Letters to the Editor

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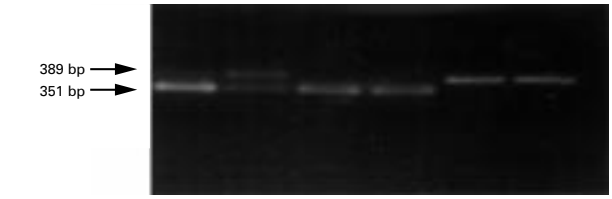
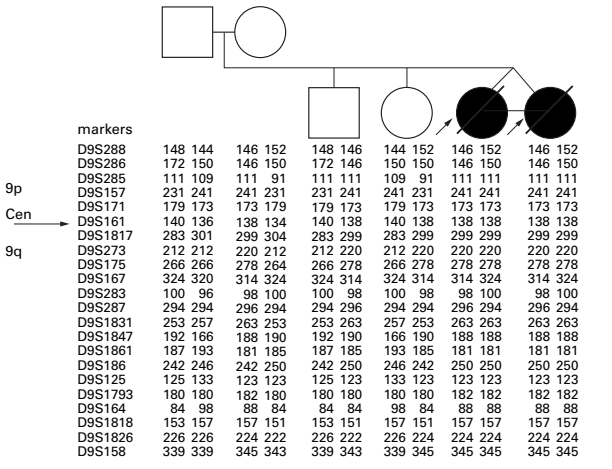
Leigh syndrome transmitted by uniparental disomy of chromosome 9

EDITOR—Severe, isolated, and generalised deficiency of complex IV (cytochrome c oxidase, COX) can result in Leigh syndrome (LS) (MIM 256000), an early onset mitochondrial disorder characterised by rapidly progressive, symmetrical degeneration of the brain stem, diencephalon, and basal ganglia.[1 2] *SURF-1*, a gene located on chromosome 9q34, has recently been identified as the gene responsible for numerous cases of LS^{COX}.[3 4]

SURF-1 associated LS^{COX} is usually inherited as an autosomal recessive trait. We report here a homozygous loss of function mutation of *SURF-1* in two monozygotic LS^{COX} female twins, owing to uniparental disomy of two almost identical maternal chromosomes 9.

The probands were born to non-consanguineous parents at 33 weeks of gestation by caesarean section. The mother was 46 years old. The pregnancy was uneventful until the 24th week, when persistent uterine contractions ensued. Two older sibs of the probands are alive and healthy. The family history was negative for neurological or metabolic disorders. Birth weight and body length were <3rd centile and the Apgar score was 1-8 in both patients. Growth rate and psychomotor development were regular during the first 8 months of life. During the following months the patients developed a rapidly progressive clinical syndrome characterised by failure to thrive, psychomotor regression, hypotonia, ophthalmoparesis, mild bilateral optic atrophy, and ataxia. At 18 months both patients had mild lactic acidosis. MRI showed symmetrical paramedian lesions in the mesencephalon and brain stem, as typically found in LS. Both patients died of respiratory failure in the third year of life.





	markers															
	D9S288	148	144	146	152	148	146	144	152	146	152	146	152
	D9S286	172	150	146	150	172	146	150	150	146	150	146	150
	D9S285	111	109	111	91	111	111	109	91	111	111	111	111
9p	D9S157	231	241	241	231	231	241	241	231	241	241	241	241
	D9S171	179	173	173	179	179	173	179	173	173	173	173	173
Cen →	D9S161	140	136	138	134	140	138	140	138	138	138	138	138
	D9S1817	283	301	299	304	283	299	283	299	299	299	299	299
9q	D9S273	212	212	220	212	212	220	212	220	220	220	220	220
	D9S175	266	266	278	264	266	278	266	278	278	278	278	278
	D9S167	324	320	314	324	324	314	324	314	314	324	314	324
	D9S283	100	96	98	100	100	98	100	98	98	100	98	100
	D9S287	294	294	296	294	294	296	294	294	296	294	296	294
	D9S1831	253	257	263	253	253	263	257	253	263	263	263	263
	D9S1847	192	166	188	190	192	190	166	190	188	188	188	188
	D9S1861	187	193	181	185	187	185	193	185	181	181	181	181
	D9S186	242	246	242	250	242	250	246	242	250	250	250	250
	D9S125	125	133	123	123	125	123	133	123	123	123	123	123
	D9S1793	180	180	182	180	180	180	180	180	182	182	182	182
	D9S164	84	98	88	84	84	84	98	84	88	88	88	88
	D9S1818	153	157	157	151	153	151	157	151	157	157	157	157
	D9S1826	226	226	224	222	226	222	226	224	224	224	224	224
	D9S158	339	339	345	343	339	343	339	345	345	345	345	345

Figure 1 (Top) Mutation specific RFLP analysis of exons 6+7 of the SURF-1 gene, amplified as described in reference 3. After digestion with BsiWI, the 389 bp wild type fragment is cut into two 351 and 38 bp fragments, while an intact fragment is obtained in the presence of the 751C>T mutation. (Middle) Pedigree of the family. (Bottom) Haplotype reconstruction of chromosome 9 specific microsatellite markers. The list and order of the markers along chromosome 9 are also indicated.

Needle muscle biopsies performed at 24 months of age showed a diffuse reduction of the histoenzymatic reaction to COX. Biochemically, COX activity in muscle homogenates was 12.1 nmol/min/mg in one patient and 3.6 nmol/min/mg in the second (normal values 68 ± 20), while the activities of the other respiratory complexes were all normal. The COX defect was also detected in cultured fibroblasts of one patient (0.4 nmol/min/mg, normal value 25 ± 11), but this assay was not performed in the second patient. Specific activities of the respiratory complexes in a muscle homogenate of the mother were all normal.

Automated sequence analysis of the nine exons of the *SURF-1* gene in the probands showed the presence of a previously reported[3] homozygous frameshift mutation (751C>T). This mutation destroys a *Bsi*WI restriction site, which is present in the wild type gene. *Bsi*WI RFLP analysis showed a heterozygous mutation in the mother, while no mutation was detected in the probands' father, sister, or brother (fig 1). A de novo mutation in the paternal chromosome 9 identical to the mutation carried by the mother was considered unlikely. Non-paternity was excluded by linkage analysis with numerous microsatellite markers. To test the hypothesis of chromosome 9 specific paternal non-contribution, we then analysed three STSs (D9S1831, D9S1826, and D9S158), flanking the *SURF-1* locus at 9q34. All three markers showed the presence of one maternal allele only, while the paternal allele was consistently absent. To verify whether the paternal non-contribution was the result of a microdeletion at 9q34, the cosmid P117B6, which contains the *SURF-1* gene,[5] was used as a probe in FISH experiments[3 6] on metaphases from one proband. The probe detected two comparable signals on both chromosome 9 homologues (fig 2). These results excluded the presence of a deletion in a paternal chromosome, suggesting instead a mechanism of uniparental disomy (UPD) of two maternal chromosomes. To test this hypothesis, additional microsatellites distributed along the whole of chromosome 9 were analysed for a total of 22 markers (fig 1). With the exception of two small regions (D9S288-D9S286, and D9S167-D9S283-D9S287, see fig 1) the alleles were all homozygous; in 15 instances the obligate contribution of the paternal allele was unequivocally missing. In particular, homozygosity was detected for 10/10 markers encompassing the *SURF-1* locus, in the interval defined by markers D9S1831-D9S158. We conclude that loss of the contribution of a second normal *SURF-1* allele has led to the manifestation of LS in our patients.

UPD is defined as the exceptional inheritance of a pair of chromosomes from one parent only, as the result of gamete complementation, chromosome loss in trisomy, or duplication in monosomy. In isodisomy, the uniparental pair is a duplicate of the same chromosome DNA template, and causes an increased risk of a recessive disorder by reduction to homozygosity.[7] In our patients the presence of two small heterozygous regions can be explained as the result of two crossing over events in otherwise identical maternal chromosomes 9. These data indicate that the double maternal contribution was the result of a non-disjunction

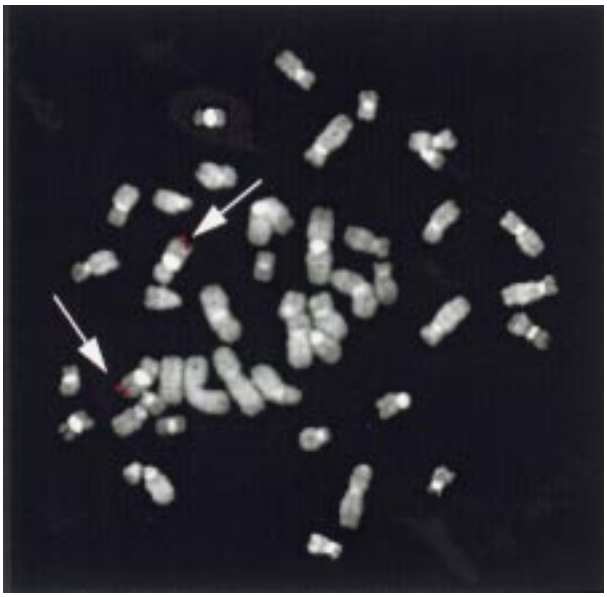


Figure 2 FISH of DAPI stained metaphases from a fibroblast cell culture of a patient. FISH was performed as described in references 3 and 6. Red signals correspond to cosmid P117B6, which contains the entire SURF-1 gene.

which occurred at the second meiotic division, with maintenance of euploidy in the zygote by elimination of the paternal contribution. The age of the mother (46 years) could have favoured the non-disjunction event in our patients, as it is known that the risk of such an event increases with maternal age.

In addition, the haplotype reconstruction showed evidence of two recombinant events in the probands' sibs, close to the *SURF-1* locus (fig 1). The brother shares with the probands the maternal allele for D9S186, but not that for D9S1861; the sister shares with the probands the maternal alleles for D9S1826 and D9S158, but not that for D9S1818. Since both sibs are homozygous wild type for *SURF-1*, these recombination events indicate that the disease locus is contained within the interval between markers D9S1861 and D9S1826.[3]

UPD may also cause functional balance disruption of imprinted genes.[7] The existence of imprinted genes on chromosome 9 is controversial, but it seems unlikely.[8–10] Our patients did not show gross dysmorphic features or malformations apart from LS. With the limitations because of the brief survival and severe phenotype, this observation suggests that chromosome 9 does not contain maternally imprinted genes crucial for embryonic development.

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A case of Williams syndrome with a large, visible cytogenetic deletion

EDITOR—Williams syndrome (WS) is generally characterised by mental deficiency, gregarious personality, dysmorphic facies, supravalvular aortic stenosis (SVAS), and idiopathic infantile hypercalcaemia. Patients with WS show allelic loss of *STX1A*,[1] elastin (*ELN*),[2 3] and *LIMK1*,[4] with most exhibiting a submicroscopic deletion at 7q11.23, detectable by FISH.[3 5] The common deletion size is about 1.5 Mb.[6] Previous studies have shown that WS patients have consistent deletion sizes and share common proximal and distal breakpoints.[7 8] Here we report a patient who has a large, atypical, visible chromosomal deletion of 7q11.2 and features consistent with, and in addition to, those typically seen in Williams syndrome.

The patient was originally referred to the genetics clinic at 5 months of age for evaluation of global developmental delay and dysmorphic features. She was delivered at 37 weeks' gestation by caesarean section weighing 2350g (<5th centile). The initial course included a history of poor feeding in the newborn period. Clinical examination showed macrocephaly, cutaneous haemangioma, and craniofacial features consisting of a large anterior fontanelle, frontal bossing, depressed nasal bridge, cup shaped ears, hypertelorism, and prominent lips (fig 1A). Neurological examination showed generalised hypotonia with heel cord and hamstring tightness. CT scan of the head and renal ultrasound were normal. Because of a grade III/VI systolic murmur, echocardiogram was performed, which showed a slightly thickened aortic valve. Cytogenetic analysis showed a 46,XX karyotype.

Re-evaluation at 4 years of age, showed short stature (90 cm, <3rd centile), continued significant developmental delay, and coarsened facial features with stellate irides. Repeat echocardiogram showed moderately severe supravalvular aortic stenosis. Cardiac catheterisation confirmed these findings without involvement of the leaflets or the rest of the ascending aorta, and no evidence of aortic insufficiency. Ophthalmological examination showed bilateral exotropia and hyperopia requiring corrective lenses