Bloom Syndrome and Maternal Uniparental Disomy for Chromosome 15

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Summary

Bloom syndrome (BS) is an autosomal recessive disorder characterized by increases in the frequency of sister-chromatid exchange and in the incidence of malignancy. Chromosome-transfer studies have shown the BS locus to map to chromosome 15q. This report describes a subject with features of both BS and Prader-Willi syndrome (PWS). Molecular analysis showed maternal uniparental disomy for chromosome 15. Meiotic recombination between the two disomic chromosomes 15 has resulted in heterodisomy for proximal 15q and isodisomy for distal 15q. In this individual BS is probably due to homozygosity for a gene that is telomeric to D15S95 (15q25), rather than to genetic imprinting, the mechanism responsible for the development of PWS. This report represents the first application of disomy analysis to the regional localization of a disease gene. This strategy promises to be useful in the genetic mapping of other uncommon autosomal recessive conditions.

Introduction

Bloom syndrome (BS; McKusick 210900) is a rare autosomal recessive disorder characterized by proportional dwarfism, skin rash, and a dysmorphic facies. Patients have an immune deficiency, a tendency to develop non–insulin-dependent diabetes mellitus, and a markedly increased incidence of a range of malignancies (German and Passarge 1989). BS has been described in several racial groups but is most common in Ashkenazi Jews. Only one complementation group has been identified, suggesting that a single locus is responsible for the disorder (Weksberg et al. 1988).

A variety of chromosomal abnormalities have been described in subjects with BS, including multiple nonspecific chromosomal breaks and a markedly elevated frequency of sister-chromatid exchange (SCE) (Kuhn and Therman 1986). The mechanisms responsible for these abnormalities are not known. An increased rate of somatic recombination and mutation at tandem repeat loci has been reported (Groden and German 1992) and may be associated with the high incidence of malignancy that occurs in these patients. Several abnormalities of enzymes involved in DNA repair and synthesis have been described (German and Passarge 1989); however, none of these are pathognomonic for the condition.

Fusions between cell lines derived from subjects with BS and from normal individuals have demonstrated correction of elevated SCE (Weksberg et al. 1988). This complementation was reproduced by the introduction of chromosome 15 (but not other chromosomes) into a BS cell line, by microcell-mediated chromosome transfer (McDaniel and Schultz 1992). Complementation was not observed on the introduction of a chromosome derivative that retained only the proximal part of the long arm of chromosome 15. These observations suggest that the abnormal gene product associated with BS is coded on distal chromosome 15q.

The association of Prader-Willi syndrome (PWS) with maternal uniparental disomy (UPD) for chromosome 15 is well documented (Nicholls et al. 1989; Mascari et al. 1992). PWS may be due to either a deletion of the paternal copy of 15q11-q13 or maternal UPD for the entire chromosome. It is believed that PWS occurs in the absence of an imprinted gene (or genes) expressed from the paternally inherited chromosome 15 homologue. This report describes a subject with features of both BS and PWS and maternal UPD for chromosome 15. Analysis of the disomic chromosomes in this individual has allowed the position of the BS locus to be assigned to 15q25→qter.

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Subject, Material, and Methods

Case Report

The case described here was the first child of a nonconsanguineous union between a 38-year-old woman with Ashkenazi Jewish ancestry and a 35-year-old Caucasian male. His mother had previously given birth to a healthy female infant by a different father. The subject was delivered at 38 wk gestation following a pregnancy that was complicated by a failed amniocentesis for advanced maternal age. Poor fetal growth was noted at 6 mo gestation. A birth weight of 1.9 kg, length of 44 cm, and head circumference of 32 cm were consistent with symmetrical intrauterine growth retardation. His postnatal course featured persistent hypotonia, a requirement for feeding by nasogastric tube, failure to thrive, and developmental delay. He remained small, and at 22 mo of age all growth parameters were below the 3d percentile. Abnormal clinical features included a "pinched" beaklike nose, high-pitched voice, hypotonia, mild developmental delay, and bilateral undescended testicles. He had an erythematous rash in a butterfly distribution over his cheeks and nose, which was exacerbated by exposure to sunlight. When most recently examined at 33 mo of age, his height and head circumference remained below the 3d percentile but his weight was on the 10th percentile. There was no hyperphagia. He had achieved some developmental milestones but had not yet acquired distinct speech. He was susceptible to upper-respiratory-tract infections and had had one episode of pneumonia.

Cytogenetic Analysis

High-resolution chromosome G-banding was performed on peripheral blood lymphocytes according to the method of Yunis (1976). SCEs were demonstrated, as described by Perry and Wolff (1974), on 72-h phytohemagglutinin-stimulated lymphocyte cultures.

DNA Polymorphism Studies

High-molecular-weight genomic DNA was extracted from peripheral white blood cells from the proband and his parents, by standard methods. Family studies were performed using a variety of RFLP, VNTR, and simple sequence repeat (SSR) polymorphisms mapping to chromosome 15. The polymorphic markers used in this study are described in the Genome Data Base.

RFLP (D15S9 [ML34/*ScaI*], D15S11 [IR4-3R/*StyI*], and D15S10 [TD3-21/*TaqI*]) and VNTR markers (D15S24 [CMW-1/*TaqI*] and D15S86 [MS620/*TaqI*]) were examined by the digestion of 10 μg of DNA with the stated restriction endonuclease (Boehringer-Mannheim) and by electrophoresis through agarose gels before Southern transfer onto Hybond-N membranes (Amersham). DNA probes were labeled with [α-^{32}P]dCTP by nick translation. Membranes were hybridized and washed as described elsewhere (Trent et al. 1991). Autoradiography was performed at −70°C with two intensifying screens, typically for 4–7 d.

SSR polymorphisms were analyzed by one of two methods. Loci at D15S113, GABRB3, GABRA5, ACTC, and THBS1 involved PCR amplification of 25 ng of DNA, according to published conditions. Eight-microliter aliquots of the PCR products were electrophoresed on nondenaturing polyacrylamide gels. Alleles were detected by silver staining (Budowle et al. 1991). Loci at D15S122, CYP19, D15S103, D15S108, D15S110, D15S95, D15S111, FES, D15S100, D15S107, and D15S87 were amplified by PCR and PAGE of ^{32}P end-labeled products, by modifications to the methods of Weber and May (1989). A single oligonucleotide primer at each locus was radiolabeled at the 5′-terminus with [γ-^{32}P]ATP by using T4 polynucleotide kinase. Reactions contained 30 ng of genomic DNA and 250 nM of each oligonucleotide primer in 200 μM each of dATP, dCTP, dGTP, and dTTP; 50 mM KCl; 10 mM Tris HCl (pH 9.0); 1.5 mM $MgCl_2$; and 0.1% Triton X-100. All reaction components were premixed in a 15-μl volume in microtiter plates and were overlaid with 50 μl of heavy mineral oil. Samples were heated to 94°C for 5 min prior to the addition of 0.17 units of *Taq* DNA polymerase. PCR amplification conditions were as follows: denaturation at 94°C for 1 min, annealing at the calculated primer-melting temperature for 30 s, and extension at 72°C for 45 s, repeated 27 times and followed by a final extension step for 10 min. The addition of 0.15 units of Perfect Match enhancer (Stratagene) was found to eliminate artifactual bands that were sometimes present in the amplification of the SSR at D15S87. After amplification, an equal volume of formamide containing xylene cyanol and bromophenol blue (0.01%) was added to the reaction, and the samples were denatured (95°C for 5 min) and chilled. Three-microliter aliquots were electrophoresed on denaturing polyacrylamide gels (5%–8%), which were processed and autoradiographed according to standard procedures (Sambrook et al. 1989).

SSR alleles were arbitrarily assigned values within the family. RFLP and VNTR alleles were named according to standard nomenclature.

Physical Localization of Polymorphic Loci

Microsatellite DNA polymorphisms were localized on chromosome 15 with somatic cell hybrids from the NIGMS mutant cell repository and by analysis of patients carrying previously characterized chromosomal rearrangements (Rogan et al., submitted). The following cell lines and patient material were used: GM10659, t(15; 17)(q22.3;q22); GM10664, t(X;15)(q25;q25); GM11418, +15; GM10500, t(15;17)(q22;q11.2); a spontaneous abortus with an unbalanced chromosome 15 translocation, 46,XY,15,+der(15)t(4;15)(p15.2;q26); and cell lines derived from PWS patients, with interstitial deletions

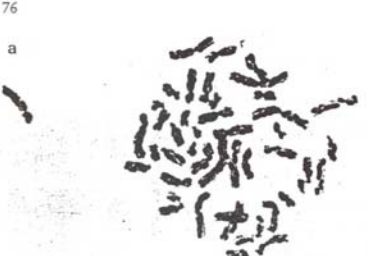

Figure 1 *a*, Metaphase spread showing increased SCE in the index case. *b*, Partial karyotype with extra unidentified centric fragment (*arrowhead*). *c*, Partial karyotype with triradial form (*arrowhead*).

del(15q)(q11-q13). A hybrid cell line lacking chromosome 15, but retaining human chromosomes 3 and X (NAO7297), was used as a negative control for these experiments.

Results

Cytogenetic Analysis

In view of the patient's small stature and characteristic facial rash, high-resolution chromosome analysis and SCE studies were performed (fig. 1*a*). Analysis of 100 cells demonstrated an average of 65 SCEs/cell (compared with 6–8 SCEs per normal cell). Twenty percent of solid-stained metaphases in nonsynchronized 72-h cultures showed the presence of an extra unidentified centric fragment (fig. 1*b*). In one cell a triradial figure was present (fig. 1*c*). These changes, in particular the elevated SCE, were consistent with a diagnosis of BS.

High-resolution chromosome G-banding (>800-band level) did not reveal the presence of a microscopic deletion involving 15q11-q13. There was no evidence of an unbalanced translocation resulting in the loss of the terminal portion of chromosome 15q.

DNA Polymorphism Analysis

The presence of neonatal hypotonia and feeding difficulties, hypogonadism manifested by cryptorchidism, and global developmental delay led to a clinical suspicion that the subject might have PWS. DNA tests were initiated in an attempt to identify a molecular abnormality involving chromosome 15. The results of these studies are presented in table 1. The subject was heterozygous at multiple loci within 15q11-q13, excluding the presence of a large interstitial deletion in this region.

At several loci (D15S113, D15S122, ACTC, CYP19, D15S103, D15S108, D15S95, D15S111, D15S107, and D15S86), the proband had inherited only maternal alleles, demonstrating the presence of maternal UPD for chromosome 15. He had inherited both maternal alleles (maternal uniparental heterodisomy) at D15S113, D15S122, ACTC, CYP19, D15S103, D15S108, and D15S95 but only one maternal allele at D15S111, D15S107, and D15S86 (maternal uniparental isodisomy). Examples of these patterns are shown in figure 2. Several loci displayed polymorphisms that were not, by themselves, informative for the parental origin of the chromosomes 15 but were used to infer the presence of uniparental heterodisomy or isodisomy by comparing the alleles seen in the proband with those found in the mother. For example, at GABRB3 the proband was heterozygous for the same alleles as his mother,

Table 1

DNA Polymorphism Studies

Locus	Proband*	Father	Mother
15q11–q13:			
D15S9	A1A2	A1A2	A1A2
D15S11	B1B4	B3B4	B1B4
D15S113	2,3	1,4	2,3
D15S10	A1A2	A1A1	A1A2
GABRB3	2,3	1,3	2,3
GABRA5	1,2	1,2	1,2
D15S122	1,2	3,4	1,2
15q13:			
D15S24	A3A3	A1A3	A3A3
15q14:			
ACTC	2,4	1,3	2,4
15q15:			
THBS1	2,2	1,2	2,2
15q21.1:			
CYP19	1,1	2,3	1,1
D15S103	1,2	3,4	1,2
15q21.1→q22:			
D15S108	1,2	3,3	1,2
D15S110	1,2	1,2	1,2
15q25:			
D15S95	1,2	3,4	1,2
D15S111	1,1	2,3	1,2
15q26.1→qter:			
FES	1,1	1,2	1,1
D15S100	1,1	2,2	1,1
D15S107	1,1	2,3	1,3
D15S87	1,1	1,2	1,3
D15S86	A5A5	A1A3	A1A5

* Boldface indicates marker informative for the presence of maternal UPD. Underling indicates reduction to homozygosity.

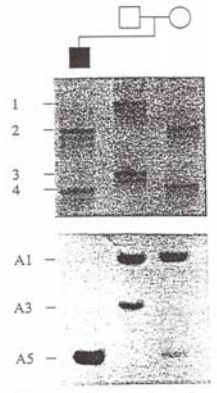

Figure 2 Examples of family polymorphism patterns. *Upper panel*, Maternal uniparental heterodisomy at ACTC. *Lower panel*, Maternal uniparental isodisomy at D15S86.

suggesting heterodisomy, while at D15S87 the proband had inherited only one of the two maternal alleles, implying isodisomy at this locus.

Chromosomal Localization of Polymorphic Markers

Studies of DNA from somatic cell hybrids and patients with telomeric deletions of chromosome 15 were used to establish the regional localization of a number of the polymorphic markers examined in this report (table 2). D15S108 and D15S110 amplified DNA from hybrids containing 15pter→q22 and 15pter→q25 but not 15q22.3→qter. These markers detected two alleles in PWS subjects with interstitial deletions involving 15q11-q13. D15S108 and D15S110 are distal to CYP19 and D15S103 (National Institutes of Health/Centre d'Étude du Polymorphisme Humain Collaborative Mapping Group 1992), suggesting that these two loci are located within the interval 15q21.1-q22.

D15S95 and D15S111 are present in a hybrid containing 15q22.3→qter but absent in a cell line carrying the 15pter→q25 interval. In addition, both markers exhibited heterozygous inheritance in two unrelated individuals known to carry hemizygous 15q26.1→qter deletions. These results place D15S95 and D15S111 within 15q25.

A single allele was inherited at D15S100, D15S107, and D15S87 in several patients hemizygous for 15q26.1→qter (Rogan et al., submitted), placing these markers distal to D15S95 and D15S111. This corresponds with the locus order of the chromosome 15 genetic map (Barnes et al. 1992; Rogan et al., submitted). Since D15S86 is closely linked to D15S87 and is distal to this marker, it is also likely to reside within 15q26→qter. The chromosomal locations of the polymorphic markers examined in this study and the region of homozygosity are indicated in figure 3.

Discussion

The subject described in this study had the clinical and cytogenetic features of BS and maternal UPD for chromosome 15. While definitive clinical diagnosis of PWS can be difficult in young children, this subject met the consensus criteria proposed for patients <3 years of age, with four points for clinical features (neonatal hypotonia, feeding difficulties in infancy, hypogonadism, and developmental delay) and one point for the presence of maternal UPD for chromosome 15 (Holm et al. 1993). Several of the phenotypic features often seen in PWS may have been modified by the coexistence of BS (e.g., dysmorphic facial features), making the diagnosis of PWS less clear-cut than might otherwise have been the case.

Conventional genetic approaches to identifying the location of the BS gene by standard linkage analysis have

Table 2

Regional Localization of Chromosome 15 Markers

Locus	Hybrid Cell Lines[a]					Patients[b]		Regional Assignment
	GM11418, +15	GM10659, 15q22.3→qter	GM10500, 15pter→q22	GM10664, 15pter→q25	NAO7297, +3, +X	Unbalanced translocation, t(4,15)(p15.2;q26)	PWS deletion patients, 15q11–q13	
D15S108	+	−	+	+	−	+,+	+,+	15q13–q22
D15S110	+	−	+	+	−	+,+	+,+	15q13–q22
D15S95	+	+	−	−	−	+,+	+,+	15q25
D15S111	+	+	−	−	−	+,+	+,+	15q25

[a] + = Amplification product detected; and − = no amplification product detected.
[b] +,+ = Heterozygous.

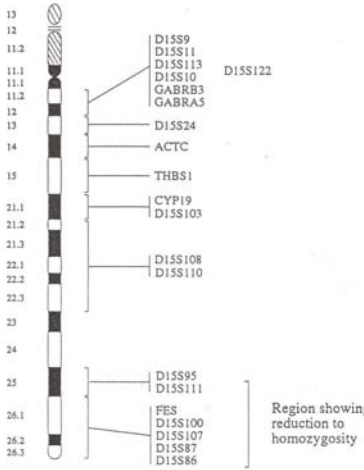

Figure 3 Ideogram of chromosome 15, indicating regional localization of the polymorphic markers used in this study. The relative position, with respect to the other loci, of D15S122 within 15q11-q13 has not yet been determined.

been hindered by the rarity of the condition and the absence of large kindreds with multiple affected subjects. The expression of rare disorders with a recessive mode of inheritance is expected to occur more often than predicted from the population allele frequencies, when both alleles show identity by descent (Lander and Botstein 1987). Although the concept of homozygosity through identity by descent was developed from a consideration of consanguineous matings, homozygous recessive alleles are in fact more likely to occur by uniparental isodisomy than by any consanguineous relationship. Isodisomy is most likely to occur in meiosis II nondisjunction, in which the constitutional genotype is homozygous in the absence of crossing over. Isodisomic domains in chromosomes that have experienced meiosis I nondisjunction can only develop as a consequence of recombination between the two maternal chromosomes.

Homozygosity for autosomal recessive alleles in the presence of UPD was predicted on theoretical grounds, in general terms, by Engel (1980) and more specifically, in PWS, by Nicholls (1991). It has been reported in humans for several genes or conditions that had been mapped to a variety of chromosomes, including cystic fibrosis (Spence et al. 1988; Voss et al. 1989), deficiency of the fourth component of the complement pathway (Welch et al. 1990), a mutation in the gene for type I procollagen (Spotila et al. 1992), and α-thalassemia (Beldjord et al. 1992).

The subject described in this study is heterozygous for markers spanning most of chromosome 15 and exhibits isodisomy only for telomeric loci. This pattern is most consistent with meiosis I nondisjunction, with reduction to homozygosity for markers on the telomeric portion of the chromosome following meiotic recombination (Rogan et al. 1992; Robinson et al. 1993).

The density of the informative markers surveyed is likely to detect most or all double recombinant intervals on the maternally disomic chromosomes in the BS-PWS individual presented here. Additional isodisomic regions are unlikely to be found in this patient, since they would have to originate from otherwise undetected double-exchange events. In a study examining meiotic recombination events in 56 patients with UPD (Rogan et al. 1992), none exhibited more than one exchange between any of the informative intervals observed in the BS-PWS patient. The longest genetic interval (7.7 cM) between two heterozygous markers within the domain bounded by D15S9 and D15S95 was between D15S108 and D15S110. It is unlikely that an isodisomic interval is present between these two loci ($P = .0099$) (Chakravarti and Slaugenhaupt 1987). The results presented here suggest that a single crossover occurred on this chromosome within the 6-cM interval separating D15S95 and D15S111. Both of these markers have been mapped to 15q25. In the patient, homozygous sequences distal to and including 15q25 are thus likely to contain the gene responsible for BS.

Using a strategy similar to that described by Lander and Botstein (1987), linkage analysis was recently performed in a number of non-Ashkenazi BS families in which the offspring were the result of consanguineous marriages. Linkage was established between the locus responsible for BS and FES and was excluded for intervals proximal to D15S26 and distal to D15S87 (Ellis et al. 1992). Comparison of the results obtained in the analysis of the individual described in this report and the results obtained by Ellis et al. may be combined to indicate that the gene responsible for BS is likely to be located between D15S95 and D15S87. This genetic interval is ~6 cM shorter than that arrived at by either study considered in isolation.

In contrast to the gene(s) responsible for PWS, there is no evidence that the BS locus is imprinted. Although not reported specifically in patients with maternal UPD, SCE occurred at normal levels in PWS subjects lacking chromosomal abnormalities (Butler and Jenkins 1987). The majority of nondeleted PWS subjects have maternal UPD for chromosome 15 (Mascari et al. 1992). The level of SCE has not been reported in subjects with Angelman syndrome (AS) and paternal UPD for chromosome 15, but it is normal in hydatidiform moles, which receive their chromo-

somal complement exclusively from the paternally derived haploid genome (Becker et al. 1992). Furthermore, apart from the occurrence of hypopigmentation in subjects with deletions involving 15q11-q13 (Rinchik et al. 1993), both PWS and AS are phenotypically indistinguishable, whether they have been caused by deletions of 15q11-q13 or by UPD for chromosome 15. These observations imply that imprinted loci on chromosome 15 that are associated with easily recognized phenotypes map to 15q11-q13.

The possible similarity between BS and the occurrence of instability of DNA sequences at microsatellite repeats in some instances of hereditary nonpolyposis cancer of the colon has been noted (Aaltonen et al. 1993). Genetic loci associated with microsatellite instability in familial colorectal cancer have been mapped to chromosomes 2 (Peltomaki et al. 1993) and 3 (Lindblom et al. 1993). The gene responsible for the chromosome 2 form of this disorder has very recently been shown to be the DNA mismatch repair enzyme *hMSH2* (Fishel et al. 1993; Leach et al. 1993). Whether related gene products might be associated with the pathogenesis of other malignant conditions or BS remains to be seen.

UPD has now been reported for several human chromosomes, and it almost certainly occurs more frequently than has been recognized in the past. This case represents the first report of the use of disomy analysis in the regional localization of a disease gene. The search for individuals with uncommon autosomal recessive disorders due to uniparental isodisomy will assist the chromosomal mapping of these conditions.

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References

Aaltonen LA, Peltomaki P, Leach FS, Sistonen P, Pylkkanen L, Mecklin J-P, Jarvinen H, et al (1993) Clues to the pathogenesis of familial colorectal cancer. Science 260:812–816

Barnes R, Dunn C, Osbourne-Lawrence S, Weiss L, Bowcock A (1992) Progress in the construction of a framework genetic linkage map of chromosome 15. Am J Hum Genet Suppl 51: A182

Becker RA, Surti U, Wenger SL (1992) Sister chromatid exchange and chromosome breakage in complete hydatidiform moles. Cancer Genet Cytogenet 62:53–57

Beldjord C, Henry I, Bennani C, Vanhaeke D, Labie D (1992) Uniparental disomy: a novel mechanism for thalassemia major. Blood 80:287–289

Budowle B, Chakraborty R, Giusti AM, Eisenberg AJ, Allen RC (1991) Analysis of the VNTR locus D1S80 by the PCR followed by high-resolution PAGE. Am J Hum Genet 48:137–144

Butler MG, Jenkins BB (1987) Sister chromatid exchange analysis in the Prader-Labhart-Willi syndrome. Am J Hum Genet 28: 821–827

Chakravarti A, Slaugenhaupt SA (1987) Methods for studying recombination on chromosomes that undergo nondisjunction. Genomics 1:35–42

Ellis NA, Roe AM, Otterud B, Leppert M, German J (1992) Homozygosity mapping of the Bloom's syndrome locus. Am J Hum Genet Suppl 51:A187

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

Fishel R, Lescoe MK, Rao MRS (1993) The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. Cell 75:1027–1038

German J, Passarge E (1989) Bloom's syndrome. XII. Report from the registry for 1987. Clin Genet 35:57–69

Groden J, German J (1992) Bloom's syndrome. XVIII. Hypermutability at a tandem-repeat locus. Hum Genet 90:360–367

Holm VA, Cassidy SB, Butler MG, (1993) Prader-Willi syndrome: consensus diagnostic criteria. Pediatrics 91:398–402

Kuhn EM, Therman E (1986) Cytogenetics of Bloom's syndrome. Cancer Genet Cytogenet 22:1–18

Lander ES, Botstein D (1987) Homozygosity mapping: a way to map human recessive traits with the DNA of inbred children. Science 236:1567–1570

Leach FS, Nicolaides NC, Papadopoulos N, Liu B, Jen J, Parsons R, Peltomaki P, et al (1993) Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. Cell 75:1215–1225

Lindblom A, Tannergard P, Werelius B, Nordenskjold M (1993) Genetic mapping of a second locus predisposing to hereditary non-polyposis colon cancer. Nature Genet 5:279–282

McDaniel LD, Schultz RA (1992) Elevated sister chromatid exchange phenotype of Bloom syndrome cells is complemented by human chromosome 15. Proc Natl Acad Sci USA 89:7968–7972

Mascari MJ, Gottlieb W, Rogan PK, Butler MG, Waller DA, Armour JAL, Ladda RL, et al (1992) The frequency of uniparental disomy in Prader-Willi syndrome: implications for molecular diagnosis. N Engl J Med 326:1599–1607

National Institutes of Health/Centre d'Étude du Polymorphisme Humain Collaborative Mapping Group (1992) A comprehensive genetic linkage map of the human genome. Science 258:67–86

Nicholls RD (1991) Uniparental disomy as the basis for an association of rare disorders. Am J Med Genet 41:273–274

Nicholls RD, Knoll JH, Butler MG, Karam S, Lalande M (1989) Genetic imprinting suggested by maternal heterodisomy in nondeletion Prader-Willi syndrome. Nature 342:281–285

Peltomaki P, Aaltonen LA, Sistonen P, Pylkkanen L, Mecklin J-P, Jarvinen H, Green JS, et al (1993) Genetic mapping of a locus predisposing to human colorectal cancer. Science 260: 810–812

Perry P, Wolff S (1974) New Giemsa method for differential staining of sister chromatids. Nature 201:156–158

Rinchik EM, Bultman SJ, Horsthemke B, Lee ST, Strunk KM, Spritz RA, Avidano KM, et al (1993) A gene for the mouse pink-eyed dilution locus and for human type II oculocutaneous albinism. Nature 361:72–76

Robinson WP, Bernasconi F, Mutirangura A, Ledbetter DH, Langlois S, Malcolm S, Morris MA, et al (1993) Nondisjunction of chromosome 15: origin and recombination. Am J Hum Genet 53:740–751

Rogan PK, Driscoll DJ, Papenhausen PR. Molecular genetic analysis of 15q loci in patients with del 15q26→qter and Russell-Silver syndromes (submitted)

Rogan PK, Mascari MJ, Ladda RL, Nicholls RD (1992) Genetic mapping with Prader-Willi patients carrying recombinant disomic chromosomes. Am J Hum Genet Suppl 51:A46

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2d ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

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

Spotila LD, Sereda L, Prockop DJ (1992) Partial isodisomy for maternal chromosome 7 and short stature in an individual with a mutation at the COL1A2 locus. Am J Hum Genet 51:1396–1405

Trent RJ, Volpato F, Smith A, Lindeman R, Wong MK, Werne G, Haan E (1991) Molecular and cytogenetic studies of the Prader-Willi syndrome. J Med Genet 28:649–654

Voss R, Ben-Simon E, Avital A, Godfrey S, Zlotogora J, Dagan J, Tikochinski Y, et al (1989) Isodisomy of chromosome 7 in a patient with cystic fibrosis: could uniparental disomy be common in humans? Am J Hum Genet 45:373–380

Weber JL, May PE (1989) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 44:388–396

Weksberg R, Smith C, Anson-Cartwright L, Maloney K (1988) Bloom syndrome: a single complementation group defines patients of diverse ethnic origin. Am J Hum Genet 42:816–824

Welch TR, Beischel LS, Choi E, Balakrishnan K, Bishof NA (1990) Uniparental isodisomy 6 associated with deficiency of the fourth component of complement. J Clin Invest 86: 675–678

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