Characterization of an Unusual and Complex Chromosome 21 Rearrangement Using Somatic Cell Genetics and Cloned DNA Probes

Margaret L. Van Keuren, Gordon D. Stewart, Cynthia M. Bradley, David M. Kurnit, Rachael L. Neve, Paul C. Watkins, Rudolph E. Tanzi, James F. Gusella, and David Patterson

Department of Pediatrics and Communicable Diseases, University of Michigan and Howard Hughes Medical Institute (M.L.V., G.D.S., D.M.K.) and Department of Human Genetics, University of Michigan Medical Center, Ann Arbor, Michigan (D.M.K.); Cytogenetics Laboratory, Children's Hospital and Medical Center, Seattle (C.M.B.); Genetics Division, The Children's Hospital, (R.L.N.) and Neurogenetics Laboratory, Massachusetts General Hospital and Department of Genetics, Harvard Medical School, Boston (R.E.T., J.F.G.); Integrated Genetics, Inc., Framingham, Massachusetts (P.C.W.); The Eleanor Roosevelt Institute for Cancer Research and Departments of Biochemistry, Biophysics and Chemistry, and of Medicine, University of Colorado Health Sciences Center, Denver (D.P.)

In a previous case of a newborn infant with typical Down syndrome, chromosome analysis indicated the presence of an unusual and complex translocation of chromosome 21. The patient's cells contained one normal chromosome 21 and a rearranged, F group-sized submetacentric chromosome. This abnormal chromosome appeared to involve duplication of the distal portion of 21q with translocation to the short arm, and a deletion of C-band-positive centromeric heterochromatin. Using linearly ordered cloned DNA probes, we report the detailed molecular examination of this abnormal chromosome, which has been isolated on a hamster background in a hybrid cell line. Both short arm and pericentromeric sequences are present on this chromosome, as well as distal 21q sequences. However, a substantial portion of proximal 21q is deleted. The distal boundary of this deleted section can be pinpointed within the region between two loci (D21S8 and D21S54), a distance of about 5,000 kb. This study illustrates the power of using precisely mapped, linearly ordered DNA probes to characterize this type of rearrangement. In addition, this hybrid cell line can also be used as a member of a mapping panel to map DNA sequences regionally on chromosome 21.

KEY WORDS: human chromosome 21, Down syndrome, somatic cell hybrids, trisomy 21

INTRODUCTION

Down syndrome most commonly results from trisomy 21. Few cases are due to translocation of all or a portion of 21q to another chromosome. Recently, an unusual and complex translocation chromosome 21 was described in a Down syndrome patient [Bradley et al., 1986]. Neonatally, the child presented with face and dermatoglyphics typical of Down syndrome. The child suddenly died at 7 months. Chromosome analysis of lymphocytes, fibroblasts, and other tissue demonstrated mosaicism for two cell lineages. One cell line was a 46,XX,-21,+t(21;21) containing a structurally abnormal submetacentric chromosome thought to represent a non-Robertsonian translocation. The other cell line, comprising about 50% of cells examined, had the same abnormal chromosome and an additional small dot-like marker chromosome. The proposita's mother and sister, both phenotypically normal, had 47 chromosomes, with an extra bisatellited marker in addition to the normal chromosomes. The father and two maternal aunts had normal chromosomes.

The abnormal submetacentric chromosome in the proposita is the size of an F chromosome and appears to be the result of a non-Robertsonian translocation involving two chromosome 21s. The centromere appears to be less constricted than in other chromosomes, and CBG banding did not detect centromeric constitutive heterochromatin. Although the exact breakpoints involved in the translocation are indeterminate by cytogenetics, it appears that distal 21q is duplicated as evidenced by distinctive R-bands (by RFA technique) on both ends of the abnormal chromosome. The chromosome is interpreted to be a duplication of the distal portion of 21q, including

band 21q22, translocated to the short arm. C-band-positive centromeric heterochromatin is deleted. However, since the chromosome was stable and present in every cell examined, it presumably has a functional centromere. The proposita's fibroblasts demonstrated a gene dosage effect for the enzyme GARS (phosphoribosylglycineamide synthetase), which maps to 21q22.1 [Moore et al., 1977; Chadefaux et al., 1984]. This is consistent with a duplication of the region of chromosome 21 containing the gene.

The small bisatellited marker found in the mother and sister was present in all cells examined. C-banding showed that it has a large heterochromatic region. It is unclear if the proposita's t(21;21) chromosome was derived from the same event that produced this bisatellited marker. A partial karyotype of the translocation chromosome and the bisatellited marker is shown in Figure 1.

Bradley et al. [1986] also reported the production of a Chinese hamster ovary (CHO) hybrid cell line that contains the proposita's abnormal translocation chromosome as the only human chromosome. Isozyme analysis demonstrated that this hybrid cell line expressed human SOD-1 (superoxide dismutase, soluble form), a chromosome 21-encoded gene mapped to 21q22.1 [Sinet et al., 1976], in addition to hamster SOD-1. The hybrid cells also displayed human GARS activity.

We have extended the analysis of the hybrid cell line containing this unusual chromosome with the use of molecular probes whose subregional chromosome location and/or linkage order is known.

MATERIALS AND METHODS
Cell Lines

The cell line ACEM2-90 was derived by fusing fibroblasts from the proposita with the Chinese hamster ovary (CHO-K1) cell line mutant Ade⁻C. Ade⁻C is deficient in GARS activity (E.C.6.3.4.13) and requires exogenous purines for growth [Oates and Patterson, 1977]. The human GARS gene is located on chromosome 21 [Moore et al., 1977], in band q22.1 [Chadefaux et al., 1984]. ACEM2-90 was grown in Ham's F12D medium [Kao and Puck, 1974] with 7% (v/v) dialyzed fetal calf serum; this medium lacks purines, and therefore hybrid cells must retain the human GARS gene for growth. ACEM2-90 contains the proposita's unusual translocation chromosome as the only human chromosome. The derivation and characterization of this cell line are described elsewhere [Bradley et al., 1986].

Cytogenetic Analysis

The chromosome constitution of the ACEM2-90 cell hybrid was verified by cytogenetic analysis prior to DNA extraction. Following GTG banding [Seabright, 1971], selected metaphases were photographed, and the slides were destained in methanol/acetic acid 3:1 (v/v) and restained using the Giemsa-11 (G-11) technique [Alhadeff et al., 1977; Friend et al., 1976]. With G-11 staining, human chromosomes appear blue, while the CHO chromosomes appear magenta. The human chromosomes were then identified in the G-banded photographs.
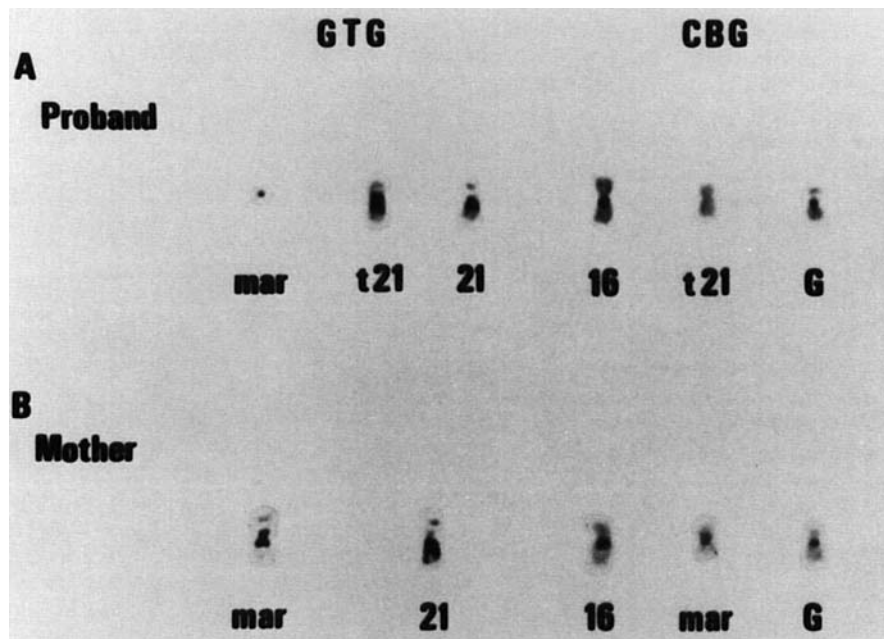

Fig. 1. A: Composite of cytogenetic results of the proposita showing GTG [Seabright, 1971] and CBG [Sumner, 1972] banding of dot-like marker (mar), normal 21 or G group, and abnormal translocation (t21). B: The bisatellited marker of the mother is shown with a normal 21 or G group, and with chromosome 16 for comparison of CBG banding of constitutive heterochromatin.

Molecular Probes

The anonymous human DNA probes pPW235D, pPW-233F, pPW228C, pPW236B, pPW245D, pPW267C, pPW231C, pPW513-5H, pPW524-5P, pPW523-10B, and pPW520-10R have been described previously [Watkins et al., 1985a,b]. All map uniquely to chromosome 21, with the exception of pPW235D, which has related sequences on several other chromosomes. These probes have been mapped to the following locations: pPW235D—21p11.2; pPW233F, pPW228C, pPW236B, pPW245D, pPW267C, pPW513-5H, and pPW524-5P—21q (21q22.3 excluded); pPW523-10B and pPW231C—21q22.3; and pPW520-10R—distal 21q22.3 [Van Keuren et al., 1986]. Recently more precise localization has been determined by in situ hybridization for pPW228C and pPW236B to 21q11.2→21q21, pPW245D to 21q21.1→21q22.11, pPW513-5H to 21q21.3→21q22.11 [Münke et al., 1988], and for pPW-524-5P to 21q21→21q22.1 [Nakai et al., 1987].

The probe C_{HB} (kindly provided by R. Schmickel) is a cloned section of the human rRNA gene cluster [Erickson et al., 1981], which is located on the stalk of the short arm of the acrocentric chromosomes [Schmickel and Knoller, 1975]. Repetitive, human-specific, alphoid centromeric DNA probes used included p82H [Mitchell et al., 1985] (a gift from A. Mitchell) and Xba14 [Sylvester et al., 1985] (a gift from J. Sylvester).

pUNC724 is an Hela cDNA clone, which detects a low-order repeated family of DNA sequences [Kurnit et al., 1984] that map to the pericentromeric region of the acrocentric chromosomes. The ACEM2-90 hybrid was shown to contain DNA sequences detected by pUNC724, which localize to 21p regions above and below the ribosomal DNA locus [Kurnit et al., 1986].

The human DNA probe p21-4U is a single copy sequence related to the 724 family of sequences [Kurnit et al., 1984, 1986]. It localizes to 21q11 by in situ hybridization (unpublished data, U. Tantravahi and D. Kurnit). The cloned probes p21-7U and p21-5L were isolated from a cDNA library; p21-7U maps to 21q22.3, and p21-5L maps to 21q22 above the 21q22.3 band [Neve et al., 1986].

The anonymous DNA probes pGSM21, pGSE9, pGSH8, pGSE8, and pGSB3 are specific for human chromosome 21 [Stewart et al., 1985]. Their map locations are as follows: pGSM21, pGSE9—21q11.1→21q21; pGSH8—proximal 21q22.3; pGSE8 and pGSB3-distal 21q22.3 [Stewart et al., 1985; Neve et al., 1986].

Some of these probes have been ordered by linkage analysis [Tanzi et al., 1988; Watkins et al., 1987] using restriction fragment length polymorphisms in a large Venezuelan kindred. The linear order, where known, is indicated in Table I.

Southern Blot Analysis

The presence or absence of DNA fragments detectable with the cloned DNA probes listed above was determined by Southern blot analysis. In each case, one or more positive controls were included. Various CHO × human hybrid cells containing an intact chromosome 21 or 21q

TABLE I. Results of Southern Blot Analysis of Cloned DNA Probes With ACEM2-90

Probe name	Locus symbol	Present (+) or absent (−) in ACEM2-90	Chromosome location
pPW235D	D21S5	+	21p11.2
C_{HB}		+	21p12
pUNC724	DNF19S4	+	21p11.2→21p13
Xba14		+	21cen
p82H		+	21cen
p21-7U	D21S78	+	21q22.3
p215L	D21S77	−	21q11.1→21q22.2
pPW267C	D21S12	−	21q11.1→21q22.2
Probes ordered by linkage in addition to physical analysis[a]			
1. pGSM21	D21S13	−	21q11.1→21q21.2
p21-4U	D21S110	−	21q11
2. pGSE9	D21S16	−	21q11.1→21q21.2
pPW233F	D21S4	−	21q11.1→21q22.2
3. pPW228C	D21S1	−	21q11.2→21q21
pPW236B	D21S11	−	21q11.2→21q21
4. pPW245D	D21S8	−	21q21→21q22.11
5. pPW513-5H	D21S54	+	21q22.13→21q22.11
6. SOD1		+	21q22.1
7. pPW524-5P	D21S58	+	21q21→21q22.1
8. pGSH8	D21S17	+	21q22.3
9. pPW523-10B	D21S57	+	21q22.3
10. pPW231C	D21S53	+	21q22.3
11. pGSE8	D21S15	+	21q22.3
12. pGSB3	D21S19	+	21q22.3
13. pPW520-10R	D21S56	+	21q22.3

[a] The order of the two probes each listed in Nos. 1–3 has not been determined by linkage analysis.

as the only human chromosome material were used for this purpose; these control hybrids are described in Van Keuren et al. [1986]. High molecular weight DNA was prepared from ACEM2-90 and control cells as described [Gusella et al., 1979]. DNA was digested to completion with the restriction enzyme appropriate for each probe, separated by agarose gel electrophoresis, and transferred to nitrocellulose as described [Southern, 1975]. Cloned probes were nick-translated [Rigby et al., 1977] with ^{32}P and hybridized to the nitrocellulose-bound DNA at 42°C with 50% formamide. Nitrocellulose filters were washed as described [Drabkin et al., 1985], dried and exposed to Kodak XAR X-ray film. In some cases, labeling was done by oligodeoxynucleotide priming [Feinberg and Vogelstein, 1983], and DNA was transferred to Genatran nylon membrane (Plasco, Woburn, MA) as described [Neve et al., 1986].

RESULTS AND DISCUSSION

Cytogenetic analysis confirmed the proposita's unusual translocation chromosome as the only human chromosome in ACEM2-90 hybrid cells. A metaphase spread of ACEM2-90 is shown in Figure 2. The human t(21;21) was present in 100% of cells examined, an expected result because of the purine prototropy selection system uti-lized. It was previously hypothesized on the basis of cytogenetic analysis that this chromosome contains a duplication of a distal section of 21q, including all or most of band 21q22 translocated onto 21p, with deletion of centromeric heterochromatin.

The presence or absence of cloned DNA probes in ACEM2-90 was determined by Southern blot analysis (Table I). These data give insight into the composition of the complex, rearranged t(21;21) chromosome, which is the apparent cause of Down syndrome in the proposita. This chromosome appears to contain a substantial part, if not all, of 21p. Human rRNA sequences are present even though the translocation chromosome was NOR-negative by silver staining in the patient's cells [Bradley et al., 1986]. Moreover, the chromosome does contain centromeric material as indicated by the presence of DNA sequences homologous to Xba14 and p82H. It is likely that the t(21;21) has a functional centromere since the chromosome appears to be present in all cells of the proposita examined, and since it appears to distribute to both daughter cells during mitosis in the ACEM2-90 hybrid. Nonetheless, the centromere in this chromosome may not be entirely normal, since we have observed a tendency for the chromosome to be lost from or to rearrange in hybrid cells. Cytogenetic analysis [Bradley
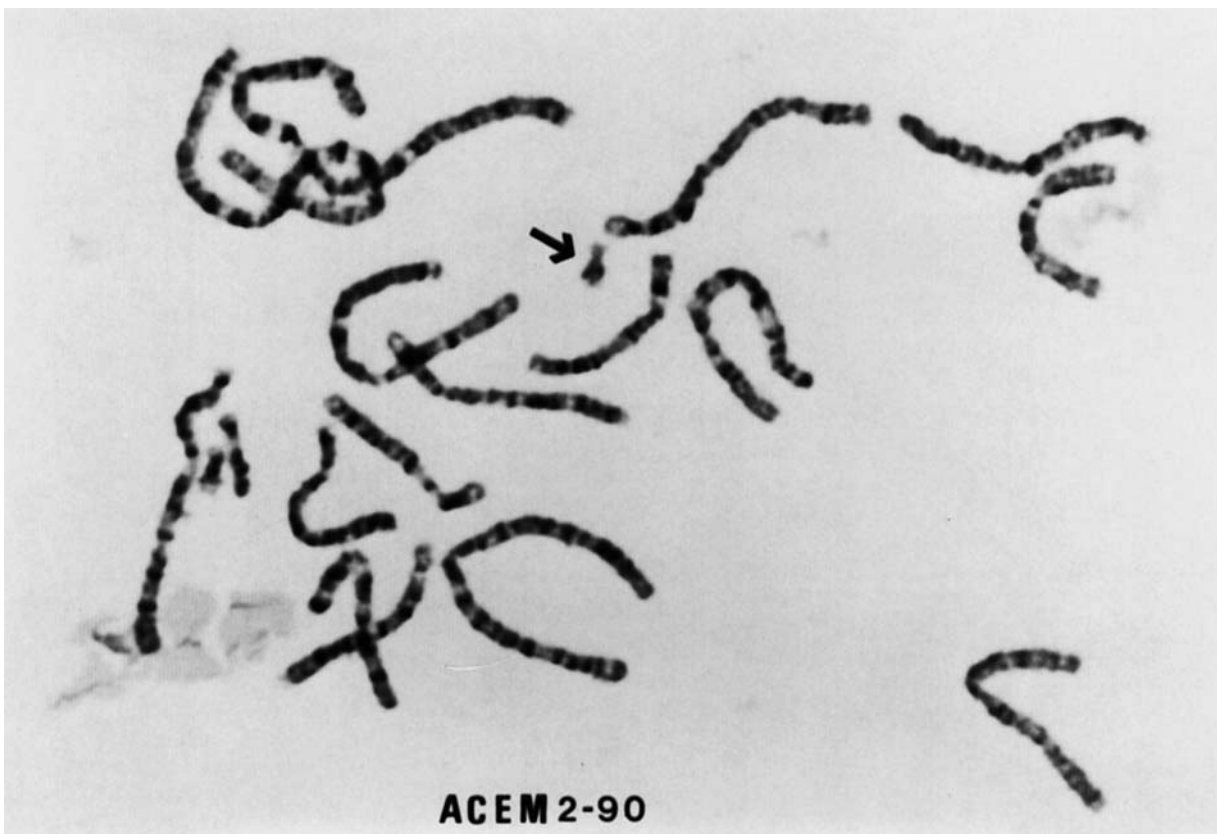

Fig. 2. GTG banding of the CHO × human hybrid ACEM2-90. The arrow indicates the only chromosome that stained blue with subsequent G-11 staining, indicating its human origin. This cell line carries the t(21;21) as the only human chromosome.

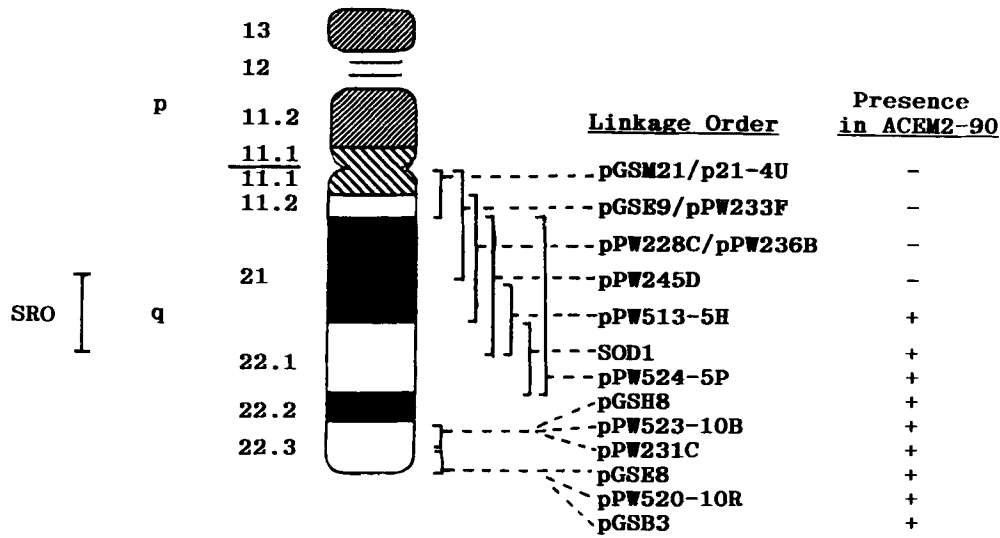|	Linkage Order	Presence in ACEM2-90
	pGSM21/p21-4U	−
	pGSE9/pPW233F	−
	pPW228C/pPW236B	−
	pPW245D	−
	pPW513-5H	+
	SOD1	+
	pPW524-5P	+
	pGSH8	+
	pPW523-10B	+
	pPW231C	+
	pGSE8	+
	pPW520-10R	+
	pGSB3	+

Fig. 3. Schematic representation of human chromosome 21 with physical location of the DNA probes, which have been ordered by linkage analysis and their presence (+) or absence (−) in the ACEM2-90 hybrid cell line. Also indicated is the smallest region of overlap (SRO) for the distal breakpoint area that can be determined from the physical map of these probes.

et al., 1986] provides further evidence of the centromere's abnormal nature as indicated by the lack of C-banding.

It appears that a contiguous section of proximal 21q (linked probes 1-4, Table I) is deleted in this chromosome. This section extends down through all or most of band 21q21. A contiguous section of distal 21q, including all or most of 21q22, appears to be present in this chromosome (linked probes 5-11, Table I). Figure 3 depicts these results.

The hypothesized loss of heterochromatin material proximal to the centromere on the long arm of the chromosome that was indicated by cytogenetic analysis is supported by our data. As yet, we have been unable to confirm deletion of any short arm material from this abnormal chromosome. The presence of a large fraction of the region of chromosome 21, namely band q22, previously shown to be significant for the development of Down syndrome [Niebuhr, 1974; Williams et al., 1975] is also confirmed. Previously it was only possible to show the presence of human superoxide dismutase and human GARS on this chromosome [Bradley et al., 1986]. We demonstrate here the presence of the pathogenetic region of chromosome 21 on this unusual chromosome, consistent with the hypothesized structure of the chromosome gathered from cytogenetic data alone [Bradley et al., 1986]. The combination of somatic cell genetic, classical genetic, cytogenetic, and molecular genetic techniques provides a robust approach for analysis of complex chromosomal rearrangements. We conclude that the 21q breakpoint at the distal boundary of the deleted section must lie between loci identified by the probes pPW245D and pPW513-5H, a segment that is proximal to the SOD1 locus. These probes are tightly linked (θ = 0.04, z = 6.60, P.C. Watkins, unpublished) and therefore confine the breakpoint to a narrow region of chromosome 21, on the
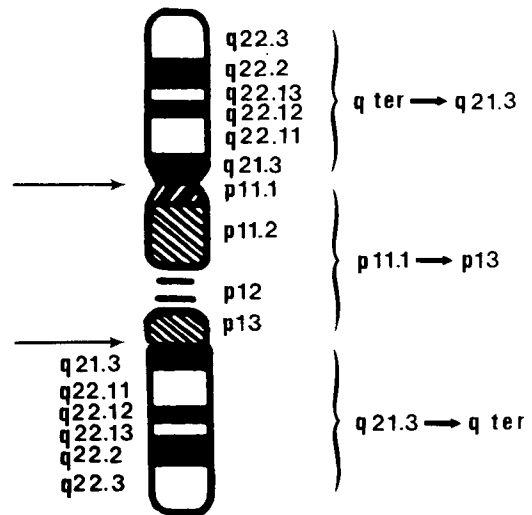

Fig. 4. A proposed schematic diagram of the abnormal chromosome in the proposita, designed to fit the cytogenetics and molecular evidence described herein. The breakpoints (indicated by arrows) may be anywhere between q21.2 and halfway down band q22.1 and are possibly different in the two segments.

order of around 5,000 kb. We propose that based on cytogenetic and molecular evidence described herein, the rearranged chromosome can be described as 21(qter→ 21.3 or q22.1::p11→p13::q21.3 or q22.1→qter) (Fig. 4).

Because a number of the DNA probes used in this study detect restriction fragment length polymorphisms (RFLPs), it is possible to gain further insight into the mechanism by which this complex rearrangement may

have occurred. Assuming that the distal 21q section present on the t(21;21) is duplicated, it is possible to calculate the odds in favor of this duplicated area originating from only one chromosome 21 homologue, rather than both, during the rearranging event. A preliminary examination was made with some of the 21q probes for which sequences were detected in the ACEM2-90 hybrid. In each case, no heterozygosity was detected by Southern blot analysis. As shown by Table II, the random probability of homozygosity for each of the six alleles examined is 4.2%. Therefore, if the distance covered by these loci is indeed duplicated in the t(21;21), it is 95.8% certain that only one chromosome 21 homologue was involved in the rearrangement. Further information could be gained if DNA samples from the parents of the child were examined and found to possess different alleles of the polymorphic probes used in this study.

These results do not demonstrate whether this abnormal chromosome in fact contains two copies of the 21q22→21qter region of chromosome 21, nor do they give a clear indication about the nature of the bisatellited marker chromosome observed in the mother and sib of the proposita. Nevertheless, certain predictions regarding these points can be made and tested experimentally on the basis of the data presented here. First, we would predict that in situ hybridization analysis to metaphase chromosomes of the ACEM2-90 hybrid should reveal the presence of two copies of at least some of the 21q22-specific probes used in this study, one at each end of the ACEM2-90 human chromosome. Similarly, we predict that short arm probes and centromere probes appear only in the short arm and pericentromeric region of this abnormal chromosome. Finally, we should be able to examine by in situ hybridization to metaphase chromosomes whether the bisatellited marker chromosome observed in the mother and sib of this patient indeed contains chromosome 21-specific sequences not found on the chromosome in the ACEM2-90 hybrid. It might even be possible to determine whether these DNA sequences have been duplicated in this marker chromosome. This would unambiguously determine whether the marker chromosome is indeed derived from chromosome 21. These studies coupled with additional studies with different chromosome 21 DNA-specific sequences as they

become available should give us an unprecedented understanding of how this chromosome rearrangement may have taken place in this family.

ACKNOWLEDGMENTS

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TABLE II. Chromosome 21 DNA Probes Used to Test Homozygosity of the t(21;21) in ACEM2-90 Hybrid*

DNA probe	Enzyme	Minor allele frequency[a]	Probability of homozygosity[b]
pPW524-5P	PstI	0.46	.50
pGSH8	BglII	0.43	.51
pPW231F	TaqI	0.10	.82
pGSE8	MspI	0.46	.50
pGSB3	PstI	0.20	.80
pPW520-10R	ApaI	0.50	.50

*Random probability of homozygosity at each allele equals (.50)(.51)(.82)(.50)(.80)(.50)= .042, or 4.2%
[a] Human Gene Mapping 9 [1987]
[b] Probability of homozygosity = 1 − probability of heterozygosity, and probability of heterozygosity = 2pq, where p and q represent major and minor allele frequencies, respectively.

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