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Genetics

Analysis of a break in chromosome 14 mapping to the region of the immunoglobulin heavy chain locus

(restriction fragment length polymorphisms/immunoglobulin γ heavy chain genes/DNA rearrangements)

PETER S. LINSLEY*, N. TORBEN BECH-HANSEN*, LOUIS SIMINOVITCH*†, AND DIANE W. COX*†‡

*Research Institute, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada; and Departments of †Medical Genetics and ‡Paediatrics, University of Toronto, Toronto, Ontario M5G 1X8 Canada

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ABSTRACT **We have detected restriction fragment length polymorphisms associated with the immunoglobulin heavy chain C_γ genes. DNA from both parents of an individual having an unbalanced rearrangement of the long arm of chromosome 14, region q32 [Cox, D. W., Markovic, V. D. & Teshima, I. E. (1982) *Nature (London)* 297, 428–430], revealed distinctive patterns of *Bam*HI fragments which hybridized with cloned probes from the $C_{\gamma 2}$–$C_{\gamma 4}$ gene cluster. The number of hybridizing fragments in both cases (five) equaled the number of known C_γ genes. Pedigree and densitometric analyses indicated that the proband did not have any maternal complement of C_γ gene-hybridizing fragments. Included on the deleted chromosomal segment was a C_γ gene having properties of the previously reported C_γ pseudogene. We also examined DNA from this family with a probe for the highly polymorphic locus *D14S1*, which recently was demonstrated to be tightly linked to the $C_{\gamma 1}$ gene locus [Balazs, I., Purrello, M., Rubinstein, P., Alhadeff, B. & Siniscalco, M. (1982) *Proc. Natl. Acad. Sci. USA* 79, 7395–7399]. *Eco*RI and *Eco*RI–*Bam*HI fragments from both parents hybridized with a probe for this locus in DNA from the proband, indicating that, unlike the C_γ gene family, *D14S1* was not deleted from the abnormal chromosome. Thus, the chromosomal breakpoint in the proband lies within region 14q32 between the two tightly linked markers, *D14S1* and the $C_{\gamma 1}$ heavy chain gene locus. The *D14S1* locus must lie proximal to the centromere relative to the C_γ gene family. The genetic variability detected with C_γ gene probes may prove useful for genetic analysis of structural rearrangements involving this region of chromosome 14.**

Certain human leukemias and lymphomas are regularly associated with consistent chromosomal translocations (1, 2). Translocations observed in Burkitt lymphomas and acute lymphocytic leukemias of B-cell origin frequently involve the joining of a portion of chromosome 8 to one of the three immunoglobulin chain-encoding chromosomes, number 2, 22, or, most commonly, number 14 (2, 3). The breakpoint on chromosome 14 is within band 14q32, a region also frequently involved in chromosomal rearrangements found in nonmalignant lymphocytes. Region 14q32 was found to be a "hot spot" for spontaneous chromosomal breaks and rearrangements in cultured lymphocytes (4). Also, clonal populations of lymphocytes having chromosomal rearrangements involving this region have been observed in some patients having the inherited disorder ataxia telangiectasia (reviewed in ref. 5).

A possible reason for the involvement of region 14q32 in chromosomal rearrangements is suggested by two recent studies demonstrating that this is the site of the immunoglobulin heavy chain genes. Cox *et al.* (6) reported on the family of an individual having a ring chromosome 14, in which one breakpoint was localized to region 14q32.3. The affected individual did not express the C_γ allotypic heavy chain markers, GM, of the maternal haplotype, indicating that chromosomal material distal to 14q32.2 is required for expression of C_γ region genes. In a parallel study, Kirsch *et al.* (7) mapped a cloned $C_{\gamma 4}$ gene to 14q32 using *in situ* chromosome hybridization. Heavy chain genes are known to undergo two types of somatic recombination: V-D-J joining is necessary for formation of a functional immunoglobulin gene (8, 9) and recombination also is thought to accompany the heavy chain class switch (9–11). Localization of the heavy chain gene cluster to a region of frequent chromosomal breaks suggests that abnormalities involving region 14q32 may result from aberrant heavy chain gene rearrangements (7).

Another site of interest in this region of frequent chromosomal rearrangements is the highly polymorphic locus *D14S1*. This locus, revealed as a pattern of restriction fragments hybridizing with the probe pAW101 (12), recently was assigned to the long arm of chromosome 14, between 14q21 and 14qter (13), and more precisely to 14q32 (14). The latter study also demonstrated tight (3–12%) linkage between *D14S1* and the $C_{\gamma 1}$ markers of the GM system. DNA rearrangements are apparently the basis for the extensive polymorphism of *D14S1* (12).

We have undertaken a study of the involvement of the heavy chain region in the ring chromosome abnormality discussed above. Here, we report results of this analysis, using as probes cloned fragments of C_γ heavy chain genes and of the tightly linked restriction fragment polymorphism (RFLP) marker *D14S1*.

MATERIALS AND METHODS

Clinical Material. The family of the proband was described (family 1) in a recent report (6). The proband had the karyotype 46XY, ring 14, and did not express IgG of the maternal GM allotype. Neither GM^a nor GM^x, which are markers for the $C_{\gamma 1}$ locus, nor GM^g, specific for the $C_{\gamma 3}$ locus, could be detected in the proband, although all were present in his mother. In this study, we have examined the proband, his parents, and their relatives.

DNA Preparation. Peripheral blood leukocytes, obtained from 20–40 ml of heparinized whole blood, were used as a source of DNA. High molecular weight DNA was extracted essentially as described (15). When leukocyte DNA was digested with *Bam*HI or *Eco*RI and hybridized with a probe specific for human J_H sequences (16), only germ line-sized hybridizing fragments could be detected (unpublished data), indicating that the germ line configuration of immunoglobulin genes is represented in these samples.

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Abbreviations: RFLP, restriction fragment length polymorphism; kb, kilobase pair(s); $C_{\psi\gamma}$, C_γ pseudogene.

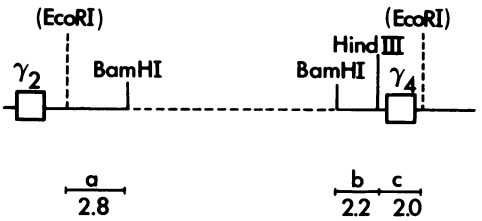

FIG. 1. Partial restriction map of the chromosomal region containing the C_γ gene probe used in this study. For a more complete map, refer to ref. 18. The *Eco*RI sites indicated were generated during construction of the DNA library used for cloning and are not present in genomic DNA. Additional restriction sites in the region encompassed by the dotted horizontal line are not shown. Sizes of probes a, b, and c are shown in kb.

Hybridization Probes. Genomic fragments of the $C_{\gamma 2}$–$C_{\gamma 4}$ gene cluster (17, 18), subcloned in pBR322, generously were provided by Jay Ellison in the laboratory of Leroy Hood (California Institute of Technology). pAW101 (12) was a gift from Arlene Wyman (Massachusetts Institute of Technology). The human growth hormone cDNA clone (19) was obtained from Alan Berstein (Ontario Cancer Institute).

C_γ gene probes used in this study were derived from three separate subclones and are indicated in Fig. 1. Probe a is a 2.8-kilobase-pair (kb) *Eco*RI–*Bam*HI fragment derived from a subcloned 3.0-kb *Eco*RI–*Hin*dIII segment of the previously described clone 24B (18), which lies 3′ to the $C_{\gamma 2}$ coding region. Probe b is a 2.2-kb *Bam*HI-*Hin*dIII fragment derived from a 3.5-kb *Hin*dIII fragment of clone 24B, lying 5′ to the $C_{\gamma 4}$ coding region. Probe c is a 2.0-kb *Hin*dIII–*Eco*RI fragment of clone 24B which contains the $C_{\gamma 4}$ coding region. After restriction enzyme digestion of the appropriate plasmids, fragments to be used as probes were separated by agarose gel electrophoresis and removed from the gel by electroelution. DNA fragments then were purified by phenol extraction, ethanol precipitation, and finally, labeled by nick-translation (20) to a specific activity of 1–5 × 10^{-8} cpm/μg, by using a commercially supplied kit (Amersham).

Restriction Enzyme Digestions and Gel Electrophoresis. High molecular weight DNA preparations were digested overnight at 37°C with an excess of enzyme, in buffers recommended by the suppliers (Bethesda Research Laboratories and Boehringer Mannheim). These conditions of enzyme excess and long incubation time ensured complete digestion and gave consistently reproducible results. Aliquots of digested DNA samples (≈5 μg) were separated on agarose gels (0.5%, unless otherwise indicated) by electrophoresis at 0.5–1.5 V/cm for 24–72 hr.

Blot Hybridization Analysis. Separated DNA fragments were transferred to nitrocellulose by using published procedures (21, 22). Hybridization conditions were essentially as described (23), except that the final washing was for 1 hr at 60°C in a solution containing 15 mM NaCl, 1.5 mM sodium citrate, and 0.1% $NaDodSO_4$. Hybridizing bands were revealed by autoradiography at −70°C by using intensifying screens.

Densitometric Analysis. Autoradiograms were scanned with a Joyce–Loebl densitometer, and band intensities were estimated as peak heights. To obtain an estimate of the relative amounts of DNA in each lane, the original membrane was hybridized with a cDNA probe for human growth hormone. This detects a gene family mapping to chromosome 17 (24, 25) and reveals hybridizing *Bam*HI fragments of 8.2, 6.7, 5.3, 3.8, 3.0, and 1.2 kb (26). The 3.0- and 1.2-kb bands were chosen as standards and an average of the relative intensities of these bands was determined. This value then was used to normalize the measured intensities of bands hybridizing with C_γ gene probes.

RESULTS

Analysis of a RFLP Detected with C_γ Gene Probes. Fig. 2*A* shows the results of an experiment in which leukocyte DNA from the father, proband, and mother was digested with *Bam*HI and analyzed by blot hybridization by using probe a. Hybridizing fragments of 13.5, 12.5, 11.8, 9.4, and 8.8 kb in DNA from the father and fragments of 25, 12.5, 11.8, 10.0, and 9.0 from the mother were detected. These same fragments also hybridized with probe c (data not shown), and four of the five from each parent hybridized with probe b (Fig. 2*B*).

Studies of amino acid and DNA sequences (17, 18, 27–29) indicate that sequence homology extends through the coding regions and into the 3′ flanking sequences of all five cloned C_γ genes ($C_{\gamma 1}$, $C_{\gamma 2}$, $C_{\gamma 3}$, $C_{\gamma 4}$, and C_γ pseudogene, $C_{\psi\gamma}$). Four of the five also show homology in their 5′ flanking sequences, with the exception being the $C_{\psi\gamma}$ (28). Consequently, because probes b and c are from the same genomic *Bam*HI fragment and probe a is from an apparently homologous *Bam*HI fragment, all would be expected to give similar patterns of hybridization. As can be seen in Fig. 2 *A* and *B*, this generally was true; an important exception was noted with the 5′ flanking sequence probe (probe b) which failed to detect the paternal 8.8-kb and maternal 10.0-kb *Bam*HI fragments (Fig. 2*B*). Additional small, faintly hybridizing bands not detected with the $C_{\gamma 4}$ coding region probe, probe c, were revealed with probes a and b. These presumably were due to the presence of additional *Bam*HI sites within the sequences adjacent to some C_γ genes.

Because five known C_γ genes, $C_{\gamma 1}$, $C_{\gamma 2}$, $C_{\gamma 3}$, $C_{\gamma 4}$, and $C_{\psi\gamma}$, have been cloned (17, 18, 28, 29) and because *Bam*HI does not cut within sequences expected to be homologous to probes a, b, and c from any of these, it is likely that each of the five *Bam*HI fragments detected in the parental DNAs contains a different C_γ gene. This conclusion is supported by the finding that the genomic *Bam*HI fragments containing several C_γ genes are similar in size to the fragments depicted in Fig. 2. Thus, 11.6-, 10.0-, and 8.6-kb *Bam*HI fragments have been found in genomic clones containing $C_{\gamma 1}$, $C_{\psi\gamma}$, and $C_{\gamma 4}$ genes, respectively (18, 29). Direct evidence as to the identity of the C_γ genes contained in one of the hybridizing bands seen in DNA from each of the parents is provided by the experiment shown in Fig. 2*B*. Both the 8.8-kb fragment and the maternal 10.0-kb fragment failed to hybridize with a probe specific for the 5′ flanking sequence of the $C_{\gamma 4}$ gene (probe b). Because the cloned $C_{\psi\gamma}$ gene has a 5′ flanking sequence distinct from

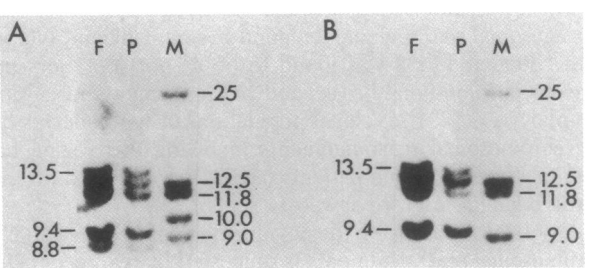

FIG. 2. Polymorphic pattern of *Bam*HI fragments hybridizing with C_γ gene probes. DNA samples were digested with *Bam*HI and analyzed by blot hybridization. Numbers indicate fragment sizes in kb. Samples from the father (F), proband (P), and mother (M) were analyzed. (*A*) Hybridization probe a was used. (*B*) The nitrocellulose membrane depicted in *A* was washed at 65°C in a solution of 60% formamide to remove previously hybridized probe and was rehybridized with probe b.

the other C_γ genes (28), both the 8.8- and 10.0-kb fragments most likely contain $C_{\psi\gamma}$, and the difference in size between these fragments is due to a polymorphic restriction site adjacent to this gene. The additional fragment size differences seen in the parental DNAs are also likely due to polymorphic sites adjacent to other C_γ genes.

Because the parental samples revealed a number of *Bam*HI-hybridizing fragments equal to the number of C_γ genes, and only one $C_{\psi\gamma}$-containing fragment, it seemed likely that both parents were homozygous for three C_γ gene RFLPs. To determine if intermediate combinations of these RFLPs were possible, we examined DNA from 20 normal, unrelated individuals (data not shown; see also Fig. 3). Samples for all individuals tested showed both the 11.8- and 12.5-kb bands. Various combinations of the other fragments were seen and the total number of fragments from a single individual ranged from a minimum of five to a maximum of eight. Because individuals exhibiting fewer than five fragments were not detected, it is likely that the minimal number of fragments comprising a "haplotype" in the population equals five. The maximal number of fragments observed in any individual (eight) equals the predicted maximal number of fragments that can arise from two genes flanked by constant restriction sites and three genes flanked by sites that can exist in either of two configurations. These observations suggest that individuals yielding simple five-band patterns (like the parents of the proband) are homozygous for their respective RFLPs and that the more complex patterns arise from individuals heterozygous for one or more RFLPs.

Inheritance of *Bam*HI RFLPs Detected with C_γ Gene Probes. Restriction fragment variations reported to date are inherited in a Mendelian fashion (12, 30, 31) and, if the parents of the proband were indeed homozygotes for their RFLPs, we would predict DNA from the proband to contain hybridizing fragments from both parents. This was not the case, because three of the five fragments from the mother were absent from the proband (Fig. 2A). This finding suggested that the proband had sustained a deletion of the chromosomal segment containing the maternal C_γ genes. To confirm the deletion of C_γ genes from the proband, we analyzed DNA from other relatives, as shown in Fig. 3. The hybridizing fragments found in each individual were tabulated and compared with GM markers in Table 1. The interpretation of these patterns, as discussed below, is based on the rarity of crossing over within the C_γ gene family (32).

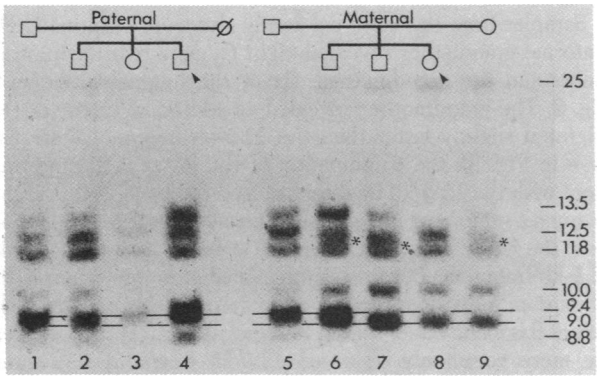

FIG. 3. Inheritance of C_γ gene RFLPs through the extended family of the proband. DNA samples from individuals having the indicated relationships to the proband were digested with *Bam*HI and analyzed by blot hybridization by using probe c. Arrowheads indicate the parents of the proband. Numbers indicate fragment sizes in kb. The original x-ray film shows more clearly the presence of the 25-kb fragment in all individuals, except for individual 4 (father of the proband; lane 4). The decreased intensity of fragments shown in the sample from individual 3 (lane 3) is attributable to a decreased amount of DNA run in this lane. Horizontal lines indicate the approximate centers of the 9.4- and 9.0-kb bands. The size of fragments in this region was confirmed by a separate experiment (using probe b) in which better resolution was achieved. The 12.5-kb region in individuals 6, 7, and 9 (lanes 6, 7, and 9, respectively) appears somewhat broad in this experiment due to the presence of an additional band (marked with *) migrating slightly ahead of the 12.5-kb fragment.

DNA from the paternal grandfather revealed all eight fragments shown in Fig. 2. Two offspring (individuals 2 and 3) revealed the same fragments as did the father, whereas individual 4 (father of the proband) showed the simple five-fragment pattern shown previously in Fig. 2. A comparison of C_γ gene RFLPs with GM markers (Table 1) indicates that a haplotype consisting of a maximum of three RFLPs (13.5-, 9.4-, and 8.8-kb fragments) segregated with $GM^{f;b}$ from the paternal grandfather. Because individual 4 is homozygous for $GM^{f;b}$, the most likely interpretation is that three identical C_γ gene RFLPs segregated with $GM^{f;b}$ from both paternal grandparents.

Table 1. C_γ restriction fragments in relatives of the proband

	Distribution of fragments*									
	Paternal				Maternal					
Fragment length, kb	1 (fb/ag)	2 (fb/ag)	3 (fb/fb)	4 (fb/fb)	5 (fb/ag)	6 (fb/ab)	7 (ag/ab)	8 (ag/axg)	9 (ab/axg)	Proband (fb/—)
25	+	+	+	−	+	+	+	+	+	−
13.5	+	+	+	+	+	+	+	−	+	+
12.5	+	+	+	+	+	+†	+†	+	+†	+
11.8	+	+	+	+	+	+	+	+	+	+
10.0	+	+	+	−	+	+	+	+	+	−
9.4	+	+	+	+	+	+	−	−	−	+
9.0	+	+	+	−	+	+	+	+	+	−
8.8	+	+	+	+	+	+	−	−	−	+

Presence or absence of a particular hybridizing fragment in Fig. 3 (or Fig. 2 for the proband) is indicated as + or −, respectively. GM markers were reported previously or determined as described (6). Presence or absence of the 9.4- and 9.0-kb fragments in all samples was confirmed in a separate experiment, as described in the legend to Fig. 3.

*GM haplotypes are given in parentheses.

†Indicates the presence of an additional fragment in this region (see text for discussion). For simplicity, both fragments were tabulated as 12.5 kb in length.

Samples from the maternal family also were examined. The maternal grandfather revealed eight C_γ gene hybridizing fragments and the grandmother, six of the fragments shown in Fig. 2. The grandmother revealed an additional fragment that migrated slightly faster than the 12.5-kb fragment (indicated by * in Fig. 3); the combination of the faster migrating fragment with the 12.5-kb fragment caused the band in this region to appear somewhat broader than normal (see Fig. 3). We believe the faster migrating fragment to be a rare variant of the 12.5-kb fragment, because it was not seen in any other of the 20 random individuals examined to date. In this pedigree, the variant fragment was observed to segregate with $GM^{a;b}$, whereas the more commonly observed 12.5-kb fragment segregated with $GM^{ax;g}$.

The 8.8-kb fragment containing $C_{\psi\gamma}$ and the 9.4-kb fragment from the maternal grandfather clearly segregated with $GM^{f;b}$; these fragments also segregated with $GM^{f;b}$ in the paternal family. By process of elimination, the 10.0-kb fragment (containing $C_{\psi\gamma}$) and the 9.0-kb fragment seen in the maternal grandfather must segregate with $GM^{a;g}$. This pattern of segregation confirms the allelic nature of the 8.8- and 10.0-kb fragments containing $C_{\psi\gamma}$.

The overall pattern of inheritance of C_γ gene RFLPs through this family is consistent with segregation of haplotypes consisting of the 25-, 12.5-, 10.0-, and 9.0-kb polymorphic fragments, as well as the 11.8-kb invariant fragment, with both of the GM haplotypes, $GM^{a;g}$ and $GM^{ax;g}$, found in the mother of the proband. The inheritance of these RFLPs clearly is inconsistent with the pattern of RFLPs seen in the proband, indicating that the chromosomal deletion sustained by the proband resulted in loss of three C_γ genes associated with RFLPs.

Densitometric Analysis of C_γ Gene Hybridization Pattern in the Proband. The experiment shown in Fig. 2*B* demonstrates that C_γ-hybridizing fragments in DNA from the proband were present at decreased intensities relative to those from both parents. These differences were observed consistently in four separate experiments. Examination of ethidium bromide staining patterns indicated that these differences were not attributable to differences in the amount of DNA loaded.

To quantitate the relative band intensities in the proband, we performed a quantitative analysis of the autoradiogram depicted in Fig. 2*A*. Intensities were estimated by densitometric analysis and then were normalized for amounts of DNA loaded by comparison with intensities of hybridization (determined on the same membrane) with a probe for a gene family localized to a chromosome unaffected by the deletion from the proband, as described in *Materials and Methods*. As shown in Table 2, the normalized values for the common parental hybridizing fragments of 11.8- and 12.5-kb were nearly identical, whereas those for all fragments from the proband were decreased and ranged from 43% to 73% (with a mean of 56%) of the paternal values. Determination of gene dosage by using this type of analysis is difficult because of technical uncertainties, including nonlinearity of film response and unknown efficiencies of fragment transfer to nitrocellulose. Nevertheless, it is clear that the band intensities determined for the proband are significantly less than those for both parents. The most likely interpretation of this finding is that C_γ gene-hybridizing fragments are present in the proband at decreased copy number.

Because DNA from both parents contained 11.8- and 12.5-kb bands, the proband conceivably could have inherited copies of these from both parents. This is unlikely, because these band intensities in the proband were decreased (Table 1). Also, the 11.8- and 12.5-kb bands would then have been present at increased intensities relative to the 13.5-kb band. However, the intensities of the 11.8- and 12.5-kb bands, relative to the 13.5-kb band, were identical in both the father and proband.

Table 2. Quantitative analysis of C_γ gene-hybridizing *Bam*HI fragments

Band size, kb	Normalized peak height (arbitrary units)		
	Father	Son	Mother
13.5	9.9	4.4 (44)	−*
12.5	10.7	6.1 (57)	9.2 (86)
11.8	10.8	6.9 (64)	9.7 (90)
9.4	10.1	7.2 (72)	−*
8.8	5.2	2.2 (43)	−*

Band intensities on the autoradiogram reproduced in Fig. 2*A* were determined and normalized for recovery. Recoveries of DNA in samples from the father, proband, and mother were estimated as 1.0, 0.62, and 0.38, respectively, and intensities of C_γ gene-hybridizing fragments then were normalized to these values. Each number in parenthesis represents the percentage of the value determined for the corresponding band in the father.
* Indicates the absence of a particular band.

Therefore, several lines of evidence indicate location of C_γ genes on the chromosome fragment deleted during ring chromosome formation. Three of five maternal *Bam*HI fragments hybridizing with C_γ gene probes were missing from the proband; the 10.0-kb $C_{\psi\gamma}$-containing fragment was absent, as were two others containing genes of unknown identity. Three of five bands from the proband clearly are paternal in origin, but were present at a decreased intensity, suggesting a lower decreased copy number. The two hybridizing fragments from the proband, which could have been derived from either parent also were present at decreased intensities and in relative amounts identical to those from the father. Thus, the most likely explanation for the previously reported lack of IgG of the maternal GM allotypes in the proband (6) is deletion of the entire maternal C_γ gene cluster.

Detection of Both Parental Alleles of *D14S1* in the Proband. Because the chromosomal breakpoint in the proband of our study is localized to the same general region of chromosome 14 as the RFLP, *D14S1* (12, 14), we determined if one of the parental *D14S1* alleles also was missing from the proband. As shown in Fig. 4, the father is heterozygous at the *D14S1* locus, because two *Eco*RI fragments of 22 and 15 kb hybridized with the *Eco*RI insert from pAW101. The mother was apparently homozygous for this marker because only a single *Eco*RI fragment (16 kb) was detected. The proband exhibited alleles derived from both parents, a 15-kb fragment from the father and a 16-kb fragment from the mother. Similar results were obtained when DNA was first digested with *Eco*RI and then with *Bam*HI before analysis (Fig. 4). In this case, fragments of 14.5 kb, derived from the father, and 15 kb, from the mother, were detected in the proband. Be-

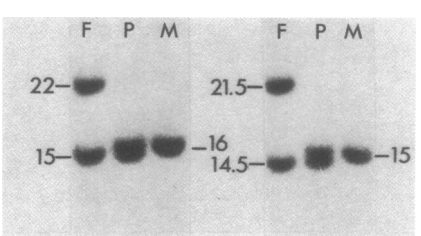

FIG. 4. Pattern of restriction fragments hybridizing with pAW101. DNA samples from the father (F), proband (P), and mother (M) were digested with *Eco*RI (*Left*) or digested sequentially with *Eco*RI and *Bam*HI (*Right*) and were subjected to electrophoresis on a 0.4% agarose gel. Blot hybridization analysis was performed, by using the *Eco*RI insert from pAW101 as probe. Numbers indicate fragment sizes in kb.

cause alleles at *D14S1* from both parents were detected in the proband, this marker was not deleted during ring chromosome formation.

DISCUSSION

In this study, we have shown that the C_γ gene cluster lies distal to band 14q32.2 on chromosome 14, thereby confirming the assignment made previously on the basis of lack of IgG expression (6). It is noteworthy that $C_{\psi\gamma}$ was deleted along with other C_γ genes from the proband; therefore, it must occupy the same region of chromosome 14 as the other C_γ genes. Thus, $C_{\psi\gamma}$ was not dispersed to a chromosomal location distant from other members of its family, as was a pseudogene of the λ light chain family (33). Another important finding is that the C_γ gene cluster was deleted from the proband, whereas the *D14S1* locus was not. This establishes the linear ordering of the two loci; the *D14S1* locus must lie proximal to the centromere relative to the C_γ gene family.

By gene dosage and somatic cell hybrid analyses, Balazs *et al.* (14) demonstrated that the *D14S1* locus was absent from the translocation chromosome, 14pter→14q32::Xq13→Xqter. Data presented in this paper, when taken together with those of Balazs *et al.*, indicate that chromosome breaks in region 14q32 occur in at least two distinct sites. One of these, described previously (14), is proximal to the centromere relative to *D14S1*. The second, found in the ring chromosome from the proband of this study, lies between *D14S1* and the C_γ gene family. From the recombination frequency of 3–12% determined for the *D14S1* and $C_{\gamma 1}$ loci (14) and from the estimate that, on the average, one unit of meiotic recombination represents a distance of $\approx 10^6$ base pairs (14, 30), it can be estimated that our results localize the breakpoint in the ring chromosome to a stretch of DNA from 3 to 12×10^6 base pairs in length. By using additional probes from this region, it may be possible to define more precisely this chromosomal breakpoint.

The demonstration of different sites of chromosomal recombination within 14q32 is not incompatible with the suggestion that certain chromosomal rearrangements have their molecular basis in aberrant heavy chain gene rearrangements. The heavy chain gene cluster is likely to be quite large—the analogous region in mouse extends more than 200 kb (34)—and contains many potential sites for recombination. For example, recombination conceivably could occur at a switch region lying 5′ to any of the functional heavy chain genes (10, 11, 35, 36). Further experimentation may clarify the relationship of sites of chromosomal recombination to regions characterized by frequent molecular rearrangements.

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