

## CASE REPORT

# Microphthalmia with linear skin defects (MLS) syndrome evaluated by prenatal karyotyping, FISH and array comparative genomic hybridization

Colyn Cargile Cain<sup>1</sup>, Daniel Saul<sup>1</sup>, Lisa Attanasio<sup>1</sup>, Erin Oehler<sup>1</sup>, Ada Hamosh<sup>2</sup>, Karin Blakemore<sup>1</sup> and Gail Stetten<sup>1,2\*</sup><sup>1</sup>Department of Gynecology and Obstetrics, Johns Hopkins University School of Medicine, Baltimore, MD, USA<sup>2</sup>Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, MD, USA

**Objective** To explore the utility of comparative genomic hybridization to BAC arrays (array CGH) for prenatal diagnosis of microphthalmia and linear skin defects syndrome.

**Methods** We used karyotype analysis, FISH and array CGH to investigate an X;Y translocation. Replication studies were done on cultured amniocytes and lymphoblasts.

**Results** We describe a severe case of MLS syndrome that presented prenatally with multiple anomalies including cystic hygroma, microphthalmia, intrauterine growth restriction and a complex congenital heart defect. Cytogenetic analysis of amniocytes revealed an unbalanced *de novo* translocation between chromosomes X and Y [karyotype 46,X,der(X)t(X;Y)(p22.3;q11.2).ish der(X)(DXZ1+,DMD+,KAL-,STS-,SRY-),22q11.2(Tuple1×2)]. MLS diagnosis was made at birth and the prenatal karyotype was confirmed. Replication studies showed the derivative X chromosome was the inactive X. Array CGH confirmed the X and Y imbalances seen in the karyotype and also showed twelve BACs in the MLS region were deleted as a result of the translocation. FISH with BAC clones verified the array findings and placed the X breakpoint in Xp22.2, resulting in the amended karyotype, 46,X,der(X)t(X;Y)(p22.2;q11.2).ish der(X)(DXZ1+,DMD+,KAL-,STS-,SRY-),22q11.2(Tuple1×2) arr cgh Xp22.3p22.2(LLNOYCO3M15D10→GS1-590J6)×1,Yq11.22q23(RP11-20H21→RP11-79J10)×1.

**Conclusion** The sensitivity of array CGH was valuable in detecting monosomy of the MLS critical region. Array CGH should be considered for the prenatal diagnosis of this syndrome. Copyright © 2007 John Wiley & Sons, Ltd.

KEY WORDS: MLS syndrome; X;Y translocation; FISH; array CGH

## INTRODUCTION

Microphthalmia with linear skin defects (MLS) syndrome (MIM 309 801) is a rare, X-linked dominant, developmental disorder associated with monosomy of the Xp22.3 region. MLS syndrome, also known as syndromic microphthalmia 7 and MIDAS syndrome, is characterized by linear areas of hypoplastic skin located on the head and neck, and eye findings including microphthalmia, sclerocornea and corneal opacities. Additional features include agenesis of the corpus callosum, ventriculomegaly, microcephaly, congenital heart defects, developmental delay and short stature (Al-Gazali *et al.*, 1990; Temple *et al.*, 1990; Happle *et al.*, 1993; Lindsay *et al.*, 1994; Zvulunov *et al.*, 1998).

The majority of MLS patients are observed to have deletions or unbalanced translocations involving the distal short arm of the X chromosome. The minimal region

of monosomy associated with the MLS phenotype lies in Xp22.2 and has been defined by cytogenetic studies and breakpoint mapping on ten MLS patients and two non-MLS patients with X;Y translocations (Wapenaar *et al.*, 1993, 1994). Four MLS patients have been reported with a normal karyotype (Cox *et al.*, 1998; Morleo *et al.*, 2005). Extensive studies of these four patients using array comparative genomic hybridization (CGH), Southern blotting and mutation analysis have failed to show any cryptic rearrangements, small deletions or point mutations in genes within the MLS critical interval (Morleo *et al.*, 2005).

In this report, we used karyotype analysis, fluorescence *in situ* hybridization (FISH) and array CGH to characterize a *de novo* unbalanced translocation, der(X)t(X;Y)(p22.2;q11.2), diagnosed in a 22 week fetus with ultrasound anomalies including cystic hygroma, microphthalmia, intrauterine growth restriction and a complex congenital heart defect. A diagnosis of MLS syndrome is typically made postnatally by observing the phenotype of the patient. MLS syndrome could present prenatally through ultrasound anomalies

\*Correspondence to: Gail Stetten, Park SB-202, Johns Hopkins Medical Institutes, 600 N. Wolfe Street, Baltimore, MD 21287, USA. E-mail: gstetten@jhmi.edu

and possibly an X chromosome abnormality, but no commercial FISH probes are available to detect the deletion of the MLS critical region that occurs in most patients. We propose that array CGH should be considered to detect monosomy of the MLS critical region at Xp22.2 when a prenatal diagnosis of MLS syndrome is suspected.

### CLINICAL REPORT

A 24-year old, para 3013, Caucasian female in good health was referred for genetic counseling with her 43-year old Caucasian husband following a fetal anatomy sonogram which identified multiple congenital anomalies. The patient had declined maternal serum screening. The couple is nonconsanguineous and had a healthy 4 year old child and one first trimester miscarriage. The patient had two children and her husband had four children by previous unions. Her family history was remarkable for a first cousin with a congenital heart defect. Her husband's history was notable for a niece with a congenital heart defect and a maternal first cousin with Down syndrome. Both family histories were otherwise unremarkable for birth defects, mental retardation, fetal wastage and known hereditary disorders.

The patient underwent an ultrasound study at 22 weeks' gestation that revealed a complex fetal cardiac defect involving an atrioventricular canal defect, coarctation of the aorta and aortic stenosis/atresia. In addition, dangling choroid plexus, a 10 mm posterior nuchal cystic hygroma, bilateral microphthalmia, fifth

finger clinodactyly of the left hand, a single umbilical artery, and bone bright echogenic foci in the liver were noted. All fetal biometric parameters were consistent with only 20 weeks' gestation. Amniocentesis was performed for karyotype, cytomegalovirus (CMV) PCR and viral culture. The amniotic fluid CMV PCR and viral culture were normal and amniotic fluid Alpha-fetoprotein AFP was 0.65 MoM. The karyotype revealed an X;Y translocation which, along with the sonographic findings, raised the likelihood of MLS syndrome. A review of the literature revealed case reports of MLS with similar features, but none as severe as the anomalies observed in this case. The family was counseled that the phenotype at birth could include some or all of the features of MLS syndrome, as well as the possibility of Turner syndrome, and/or Madelung deformity. The couple elected to continue the pregnancy.

An ultrasound study at 28 3/7 weeks' gestation was remarkable for a 3 week lag in fetal growth with mild bilateral cerebral ventriculomegaly and bilateral dangling choroid plexi, possible agenesis of the corpus callosum, skin thickening over the cervical spine (resolving cystic hygroma), the complex cardiac abnormality (Figure 1(a)), and an abnormal face with bilateral microphthalmia (Figure 1(b)), anteverted nostrils and low set ears (Figure 1(c)). Serial sonograms at 33 1/7 and 36 1/7 weeks' gestation showed progressive though mild cerebral ventriculomegaly and asymmetric fetal growth restriction (<3rd percentile). The umbilical artery Doppler indices remained normal.

The pregnancy resulted in a vaginal delivery of a female infant at 39 weeks with a birth weight of 2480 g,

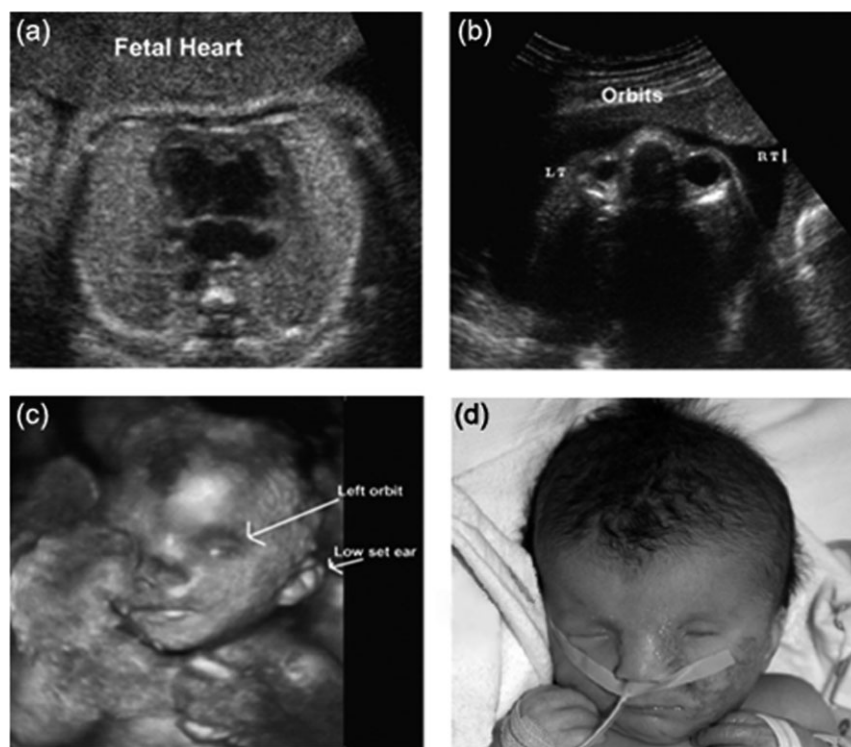


Figure 1—Ultrasound images at 28 3/7 weeks' gestation showing the (a) fetal heart, (b) ocular orbits and (c) three-dimensional image of the fetal face. Linear skin defects on the face typically seen in MLS patients are notable after birth (d)

and APGARS of 9 and 9 at 1 and 5 min, respectively. Physical examination showed abnormal facial features, including bilateral microphthalmia (more pronounced on left than right), corneal clouding on the right (unable to evaluate the left), facial skin lesions extending from the nose to the cheeks (Figure 1(d)), prominent nasal bridge with a high, broad nasal root, and a depression of the anterior fontanelle near the forehead. In addition, the infant had a webbed neck, high arched palate, imperforate anus with perineal fistula, tapered fingers, fifth finger clinodactyly, bilateral single palmar crease, and decreased lower extremity tone. An echocardiogram performed on day one of life demonstrated mitral atresia *versus* complete atrioventricular canal, aortic atresia, severe ascending aorta hypoplasia, and no connecting vein. MLS syndrome was diagnosed at birth from the physical examination and abnormal karyotype.

Given the extent of the anomalies, particularly the severe cardiac defect, the family decided to discontinue life support, and the infant died on day five of life. The family declined autopsy.

## MATERIALS AND METHODS

### Conventional cytogenetics

Chromosome analyses at approximately the 500-band level were performed on cultured amniotic fluid cells from the fetus and lymphocytes from the infant and the parents. A lymphoblast cell line was established from blood obtained at birth. Replication studies were carried out on cultured amniocytes and the lymphoblast cell line from the infant following a 6 hour BrdU pulse (Pai and Thomas, 1980).

### Fluorescent *in situ* hybridization (FISH)

Commercial FISH probes were obtained from Abbott Molecular and used according to the manufacturer's instructions. DNA from BAC clones obtained from Spectral Genomics, Inc. and Roswell Park Cancer Institute were labeled by nick-translation using Spectrum Orange dUTP or Spectrum Green dUTP (Abbott Molecular) and hybridized to metaphase chromosome preparations (Pinkel *et al.*, 1986). All images were captured using a Zeiss Axioskop equipped with Cohu camera and CytoVision imaging software (Applied Imaging).

### Comparative genomic hybridization to BAC arrays (array CGH)

Array CGH was performed according to manufacturer's instructions on two genomic DNA arrays, Constitutional Chip (CC) 3.0 and Spectral Chip (SC) 2600 (Spectral Genomics Inc., Houston, TX USA), containing BAC clones spotted in triplicate and duplicate, respectively. The CC has 606 BACs mapped to 41 unique subtelomeres and 57 chromosomal regions known to be involved in structural defects associated with specific

genetic syndromes, whereas the SC 2600 has 2621 BACs more evenly spaced throughout the genome at about a 1 megabase (Mb) resolution. The location of the BAC clones on the CC and SC is based on National Center for Biotechnology Information NCBI Human Genome build 35.1 (<http://www.ncbi.nlm.nih.gov/mapview>).

Briefly, genomic DNA prepared from the lymphoblast culture (PureGene Blood Kit, Gentra Systems, Inc. Minneapolis, MN) and normal male and female reference DNA (Promega Corporation, Madison, WI) were sonicated to obtain an average fragment size of 400–2000 base pairs and random prime labeled (BioPrime Labeling Kit, Invitrogen, Germantown, MD) with Cyanine 3 (Cy3) and Cyanine 5 (Cy5) dCTP (PerkinElmer Inc., Wellesley, MA). The patient DNA labeled with Cy5 was precipitated with reference DNA labeled with Cy3, and vice versa. Both combinations were cohybridized to the CC and SC, and washed according to the manufacturer's protocol.

The hybridized slides were simultaneously scanned at wavelength 635 nm and 532 nm using a GenePix 4000B scanner (Axon Instruments, Molecular Devices Corporation, Sunnyvale, CA, USA). The scanned images were imported into GenePix Pro 6.0 (Molecular Devices) and the ratio of Cy5 to Cy3 fluorescence intensities for each set of BACs were calculated and averaged. These ratios were uploaded into SpectralWare software (Spectral Genomics, Inc.), normalized with linear regression algorithms and plotted on a log<sub>10</sub> scale graph according to the BAC chromosomal location.

## RESULTS

### Cytogenetic studies

An X;Y translocation was seen in 27 colonies studied from five separate cultures of amniocytes and no mosaicism was detected (Figure 2(a)). Quinacrine and DAPI fluorescence confirmed the presence of Yq material on Xp. FISH studies indicated the derivative X chromosome was missing the steroid sulfatase (STS) and Kallmann (KAL) regions in Xp22.3 (Table 1). The SRY locus at Yp12 was also deleted as a result of the translocation (data not shown). The Duchenne muscular dystrophy (DMD) locus at Xp21.1 (Table 1), the X centromere and the DiGeorge critical region (22q11.2) were present by FISH analyses resulting in the karyotype 46,X,der(X)t(X;Y)(p22.3;q11.2).ish der(X)(DXZ1+,DMD+,KAL-,STS-,SRY-,22q11.2(Tuple1X2)). Chromosome analysis performed on lymphocyte cells from the fetus confirmed the 46,X,der(X)t(X;Y)(p22.3;q11.2) karyotype. A total of 100 cells were scored and no mosaicism was detected. Parental karyotypes were normal, indicating the rearrangement was a *de novo* event.

Replication studies on cultured amniocytes and lymphoblasts indicated the derivative X chromosome was late replicating in 20 of 20 spreads from each tissue, and hence the inactive X chromosome (data not shown).

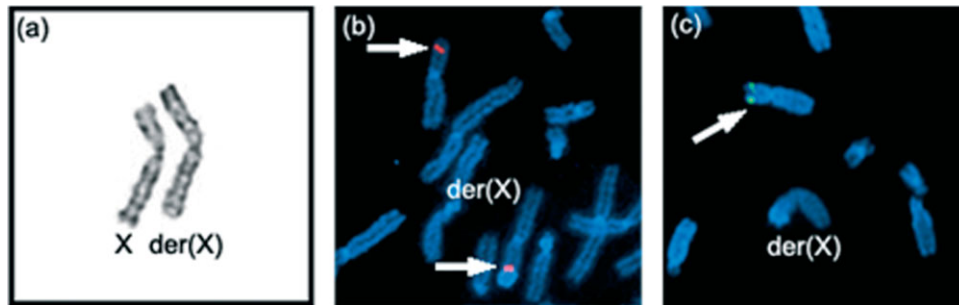


Figure 2—(a) G-banded normal X and derivative X [der(X)] chromosomes. Partial metaphase spreads following FISH with (b) RP11-90F9 (red) showing signal on the normal X and der(X) (arrows) and (c) RP11-382D24 (green) showing signal only on the normal X chromosome (arrow). These FISH experiments map the breakpoint to a 1 Mb region proximal to the MLS critical region between BAC clones RP11-382D24 and RP11-90F9.

Table 1—Array CGH and FISH data for BAC clones on the Spectral chip(SC) and Constitutional chip(CC). MLS (mcr) refers to the MLS minimal critical region.

BAC clone ID	Array	Array copy number	FISH		Chromosome band	Disorder
			der(X)	X		
LLNOYCO3M15D10	SC/CC	Loss			Xp22.33/Yp11.32	
LLNOYCO3M34F5	SC/CC	Loss			Xp22.33/Yp11.32	
RP11-839D20	SC/CC	Loss			Xp22.33/Yp11.32	
509O10	SC/CC	Loss			Xp22.33/Yp11.32	
GS-98-C4	SC/CC	Loss			Xp22.33/Yp11.32	
GS-839-D20	CC	Loss			Xp22.33/Yp11.32	
RP5-1123N13	SC/CC	Loss			Xp22.33/Yp11.32	
RP4-617A9	SC/CC	Normal			Xp22.33	
RP11-315B16	SC/CC	Normal			Xp22.32	
RP11-366M24	SC	Normal			Xp22.31-p22.32	
RP11-294K6	SC	Normal			Xp22.31	
RP11-483M24	CC	Normal			Xp22.31	STS
GS1-227L7	CC	Normal	–	+	Xp22.31	STS
RP11-143E20	SC/CC	Normal			Xp22.31	
CTB-9P2	SC	Normal			Xp22.31	
RP11-589J20	CC	Normal			Xp22.31	KAL
RP11-606J16	CC	Normal	–	+	Xp22.31	KAL
RP11-383I22	SC	Normal			Xp22.31	
GS1-214D18	SC	Normal			Xp22.31	
RP11-451G24	SC	Normal			Xp22.2-p22.31	
239F12	CC	Normal			Xp22.2	MLS
232B2	CC	Normal			Xp22.2	MLS
8D11	CC	Normal			Xp22.2	MLS
RP11-89B5	SC	Normal	–	+	Xp22.2	MLS (mcr)
RP1-185L21	CC	Normal			Xp22.2	MLS (mcr)
RP1-134G1	CC	Normal			Xp22.2	MLS (mcr)
RP1-39D12	CC	Normal			Xp22.2	MLS (mcr)
GS-602M16	CC	Normal	–	+	Xp22.2	MLS (mcr)
55C5	CC	Normal			Xp22.2	MLS (mcr)
RP1-27C22	CC	Normal			Xp22.2	MLS (mcr)
CT-285I15	CC	Normal			Xp22.2	MLS (mcr)
GS-590J6	CC	Normal	–	+	Xp22.2	MLS (mcr)
RP11-382D24	—	Not on arrays	–	+	Xp22.2	
RP11-90F9	SC	Gain	+	+	Xp22.2	
GS-607H18	SC	Gain	+	+	Xp22.2	
RP5-958B3	SC	Gain	+	+	Xp22.13	
RP1-245G19	SC	Gain			Xp22.13	
RP11-345L8	SC	Gain			Xp22.12	
RP11-507P24	SC	Gain			Xp22.12	
RP11-497C10	SC	Gain	+	+	Xp22.11-p22.12	

Table 1—(Continued)

BAC clone ID	Array	Array copy number	FISH		Chromosome band	Disorder
			der(X)	X		
ICRFC104A0359	SC	Gain			Xp22.11	
RP11-79B3	SC/CC	Gain	+	+	Xp22.11	
RP11-487M22	SC/CC	Gain	+	+	Xp21.3	
RP6-27C10	SC	Gain			Xp21.3	
CTB-229E10	SC	Gain			Xp21.2	
AD8E10	CC	Gain			Xp21.2	
GS1-484O17	CC	Gain			Xp21.2	
RP11-89L23	SC/CC	Gain			Xp21.2	
RP11-242C19	CC	Gain			Xp21.2	
RP11-46A23	CC	Gain			Xp21.2	DMD
RP11-338L12	CC	Gain			Xp21.2	DMD
RP11-122N14	SC/CC	Gain			Xp21.1-p21.2	DMD
RP11-124H12	SC/CC	Gain			Xp21.1	DMD
222F5	CC	Gain			Xp21.1	DMD
RP11-377J16	CC	Gain			Xp21.1	DMD
RP11-509C1	CC	Gain	+	+	Xp21.1	DMD
RP4-639D23	CC	Gain			Xp21.1	DMD
RP5-1147O16	SC/CC	Gain			Xp21.1	DMD
RP11-357F9	CC	Gain			Xp21.1	DMD
RP5-1174H9	CC	Gain			Xp21.1	DMD
36 000 000	CC	Gain			Xp21.1	DMD
RP4-672M15	CC	Gain			Xp21.1	DMD
RP4-769D20	SC	Gain			Xp21.1	DMD
RP11-70D7	SC	Gain			Xp21.1	
RP13-46M24	SC	Gain			Xp21.1	
RP11-495K15	SC	Gain			Xp11.4-p21.1	

## Array CGH

The graphs representing the Cy5 to Cy3 ratio values of BAC clones mapping to the X chromosome from the CC and SC hybridizations are shown in Figure 3(a), (b). When male reference DNA is used, BAC clones from the pseudoautosomal regions of the X and Y chromosome, which are deleted in the derivative X chromosome, show loss of copy number. BACs mapping to regions of the X chromosome proximal to the pseudoautosomal region but missing due to the translocation do not deviate significantly from a normal one to one ratio, whereas BACs from regions retained in the derivative X show an overall gain in copy number (Figure 3(a), (b), Table 1). The array data refined the breakpoint in the derivative X chromosome to Xp22.2 (Table 1), while the Y chromosome breakpoint was more precisely defined as an interval spanning Yq11.221 to Yq11.222 (data not shown).

Array and FISH data for BAC clones mapping to Xp22.31-p21.1 on both the CC and SC are shown in Table 1. BAC clones that span the STS and KAL loci on the CC show a normal one to one ratio, whereas clones from the DMD locus showed ratios consistent with gain in copy number, which support the FISH findings for these regions (Table 1). The CC has eight BACs and the SC has one BAC in the MLS minimal critical region that show normal copy number and therefore are deleted as a result of the X;Y translocation (Table 1). Incorporating the new information provided by array CGH, the amended karyotype

is 46,X,der(X)t(X;Y)(p22.2;q11.2).ish der(X)(DXZ1+, DMD+,KAL-,STS-,SRY-),22q11.2(Tuple1 × 2) arr cgh Xp22.33p22.2(LLNOYCO3M15D10→GS1-590J6) × 1,Yq11.222q23(RP11-20H21→RP11-79J10) × 1. FISH with BAC clones within the minimal critical region and near the derivative X breakpoint from the CC and SCs was used to confirm the array findings (Figure 2(b), Table 1). BAC clone GS-602M16 containing the *HCCS* gene, implicated in MLS syndrome, is deleted in the derivative X by array CGH and FISH (Table 1). An additional clone from Roswell Park Cancer Institute (RP11-382D24) was included in FISH mapping experiments to help refine the breakpoint (Figure 2(c)). These experiments placed the breakpoint in a 1 Mb region proximal to the MLS critical region in Xp22.2 between BAC clones RP11-382D24 and RP11-90F9 (Table 1).

## DISCUSSION

MLS syndrome, characterized by microphthalmia, sclerocornea, and dermal aplasia of the face and neck, was first described in females with deletions or translocations involving the short arm of the X chromosome (Al-Gazali *et al.*, 1990; Temple *et al.*, 1990; Happle *et al.*, 1993; Lindsay *et al.*, 1994). The disease is believed to be lethal in the male hemizygous state (Prakash *et al.*, 2002), however, several males with MLS syndrome have been reported with Xp;Yp translocations (Lindsay *et al.*, 1994; Paulger *et al.*, 1997; Kobayashi *et al.*, 1998; Stratton *et al.*, 1998; Kono *et al.*, 1999; Anguiano *et al.*, 2003).

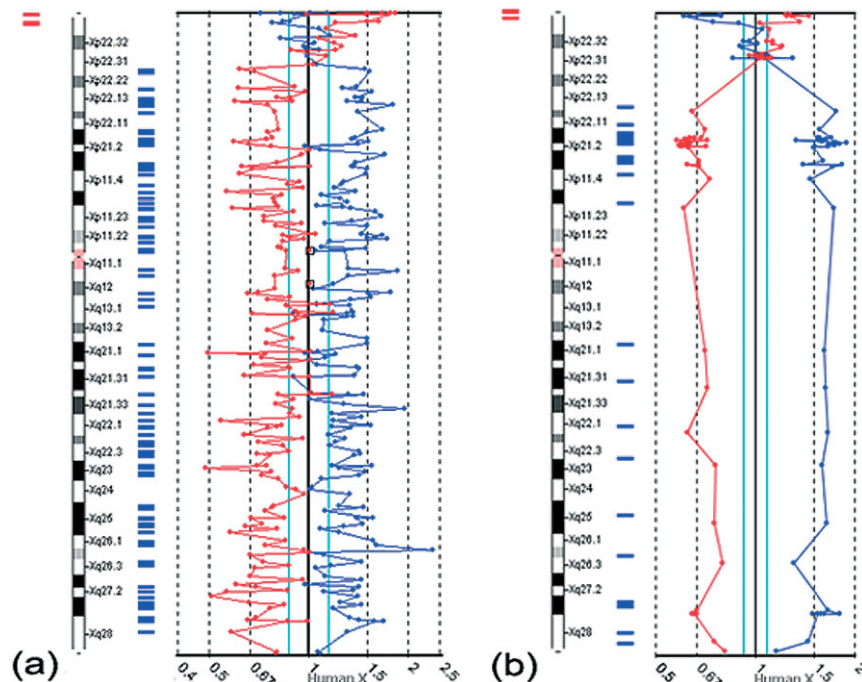


Figure 3—Array CGH ratio profiles of the X chromosomes from the (a) Spectral and (b) Constitutional Chip when normal male DNA is used as the reference. Blue line plots represent the ratios for each BAC clone when the patient DNA is labeled with Cy5 and the reference in Cy3. Red line plots represent the ratios when the patient DNA is Cy3 and reference is Cy5. A true DNA copy number change is represented by an equal and statistically significant migration of both ratios away from 1. Blue tick marks on the right side of the X chromosome ideogram identify BAC clones that show gain of those regions, while red ticks signify loss.

We present a case of severe MLS syndrome that was ascertained prenatally, fully diagnosed at birth with death occurring at five days of life. Linear skin defects and eye abnormalities typically seen with MLS syndrome were present, as well as central nervous system involvement, including agenesis of the corpus callosum and ventriculomegaly. In addition, our patient had a severe structural cardiac defect, a rarer feature of MLS syndrome (Morleo *et al.*, 2005). Prenatal karyotype analysis showed an X;Y translocation that was confirmed and further characterized by array CGH and FISH. Array CGH showed twelve BAC clones representing the MLS critical region were deleted as a result of the translocation.

The minimal monosomic interval leading to MLS syndrome has been mapped to a 610 kb region in Xp22.2 (Wapenaar *et al.*, 1993, 1994) that contains three genes; *MIDI*, *HCCS*, and *ARHGAP6* (reviewed in Morleo *et al.*, 2005). Recently, Wimplinger *et al.* (2006) identified *de novo* point mutations in the *HCCS* gene in two unrelated females that have MLS and a normal karyotype, as well as a third family where a submicroscopic deletion of *HCCS* segregated in a mother and two daughters displaying MLS phenotypic variability. Array CGH and FISH analysis of our patient show the minimal critical region including the *HCCS* gene (BAC clone GS-602M16) are deleted as a result of the X;Y translocation. *HCCS* encodes the mitochondrial holoenzyme *c*-type synthase, which catalyzes the covalent attachment of heme to both apocytochrome *c* and *c*<sub>1</sub>, necessary precursors utilized in the mitochondrial respiratory chain (Bernard *et al.*, 2003).

The deletion we describe has a breakpoint within the same interval in Xp22.2 as the two largest deletions reported in MLS patients (Lindsay *et al.*, 1994, reviewed in Morleo *et al.*, 2005). It appears the size of X chromosome deletion does not correlate with severity of the MLS phenotype since a patient with one of the largest deletions reported showed only linear skin defects with no other reported features of MLS syndrome (Lindsay *et al.*, 1994).

Clinical variability observed in MLS patients, including identical twins (Cape *et al.*, 2004), suggests patterns of X-inactivation may play a role in the development of the MLS phenotype (reviewed in Van den Veyver, 2001). In the case presented, replication studies indicated the derivative X chromosome was the inactive X in all amniocytes and lymphocytes observed. A similar skewed X-inactivation pattern has been reported in several individuals with MLS syndrome and a derivative X chromosome (Ogata *et al.*, 1998; Anguiano *et al.*, 2003). However, X-inactivation ratios may vary between different tissues within an individual (Gale *et al.*, 1994; Azofeifa *et al.*, 1996). The severe MLS phenotype reported here may be due to inactivation of the normal X chromosome in tissues where functional nullisomy of the *HCCS* gene is not well tolerated.

The introduction of the array CGH technique into the cytogenetics laboratory allows for rapid, sensitive, high-resolution analysis of the genome (Pinkel *et al.*, 1998, reviewed in Shaffer and Bejjani, 2004). The case we present illustrates that array CGH is the best method of diagnosis whenever MLS syndrome is suspected prenatally. However, patients should be warned that

not every case of MLS syndrome will be picked up since the commercial BAC arrays are not designed to detect very small deletions or mutations in genes or regulatory elements that may lead to MLS syndrome in some patients.

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