Am. J. Hum. Genet. 45:373–380, 1989

Isodisomy of Chromosome 7 in a Patient with Cystic Fibrosis: Could Uniparental Disomy Be Common in Humans?

Ruth Voss,*,[1] Elena Ben-Simon,* Avraham Avital,† Simon Godfrey,† Joel Zlotogora,* Judith Dagan,* Yaron Tikochinski,‡ and Jossi Hillel§

*Department of Human Genetics, Hadassah Hebrew University Medical Center, Ein-Karem, Jerusalem; †Department of Pediatrics, Hadassah Hebrew University Medical Center. Mount Scopus, Jerusalem; ‡Department of Genetics, Institute for Life Sciences, The Hebrew University of Jerusalem, Jerusalem; and §Genetics and Animal Breeding Unit, Faculty of Agriculture, The Hebrew University, Rechovot, Israel

Summary

Maternal isodisomy for chromosome 7 was observed in a 4-year-old cystic fibrosis patient with very short stature. In an examination of 11 DNA polymorphisms spanning the entire length of chromosome 7, no paternal contribution could be shown in seven informative loci. Paternity was examined with probes for five polymorphic loci on the Y chromosome, for the pseudo beta-globin locus on chromosome 11 and by Jeffreys's hypervariable probes. The results with the latter gave a probability of 3.7×10^{-9} for nonpaternity. Chromosomal examination revealed a centromeric heteromorphism of chromosome 7 in the mother, for which the proband was homozygous. Isodisomy of the patient was thus shown for the entire length of a maternal chromosome 7. The mechanisms leading to this isodisomy involve at least two events of abnormal cell division, events that may be meiotic, postzygotic, or both. This proband is the second reported maternal isodisomy; both were detected through homozygosity for CF. Both patients had short stature, which could have been caused by parental imprinting, since similar results have been observed in isodisomic mice. Homozygosity due to uniparental descent in man should be kept in mind as a mechanism for recessive disorders, especially for chromosome 7.

Introduction

The concept of uniparental disomy (UPD) in man, i.e., that two homologous chromosomes originate from one parent, and of isodisomy, in which the two chromosomes are identical, was defined by Engel (1980). Uniparental disomy has been reported in Robertsonian translocations in mice and in man (Kirkels et al. 1980; Palmer et al. 1980; Cattanach and Kirk 1985). In somatic cells, homozygosity for one parental allele or isodisomy of a chromosome may be a cause of malignant disease, e.g., in retinoblastoma (Cavenee et al. 1983).

The theoretical expected frequency of UPD in zygotes was estimated by Engel (1980) to be around the order of 3×10^{-4}. Therefore the frequency of individuals with UPD in the population could be relatively high. Yet, so far, only one individual with UPD and a normal karyotype has been described (Spence et al. 1988). In this case a UPD for chromosome 7 was the etiological cause for cystic fibrosis (CF)—an autosomal recessive disease whose gene is located at 7q31 (Estivill et al. 1987; Spence and Tsui 1987). The recognition of UPD in individuals may have both clinical and theoretical importance. In the following we describe a second individual affected with CF caused by maternal isodisomy for chromosome 7.

Received February 3, 1989; revision received April 28, 1989.

Address for correspondence and reprints: Tirza Cohen, M.D., Department of Human Genetics, Hadassah Medical Center, P.O. Box 12000, Jerusalem 91120, Israel.

1. We deeply mourn the death of Dr. R. Voss on August 7, 1988.

0002-9297/89/4503-0006$02.00

Patient, Material, and Methods

Patient

Fifty-five families were examined in our laboratory for the diagnosis of CF with DNA markers. In one of the families examined the proband was a boy aged 4

Table 1

List of DNA Markers

Chromosome Region	Symbol	Probe	Enzyme	Polymorphism			Reference
				Allele Symbol	Size (kb)	Frequency	
7pter-p14	D7S135	TM102L	*Taq*I	A1	12	.57	Tsui et al. 1987
				A2	8.5	.43	
7p	D7S150	TS194	*Taq*I	A1	1.5	.72	Tsui et al. 1987
				A2	1.0	.28	
7cen-q22	D7S129	TG16	*Eco*RI	A1	2.2	.70	Tsui et al. 1987
				A2	1.0	.30	
7q 31	D7S126	SC33	*Eco*RI	A1	4.5		Tsui et al. 1987
				A2	4.0		
				A3	3.5		
7q 31	MET	met H	*Taq*I	A1	7.5	.61	White et al. 1985
				A2	4.0	.39	
7q 31	MET	met D	*Taq*I	C1	6.0	.77	White et al. 1985
				C2	4.4	.23	
7q 31	MET	met D	*Ban*I	D1	7.6		White et al. 1986
				D2	6.8		
7q 31	D7S23	KM19	*Pst*I	C1	7.8	.71	Estivill et al. 1987
				C2	6.6	.29	
7q 31	D7S23	XV-2c	*Taq*I	A1	2.1	.47	Estivill et al. 1987
				A2	1.4	.53	
7q22-q32	D7S8	pJ3.11	*Msp*I	A1	4.2	.41	Bartels et al. 1986
				A2	1.8	.59	
7q34-qter	D7S392	TM196	*Pst*I	A1	4.2		Tsui et al. 1987
				A2	3.7		
7q36-qter	D7S22	pλg3	*Hin*fI				Wong et al. 1987
11p15.5	HBBPI		*Hin*cII	A1	4.6,3.0		Antonarakis et al. 1982
				A2	7.6		
				A3	6.0		
				A4	3.3		
Yq11	DYS1	49f	*Taq*I	A0	–	.04	Ngo et al. 1986
				A1	10	.04	
				A2	12	.18	
				A3	15	.68	
				A4	17	.04	
				C0	. . .	.64	
				C1	8.1	.36	
				D0	. . .	.23	
				D1	7.2	.59	
				D2	6	.23	
				F0	. . .	.03	
				F1	5.4	.97	
				I0	. . .	.64	
				I1	3.5	.36	
HVR mini-satellites		33.15	*Hin*fI				Jeffreys et al. 1985
		33.6	*Hin*fI				Jeffreys et al. 1985

years with severe growth retardation. The parents were of Ashkenazi origin and had four children. The patient was the second child, born when the father was 30 years old and when the mother was 25 years old. There was no family history of CF or of any other major disease. The proband was born in the 38th week of pregnancy and was small for gestational age (1,770 g). He remained small; at age 4 years his stature was 87 cm (50% for age 2 years). CF was diagnosed in infancy.

DNA Isolation and Southern Blot Analysis

High-molecular-weight genomic DNA was extracted

from whole blood after separation of white-blood-cell nuclei with 0.2% Nonidet P40 in 0.9% NaCl; lysis was performed with 7 M urea and 2% SDS in lysing buffer (0.01 M tris-HCl, pH 7.5, 0.3 M NaCl, 0.01 M EDTA). Proteinase K digestion and DNA extraction were performed by standard methods (Maniatis et al. 1982). From each individual 10 μg DNA were digested with the relevant restriction enzyme and were separated by electrophoresis in 0.7% agarose gel and were blotted onto nitrocellulose filters (Southern 1975). Filters were prehybridized in 10 × Denhardt's solution, 5 × SSC, 0.1% SDS, 6% polyethylene glycol, and 100 μg denaturated sonicated salmon sperm DNA/ml at 65°C for 2 h. Probes were labeled with (α -^{32}P) dCTP by the multiprime labeling method (Feinberg and Vogelstein 1983) for 6–14 h at room temperature. The denatured probes were added to the prehybridized filters and hybridized overnight at 65°C. Filters were washed according to recommended stringency.

For DNA fingerprinting, two samples of 10 μg DNA from each individual were digested with 25 U *Hin*fI. One set was directly loaded onto 0.9% agarose gel while the other set was recovered after three ethanol precipitations and was dissolved in 0.2 M sodium acetate. The gel was run overnight at 40 V and for an additional 5 h on 100 V, until the 2-kb marker reached 17 cm. Blotting to Hybond-N filters followed standard procedures (Jeffreys et al. 1985). The inserts of Jeffreys's probes 33.6 and 33.15 were separated in low-melting-point agarose from the recombinant M13 and were labeled with ^{32}P to a specific activity of 1.5×10^9. Prehybridization and hybridization were according to the method of Church and Gilbert (1984). Hybridization was performed at 65°C for 16 h. Filters were washed three times for 15 min each in 0.5 M $NaHPO_4$, 1% SDS, followed by two washes in 1 × SSC, 0.1% SDS for 15 min each. The filters were exposed on Fuji X-ray film by using double intensifying screens at −70°C for 48 h and for 7 d.

The DNA probes that were used are detailed in table 1.

Cytogenetic Study

Cytogenetic analysis was performed on peripheral blood lymphocytes according to standard methods (Yunis 1976).

Results

Molecular Study

DNA polymorphisms linked to the CF region on chromosome 7 were tested with the following six probes: metH, metD-*Taq*I, metD-*Ban*I, KM19, XV-2c, and J3.11 (table 1); in all of them the proband was homozygous (fig. 1). Four of the probes—metH, metD-*Ban*I, KM19, and XV-2c—were informative, and the proband showed only maternal and no paternal contribution. The results of Southern blot analysis for metH and XV-2c in the family are shown in figure 2*a*. For both probes, the proband and his mother were homozygous for allele 1 and the father for allele 2. The three siblings were heterozygotes, as expected. Analysis of the family by five additional probes spanning the length of chromosome 7 (table 1) showed again that the proband was homozygous for all alleles, including three maternal alleles (TM102L, SC33, and TM196; fig. 1) not carried by the father. The results on two of these informative probes are shown in figure 2*b*. For both probes, the father was homozygous for allele 2 and the mother was heterozygous 1–2, while the proband was homozygous for allele 1 and the three siblings had contributions from both parents. In figure 1 the haplotypes of chromosome 7

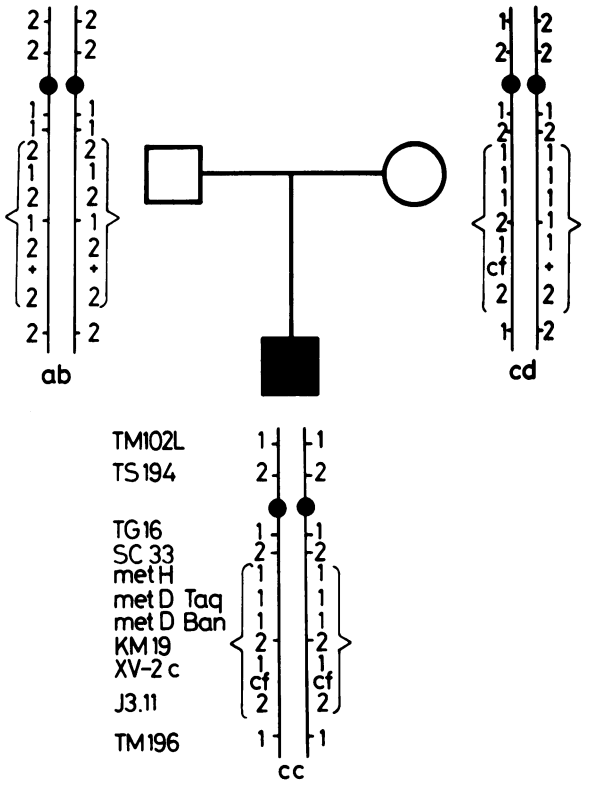

Figure 1 Haplotypes of father, mother, and proband, showing maternal isodisomy in the proband, with 11 probes for the length of chromosome 7.

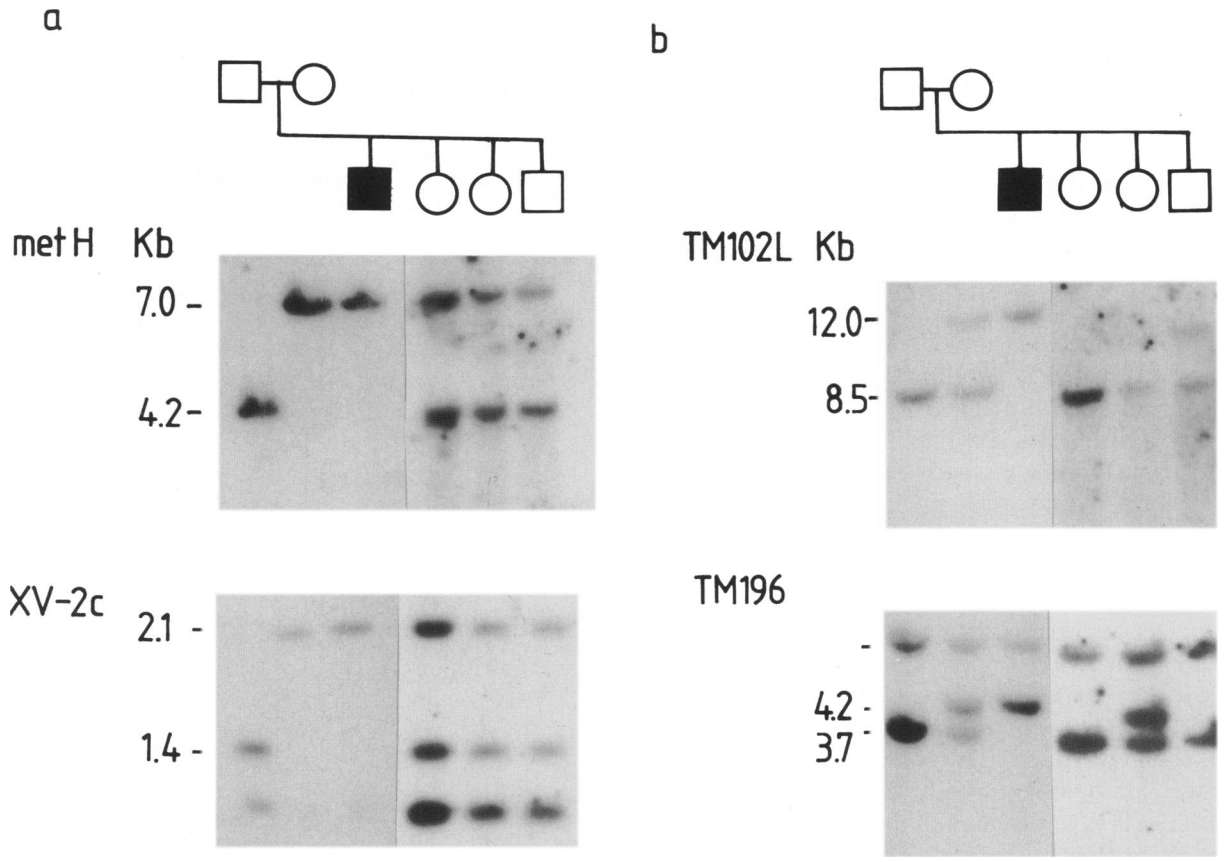

Figure 2 Autoradiograms showing results for the family, with four probes: two probes linked to the CF locus (*a*) and two probes at the distal ends of chromosome 7 (*b*). Only the proband showed maternal isodisomy.

alleles carried by the proband and by his father are shown; also shown are those carried by the mother, which have been deduced from the proband's haplotypes. Since we have not tested the mother's parents, we do not know what haplotypes she carries and whether the proband inherited nonrecombinant or recombinant haplotypes. The mother was heterozygous at three loci, and her haplotypes are different from the paternal haplotypes. The proband is homozygous for haplotype c (fig. 1). The father was homozygous for all markers tested, except for the hypervariable minisatellite probe pλg3 (table 1). He was heterozygous for a 10-kb and a 3.4-kb fragment. Neither fragment was observed in the proband; one sibling carried the 10-kb fragment, and two siblings carried the 3.4-kb fragment (data not shown).

Chromosomal Study

Chromosomal studies revealed normal karyotypes in the proband and his parents, and no mosaicism was observed. However, the mother showed a centromeric heteromorphism, possibly owing to a small pericentric inversion, in one chromosome 7 (fig. 3). The proband was homozygous for this heteromorphism. These findings were consistent with the molecular results and may in-

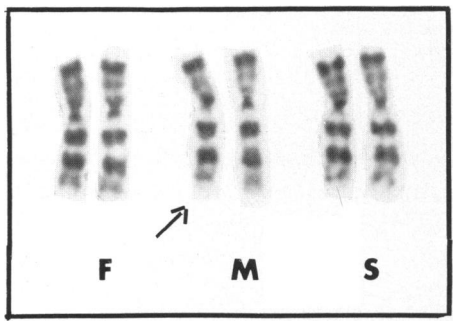

Figure 3 Chromosomes 7 of father (*F*), mother (*M*), and son (*S*). Note the centromeric heteromorphism in the left maternal and in both chromosomes of the son.

dicate nonpaternity or that the proband could be isodisomic for a maternal chromosome 7.

Paternity Testing

Nonpaternity was excluded by RFLPs on chromosomes Y and 11 and by Jeffreys's hypervariable probes (table 1). Figure 4*a* shows the results of five allelic series on chromosome Y, detected by probe 49f with *Taq*I (Ngo et al. 1986). The pattern was identical in the father and the proband. The pseudo beta probe with *Hin*cII (Antonarakis et al. 1982) was used as a chromosome 11 marker (fig. 4*b*). The father was heterozygous for the 7.6-kb and 6-kb fragments, and the mother was heterozygous for the 6-kb and for the 4.6-kb and 3-kb fragments. The proband had the paternal 7.6-kb fragment and the maternal 4.6-kb and 3-kb fragments.

DNA fingerprint patterns were prepared using *Hin*fI digests, hybridized to Jeffreys's minisatellite probes 33.6 and 33.15 (Jeffreys et al. 1985). Seventeen fragments of the proband's DNA fingerprint were analyzed using probe 33.6 (fig. 4*c*), and 18 fragments were analyzed using probe 33.15 (data not shown). Nine and five of the fragments with probes 33.6 and 33.15, respectively, were paternal specific fragments (not shared with the mother), and four and five fragments, respectively, were shared by the proband and both his parents. With both probes, no mismatches were observed; all of the proband's fragments could be traced to one or both parents. The level of band sharing between unrelated individuals within populations was estimated as .25 (Jeffreys 1987). On the basis of both this estimate and the observed level of band sharing between the proband and

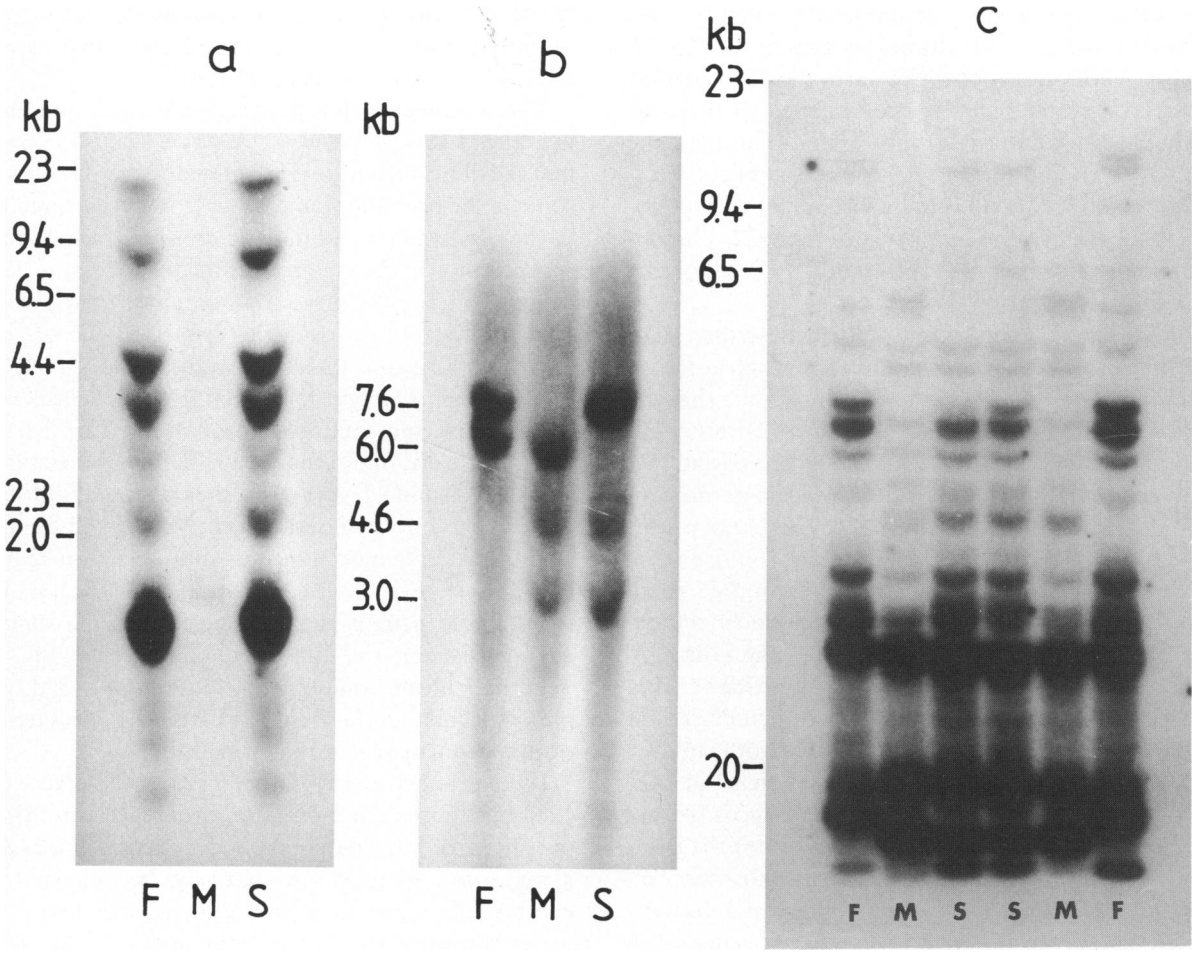

Figure 4 Autoradiograms showing that paternal alleles are present in the proband when tested by 49f probe detecting allelic Y-specific series (*a*), pseudo beta probe on chromosome 11 (*b*), and DNA fingerprint patterns with Jeffreys' probe 33.6 (*c*). The left side is shown after three ethanol precipitations of the DNA. *F* = father; *M* = mother; *S* = son.

his father, a probability (*P*) for nonpaternity was calculated (Jeffreys 1987; Helminem et al. 1988). This probability is the chance of having the observed band-sharing level between the child and his alleged father if it is assumed that they are unrelated individuals: $P = .25^{9+5} = 3.7 \times 10^{-9}$. This result indicates that nonpaternity can be excluded and that the proband is isodisomic for one maternal chromosome 7.

Discussion

In this family the proband was homozygous for maternal alleles at all 11 loci tested along chromosome 7, including the three for which the mother was heterozygous, and for the centromeric heteromorphism. Therefore the proband is isologous (has identical segments) for the length of chromosome 7.

According to Beaudet et al. (1988) the haplotypes obtained with probes XV-2c and KM19 (table 1)—i.e., haplotypes 1–2 and 2–1—have a probability of 1 in 9.5 and 1 in 1,000, respectively, to carry the CF mutation among North Americans. Extending their work to our family, we can assume that the mother's c chromosome (fig. 1), which had the 1–2 haplotype, carried the CF mutation. The proband is homozygous for this haplotype, while the father is homozygous for the 2–1 haplotype and therefore has a low probability (1:500) of carrying the CF mutation.

Our proband is the second patient described with UPD, and both have been ascertained through homozygosity for the CF gene. It is hard to postulate that the cause for UPD is inherent in the CF mutation itself, although it cannot be ruled out before more is known about the mutation. Both patients had maternal isodisomy, which may be coincidental or because of potential lethality of paternal isodisomy of chromosome 7.

The etiology of the very short stature in the patient who was reported by Spence et al. (1988) and in our patient, both with CF and maternal isodisomy, is intriguing. In the RFLP studies there was no evidence that any deletion that includes the CF locus could account for the short stature. The possibility that both children are also homozygous for a rare recessive gene, in linkage disequilibrium with the CF gene, is possible but unlikely. The most plausible explanation for the short stature may be the lack of paternal chromosome 7 alleles and therefore the lack of expression of paternally derived genes. The different expression of genes when inherited from the male versus the female parent is referred to as parental imprinting. Cattanach and Kirk (1985) showed that, in the mouse, some maternal and some paternal chromosomes are essential for the normal development of the embryo and fetus. It has been suggested that about a quarter of the mouse genome is imprinted and that the rest of the genetic material is unaffected by parental origin (Surani et al. 1988). Similar imprinting mechanisms may well be present in humans, and therefore isodisomy may either have no influence on the fetus or result in some abnormality, e.g., small size, lethality, or unusual shape. Twenty-two loci on human chromosome 7 are homologous to loci on the following mouse chromosomes: chromosome 2 (one locus) chromosome 5 (seven loci), 6 (seven loci), 11 (one locus), 12 (one locus), 13 (four loci), and 16 (one locus) (Searle et al., in press). The area of human chromosome 7 that maps the CF gene is most likely to be homologous to mouse chromosome 6. Of the mouse chromosomes with homology to human 7, chromosomes 6 and 11 demonstrate imprinting in the mouse. Chromosome 6 maternal isodisomy was shown to be lethal in the mouse, and a maternally derived disomy of chromosome 11 was associated with small size at birth (Cattanach and Kirk 1985).

The mechanism that generates an isodisomic individual will always involve at least two events of abnormal cell division. The origin of the duplicated chromosome in the proband stems either from nondisjunction in the maternal second meiotic division, from mitotic reduplication in the zygote, or from double mitotic nondisjunction. For the first two alternatives there is more than one way to generate the disomy. If the original error was a second meiotic nondisjunction, then the zygote either (1) might have been trisomic with a normal paternal contribution of chromosome 7, which was lost mitotically, or alternatively, (2) might have started out as a disomic zygote with two maternal chromosomes 7 and no paternal chromosome 7. In the first case we would expect a mosaic if no selection against trisomic cells exists. We have not ruled out the possibility that the patient is a mosaic for chromosome 7 aneuploidy, but the fact that homozygosity for one maternal chromosome 7 was observed in blood lymphocytes and that the child has CF that involves tissues of independent origin makes it unlikely.

If the primary event led to a monosomic zygote rescued by reduplication of chromosome 7, then (3) the meiotic error was a paternal nondisjunction that caused a nullisomic gamete. Alternatively, (4) only mitotic events in the zygote may have occurred with loss of the paternal chromosome 7 and reduplication of the maternal chromosome 7. This mechanism is well demonstrated for the retinoblastoma tumor evolution (Cavenee et al. 1983). (5) Double mitotic nondisjunction leading to two isodisomic cells—maternal and paternal—

must be exceedingly rare. However, if it happened early in development, with subsequent loss of the paternal 7 disomic line, the result would be what is seen in our patient.

Our results demonstrate that the proband has two identical chromosomes 7. This could have been due either to a disomic maternal gamete that did not undergo recombination of chromosome 7 during oogenesis or to a postmeiotic reduplication or nondisjunction of chromosome 7. The possible pericentric inversion in the mother could have suppressed recombination, yet all three siblings of the proband showed evidence of crossing-over. We therefore consider the possibility of a postmeiotic event to be more likely.

The frequency of UPD occurring as a result of two meiotic events, i.e., gamete complementation, was estimated by Warburton (1988) to be 1/30,000, and the expected proportion of CF patients that is due to isodisomy was estimated to be 1/10,000. We estimated that since the locus for CF has been mapped, around 2,000 families have been haplotyped on the DNA level. The fact that already two CF patients with uniparental disomy have been observed and that both had maternal isodisomy supports a mechanism involving a nullisomic sperm and reduplication of the maternal chromosome.

It is plausible that heterodisomic or isodisomic individuals, not detected through a recessive disorder but manifesting a specific phenotype, will go unnoticed. Obvious candidates for the detection of isodisomic individuals are recessive homozygotes that have only one carrier parent. With the widespread use of RFLPs in haplotyping families for specific loci, more cases are expected to be found. If one considers the number of families who have been typed for chromosome 11 because of the beta-thalassemias, the question arises whether (*a*) there were isodisomics that were not observed or (*b*) chromosome 11 is imprinted in a way that does not permit uniparental disomy. The same line of thought applies to other chromosomes—including the X chromosome, in which the hemizygous males enable easy detection of suspected individuals with uniparental disomy, i.e., affected females of healthy fathers in a recessive X-linked disease. Therefore, in the genetic counseling clinic, UPD should be considered as a possible cause of disease.

Acknowledgments

We thank the following people for permission to use their probes: Dr. G. F. Vande Woude—pmetH and pmetD; Dr. J. Schmidtke—pJ311; Dr. R. Williamson—KM19 and XV-2c; Dr. L. C. Tsui—TM102L, TS194, TG16, TM19, and TM196; Dr. J. Weissenbach—p49f; Dr. A. Oppenheim—pseudo beta-globin probe; and Dr. A. Jeffreys—probes 33.6 and 33.15. We also wish to thank Dr. M. Steinitz for help in setting up the lymphoblast lines and Israela Lerer and Drs. Judith Hall and Tirza Cohen for discussions of the manuscript. This work was supported by grants from the chief scientist, Ministry of Health, Israel, and by the Cystic Fibrosis Foundation, Israel.

References

Antonarakis SE, Boehm CD, Giardina PJV, Kazazian HH (1982) Nonrandom association of polymorphic restriction sites in the beta-globin gene cluster. Proc Natl Acad Sci USA 79:137–141

Bartels I, Grzeschik K-H, Cooper DN, Schmidtke J (1986) Regional mapping of six cloned DNA sequences on human chromosome 7. Am J Hum Genet 38:280–287

Beaudet AL, Spence JE, O'Brien WE, Estivill X (1988) Experience with new DNA markers for the diagnosis of cystic fibrosis. N Engl J Med 318:50–51

Cattanach BM, Kirk, M (1985) Differential activity of maternally and paternally derived chromosome regions in mice. Nature 315:496–498

Cavenee WK, Dryja TP, Phillips RA, Benedict WF, Godbout R, Gallie BL, Murphree AL, et al. (1983) Expression of recessive alleles by chromosomal mechanisms in retinoblastoma. Nature 305:779–784

Church GM, Gilbert W (1984) Genomic sequencing. Proc Natl Acad Sci USA 81:1991–1995

Engel E (1980) A new genetic concept: uniparental disomy and its potential effect, isodisomy. Am J Med Genet 6: 137–143

Estivill X, Farrall M, Scambler PJ, Bell GM, Hawley KMF, Lench NJ, Bates GP, et al (1987) A candidate for the cystic fibrosis locus isolated by selection for methylation-free islands. Nature 326:840–845

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Helminem P, Ehnholm C, Lokki ML, Jeffreys A, Peltonen L (1988) Application of DNA "fingerprints" to paternity determinations. Lancet 1:574–576

Jeffreys AJ (1987) Highly variable minisatellites and DNA fingerprints. Biochem Soc Trans 15:309–317

Jeffreys AJ, Wilson V, Thein SL (1985) Hypervariable "minisatellite" regions in human DNA. Nature 314:67–73

Kirkels VGHJ, Hustinx ThWJ, Scheres JMJC (1980) Habitual abortion and translocation (22q;22q): unexpected transmission from a mother to her phenotypically normal daughter. Clin Genet 18:456–461

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Ngo KY, Vergnaud G, Johnsson C, Lucotte G, Weissenbach J (1986) A DNA probe detecting multiple haplotypes of the human Y chromosome. Am J Hum Genet 38:407–418

Palmer CG, Schwartz S, Hodes ME (1980) Transmission of a balanced homologous t (22q;22q) translocation from mother to normal daughter. Clin Genet 17:418–422

Searle AG, Peters J, Lyon MF, Hall JG, Evans EP, Edwards JH, Buckle VJ Chromosome maps of man and mouse. IV. Ann Hum Genet (in press)

Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517

Spence JE, Perciaccante RG, Greig GM, Willard HF, Ledbetter DH, Hejtmancik JF, Pollack MS, et al (1988) Uniparental disomy as a mechanism for human genetic disease. Am J Hum Genet 42:217–226

Spence MA, Tsui LC (1987) Report of the Committee on the Genetic Constitution of Chromosomes 7, 8 and 9. Cytogenet Cell Genet 46:170–172

Surani MA, Reik W, Allen ND (1988) Transgenes as molecular probes for genomic imprinting. Trends Genet 4:59–62

Tsui LC (1987) Human gene mapping. Cytogenet Cell Genet 46:442–445

Warburton D (1988) Editorial: uniparental disomy: a rare consequence of the high rate of aneuploidy in human gametes. Am J Hum Genet 42:215–216

White R, Leppert M, O'Connell P, Nakamura Y, Woodward S, Hoff M, Herbst J, et al (1986) Further linkage data on cystic fibrosis: the Utah study. Am J Hum Genet 39: 694–698

White R, Woodward S, Leppert M, O'Connell P, Hoff M, Herbst J, Lalouel JM, et al (1985) A closely linked genetic marker for cystic fibrosis. Nature 318:382–384

Wong Z, Wilson V, Patel I, Povey S, Jeffreys AJ (1987) Characterization of a panel of highly variable minisatellites cloned from human DNA. Ann Hum Genet 51:269–288

Yunis JJ (1976) High resolution of human chromosomes. Science 191:1268–1270