch (CA18). Restriction fragments displaying strong hybridisation signals were gel purified, cloned into appropriately restricted pBluescript vector and sequenced.

STR Analysis

Sequences derived from CA-positive fragments were analysed for database homologies using the BLAST-N and BLAST-X algorithm as previously described.[14] Primers were designed around each uninterrupted dinucleotide repeat ($\geq$12 repeating units).

Patients

Detailed clinical presentations have been previously reported for BA649[1] and BA96.[15] A full case report of patient BA530 will be presented elsewhere (Kayserili *et al*, submitted). Details of all other cases in this study are previously unreported.

Preparation and PCR on DNA from Paraffin Embedded Tissue

Two to three 0.5 μm slices from a paraffin-embedded skin biopsy were used. The paraffin was dissolved with xylene. The tissue was pelleted, rinsed in ethanol, and desiccated. The tissue was then resuspended in 20 μl of lysis buffer (0.5 mM KCl, 0.1 mM Tris-HCl pH 8.3, 0.15mM MgCl2, 0.1 mg/ml gelatin, 0.45% Nonidet P40, 0.45% Tween 20), 0.8μl of proteinase K (10 mg/ml) added and the sample incubated at 65°C for 1 h followed by heat inactivation at 98°C for 20 min. Cell debris was briefly pelleted prior to use in amplification reactions; 0.5–2 μl of the preparation was used for amplification of the developed microsatellites.

Results

Saturation Screen for CA-type Polymorphic Dinucleotide Repeats

A restriction fragment containing a CA18 repeat was used as a probe on digests of 52 overlapping cosmids spanning the MLS region.[11] Of the 44 subclones which exhibited medium to strong hybridisation signals and from which sequence was generated, 36 contained uninterrupted CA-repeat lengths of 12 units or greater ([CA]*n*; $n \geq 12$). Six PCR-based markers other than CA repeats were developed: a GA-repeat (CxM33); a tetranucleotide repeat, (AAAC)9; a common XbaI restriction site polymorphism; and polyA stretches of 23 (CxM08); 27 (CxM27); and 30 (CxM10) nucleotides. For all these sequences primer pairs were designed.

One of the CA repeat units was revealed to correspond to DXS7108 (see Figure 1). In total, 40 novel potentially polymorphic markers, were identified. Each microsatellite was tested for its level of polymorphism directly on the patient, parent samples, and on a set of eight unrelated phenotypically normal individuals (representing 12 independent X chromosomes). Thirty-seven of the 40 newly developed markers exhibited allelic variation in the limited number of individuals examined (Table 1). Allele sizes, annealing temperature and primer sequences are available at http://www.tigem.it/TIGEM/PUBLIC/index.html.

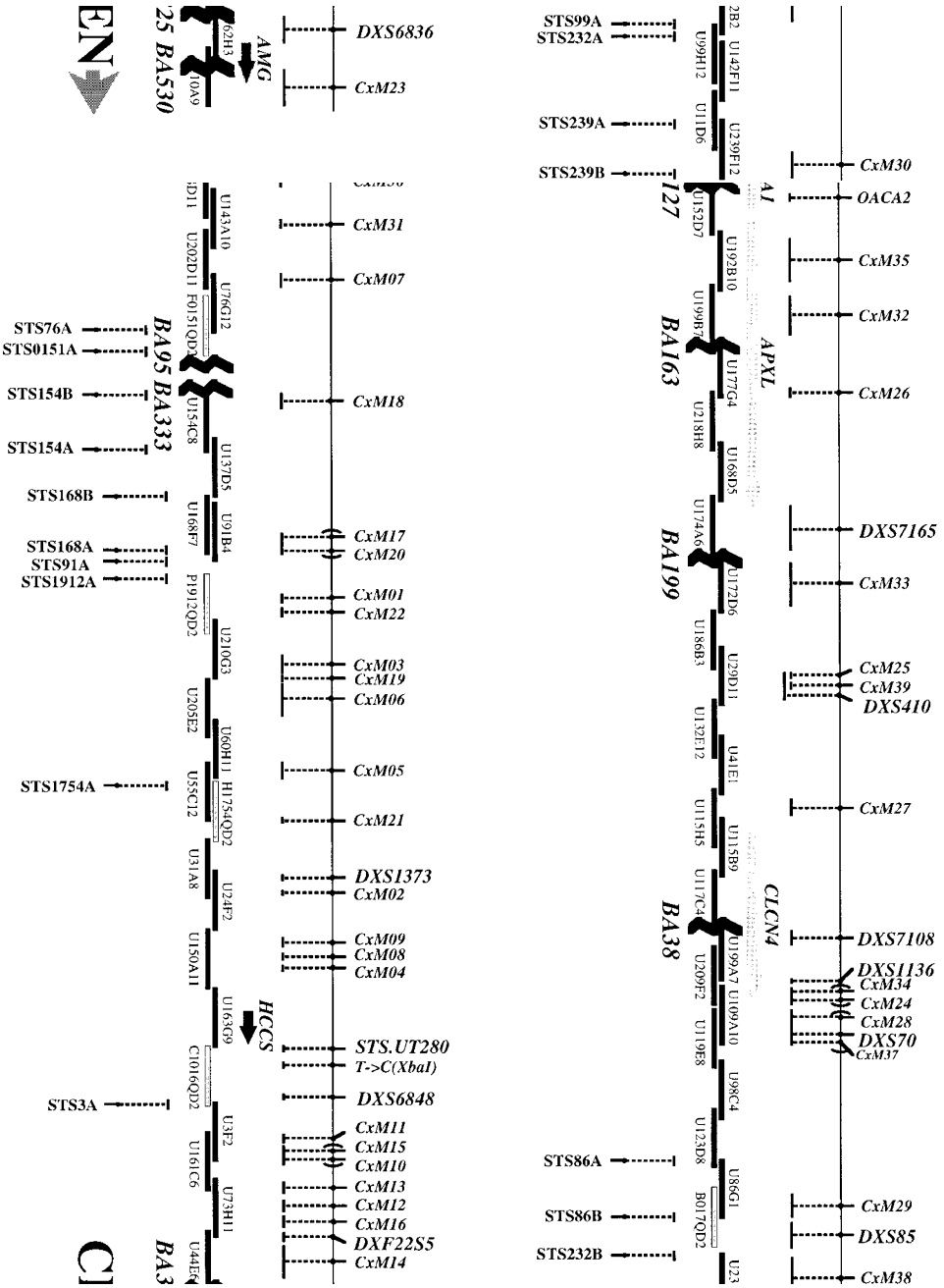

Figure 1 *Integrated physical and genetic map of 1.7 Mb distal to Amelogenin in Xp22.3. Patient breakpoints are indicated by jagged lines and underwritten 'BA' numbers. Cosmids derived from the Lawrence Livermore National Laboratories (LLNL) and Reference Library Database (RLDB) X chromosome-specific libraries are represented by filled or lightly shaded rectangles, respectively. The relative degree of overlap of each named cosmid is also depicted. The names indicated for the RLDB cosmids have been shortened for the figure: each cosmid can be correctly identified by the addition of 'ICRFc104' to the front of each supplied identification. Published genes are drawn as lightly shaded arrows – the arrowhead representing the orientation of transcription with respect to the centromere and telomere. Filled arrow denotes unpublished gene 176X (Bassi et al., in prepartaion). The regional positions of the newly developed markers (CxM) as well as STS and STR markers transposed from the YAC[13] and Généthon linkage[12] maps are indicated by proportionately sized horizontal bars above the cosmid contig. Relevant new STS markers (derived from cosmid end-fragment sequencing) are indicated similarly below the cosmid contig.*

Assembly and annotation of the 1.7 Mb Cosmid Map in Xp22.3

We have determined precise cosmid locations for 13 Xp22 loci, whose position on the YAC map of this region had previously been determined (bins 55–73 from Ferrero *et al*).[13] Ten of these 13 loci mapped to the cosmids with a relative physical order that was consistent with previous YAC data,[13] with the exception of DXS410 which was mapped distal instead of proximal to CLCN4. Locus DXS6836, was localised between DXF22S5 and AMG, two to three bin positions away from its originally reported location.[13] Four other loci investigated (DXS8051, DXS7103, DXS1313 and DXS1380) did not map within the current cosmid contig boundaries. Of note, an additional STS (HUMUT280; Genbank accession #L31676) has been fortuitously localised adjacent to DXS6848 in cosmid U188H7 (Figure 1) by homology searches from initial sequencing efforts (unpublished data).

In addition to these existing markers, the newly developed microsatellites from this region were also integrated into the cosmid map through PCR analysis of cosmid DNAs. Annotation of the cosmid map allowed us to uncover some inconsistencies, involving contigs 6 and 7 of the original contig.[11] The new cosmid order for this region (see Figure 1 has been confirmed by a combination of PCR-amplifiable STS and microsatellite markers as well as by hybridisation and cosmid fingerprinting.

To close the gaps between the contigs,[11] we have used the STS markers and end-fragment subclones closest to the reported gaps to screen the Lawrence Livermore and the RLDB X chromosome specific cosmid libraries. In total, 60 clones (50 described by Wapenaar *et al*,[11] 10 newly isolated) comprise the minimal set of overlapping cosmids in the five contigs (represented in Figure 1) necessary to cover the 1.7 Mb region.

Patient Analysis

Four different types of patient were collected for analysis: Group 1 – three patients with MLS carrying a cytogenetically detectable Xp22 abnormality – BA96, BA389 and BA530; Group 2 – two exceptional cases with MLS features but exhibiting an apparently 'normal karyotype' – BA649 and BA529; Group 3 – three karyotypically normal patients with typical Aicardi syndrome – BA306, BA336 and BA643; and Group 4 – three karyotypically normal patients with FDH – BA330, BA416 and BA639. Patients with Xp22 abnormalities had either 'pure' terminal Xp deletions or unbalanced X/autosomal translocations (producing Xp22 monosomy). It is important to point out that the two patients with MLS features but normal karyotypes show linear skin lesions strikingly similar to those observed in MLS patients with documented Xp22 abnormalities, however, they do not exhibit microphthalmia.

A total of 52 polymorphic markers, 40 of which were developed in this study, were typed on patients' DNA and on their parents (where available), to characterise the extent of the cytogenitically detectable deletions and to search for the presence of submicroscopic deletions in those patients with normal karyotypes. The analysis of cases with cytogenetically visible abnormalities (Group 1) permitted a precise assignment of the deletion breakpoints. The breakpoints of patients BA96[15] and BA389 were mapped between DXS1043 and DXS1224, and between DXS1224 and DXS1226, respectively (Table 1). In both cases, the results have been confirmed by fluorescence *in situ* hybridisation using a YAC probe (data not shown). The third MLS patient, BA530, presents with the full spectrum of diagnostic MLS features and appears to be a 'pure' terminal deletion case as suspected cytogenetically.

Samples from the two karyotypically normal MLS patients (BA649 and BA529) were initially analysed using available polymorphic microsatellites from Xp22: DXS1233, DXS1060, DXS996, DXS1223, DXS1043, DXS1224 and DXS1226. Subsequently, the microsatellites developed in this study were analysed. All the results were consistent with two alleles being present for each marker tested on both these novel MLS patients. Therefore, fully informative markers provided a rapid scanning resolution of approximately 100 kb for BA649 and 75 kb for BA529 with no Xp abnormality evident (Table 1).

Similarly, the microsatellite analysis of Aicardi and FDH syndrome patients found no evidence for submicroscopic deletions within the critical region for MLS (Table 1).

Discussion

With the ultimate goal of identifying and characterising the gene(s) contributing to the MLS phenotype, we set out to develop a near-saturation map of AC-type microsatellites which would aid in the identification of deletion boundaries by comparing patient with parental alleles.

	Group 1					Group 2						Group 3						Group 4			
Marker	**BS96**	**BS389**	**mo**	**fa**	**BA530**	**mo**	**fa**	**BA649**	**mo**	**fa**	**BA529**	**BA336**	**mo**	**fa**	**BA306**	**mo**	**fa**	**BA643**	**BA330**	**BA416**	**BA639**	***Marker***
DXS1233	C	C	BC	B	C	AC	C	√	AC	C	C	AC	C	C	C	AC	C	AC	AC	C	AC	*DXS1233*
DXS1060	F	B	A	D	A	BF	F	F	D	B	BD	DE	B	A	AB	BD	B	BD	BD	DF	AC	*DXS1060*
DXS996	H	C	AE	E	E	E	E	(E)	CG	H	GH	BD	EH	I	EI	CH	G	GH	FH	GH	CE	*DXS996*
DXS1223	G	D	DG	G	D	CF	B	BF	FG	D	DF	DE	BD	G	DG	DG	F	FG	BE	CF	AF	*DXS1223*
DXS7103	D	F	BC	E	C	E	C	EC	CE	B	BE	E	E	B	BE	E	E	E	E	BC	B	*DXS7103*
OACA2 [16]						F	G	FG	BF	E	EF	BI	F	G	FG	AC	G	CG	FG	BG	G	*OACA2*
DXS10005 (CxM35)						DH	D	√	EJ	E	EJ	CH	AD	D	AD	GH	G	GH	GH	EH	FG	*CxM35*
DXS10002 (CxM32)	F	A	G	H	G	FG	G	G	E	C	CE	BE	DG	G	G	G	G	G	G	BG	EG	*CxM32*
DXS9996 (CxM26)						BC	B	√	B	B	B	AB	BC	B	BC	B	B	B	BC	AB	BC	*CxM26*
DXS10003 (CxM33)						A	A	(A)	A	A	A	A	A	A	A	A	A	A	A	A	A	*CxM33*
DXS9995 (CxM25)						C	C	(C)	C	C	C	C	C	B	BC	BC	C	BC	C	C	BC	*CxM25*
DXS10009 (CxM39)						B	B	(B)	BC	B	BC	B	AB	B	AB	B	B	B	B	B	B	*CxM39*
DXS9997 (CxM27)						D	D	(D)	AD	A	AD	AD	C	D	CD	D	D	G	AD	D	AD	*CxM27*
DXS7108	A	B	AD	E	A	DE	D	D	BD	E	DE	CD	CE	F	CF	DE	G	EG	CE	D	BE	*DXS7108*
DXS10004 (CxM34)						AF	C	√√	CD	F	CF	CF	F	D	DF	BG	H	GH	F	EF	D	*CxM34*
DXS9994 (CxM24)	F	F	DF	F	F	F	F	(F)	F	B	BF	DF	CH	F	FH	CF	A	AC	F	CD	F	*CxM24*
DXS9998 (CxM28)	G	G	FG	B	G	G	G	(G)	G	B	BG	G	EG	F	FG	BG	D	BD	G	F	AC	*CxM28*
DXS10007 (CxM37)						C	C	(C)	CD	C	C	CF	CG	C	CG	C	F	CF	CE	F	AC	*CxM37*
DXS9999 (CxM29)	A	B	EG	E	E	BD	D	D	DE	E	DE	DG	AE	D	AD	DH	C	CE	AB	F	E	*CxM29*
DXS10008 (CxM38)						AB	B	B	B	A	AB	B	AB	B	AB	B	B	B	AB	B	B	*CxM38*
DXS10000 (CxM30)						CF	D	√√	DF	F	DF	BI	D	E	DE	DG	G	G	EF	DE	BG	*CxM30*
DXS10006 (CxM36)						B	B	(B)	AC	B	AB	C	CD	B	BC	BD	C	BC	AD	AB	BE	*CxM36*
DXS10001 (CxM31)	C	G	CE	G	C	FG	H	GH	GI	E	EG	BE	G	F	FG	CH	J	HJ	DI	G	DG	*CxM31*
DXS9977 (CxM07)	M	H	FL	E	L	DF	C	√√	BK	G	BG	EI	FI	K	IK	AL	E	AE	FM	FN	F	*CxM07*
DXS9988 (CxM18)	C	E	D	B	D	E	E	(E)	E	A	AE	CD	E	E	E	DE	E	E	DF	EF	CE	*CxM18*
DXS9987 (CxM17)	B	B	AB	A	B	A	B	AB	A	A	A	A	A	B	AB	B	A	AB	AB	A	B	*CxM17*
DXS9900 (CxM20)						A	C	AC	A	A	A	A	A	C	AC	C	A	AC	AC	A	C	*CxM20*
DXS9971 (CxM01)	C	C	BC	C	C	C	D	CD	BC	D	BC	C	AC	C	C	CD	D	CD	C	C	AC	*CxM01*
DXS9992 (CxM22)						AC	D	CD	AD	D	D	BC	BC	D	CD	D	D	CD	D	CD	DE	*CxM22*
DXS9973 (CxM03)						A	A	(A)	A	A	A	A	A	A	A	A	A	A	A	A	A	*CxM03*
DXS9989 (CxM19)						AB	B	B	AB	B	B	B	B	B	B	B	B	B	B	B	B	*CxM19*
DXS9976 (CxM06)						B	B	(B)	B	B	B	BC	B	B	B	B	B	B	B	B	AB	*CxM06*
DXS9975 (CxM05)						B	B	(B)	BD	B	BC	C	CD	B	BC	BD	C	BC	AD	AB	BE	*CxM05*
DXS9991 (CxM21)						BC	C	C	C	C	C	C	BC	C	BC	AC	C	AC	BC	C	C	*CxM21*
DXS9972 (CxM02)						D	B	BD	CD	D	CD	D	AD	D	D	BD	D	BD	BD	D	B	*CxM02*
DXS9979 (CxM09)	D	D	BD	D	B	BD	D	D	D	D	D	D	BD	D	BD	AD	D	D	AC	BD	D	*CxM09*
DXS9974 (CxM04)						B	B	B	B	B	B	B	AB	B	B	B	B	B	B	B	B	*CxM04*
DXS9978 (CxM08)						B	B	(B)	B	B	B	AB	B	B	B	B	C	BC	B	B	B	*CxM08*
T->C (Xbal)						n	x	nx	n	x	x	n	n	x	nx	n	x	x	nx	n	x	*T->C (Xbal)*
DXS9981 (CxM11)	A	C	B	B	B	B	E	BE	B	E	E(B)	B	B	E	BE	DE	B	BE	BE	AC	BE	*CxM11*
DXS9985 (CxM15)	E	A	BC	B	C	DE	N	EN	HN	N	N	DE	FG	N	GN	DL	H	HL	CM	BG	N	*CxM15*
DXS9980 (CxM10)	C	C	BC	A	C	BC	C	C	CD	D	D	B	AB	C	BC	C	C	C	C	C	C	*CxM10*
DXS9983 (CxM13)	B	B	BC	B	C	BC	B	BC	BD	B	BD	AC	BC	B	B	BC	B	B	C	B	B	*CxM13*
DXS9982 (CxM12)	A	A	A	A	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	*CxM12*
DXS9986 (CxM16)	C	C	D	D	D	D	C	CD	CD	B	BC	CD	CD	D	CD	CD	C	C	D	CD	BC	*CxM16*
DXS9984 (CxM14)	A	A	AB	A	A	A	B	√√	A	B	AB	A	AB	B	B	AB	D	BD	AB	A	BD	*CxM14*
DXS9993 (CxM23)	A	E	BC	D	CD	BC	E	BE	BD	G	BG	CD	DF	F	DF	BC	F	BG	BE	E	DF	*CxM23*
DXS1043	D	E	AD	D	D	DE	D	D	D	C	CD	D	D	D	D	CD	D	D	CD	D	BD	*DXS1043*
DXS7104	C	A	C	B	BC	C	C	(C)	CD	C	CD	C	C	C	C	C	D	CD	CD	C	C	*DXS7104*
DXS7109	C	A	C	C	C	C	C	(C)	BC	C	BC	C	C	C	C	C	B	BC	C	C	C	*DXS7109*
DXS1224	CG	E	FG	B	BG	CG	E	EG	G	D	DG	DE	G	G	G	AF	F	F	FG	G	E	*DXS1224*
DXS1226	CD	FJ	EG	C	CG	BG	C	√√	DE	E	E	EI	CI	G	GI	GH	H	GH	FG	GH	DF	*DXS1226*

Table 1 Analysis of MLS, Aicardi and FDH syndrome patients using the newly developed and publicly available polymorphic markers

Together with the two previously identified markers from the region (DXS7108 and OACA2),[16] the 40 markers generated in this study represent a combined average of one STR every 41 kb (or one AC-type STR every 47 kb). Considering the chosen threshold size of (CA) n [$n \geq 12$] for marker development, this frequency is consistent with original whole genome estimates.[17]

In addition to providing an anchored foundation for the subsequent efficient generation of its complete sequence, the direct integration of our 40 novel polymorphic markers into the recent Généthon linkage map[12] complements this broader coverage of markers and will be a valuable resource for positional cloning efforts of other disease genes mapped over this region of the X chromosome.

Three MLS cases with cytogenetically visible abnormalities in Xp22 were tested together with their parents using the microsatellites developed in this study (Table 1). In defining these breakpoints, we have identified a patient (BA530; Figure 1 and Table 1) whose deletion extends only to the Amelogenin locus. This deletion is one of the shortest known terminal deletions expressing the full spectrum of MLS clinical features, including the facial dermal aplasia, severe microphthalmia and agenesis of the corpus callosum.

One possible exception to the finding of cytogenetically visible abnormalities of Xp22 in MLS has been reported to date in the literature.[1] The availability of biopsy specimens taken from the linear skin lesions of this patient provided a unique opportunity to investigate by PCR the minimal region of deletion causing the MLS phenotype. During this study, a second 'normal karyotype' patient presenting with the characteristic facial skin lesions was brought to our attention (Dr A David, personal communication, 1996) and was incorporated in the analysis. Alelle inheritance patterns were analysed and the results showed no demonstrable Xp22 abnormality at the resolution limit defined by the physical order and informativeness of these markers (100 kb and 70 kb in BA649 and BA529, respectively). A number of explanations exist for these unique observations:

(1) these patients do not represent true examples of MLS (ie phenocopies);

(2) they have either small deletions which reside between informative markers developed in this study or point mutations in a gene from this region;

(3) they are mosaic for an Xp abnormality.

Although both patients present with the linear skin lesions restricted to the head and neck as the only truly characteristic feature of the MLS phenotype, the absence of microphthalmia does not rule out a diagnosis of MLS;[1] this paper. As further support for this, two other cases presenting with only the linear skin defects have been reported in the literature.[9,18] Both these patients have cytogenetically visible abnormalities involving Xp22 and each has had a child affected with the full spectrum of the MLS phenotype, supporting the hypothesis for a role of X-inactivation in the clinical presentation of the disorder.[6,9] That either or both of these patients may be mosaic for an Xp22 abnormality and, consequently, that a bias against detection using the polymerase chain reaction has been introduced seems unlikely (but cannot be excluded) as no cytogenetic mosaicism was detected during the

Table 1 *Continued.* Group 1: MLS patients with cytogenetic abnormalities of Xp22. Group 2: Patients with features of MLS but with normal karyotypes. Group 3: classical Aicardi syndrome patients. Group 4: patients with Focal Dermal Hypoplasia (FDH). The markers tested on each group of patients are indicated in Table 1 in an ascending order that reflects their relative physical order on the chromosome (see Figure 1): top of column represents the telomeric end; the bottom, the centromeric end. The two horizontal lines (between CxM27 and DXS7108 and between CxM14 and CxM23, represent the approximate positions of the patient breakpoints (BA38 and BA325, respectively, see Figure 1) that define the MLS critical interval designated for this study (see text for details). The total complement of alleles observed for each marker in this study were assigned letters according to decreasing size of the allele: A represents the largest allele of a given marker, B the second largest, etc. For the restriction site polymorphism, T- > C(XbaI), the letters 'x' (cut) and 'n' (not cut) in the spaces represent the status of each allele after digestion with the enzyme XbaI. Unshaded columns represent parental samples. Black spaces indicate confirmation of the presence of two alleles for a given marker, i.e. not deleted. Dark grey shaded spaces (e.g. Group 1, columns 1, 2 and 5) indicate the confirmed absence of one allele. Lightly shaded spaces represent markers which could not be confirmed to be present as one or two copies in patient samples, i.e. uninformative. Blank spaces (in Group 1 patients) indicate that the marker(s) was not examined in those individuals. Letters in brackets (BA649 column, Group 2) indicate assumed allele sizes based on uninformativeness of parental alleles and because of limiting patient tissue samples. Due to technical difficulties, in many instances a consequence of product size limitations in amplifucation of DNA from paraffin-embedded tissue, the markers in the BA649 column indicated with single (✓) or double (✓✓) checks could not be examined. Single checks imply potentially informative markers. Double checks imply fully informative markers. The reference for the previously reported marker OACA2 has been indicated.

repeated karyotype analyses on each patient. Furthermore, some of the informative marker results obtained on BA649 have been confirmed on two different affected tissue types: biopsy samples from an area of erythematous facial skin and cardiomyopathic heart taken at autopsy.

The saturation development of AC-type microsatellites surrounding the critical interval for the MLS syndrome is a unique approach that permitted the detailed molecular analysis of each X chromosome in critical patients which would have otherwise been impossible using standard techniques. Such high density microsatellite maps are very important resources for deletion scanning in any deletion-associated disease.

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