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Hunter Disease (Mucopolysaccharidosis Type II) Associated with Unbalanced Inactivation of the X Chromosomes in a Karyotypically Normal Girl

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Summary

The mechanism of profound generalized iduronate sulfatase (IDS) deficiency in a developmentally delayed female with clinical Hunter syndrome was studied. Methylation-sensitive RFLP analysis of DNA from peripheral blood lymphocytes from the patient, using *Msp*I/*Hpa*II digestion and probing with M27β, showed that the paternal allele was resistant to *Hpa*II digestion (i.e., was methylated) while the maternal allele was digested (i.e., was hypomethylated), indicating marked imbalance of X-chromosome inactivation in peripheral blood lymphocytes of the patient. Similar studies on DNA from maternal lymphocytes showed random X-chromosome inactivation. Among a total of 40 independent maternal fibroblast clones isolated by dilution plating and analyzed for IDS activity, no IDS$^-$ clone was found. Somatic cell hybrid clones containing at least one active human X chromosome were produced by fusion of patient fibroblasts with Hprt$^-$ hamster fibroblasts (RJK88) and grown in HAT-ouabain medium. Methylation-sensitive RFLP analysis of DNA from the hybrids showed that of the 22 clones that retained the DXS255 locus (M27β), all contained the paternal allele in the methylated (active) form. No clone was isolated containing only the maternal X chromosome, and in no case was the maternal allele hypermethylated. We postulate from these studies that the patient has MPS II as a result of a mutation resulting in both the disruption of the IDS locus on her paternal X chromosome and unbalanced inactivation of the nonmutant maternal X chromosome.

Introduction

Hunter disease (mucopolysaccharidosis type II), due to an inherited defect in the lysosomal catabolism of acidic glycosaminoglycans (GAG) caused by deficiency of the enzyme iduronate sulfatase (IDS) (Bach et al. 1973) is characterized by wide-spread accumulation of dermatan and heparan sulfates, resulting in typical coarse facial features, skeletal abnormalities, joint contractures, short stature, hepatosplenomegaly, deafness, and intellectual deterioration in affected males (Neufeld and Muenzer 1989). It is transmitted as an X-linked recessive disorder; carrier females generally show no clinical manifestations of the disease, though they often show decreased levels of IDS in serum (Archer et al. 1983).

Hunter disease in females is exceedingly rare. In 1977, Neufeld et al. (1977) described two karyotypically normal girls with clinical manifestations of mucopolysaccharidosis type II and IDS deficiency in a number of tissues including blood and cultured skin fibroblasts. They postulated the existence of an autosomal recessive form of the disease. However, enzyme activities in peripheral blood leukocytes and cultured skin fibroblasts from the parents were normal. One of the cases reported was subsequently shown to have multiple sulfatase deficiency (MSD), an autosomal recessive condition associated with deficiency of a number of sulfatases, including IDS (Fluharty et al. 1978). The second patient, now in her late teens, is clinically severely handicapped by skeletal deformities

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but is apparently intellectually normal (S. Cederbaum, personal communication). Although the patient does not appear to have MSD, the nature of the genetic defect causing her disease is still unclear.

In 1983, Mossman et al. (1983) reported a girl with clinical features of Hunter disease and profound IDS deficiency who was also found to have a balanced reciprocal chromosomal translocation, 46,XX,t(X; 5), apparently disrupting the Hunter gene. Although the breakpoint on the X chromosome was originally thought to be between q26 and q27, further cytogenetic analysis of the case indicated it was more distal in q28 (Roberts et al. 1989). Broadhead et al. (1986) reported another case of Hunter disease in a girl with a partial deletion of the long arm of one of the X chromosomes, resulting in nonrandom inactivation of the structurally abnormal paternal X chromosome and expression of an IDS mutation on the maternal X.

We report here observations on a case of Hunter disease in a karyotypically normal girl. Her disease appears to be the result of a mutation causing IDS deficiency as well as unbalanced inactivation of the nonmutant X chromosome.

Subject and Methods

Case Report

The patient, a developmentally delayed female child with macrocephaly, dysmorphic facies, hypotonia, hepatosplenomegaly, generalized dysostosis multiplex, and mucopolysaccharidurια, was shown to have profound deficiency of IDS activity in serum and fibroblasts (Clarke et al. 1990). However, this patient was severely developmentally delayed from early in infancy, and she exhibited hypermobility of her joints, rather than the joint-mobility limitation encountered in males with classical Hunter disease. Chromosome analyses, including high-resolution banding, were normal. Analysis of ^{35}S uptake by cultured skin fibroblasts from the patient showed accumulation of glycosaminoglycans that was corrected by coculturing with Hurler cells but not by coculturing with classical Hunter disease cell lines.

Cell Lines and Media

Cultured skin fibroblast lines were established from skin biopsies from both forearms of the proband (HSC3119), a healthy control (HSC1685), a male patient with classical Hunter disease (HSC1128), the mother of the proband (from two different biopsies, HSC2580 and HSC3222), and a female Hunter disease carrier (HSC0183). Skin fibroblasts from a patient with multiple sulfatase deficiency (GM04681) were obtained from the Coriell Institute, Camden, NJ. The cells were grown in α-MEM (Eagle, modified; University of Toronto, Toronto), supplemented with 10% FCS (CELLect Silver Fetal Calf Serum; Flow Laboratories, McLean, VA), and 1% penicillin-streptomycin (GIBCO, Grand Island, NY).

The hypoxanthine guanine phosphoribosyltransferase–deficient ($Hprt^-$) line of cultured Chinese hamster fibroblasts (RJK88) (Fuscoe et al. 1983) used for the formation of rodent-human somatic cell hybrids was provided by R. G. Fenwick, Jr. (Baylor College of Medicine, Houston) and maintained at 37°C in α-MEM supplemented with glucose (final concentration 11×10^{-3} M), 1% penicillin-streptomycin (GIBCO, Grand Island, NY), and 10% FCS (Flow Laboratories, McLean, VA).

Complementation Studies

Genetic complementation was assessed by analysis of IDS activity in hybrid somatic cell clones produced by fusion of fibroblasts by treatment with polyethylene glycol (PEG) (Davidson and Gerald 1976; Wood 1978; Gravel et al. 1979; Gravel and Leung 1983) and isolation by unit-gravity sedimentation (Chang et al. 1979). The cells in one T75 culture flask (Becton-Dickinson Labware, Lincoln Park, NJ) were harvested by trypsinization (trypsin, 2.5% [10 ×]; GIBCO, Grand Island, NY), transferred to a single roller bottle, and incubated for 2 wk in an atmosphere of 95% air/5% CO_2 which generally yielded about 25×10^6 cells/roller bottle. Aliquots (approximately 1×10^6 cells) of each of the two cell lines being fused were thoroughly mixed and plated in P60 culture dishes (60 × 15 mm) and incubated for 24 h in α-MEM containing 15% FCS. The medium was then removed, and the cells were washed twice with PBS and overlayed with 5 ml of the 40% PEG for a period not exceeding 1 min. The medium containing PEG was removed, and the cells were washed thoroughly five times in rapid succession with PBS. The final PBS wash was replaced by α-MEM containing 15% FCS, and the cells incubated for a further 48 h at 37°C in an atmosphere of 95% air/5% CO_2. The fusion procedure was repeated. Seventy-two hours after the second fusion event, the cells were harvested by trypsinization, washed with PBS, and stored at −20°C until analyzed. Parallel plates were stained with methylene blue to assess the extent of heterokaryon formation.

Cellular Cloning Studies

Monolayer cultures of maternal skin fibroblasts were established from 4-mm skin biopsies generally taken from the forearm and maintained in α-MEM containing 10% FCS at 37°C in an atmosphere of 95% air/5% CO_2. Clonal cell lines were obtained by the dilution method described by Ham and Puck (1962). The purity of the clones was confirmed by RFLP analysis (see below). Cultured skin fibroblasts from a known Hunter disease heterozygote were cloned and analyzed by the same procedures.

Biochemical Studies

IDS activity in cultured skin fibroblasts was measured according to the method of Hall et al. (1978), with the modifications suggested by Wasteson and Neufeld (1982) for measurement of the enzyme activity in serum. The substrate was O-(α-L-idopyranosyluronic acid 2-sulfate)-(1–4)-2,5-anhydro-D-[^{3}H]-mannitol-6-sulfate, approximately 450 mCi/mmol (HSC Research Development Corp., Toronto). Cultured cells were harvested by trypsinization, suspended in 0.1% Triton X-100, and frozen and thawed six times. The extracts were dialyzed overnight against 0.01 M Tris-HCl buffer, pH 7.4, containing 0.01 M NaCl at 4°C. Aliquots of the dialyzed extract (25–100 μg protein) were incubated with 0.08 nmol (30,000–40,000 cpm) of radiolabeled substrate and 10 μmol sodium acetate buffer, pH 4.5, in a final volume of 80 μl at 37°C for 23 h. The reaction was stopped by cooling on ice and addition of 1 ml 1 mM Na_2HPO_4. The reaction mixtures were applied to small columns of 0.4-ml ECTEOLA cellulose (Cellex E) equilibrated with water, and the reaction products were eluted with two 5-ml aliquots of 0.07 M sodium formate. Radioactivity in the eluate was quantitated by liquid scintillation spectrometry. In a typical assay of IDS activity in normal skin fibroblast extracts, 15%–30% of the added substrate was hydrolyzed during 23-h incubation.

Molecular Studies

The origin of the human X chromosome in peripheral blood lymphocytes, cultured fibroblasts, and rodent-human somatic cell hybrids was determined by RFLP analysis of the DXS52 locus, with the use of the highly polymorphic probe, St14–1, localized to chromosome region Xq28 (Oberle et al. 1985) and previously shown to be informative in this family (Clarke et al. 1990). Aliquots of DNA (3 μg) were digested with the restriction enzyme *Taq*I (Boehringer Mannheim), separated by electrophoresis on 0.6% agarose gels, and transferred to nylon filters (Hybond-N; Amersham) (Southern 1975). The filters were prehybridized in 5 × SSPE (Maniatis et al. 1982), 1% SDS, 40% formamide, herring sperm DNA (250 μg/ml), and 0.5% skim milk powder for 5–6 h at 42°C. Hybridization was done in the same solution, with the addition of 10% dextran sulfate, using St14–1 radiolabeled with [^{32}P]dCTP by the random-hexamer labeling technique (Feinberg and Vogelstein 1983). After probing, the filters were washed in 0.2 × SSPE, 0.1% SDS at 65°C and then were exposed to Kodak X-Omat AR film for 1–4 d at −70°C.

In order to identify the active X chromosome, use was made of a methylation-sensitive RFLP system. The probe was M27β, a 1.9-kb *Eco*RI fragment marking a hypervariable region of DNA (DXS255) mapping to Xp11.22 (Fraser et al. 1989). This region was reported to show differential methylation patterns on the active and inactive X chromosomes when the DNA was digested with *Msp*I and with *Hpa*II (Brown et al. 1990).

For methylation-sensitive RFLP analyses, total genomic DNA was extracted from peripheral blood lymphocytes, cultured skin fibroblasts, or patient-hamster somatic cell hybrids as described above. DNA samples were divided into three 3-μg aliquots. One aliquot was digested with restriction endonuclease *Msp*I, and another was digested with the methylation-sensitive endonuclease *Hpa*II, which recognizes the same restriction site as *Msp*I but cleaves the DNA only if the site is unmethylated. All samples were then redigested with *Pst*I to reduce the size of DNA fragments and facilitate their separation on agarose gels. Digested DNA was size-fractionated by electrophoresis on agarose gel and transferred to nylon filters as previously described (Greer et al. 1989). Confirmation of the presence of the HPRT locus of at least one human X chromosome in the somatic cell hybrids was done by probing the *Taq*I digests, previously probed with St14–1 (see above), with pPB 1.7, a 1.7-kb *Bam*HI/*Pst*I fragment from the first intron of the human HPRT gene (Jolly et al. 1982).

Somatic Cell hybrids

Rodent-human somatic cell hybrids were produced by brief treatment of mixed cultures with PEG (Davidson and Gerald 1976), and selection for hybrids was carried out by maintaining the cultures in HAT-ouabain

medium (10^{-4} M hypoxanthine, 1.6×10^{-5} M thymidine, 4.0×10^{-7} M aminopterin, 1.89×10^{-6} M ouabain). In brief, equal numbers (5×10^5 cells) of RJK88 Chinese hamster cells and human fibroblasts were mixed and cultured together in 60 × 15-mm petri dishes (Falcon, Lincoln Park, NJ). After 18–24 h, the medium was aspirated, and the cells were washed with α-MEM and exposed to 1.5 ml of PEG for no more than 60 s. The PEG was aspirated rapidly, and the cells were washed thoroughly with α-MEM and then maintained in α-MEM supplemented with glucose (final concentration 11×10^{-3} M), 1% penicillin-streptomycin (GIBCO, Grand Island, NY), and 10% FCS. After 24 h, the medium was replaced by HAT-oubain medium which was replaced every 2–4 d with fresh selective medium. After 2–4 wk, HAT-ouabain-resistant colonies of cells were harvested by trypsinization in cloning rings, transferred to T25 flasks, and expanded for DNA analysis.

Results

Evidence for the Presence of IDS Mutation in the Patient

The IDS activity in cultured skin fibroblasts from the patient was comparable to that in a patient with classical Hunter disease (MPS II) (table 1). Table 1 also shows the results of the analysis of IDS activities in heterokaryons formed by PEG-induced fusion of the patient's cultured skin fibroblasts with (*a*) cells from a patient with MSD (GM04681) and (*b*) cells from a male with classical Hunter disease. IDS activity in patient × Hunter heterokaryons was not significantly different from the enzyme activity in unfused mixtures of the two types of cells. In contrast, the IDS activity in heterokaryons formed by fusion of patient cells with MSD cells was increased 3–4-fold over the activities either in control fusions of the patient cells or in MSD cells alone.

Evidence That the Mother of the Patient Is Not a Carrier of the IDS Mutation

A total of 40 independent clones of maternal skin fibroblasts was isolated and analyzed for IDS activity, and not a single IDS^- clone was found. Parallel studies on cultured fibroblasts from a known Hunter carrier showed the presence of two populations of clones, 18 IDS^+ clones and eight IDS^- clones. Methylation-sensitive RFLP analysis of several of the maternal fibroblasts clones by using M27β showed that, based on differential DNA methylation patterns, there were two populations of clones (fig. 1, panel 4). One set of clones showed hypermethylation of the paternal M27β locus, and in the other set the maternal allele was hypermethylated. The results confirmed that each set of clones analyzed contained only a single active X chromosome and that two sets of clones were isolated, each with a different X chromosome active. These observations essentially eliminated the possibility that the mother of the patient is heterozygous for MPS II, since all the clones had normal IDS activity, irrespective of which of the grandparental X chromosomes was active. The results also confirmed that the mother of the patient has two active X chromosomes, thereby eliminating the possibility that she carries some X-linked mutation affecting expression of one or the other X chromosome.

Table 1

Analysis of Complementation of IDS Activity in Heterokaryons Formed by PEG-induced Fusion of Patient Fibroblasts with MSD Fibroblasts and with Hunter Disease Fibroblasts

Cell Line	IDS Activity (units[a]/mg/h)	IDS Activity Ratio (activity in heterokaryons/activity in mixed cells)		
		+MSD Cells	+Hunter Cells	+Patient Cells
Patient (HSC3119)	.76 ± 1.14 (8)	4.01 ± 1.95 (17)**	1.38 ± .76 (20)	1.36 ± 1.01 (8)
Hunter (HSC1128)	.54 ± .05 (5)	10.47 ± 7.97 (7)*	1.17 ± .46 (5)	
MSD (GM04681	2.17 ± 1.58 (3)	1.25 ± .66 (6)		
Control (HSC1685)	17.9 ± 6.0 (9)			

Note.—Data are means ± SD; numbers in parentheses are number of experiments carried out on different occasions over a period of several months. The details of the experiments are described in the text.

[a] Units are percentage of substrate hydrolyzed.

* $P < .05$ (difference between activity in mixed heterokaryons, compared with the activity in heterokaryons formed from Hunter cells alone).

** $P < .01$ (difference between activity in mixed heterokaryons, compared with the activity in heterokaryons formed from patient cells alone).

Evidence of Unbalanced X-Chromosome Inactivation in the Patient

Analysis of peripheral blood lymphocytes.—Figure 1 (panels 1–3) shows the results of the methylation-sensitive RFLP analysis of DNA extracted from peripheral blood lymphocytes from a healthy control female (panel 1), from an obligate carrier of Wiskott-Aldrich syndrome (WAS) (panel 2), from both parents (panels 3a and 3c), and from the patient (panel 3b). Both the RFLP pattern of the mother (panel 3c) of the patient and that of the healthy control individual (panel 1) showed the predicted four bands on digestion with *Pst*I/*Hpa*II that would be expected with random X inactivation. The RFLP pattern of the WAS carrier (panel 2) showed only two bands on *Pst*I/*Hpa*II digestion, consistent with nonrandom X inactivation (Greer et al. 1989). *Pst*I/*Hpa*II digestion and probing of DNA from the patient consistently showed both resistance of the paternal allele to *Hpa*II digestion and almost complete digestion of the maternal allele, indicating unbalanced X-chromosome inactivation.

Analysis of rodent-patient somatic cell hybrids.—A total of 36 independent patient-hamster (HSC3119 × RJK88) somatic cell hybrid clones were isolated, and the DNA was extracted and analyzed (table 2). The results of probing with M27β showed that the DXS255 locus was present in 22 of them. In 12 of the 22 clones in which DXS255 was retained, only the paternal allele was found; in the other 10, both paternal and maternal alleles were present. In no case was a clone identified containing only the maternal allele. In all cases, the paternal allele was hypermethylated (i.e., *Hpa*II resistant) (fig. 2).

Table 2

Molecular Analysis of Hamster-Human Somatic Cell Hybrids

X-Chromosome Complement	M27β (DXS255)	St14-1 (DXS52)
Paternal and maternal	10	11
Paternal	12	16
Maternal	0	0
Unknown[a]	14	9

[a] Neither paternal nor maternal alleles were demonstrated, though all lines were positive for human HPRT by Southern analysis (see text).

The absence of the DXS255 locus in 14 of the HAT-resistant clones suggested the possibility of chromosome fragmentation resulting in loss of the DXS255 locus (Xp21) and retention of the HPRT locus (Xq26) necessary for survival of the cell lines in HAT-selective medium. To test this possibility, the clones were probed with another X-chromosome probe, St14–1, which is specific for a highly polymorphic locus (DXS52) localized to Xq28 (Oberle et al.

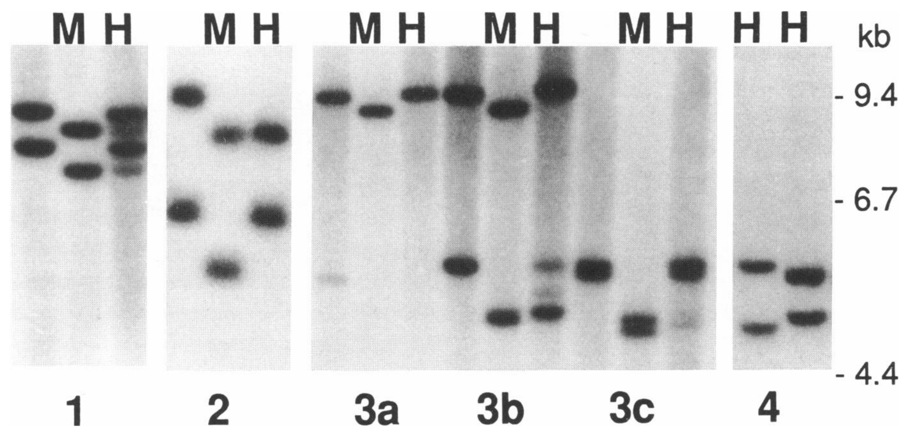

Figure 1 Autoradiograms showing X-chromosome inactivation patterns. Each set of three lanes shows the RFLP patterns obtained after digestion of DNA extracted from peripheral blood lymphocytes by *Pst*I (unlabeled lanes), *Pst*I/*Msp*I (lanes M), or *Pst*I/*Hpa*II (lanes H) and probing with M27β. Panel 1, Control female. Panel 2, Obligate carrier of WAS who was previously shown to exhibit nonrandom X-chromosome inactivation in peripheral blood leukocytes (Greer et al. 1989). Panel 3a, Father of patient. Panel 3b, Patient. Panel 3c, Mother of patient. Panel 4, RFLP patterns obtained after *Pst*I/*Hpa*II digestion of DNA extracted from two different IDS⁺ clones of maternal skin fibroblasts. The bars at the right indicate the positions and sizes of fragments of λ-phage DNA digested with *Hin*dIII. The faint band at 5.6 kb, seen only in the *Pst*I digest of the paternal DNA (panel 3a), is an unidentified artifact which was not present in any other Southern analysis of paternal lymphocytic DNA.

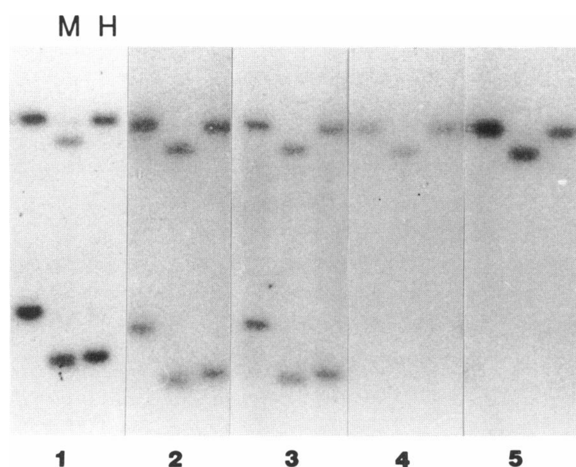

Figure 2 Analysis of paternal and maternal X-chromosome alleles in rodent-patient somatic cell hybrids. DNA was extracted from hamster-patient somatic cell hybrids produced as described in Subjects and Methods. Samples of DNA were digested with *Pst*I (left-hand lanes), *Pst*I/*Msp*I (lane M and all other middle lanes), or with *Pst*I/*Hpa*II (lane H and all other right-hand lanes) and probed with M27β. Panels 1–3, Clones showing presence of both maternal and paternal alleles. Panels 4–5, Clones showing presence of paternal allele only. In all cases the paternal allele was resistant to *Hpa*II digestion—i.e., was hypermethylated—indicating unbalanced expression of the paternal X chromosome.

1985) and previously shown to be informative in this family (Clarke et al. 1990). In the 14 DXS255-negative clones, probing with St14–1 showed the presence of the paternal DXS52 allele alone in six clones and showed both paternal and maternal alleles in an additional three clones. No clone contained the maternal allele alone. It is interesting that in three clones the DXS255 locus, but not the DXS52 locus, was retained and that in six clones neither DXS255 nor DXS52 alleles could be demonstrated. Probing with HPRT showed preservation of this locus in all clones (results not shown).

Discussion

The patient reported here has a condition biochemically indistinguishable from classical Hunter disease (mucopolysaccharidosis type II). She shows profound deficiency of IDS activity in serum, leukocytes, and cultured skin fibroblasts. She also excretes abnormal quantities of dermatan and heparan sulfates in her urine. Cultured skin fibroblasts accumulated [^{35}S]-GAG like cells from patients with classical Hunter disease, and there was no evidence of cross-correction of the defect when the patient's cells and classical Hunter disease cells were cultured together. In contrast, [^{35}S]-GAG accumulation, both by Hunter cells and by cells from the patient, was corrected by coculturing with normal cells or with Hurler disease cells. The levels of other sulfatases, including arylsulfatases A, B, and C, were normal in serum, leukocytes, and cultured skin fibroblasts, under conditions that would be expected to expose deficiencies attributable to MSD (Chang et al. 1983).

Hunter disease has been reported in girls (Neufeld et al. 1977; Mossman et al. 1983; Broadhead et al. 1986), but it is extremely rare. Some cases have been shown to be patients with MSD with particularly marked deficiency of IDS activity. However, since no deficiencies of any other of the sulfatases investigated could be demonstrated in the patient reported here, this possibility can be considered to have been eliminated. The possibility that the IDS deficiency arose as a result of either a rearrangement of the X chromosome (Mossman et al. 1983) or gross interstitial deletions involving the IDS locus is ruled out by the normal karyotype. The possibility that the disease in this girl is due to simultaneous mutations affecting both parental X chromosomes is considered extremely unlikely (Neufeld et al. 1977). Uniparental disomy, coupled with maternal heterozygosity for the Hunter mutation, was ruled out by demonstrating, by RFLP analysis, that one of the X chromosomes in the patient was of paternal origin (Clarke et al. 1990).

Clinical expression of X-linked disease in girls may occur as a result of unbalanced Lyonization in which deviation from the theoretical inactivation of equal proportions of both parental X chromosomes occurs by chance alone, especially if the precursor cell pool is small. Although this is no doubt the explanation of clinical disease occurring in some female carriers of Duchenne muscular dystrophy, for example, it is considered very unlikely in the present case. Presumably as a result of in vivo complementation, female carriers of the Hunter mutation never appear to display even subtle clinical abnormalities. Lyonization that is sufficiently unbalanced to cause virtual absence of enzyme activity would be statistically highly improbable. This makes the combination of (*a*) profoundly unbalanced Lyonization by chance alone and (*b*) de novo mutation of IDS, another unlikely event, extremely unlikely.

The results of the methylation-sensitive RFLP analyses of peripheral blood lymphocytes, cultured skin fibroblasts, and patient-hamster somatic cell hybrids indicate that X inactivation in the patient is unbal-

anced and that the paternal allele is active in the majority of cells. Since the mother of the patient does not show nonrandom X inactivation, this suggests that the occurrence of severe IDS deficiency in this girl is due to the presence, on her paternal X chromosome, of a mutation affecting IDS activity and also causing the paternal X chromosome to be preferentially expressed.

Structural abnormalities of the X chromosome are a well-known cause of nonrandom X inactivation in humans (Therman and Patau 1974; Gartler and Riggs 1983). In cases of balanced reciprocal X-autosome translocations, the normal X is usually inactivated. Deletions and duplications of the X tend to result in selective inactivation of the abnormal X chromosome (Therman and Patau 1974). While selective inactivation of a deleted X chromosome in females is a well-known cause of clinically significant X-linked disease, including Hunter disease (Broadhead et al. 1986), selective inactivation of the structurally normal X chromosome in females bearing such a deleted X is unusual. An interesting case has been reported recently in which an interstitial deletion of Xq27 was discovered to cause such selective inactivation of the structurally normal X chromosome (Schmidt et al. 1990). Magenis et al. (1984) reported a similar instance of selective inactivation of the structurally normal X chromosome in a girl with a duplication of Xq26.3-qter. In studies on a girl with an unusual recombinant X, Mohandas et al. (1987) showed that inactivation of the distal end of the long arm (Xq26.3-qter) occurred in spite of its being separated from the putative X-chromosome inactivation site, near Xq13 (Therman et al. 1979; Tantravahi et al. 1983; Brown et al. 1991), by a region of the chromosome that was not inactivated. This was interpreted as suggesting the existence of specific sequence information in the region, Xq26.3-qter, that is important in the spreading, if not initiation, of X-chromosome inactivation. Since the IDS gene has been localized to the same region of the X chromosome (Chase et al. 1986; Roberts et al. 1989; Le Guern et al. 1990), it is conceivable that a single submicroscopic deletion or rearrangement might cause both disruption of the IDS gene and nonrandom expression of the chromosome.

Nonrandom X-chromosome expression may also occur as a result of postinactivation selection against cell lines bearing an X-linked mutation. This is presumably both the mechanism of the apparent selective X-chromosome expression observed in the lymphocytes of women heterozygous for WAS (Prchal et al. 1980) and the explanation of the nonrandom distribution of erythrocyte cell lines in Lesch-Nyhan disease (Nyhan et al. 1970) and of B-lymphocytes in X-linked agammaglobulinemia (Conley et al. 1986). Some evidence for apparent postinactivation selection favoring survival of cultured-skin-fibroblast lineages bearing the IDS^- mutation has been reported (Booth and Nadler 1973, 1974; Donnelly and Di Ferrante 1975). However, Migeon et al. (1977) were not able to duplicate the phenomenon in comparable studies. The possibility that our patient inherited from her mother a mutation affecting X-chromosome inactivation was ruled out by the demonstration that X inactivation in the mother was apparently random.

Our interpretation of the results of these studies is that our patient has MPS II as a result of a new mutation causing both disruption of the IDS locus on her paternal X chromosome and, by involvement of other nearby loci, preferential expression of the mutant X chromosome. The patient shows many clinical features typical of Hunter disease (Clarke et al. 1990). On the other hand, she has been developmentally retarded from birth and is currently more severely mentally handicapped than most patients with uncomplicated Hunter disease. She also exhibits hypermobility of joint movements, rather than joint-mobility limitation characteristic of classical Hunter disease. These characteristics of her disease support the suggestion that the X-chromosome mutation responsible for her condition possibly involves loci other than just IDS. Future studies to characterize the mutation will be facilitated by the recent report of the isolation of IDS cDNA (Wilson et al. 1990) and may provide insight into the mechanism of X-chromosome inactivation.

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