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Uniparental Disomy as a Mechanism for Human Genetic Disease

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Summary

A female with cystic fibrosis and short stature was investigated for molecular or cytogenetic abnormalities that might explain the combined phenotype. Analysis with polymorphic DNA markers indicated that the father did not contribute alleles to the propositus for markers near the CF locus or for centromeric markers on chromosome 7. High-resolution cytogenetic analysis was normal, and the result could not be explained on the basis of nonpaternity or a submicroscopic deletion. All of the data indicate that the propositus inherited two identical copies of maternal sequences for much or all of chromosome 7. The occurrence of uniparental disomy could be explained by models postulating postfertilization error, gamete complementation, monosomic conception with subsequent chromosome gain, or trisomic conception followed by chromosome loss. Uniparental disomy in an individual with a normal chromosome analysis is a novel mechanism for the occurrence of human genetic disease.

Introduction

The locus for cystic fibrosis (CF) was mapped to the long arm of chromosome 7 in 1985 (Knowlton et al. 1985; Tsui et al. 1985; Wainwright et al. 1985; White et al. 1985). Because patients with rare cytogenetic aberrations were helpful for mapping and cloning the human genes for Duchenne muscular dystrophy, chronic granulomatous disease, and retinoblastoma (Orkin 1986), patients with CF and additional abnormalities were studied in an attempt to identify cytogenetic or molecular alterations of chromosome 7. A patient was found with short stature and CF who demonstrates unusual transmission of DNA markers on chromosome 7. Although results of chromosome analysis were normal, molecular studies indicated that the child inherited two copies of maternal DNA with no paternal contribution for all or part of chromosome 7.

The presence of two chromosomes from one parent in a disomic cell line is termed uniparental disomy (Engel 1980; Niikawa and Kajii 1984). Uniparental disomy is one form of aberrant origin for disomic cells, and in the present article the term pseudodisomy is used to describe disomic cells arising by means of aberrant mechanisms, whether of uniparental or diparental origin. Uniparental disomy can involve homozygosity for the chromosome, and the term isodisomy has been suggested for this phenomenon (Engel 1980). This is the first documentation of uniparental disomy in a human individual with a normal chromosome analysis. This type of abnormality usually would be undetected by current clinical methods, and the observation has numerous implications for human genetic disease.

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Material and Methods

Clinical Information

This patient (A.B.) was reported previously at age 7 years to have short stature, CF, and growth-

hormone deficiency (Hubbard et al. 1980). The long-term response to growth-hormone administration was poor. At age 14 years she experienced menarche, and her height was 52 inches at 16 years of age. There is a slight leg-length discrepancy, and she uses a ¼-inch lift on the right shoe. Asymmetry might suggest a diagnosis of Russell-Silver syndrome, but other features, such as triangular facies, are not present. The patient is of normal intelligence, and she has three older normal siblings. The mother of A.B. was age 36 years when the affected child was born, was diagnosed with lymphoma 6 mo after delivery, and died at age 39 years.

Cytogenetic analysis was performed on both phytohemagglutinin-stimulated lymphocytes and cultured skin fibroblasts. High-resolution studies were performed on peripheral lymphocytes after methotrexate synchronization (Yunis 1976).

Molecular Studies

Isolation of genomic DNA from peripheral blood, as well as Southern blotting with conventional markers, was performed in Houston by using modifications of standard techniques (Maniatis et al. 1982; Aldridge et al. 1984; Spence et al. 1986). For the patient, DNA also was isolated from cultured lymphoblasts and from cultured skin fibroblasts. DNA probes for chromosome 7 included the following: *met*H and *met*D provided by R. White and G. Vande Woude (White et al. 1985); J3.11(D7S8), B79a (D7S13), 7C22(D7S16), and J5.11(D7S10) provided by R. Williamson and J. Schmidtke (Estivill et al. 1986; Scambler et al. 1986); 917(D7S15) provided by H. Donis-Keller (Tsui et al. 1985); M102L and M60 short-arm probes for chromosome 7 provided by L.-C. Tsui (personal communication); NPY provided by M. Meissler (Meissler et al. 1987); Hf-32 (COL1A2) provided by F. Ramirez (Myers et al. 1981); and pASSψC7 (ASSP11) from this laboratory. Information for most other DNA probes is included in Human Gene Mapping 8 (Willard et al. 1985). Five variable number tandem repeat (VNTR) probes —including hINS 5′ 2.7 from the insulin locus (Rotwein et al. 1986), 213-274C from chromosome 5 (Overhauser et al. 1987), and three probes (YNZ2, YNZ22.1, and THI16) from Nakamura and colleagues (Nakamura et al. 1987)—were used to evaluate paternity. The following X-linked probes were used for paternity analysis (Willard et al. 1985; Hejtmancik et al. 1986): L1.28(DXS7), X65H7 (DXS72), TAK2(DXYS13), 58C(DXS99), 46d (DXS19), X13(DXS15), C7(DXS28), 754 (DXS84), and the tightly linked group including XJ1.1, pERT 87-15, and pERT 87-30(DXS164). Southern blotting analysis using alphoid repeat probes was performed in Toronto according to a method described elsewhere (Willard et al. 1986; Waye et al. 1987). The pMGB7 clone (Waye et al. 1987), containing alpha satellite DNA from chromosome 7, was used as probe, and filters were washed at high stringency to ensure specific hybridization to chromosome 7 (Willard et al. 1986; Waye et al. 1987).

Results

Initial Molecular Analysis

Initial Southern blotting analysis was performed with the *met* and J3.11 markers, which are known to be closely linked to CF (Wainwright et al. 1985; White et al. 1985). Interestingly, for three RFLPs—*met*H with *Msp*I, *met*H with *Taq*I, and *met*D with *Ban*I—the father was homozygous for one allele whereas the affected daughter, A.B., was homozygous for the opposite allele (fig. 1). Analysis of sam-

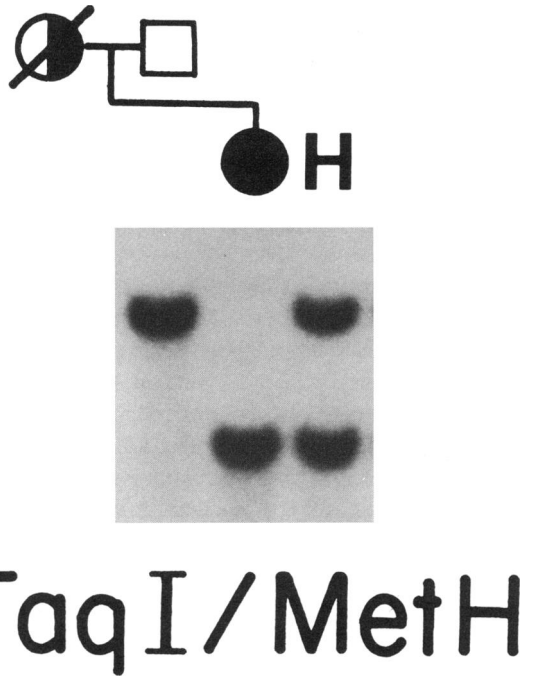

Figure 1 Lack of transmission of a paternal allele at the *met* locus. Southern blotting analysis of DNA from A.B., her father, and an unrelated individual (H) heterozygous for the RFLP is presented. DNA isolated from peripheral leukocytes was digested with *Taq*I and analyzed with the *met*H probe as described in Material and Methods.

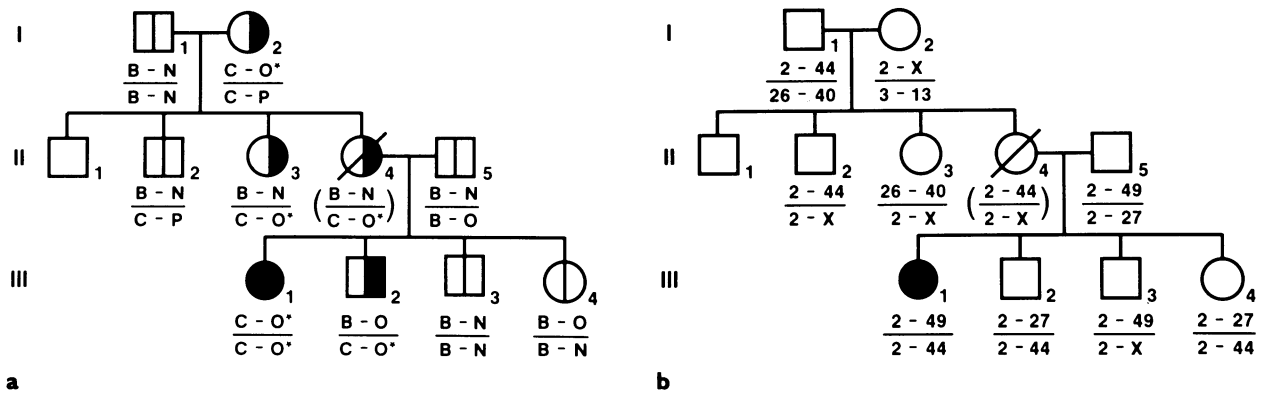

Figure 2 Genetic analysis for *met*, J3.11, and HLA in the B family. *a*, *met* Haplotypes are designated B or C, and J3.11 haplotypes are designated N, O, or P. Asterisks(*) denote haplotypes linked to CF. *b*, HLA-A and HLA-B types are presented as A-B, e.g., A2-B44/A26-B40 for the maternal grandfather. For the father of the propositus, the data are presented as A2-B49/A2-B27, although he could be A2/AX rather than A2/A2. Sibs are not depicted in birth order. Data for the mother of the propositus are deduced from data on other family members. The solid symbol indicates the presence of CF, and the half-shaded symbols in panel *a* indicate presumed CF heterozygotes.

ples of A.B.'s DNA from peripheral leukocytes, a lymphoblast cell line, and cultured skin fibroblasts revealed no evidence of a faint band of paternal origin, as might occur when chromosomal mosaicism is present. Haplotypes were prepared for four RFLPs at the *met* locus and for two RFLPs detected with J3.11 as reported elsewhere (Spence et al. 1986). The inheritance of markers tightly linked to CF appeared appropriate in the family, with the exception that the propositus did not inherit a *met* haplotype from her father (fig. 2*a*). The two explanations initially thought to be the most likely were nonpaternity or a small de novo deletion originating from the paternal chromosome 7. Cytogenetic analysis using methotrexate synchronization of the patient's peripheral lymphocytes for the purpose of high-resolution chromosome banding revealed a normal diploid karyotype. Analysis of 100 metaphases from peripheral blood and of 100 metaphases from cultured skin fibroblasts revealed no mosaicism for chromosome 7.

Southern blotting analysis using dosage techniques indicated disomic dosage for the *met* and J3.11 markers (data not shown), and, as discussed below, additional data were obtained that could not be explained by the occurrence of a submicroscopic deletion. Because the mother was deceased, paternity was analyzed using samples from numerous maternal relatives. Subsequent data indicated the importance of considering the possibility of an incestuous relationship. The results of HLA analysis, indicating that the child carried the B49 marker (which could not have been contributed by any maternal relative who was tested [fig. 2*b*]) were consistent with the assumed paternity.

The possibility that an alternative, unrelated individual was the true biological father of the affected child was investigated by analyzing the obligate possible paternal antigen types of the child by means of the red-cell genetic-marker systems ABO, Rh, MNSs, Kell, Kidd, and Duffy, in addition to the HLA system. The probability that the father contributed those obligate antigens was then compared with the probability that an untested random Caucasian man could have contributed them. Although the deceased mother's contribution was unknown for the ABO and MNSs systems, the alternative alleles were known—and a maximum probability of nonpaternity of 1×10^{-3} was calculated under the assumption that this possible random man had equal access to the mother (prior probability .5). Because of the lack of availability of the mother, probes from the X chromosome were particularly useful, since the daughter must receive all X-linked alleles located on her father's X chromosome. Based solely on X-linked DNA markers, the probability of nonpaternity was 3.5×10^{-2} when a prior probability of .5 was assumed. The patient and her father were also analyzed with five VNTR probes. When both those data collected by means of the VNTR probes and those data collected by means of an X centromeric probe (to be described below) were taken into account, the overall probability of nonpaternity was 6×10^{-7} when markers located on chromosomes other than 7 were used.

Table 1

Analysis of A.B. and Her Father by Means of RFLPs on Chromosome 7

Probe (Locus) and Enzyme	Map Location	Father	A.B.	Probability of Homozygosity[a]
*met*H:				
*Msp*I	7q22	1,1	2,2	
*Taq*I	7q22	1,1	2,2	
*met*D:				
*Taq*I	7q22	1,1	1,1	.14
*Ban*I	7q22	1,1	2,2	
J3.11 (D7S8):				
*Msp*I	7q22	1,2	1,1	
*Taq*I	7q22	1,1	1,1	
7C22 (D7S16):				
*Eco*RI	7q22	1,1	2,2	.64
ASSψC7(ASSP11):				
*Hin*dII	7p	1,1	1,1	.46
*Bgl*I	7p	1,2	2,2	
917 (D7S15):				
*Hin*dIII	7q21-q22	1,1	1,1	.38
*Hin*dII	7q21-q22	1,2	1,1	
Hf-32 (COL1A2):				
*Msp*I	7q21-q22	2,2	2,2	.70
*Stu*I	7q21-q22	2,2	2,2	
B79a (D7S13):				
*Msp*I	7q22	2,2	2,2	.55
*Hin*dIII	7q22	2,2	2,2	
λ37-6(NPY):				
*Taq*I	7p	1,1	1,1	.77
M102L:				
*Taq*I	7p	1,2	2,2	.51
M60:				
*Taq*I	7p	1,2	2,2	.66
J5.11 (D7S10):				
*Msp*I	7pter-p14	1,2	1,1	.54
Total analysis				.0008

NOTE.—Twelve DNA probes were used to detect 19 RFLPs.

[a] Probability that a random individual would be homozygous for the RFLPs. Observed homozygosity for all six RFLPs with *met* and J3.11 was used. For other RFLPs, observed homozygosity or allele frequencies were used for calculations.

Because both nonpaternity and submicroscopic deletion appeared unlikely, it was hypothesized that the patient might carry two copies of maternal DNA with no paternal contribution for much or all of human chromosome 7. Following this rationale, we analyzed DNA from the patient and her father by means of Southern blotting, using a series of RFLPs assigned to human chromosome 7 (table 1). For each RFLP, it was of interest to determine, first, whether absence of obligate paternal alleles was observed and, second, whether the patient would ever be heterozygous for polymorphisms on this chromosome. The failure of paternal transmission of an allele can only be detected in the fortuitous circumstance in which the father has no alleles in common with the daughter—e.g., when the father is homozygous for one allele and the daughter is homozygous for the opposite allele. Thus uniparental disomy might go undetected in the majority of analyses. There was failure of transmission of paternal alleles for *met*H, *met*D, and 7C22. In addition, it was found that the patient was not heterozygous for any of the 19 RFLPs assigned to chromosome 7. If both the linkage disequilibrium observed for RFLPs detected with each locus and any available observed heterozygosity data are taken into account, the probability that a random individual would be homozygous for all of the markers tested would be ~.0008. The striking lack of heterozygosity

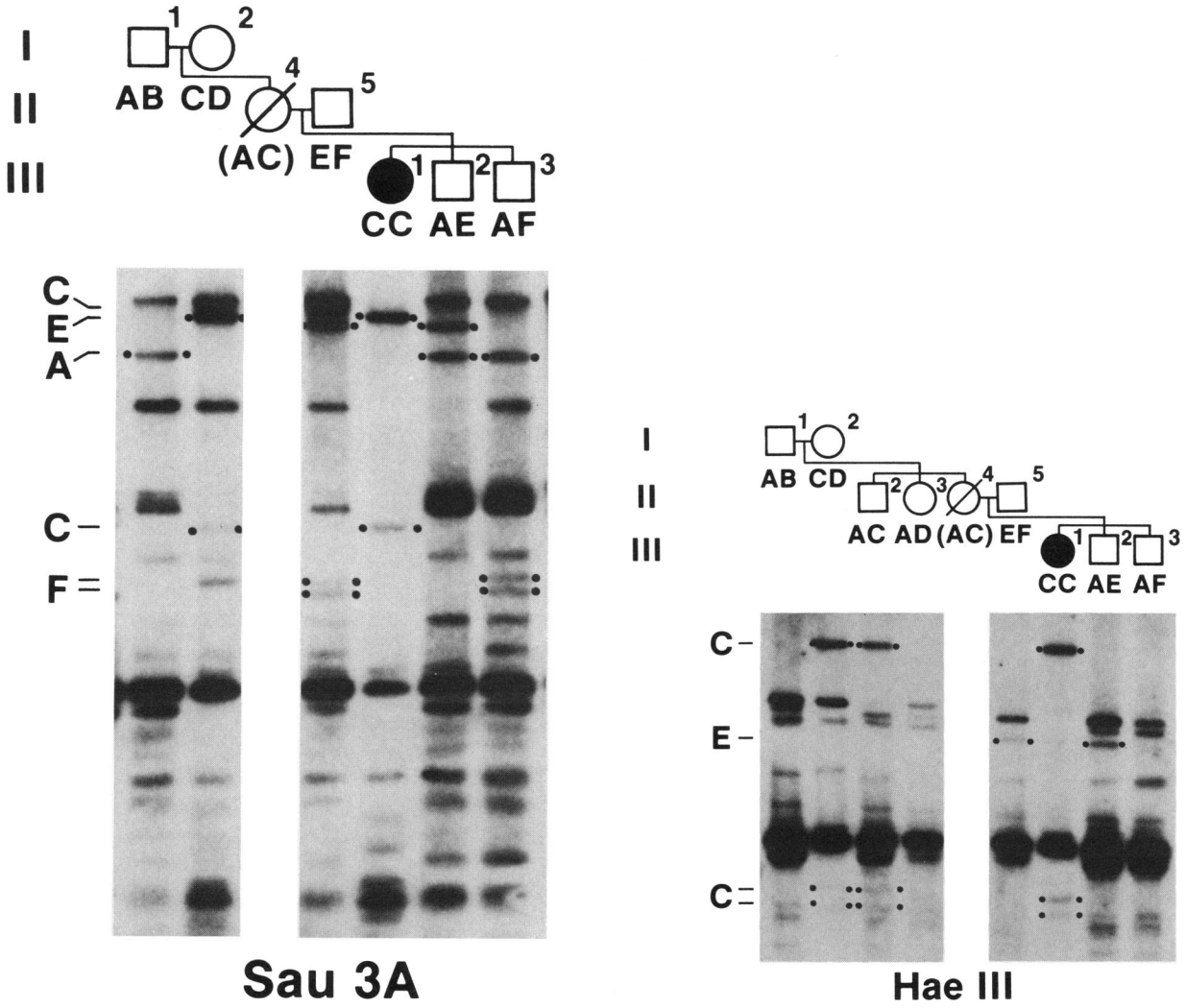

Figure 3 Southern blotting analysis of the B family by using an alphoid probe from chromosome 7. Pedigree identifications are consistent with those in fig. 2. A–F designate centromeres and unique fragments from these centromeres. DNA was digested with *Sau*3A or *Hae*III. Southern blotting analysis using alphoid repeat probes was performed as described in Material and Methods.

on chromosome 7 is consistent with an exceptional genetic circumstance.

Analysis with Alphoid Probes

Unusual segregation of chromosome 7 in this family can be assessed by means of analysis of DNA polymorphisms associated with alpha satellite (alphoid) DNA. Alpha satellite DNA is arranged as tandemly reiterated, divergent repeats of monomer length 171 bp, organized in a chromosome-specific fashion characteristic of each chromosome (Willard and Waye 1987). These repeated DNA sequences are located at the centromeric region of each chromosome, and RFLPs detected with alpha satellite DNA probes can be used as centromeric DNA markers. The pMGB7 probe (Waye et al. 1987) can be used to detect such polymorphisms specifically at the centromere of chromosome 7 (H. Willard, G. Greig, and J. Waye, unpublished data). Each centromere contains a characteristic group of repeat fragments or morphs that comprise a haplotype. The term morph implies a particular variant form that, in this case, is a repeated polymorphic fragment rather than a single-copy polymorphic fragment. Morphs unique to a particular centromere in a family can be used as DNA markers. Results of analysis of the B family by means of a chromosome 7 alphoid DNA probe are presented in figure 3. A total of six centromeres, designated A–F,

are present among the individuals included in figure 3; four of the six are from the maternal grandparents, and two are from the father of the propositus. Results of representative analyses are shown in figure 3, with morphs unique to particular centromeres being identified by the corresponding letters.

As seen in figure 3*a*, *Sau*3A digestion was used to identify morphs unique to centromeres A, C, E, and F. The C morphs are found in the maternal grandmother and in A.B., the propositus. Morphs labeled E and F mark the two centromeres of A.B.'s father. This man contributed the E haplotype to one son and the F haplotype to the other son, but he contributed neither the E nor the F haplotype to A.B., his daughter. Thus there is failure of transmission of paternal centromeric markers to the affected daughter. Figure 3*b* presents the results of a similar analysis with the enzyme *Hae*III. The morphs distinguishing the C centromere are present in the maternal grandmother, in A.B., and in a maternal uncle and a maternal aunt of the patient. Morphs from the A and E centromeres also can be traced in this analysis. The data collected by means of the alphoid probe from chromosome 7 indicated that the father did not contribute a centromeric marker to A.B., his daughter, and are consistent with the hypothesis that A.B. inherited two identical maternal centromeres that originated from her maternal grandmother.

To address the probability that a random man could have contributed an alphoid chromosome 7 haplotype identical to that contributed by the mother requires consideration of the frequency of individual morphs and haplotypes in the general population. Only limited haplotype data are available, owing to the fact that a haplotype can only be determined in this system either in large family studies or in somatic-cell hybrids. Nonetheless, on the basis of analysis of several hundred individuals with *Sau*3A, *Hae*III, and *Bam*HI (H. F. Willard, G. Greig, and J. Waye, unpublished data), we estimate conservatively that the probability of a random man possessing an alphoid chromosome 7 haplotype identical to the C centromere of the mother is $<.01$.

In addition, an alphoid probe from the X chromosome was used to evaluate paternity (Willard et al. 1986). Analysis with four restriction enzymes indicated that A.B. inherited one X centromeric haplotype found in her maternal grandmother and one from her father. The mother's X centromeric haplotypes were deduced on the basis of data from her two sons, and A.B. inherited the exact sum of the morphs from one of these haplotypes plus her father's haplotype. Conservatively, we estimate at .05 the probability that a random man would contribute the same markers as A.B.'s father. In addition, A.B. possesses X centromeric morphs in common with her father, but these are not seen in the maternal grandparents. The absence of these X-linked morphs in the maternal grandmother means that none of her sons, known or unknown, could be the father of A.B. by an incestuous mating.

Interpretation

Aberrant transmission of DNA markers was evaluated thoroughly in this patient. On the basis of analysis by means of conventional polymorphisms, DNA markers, and an alphoid probe from the X centromeric region, nonpaternity is extremely unlikely. Extensive homozygosity on chromosome 7 in the patient would not be explained by nonpaternity unless the mating was incestuous, and the possibility of close consanguinity was eliminated. Lack of paternal inheritance for markers at the midportion of the long arm of chromosome 7 and at the centromere cannot be explained by the occurrence of a de novo submicroscopic deletion. The data strongly suggest uniparental disomy for at least the segment from the centromere to q22 of chromosome 7 in this patient. In addition, there is extensive homozygosity for chromosome 7, with the possibility of isodisomy for the entire chromosome. It is likely that the mother was heterozygous for the CF mutation, but the father is unlikely to carry the CF gene.

Discussion

Analysis of a patient with short stature and CF has unveiled unusual genetic findings that are most consistent with the presence of uniparental disomy. Some mechanisms that might lead to various forms of pseudodisomy are depicted in figure 4. Postfertilization errors such as nondisjunction with reduplication, mitotic recombination, or gene conversion might lead to complete or partial isodisomy. These events would be analogous to those that have been observed in the pathogenesis of retinoblastoma (Cavenee et al. 1983), except that the event might occur in a very early embryonic stage. Mosaicism might be frequent with postfertilization errors, since there would be no selection against the original disomic line. Apart from the observations in retino-

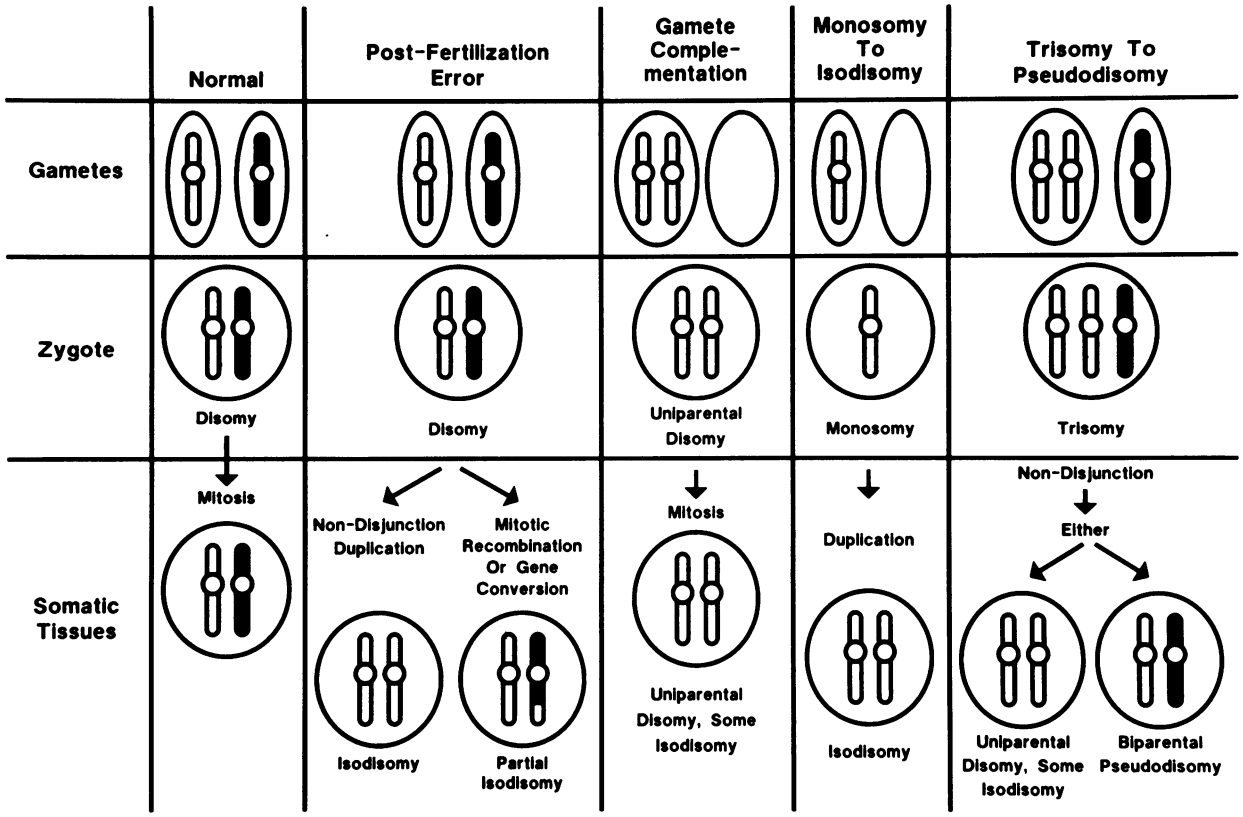

Figure 4 Proposed mechanisms that would account for the occurrence of pseudodisomy. The chromosomal composition for an autosome is depicted.

blastoma and analogous tumors, we are unaware of any precedent for such events in this context.

Gamete complementation involves a fertilization between one nullisomic gamete and one disomic gamete for the same chromosome, resulting in a zygote with uniparental disomy. Gamete complementation has been achieved in mice (Lyon 1983; Searle and Beechey 1985) by using parents with balanced translocations that predispose to disomic and nullisomic gametes. Gamete complementation is possible for some chromosomes and not others in mice, with some dependence on which parental gamete is nullisomic or disomic (see below). With gamete complementation, the centromeres would be different if the disomic gamete arose from a meiosis I error, whereas the centromeres would be identical if the disomic gamete arose from a meiosis II error. Varying extents of isodisomy are possible with gamete complementation, depending both on where the meiotic error occurs and on the number of crossovers occurring during meiosis. No mosaicism would be expected with gamete complementation.

Pseudodisomy could arise after aneuploid conception. For a monosomic conception, duplication of the single chromosome could lead to complete isodisomy. Although mosaicism might occur, monosomic cells might grow poorly and be rare in the mature somatic tissues. Pseudodisomy arising after trisomic conception is theoretically more complex because the contribution of the disomic gamete can vary and because nondisjunction might lead to the loss of any of the three homologous chromosomes. As discussed for gamete complementation, the contribution of the disomic gamete will vary depending on the type of meiotic error and on crossing-over. Subsequent nondisjunction could lead to uniparental disomy or to a biparental form of pseudodisomy indistinguishable from normal disomy. The uniparental disomy would have the same potential for partial isodisomy as occurs with gamete complementation. Mosaicism might be variable when pseudodisomy arises from trisomy, since many different trisomic cells can persist in somatic tissues. There is evidence that most instances of mosaic trisomy involve meiotic

errors with trisomic conception (Hassold 1982), and mosaic uniparental disomy for chromosome 21 has been reported (Niikawa and Kajii 1984). Transmission of a balanced 22;22 Robertsonian translocation in man may represent an example of gamete complementation or of chromosome loss after trisomic conception (Kirkels et al. 1980; Palmer et al. 1980).

Pseudodisomy can lead to a pathological outcome by way of various mechanisms. If pseudodisomy involves homozygosity, i.e. isodisomy, there will be an increased risk of recessive genetic disorders. If a mechanism involves chromosomal mosaicism and aneuploidy, as in the case of monosomic or trisomic conception, there is the potential for pathologic effects arising from the mosaic aneuploidy, depending in part on the chromosome involved and on the time of chromosomal loss or gain. A third pathological mechanism might involve requirements for chromosomal contributions from each parent. The inability to detect gamete complementation for certain chromosomes in mice is presumed to be due to lethality caused by lack of contribution from one parent. There is evidence that certain functional genes must be maternally inherited and that other functional genes must be paternally inherited (Lyon 1983; Cattanach and Kirk 1985). Although lethality might prevent the occurrence of gamete complementation or isodisomy for certain chromosomes, sublethal pathologic effects are known to occur.

It is uncertain whether any form of pseudodisomy might be a reasonably frequent cause of human disease, but we believe that the potential for pseudodisomy after aneuploid conception may be significant. Nullisomic gametes are competitive for fertilization in the mouse, and monosomic conceptions usually die prior to implantation (Epstein 1986). Trisomy is a frequent finding in human spontaneous-abortion material, but monosomy is rare (Hassold and Jacobs 1984). On the basis of these findings and limited information regarding chromosome composition of human gametes and early embryos (Martin 1985; Wramsby et al. 1987), we believe that monosomic conception with preimplantation loss could be reasonably frequent in humans. If monosomic cells were overgrown by pseudodisomic cells, this phenomenon would go undetected by current medical genetic diagnostic techniques. There is evidence in cultured somatic cells that the change from monosomy to pseudodisomy may occur readily (Cox et al. 1976; Eves and Farber 1983). Trisomic conception leading to pseudodisomy also could be a significant problem. Considerable insight into this question might be gained from detailed molecular analysis of prenatally diagnosed instances of mosaic trisomy or mosaic monosomy in which the infant is born with a normal diploid karyotype.

The observations in the patient described here could be explained by several of the mechanisms depicted in figure 4. The uniparental origin of the centromere, the lack of heterozygosity on chromosome 7, and a failure to detect mosaicism lead us to favor monosomic conception as the explanation for the findings in this patient, although trisomic conception followed by chromosomal loss and other mechanisms also are tenable. It is of interest that the mother was age 36 years at the time of delivery. For a monosomic conception, a paternal error is implicated in this case; but a maternal error is implicated for a trisomic conception. The short stature might be due to either embryonic chromosomal mosaicism or to a second recessive genetic disorder on chromosome 7.

The detection of this patient suggests that related phenomena can be found in human genetics. Patients with more than one recessive genetic disorder or patients with a recessive genetic disorder and a sporadic syndrome are suitable for investigation. Isodisomy should also be considered (1) in cases of an apparent new mutation leading to a recessive disorder when only one parent is a heterozygote and (2) in cases of females affected with X-linked recessive disorders (Lusher et al. 1969; Neufeld et al. 1977). The phenomena of human pseudodisomy and uniparental disomy can be evaluated thoroughly by using polymorphic DNA markers and chromosome-specific alpha satellite probes.

Note added in proof: When the XV-2c and *Taq*I were used to detect an RFLP in linkage disequilibrium with the CF mutation (Estivill et al. 1987), the patient was homozygous for the 1 allele and the father was homozygous for the 2 allele. Similarly, when the KM-19 probe and *Pst*I (Estivill et al., personal communication) were used, the patient was homozygous for the 2 allele and the father was homozygous for the 1 allele. These results are in agreement with the observed linkage disequilibrium and are consistent with the interpretation of uniparental disomy in this patient.

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