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Paternal Isodisomy for Chromosome 5 in a Child with Spinal Muscular Atrophy

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Summary

Paternal isodisomy for chromosome 5 was detected in a 2-year-old boy with type III spinal muscular atrophy (SMA), an autosomal recessive degenerative disorder of alpha motor neurons, known to map to 5q11.2-13.3. Examination of 17 short-sequence repeat polymorphisms spanning 5p15.1-15.3 to 5q33.3-qter produced no evidence of maternally inherited alleles. Cytogenetic analysis revealed a normal male karyotype, and FISH with probes closely flanking the SMA locus confirmed the presence of two copies of chromosome 5. No developmental abnormalities, other than those attributable to classical childhood-onset SMA, were present. While the absence of a maternally derived chromosome 5 could have produced the symptoms of SMA through the mechanism of genomic imprinting, the lack of more global developmental abnormalities would be unusual. Paternal transmission of two copies of a defective gene at the SMA locus seems to be the most likely cause of disease, but proof of this will have to await the identification of the SMA gene. While uniparental isodisomy is a rare event, it must be considered as a possible mechanism involved in SMA when conducting prenatal testing and counseling for this disorder.

Introduction

Uniparental disomy (UPD) is the inheritance of two homologous chromosomes from one parent in a disomic cell line (Engel 1980). Cases of UPD have been reported for chromosome 6 (Welch et al. 1990), chromosome 7 (Spence et al. 1988; Voss et al. 1989; Spotila et al. 1992), chromosome 9 (Willatt et al. 1992), chromosome 11 (Beldjord et al. 1992), chromosome 14 (Temple et al. 1991; Pentao et al. 1992), chromosome 15 (Nicholls et al. 1989; Malcolm et al. 1991), chromosome 16 (Bennett et al. 1992; Dworniczak et al. 1992), chromosome 21 (Blouin et al. 1993), and chromosome 22 (Kirkels et al. 1980; Palmer et al. 1980). UPD is often associated with developmental abnormalities and has provided evidence for genomic imprinting in humans by demonstrating the importance of parental origin in UPD, as in the association of paternal UPD of chromosome 15 with Angelman syndrome (Malcolm et al. 1991) and the association of maternal UPD of chromosome 15 with Prader-Willi syndrome (Nicholls et al. 1989).

UPD can cause autosomal recessive disorders by the transmission of two copies of a defective gene from a single parent. This was first proposed as an explanation for two cases of cystic fibrosis (Spence et al. 1988; Voss et al. 1989), and subsequently reported for complement C4 deficiency (Welch et al. 1990), thalassemia (Beldjord et al. 1992), and rod monochromacy (Pentao et al. 1992). For most of these reports, however, there was evidence of malformations or defects in growth and development which could not be fully attributed to the identified autosomal recessive disorder. It has been postulated that these additional defects arose through the action of genomic imprinting on other genes on the UPD chromosomes.

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Spinal muscular atrophy (SMA) describes a group of heritable diseases characterized by selective degeneration of the alpha motor neuron. Most cases present with symmetrical weakness and atrophy of limb muscles but with no spasticity, hyperreflexia, or sensory loss. Electromyography (EMG) reveals denervation with normal conduction times, while muscle biopsy typically shows grouped atrophy and fiber-type grouping. The cerebrospinal fluid is normal, and creatine kinase levels are seldom very elevated, measuring within the normal range in over half of all patients (Brooke 1985).

Childhood-onset SMA usually displays an autosomal recessive pattern of inheritance, affecting 1 in 10,000 live births, with a carrier frequency of approximately 1 in 50 (Pearn et al. 1973; Pearn 1978). Late-onset (i.e., at 15 to 55 years of age) cases are more heterogeneous with regard to mode of inheritance, and exhibit autosomal dominant (Pearn et al. 1978) and X-linked recessive (Kennedy et al. 1968), in addition to autosomal recessive, patterns. The childhood-onset form of SMA has been genetically mapped to chromosome 5q11.2-13.3 (Brzustowicz et al. 1990; Melki et al. 1990; Daniels et al. 1992), and linkage-based prenatal testing is currently being conducted for this disorder. While performing routine prenatal and informativeness tests for families with a previous child affected with SMA, a case of SMA associated with paternal isodisomy for chromosome 5 was identified.

Material and Methods

Clinical Information

The proband was a 2.5-year-old male, referred for evaluation of an abnormal gait and difficulty in rising to standing and in climbing stairs, first noted at 2 years of age. He was born at 35 wk gestation to a 29-year-old, gravida 1, para 1 mother and a 36-year-old father, and he weighed 5 pounds 13 ounces. The prenatal period was complicated by HELLP syndrome (severe hemolysis, elevated liver enzymes, low platelet count, and preeclampsia) in the mother. Early developmental milestones were reportedly normal. He followed light and smiled in the first 2 mo, sat unassisted by 6 mo, stood by 1 year, and walked between 12 and 15 mo. He spoke in three-word sentences by age 2 years.

Physical examination revealed no dysmorphic features. Height was at the 90th percentile, with weight and head circumference each at the 75th percentile. Cranial nerve examination was normal, with no facial weakness or fasciculations of the tongue. There was hypotonia at the shoulders and mild proximal weakness of the lower extremities. He exhibited a Gower sign and walked with a waddling gait. The only obtainable deep-tendon reflexes were in the ankles. Babinski signs were absent.

Laboratory studies revealed mildly elevated creatine phosphokinase of 360 U/liter (normal is 0–235 U/liter). Motor and sensory nerve conduction studies of a lower extremity were normal. EMG showed chronic denervation and reinnervation of motor-unit potentials, with reduced neurogenic recruitment and increased amplitude of motor-unit potentials, which is consistent with a disorder of motor neurons. Muscle biopsy revealed extensive group atrophy involving major portions of, or entire, fascicles. The atrophic fibers were extremely small and round, with the remaining fibers extremely hypertrophic and mostly of type I. These findings were consistent with the diagnosis of SMA.

Molecular Analysis

Genomic DNA was isolated from blood samples (Maniatis et al. 1982) from the proband and his parents. All three samples were genotyped for 17 short-sequence repeat polymorphisms (SSRPs), 6 from the 5q11.2-13.3 region and 11 from the remainder of the chromosome, spanning 5p15.1-15.3 to 5q33.3-qter (table 1). All SSRPs used have been described elsewhere (D5S76/lambda 599-Ha by Sherrington et al. [1991]; D5S125/EF by Morrison et al. [1992]; D5S435/VS19 by Soares et al. [1993]; MAP1B-5′ and MAP1B-3′ by Brzustowicz et al. [1992]; D5S204/6741GT by Mankoo et al. [1990]; and the remainder by Weber et al. [1991]). They were amplified using published cycle parameters, in 10-μl reactions containing 40 ng of each genomic DNA, 10 mM Tris-Cl, 1.5 mM $MgCl_2$, 50 mM KCl, 200 μM dATP, 200 μM dTTP, 200 μM dGTP, 2.5 μM dCTP, 25 nM ^{32}P-α-dCTP, 10 pmol of each primer, 0.25 units of *Taq* DNA polymerase (Boehringer Mannheim) and 1 mg gelatin/ml. PCR products were separated on 6% acrylamide (19:1 bis) nondenaturing gels, dried onto Whatman 3MM paper, and autoradiographically visualized by a 1–16-h room-temperature exposure to Kodak X-AR film.

Cytogenetic and FISH Studies

Cytogenetic analysis was performed using heparinized whole blood, cultured for 72 h at 37°C in RPMI 1640 supplemented with 17% FBS and antibiotics. T-cell mitosis was stimulated with phytohemagglutinin.

Table 1

Short-Sequence Repeat Polymorphisms Analyzed

Locus	Heterozygosity	Location	Result
D5S117	.55	p15.1-p15.3	Uninformative
D5S208	.69	p15.1-p15.3	No maternal allele
D5S111	.53	p13.1-p14.1	No maternal allele
D5S108	.51	p13.1-p14.1	No maternal allele
D5S76	.76	cen-q11.2	No maternal allele
D5S118	.56	cen-q11.2 or q13.3-q15	No maternal allele
D5S107	.82	q11.2-q13.3	No maternal allele
D5S125	.50	q11.2-q13.3	Possibly Mendelian
D5S435	.73	q11.2-q13.3	No maternal allele
MAP1B-5′	.61	q11.2-q13.3	Possibly Mendelian
MAP1B-3′	.65	q11.2-q13.3	Possibly Mendelian
D5S204	.88	q11.2-q13.3	No maternal allele
IL9	.62	q22.3-q31.3	No maternal allele
D5S210	.75	q22-q22.3 or q31.3-q33.3	Possibly Mendelian
D5S209	.71	q22-q22.3 or q31.3-q33.3	No maternal allele
D5S119	.49	q22-q22.3 or q33.1-q33.3	Possibly Mendelian
D5S211	.72	q33.3-qter	No maternal allele

NOTE.—Results are listed as *uninformative* if the parents and child all appear homozygous for the same allele, as *no maternal allele* if the child appears homozygous for a paternal allele which is not present in the mother, and as *possibly Mendelian* if the child appears homozygous for an allele present in both parents.

Two and one-half hours prior to culture harvest, ethidium bromide was added, to a final concentration of 20 μg/ml. Twenty minutes prior to culture harvest, colcemid was added, to a final concentration of 0.1 μg/ml. Cells were pelleted and exposed to a hypotonic solution of 0.075 M KCl for 13 min at 37°C, pelleted again, and fixed in three changes of 3:1 methanol:acetic acid. The cell pellet was then placed at −20°C for 30 min, and a suspension of cells in a fixative was dropped onto clean wet slides. Slides were aged overnight at 60°C and were G-banded with trypsin and Giemsa. Metaphases were photographed with Kodak 2415 technical pan film. FISH was performed according to the protocol of Klinger et al. (1992), using probes cos 16 and ϕ98-36 (isolated from YAC 38 and YAC 98, respectively, described by Kleyn et al. 1993), which flank the SMA locus and are separated by approximately 1.5 Mb.

Results

The proband and his family were initially referred for genotype analysis as part of linkage-based SMA informativeness testing for a pending pregnancy. Review of all clinical material prior to DNA testing revealed no unusual features in the presentation or course of the proband's illness, and he was felt to meet international consensus criteria (Gilliam and Brzustowicz 1992) for SMA type III. The family was initially analyzed with six SSRP markers from the SMA region of chromosome 5, but the lack of Mendelian inheritance at three of

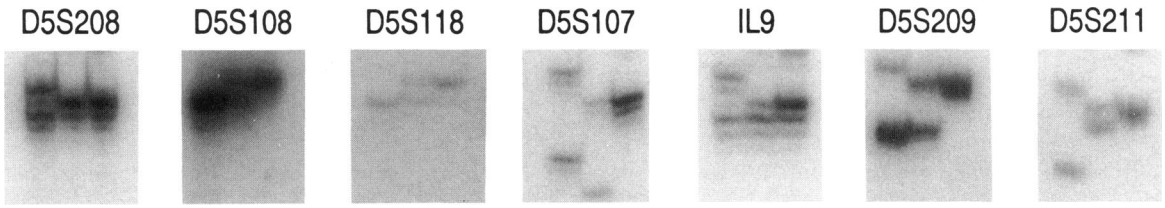

Figure 1 SSRP results demonstrating the lack of inheritance of maternal alleles for each of seven cytogenetic intervals spanning p15.1-15.3 to q33.3-qter. For each locus the three samples are mother, father, and child, loaded from left to right.

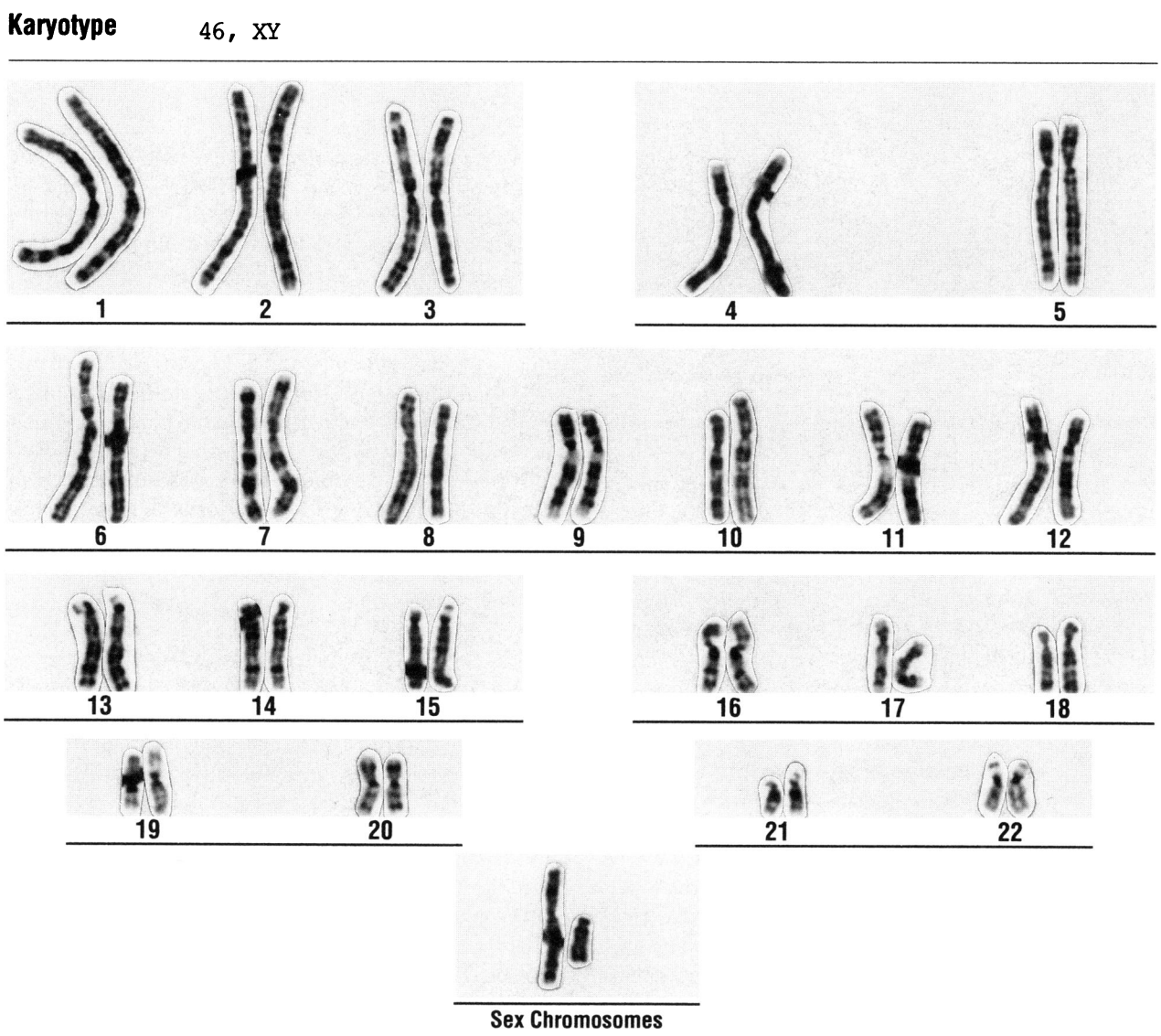

Figure 2 Karyotype of the proband, revealing a normal 46, XY with no visible deletions

those loci triggered a more extensive genetic investigation.

The results of the SSRP analysis are summarized in table 1. For all 17 markers used, amplification of the proband's DNA produced only a single allele. For six of the loci, the allele present in the proband was present in both the mother and father, and whether the patient was hemizygous or homozygous could not be determined. For the remaining 11 loci, however, the allele present in the proband was present only in the father, with no evidence of amplification of an allele inherited from the mother. The loci demonstrating a lack of maternal transmission span chromosome 5 from p15.1-p15.3 to q33.3-qter, physically mapping to seven different cytogenetic intervals, which are listed in table 1. SSRP results from each interval are presented in figure 1. Nine additional markers, located on chromosomes 2, 4, 16, and X, were genotyped and revealed normal Mendelian transmission of maternal and paternal alleles to the proband (data not shown).

Cytogenetic analysis revealed a normal male karyotype with no visible deletions or evidence of monosomy

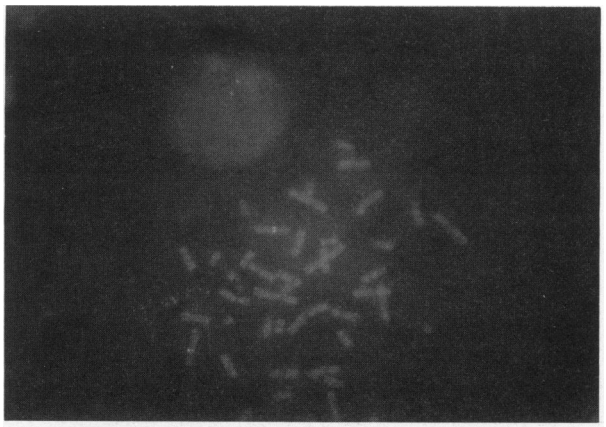

Figure 3 FISH analysis of the proband with the probe cos 16, which closely flanks the SMA locus on chromosome 5q.

5 (fig. 2). FISH analysis with probes that closely flank the SMA locus within the 5q11.2-13.3 region confirmed the presence of two copies of these flanking sequences, indicating that there is no deletion around the SMA locus (fig. 3).

Discussion

We describe a case of paternal uniparental isodisomy for chromosome 5, detected in a child with clinically typical SMA. Karyotype and FISH data from closely linked DNA markers clearly demonstrate that the proband has two copies of chromosome 5, yet the SSRP results indicate that the two chromosomes are identical at multiple loci spanning their entire length. Furthermore, although normal Mendelian inheritance was observed at loci on other chromosomes, there were no demonstrable maternally-derived alleles on the proband's chromosome 5.

While UPD is a rare genetic event, it must be considered as a possible cause of SMA when conducting prenatal testing. The chance of missing a case of UPD would be especially great if a small number of two-allele markers were used, since a child homozygous on the basis of UPD could easily appear to be conforming to Mendelian inheritance, as the probability of the parents transmitting a shared allele would be high (50% for markers with two alleles of equal frequency). Subsequent pregnancies could be assigned a mistakenly high risk for SMA. Typing multiple highly polymorphic markers, as was done in this case, should easily identify a non-Mendelian pattern of inheritance. The subsequent pregnancy in this family was assigned a very low risk for SMA, as the fetus was determined to have one maternally and one paternally derived chromosome 5, with the paternal chromosome differing from the one inherited by the proband.

Two readily evident mechanisms could relate isodisomy for chromosome 5 to SMA. First, as has been seen for several autosomal recessive disorders, one parent (in this case, the father) can be a carrier and transmit two copies of a defective gene to the proband. Alternatively, chromosome 5 may be subject to genomic imprinting, and the SMA due to the lack of a maternal contribution for the region containing the SMA gene. Mouse chromosome 11, which is involved in imprinting (Cattanach and Kirk 1985), is homologous to the human 5q23-q33 region (O'Brien et al. 1993), suggesting that a portion of 5q would be a likely candidate region for imprinting in humans (Hall 1990). However, the human region 5q11.2-13.3 that contains the SMA locus is homologous to mouse chromosome 13 (O'Brien et al. 1993), which does not appear to be subject to imprinting (Cattanach and Kirk 1985).

Even if SMA in an individual with UPD for chromosome 5 is not attributable to imprinting, other disorders of growth or development that are due to imprinting could co-occur. Mice with maternal disomy for proximal 11 are smaller than normal, while those with paternal 11 disomy are larger than normal (Cattanach and Kirk 1985). Mouse chromosome 11 is homologous with parts of human chromosomes 2, 5, 7, 16, 17, and 22 (O'Brien et al. 1993). The three reports of maternal UPD for chromosome 7 have all been characterized by small stature and retarded growth, which were not fully explained by the homozygous disease mutations (in CFTR or COL1A2) of those individuals (Spence et al. 1988; Voss et al. 1989; Spotila et al. 1992). In contrast, the reports of UPD for chromosome 22 (Kirkels et al. 1980; Palmer et al. 1980) do not report any phenotypic abnormalities, while the cases of UPD for chromosome 16 report growth retardation only at the intrauterine stage (Bennett et al. 1992; Dworniczak et al. 1992), possibly attributable to placental trisomy 16.

Like the cases of UPD for chromosome 22, this case of paternal isodisomy for chromosome 5 demonstrates no global alterations in growth. No developmental abnormalities, other than those attributable to classical childhood-onset SMA, are present. While further characterization of the SMA locus inherited from the father will have to wait until the gene involved in SMA is identified, it seems likely that the disorder in this child

is due to the paternal contribution of two copies of a defective SMA gene, with no evidence for additional abnormalities due to imprinted genes on chromosome 5.

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