Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 10609–10613, December 1991
Genetics

Detailed physical map of human chromosomal region 11q12-13 shows high meiotic recombination rate around the *MEN1* locus

(gene mapping/pulsed-field gel electrophoresis/radiation-reduced somatic cell hybrids/multiple endocrine neoplasia type 1)

MARIE JANSON*†, CATHARINA LARSSON*, BARBRO WERELIUS*, CAROL JONES‡, TOM GLASER§, YUSUKE NAKAMURA¶, C. PETER JONES‖, AND MAGNUS NORDENSKJÖLD*

*Department of Clinical Genetics, Karolinska Hospital, S-104 01 Stockholm, Sweden; ‡Eleanor Roosevelt Institute for Cancer Research, Denver, CO 80206; §Howard Hughes Medical Centre, Harvard Medical School, Boston, MA 02115; ¶Cancer Institute, Japanese Foundation for Cancer Research, Kami-Ikebunuro, Toshima-ku, Tokyo 170, Japan; and ‖Molecular Genetics Unit, Medical Research Council, Hills Road, Cambridge CB2 4QH, United Kingdom

Communicated by Rolf Luft, August 16, 1991

ABSTRACT **We have constructed a physical map of the region q12-13 on chromosome 11 by combining data generated from a panel of radiation-reduced somatic cell hybrids and pulsed-field gel electrophoresis (PFGE). Twenty different genetic markers have been sublocalized and ordered within this region and a total of 8.0 megabases has been mapped in detail using rare-cutting restriction endonucleases and PFGE. In two instances, the long-range restriction PFGE map spans the total distance between pairs of loci that have been previously mapped by genetic linkage in reference families. Comparison of this physical map with the available linkage map indicates a great variation in the recombination frequency over the region. The recombination rate is higher than expected, particularly for markers flanking the *MEN1* region. Thus, for the closest pair of linked markers on the centromeric side, one centimorgan corresponds to ≈300 kilobases, and for markers on the telomeric side, one centimorgan corresponds to ≈350–600 kilobases.**

Physical mapping of large chromosomal regions is possible due to the technique of pulsed-field gel electrophoresis (PFGE) in combination with the use of rare-cutting restriction endonucleases (1). These enzymes, with at least one CpG dinucleotide in their recognition sequence, cut infrequently in the human genome, are sensitive to methylation, and often have sites clustered in hypomethylated HTF islands. These islands have been associated with expressed sequences in mammalian genome, making them potential landmarks for human genes (2). Long-range restriction mapping can bridge the gap between the cytogenetic methods, restricted by the resolution of the light microscope, and conventional restriction mapping with an upper limit of resolution of about 50 kilobases (kb). This technique has been instrumental in constructing physical maps necessary for the identification of disease genes by positional cloning (3–6).

The chromosome region of 11q12-13 has been implicated in the pathogenesis of multiple endocrine neoplasia type 1 (MEN1), an autosomal, dominantly inherited predisposition to neoplasia in several endocrine organs, including the pituitary, the parathyroids, and the endocrine pancreas (7). Strong linkage has been established between MEN1 and a region extending 7 centimorgans (cM) on either side of the *PYGM* locus (McArdle disease), flanked by p3C7/D11S288 on the centromeric side and by INT-2 on the telomeric side within chromosome region 11q12-13 (8, 9). Comparisons of the genotype of MEN1-associated tumors and the corresponding constitutional tissue have also shown loss of heterozygosity in this region, indicating that tumorigenesis of these lesions involves unmasking of a recessive mutation at the *MEN1* locus (8, 10–12), in agreement with the two-mutation model postulated by Knudson (13). Chromosomal rearrangements involving 11q13 have also been described in chronic lymphocytic leukemia (14), prolymphocytic leukemia (15, 16), non-Hodgkin lymphoma (17), and multiple myeloma (18). A cluster region for breakpoints, BCL-1, has been identified and sequenced (19) as well as two potential oncogenes, *INT-2* (20) and *HSTF1* (21). Atopy, as defined by abnormal IgE response, rhinitis, and asthma, has also been found to be linked to a marker in this region, pMS51/D11S97 (22).

In this paper we report the physical mapping of 20 DNA markers within the region 11q12-13 by combining hybridization of DNA markers onto somatic cell hybrids and radiation-reduced somatic cell hybrids with long-range restriction mapping by PFGE. Comparisons of our PFGE data with the genetic linkage map generated by multipoint linkage analysis in reference families (23) reveal that the physical distance between meiotic crossovers varies noticeably over the region, being particularly short for paired markers flanking the *MEN1* locus.

MATERIALS AND METHODS

DNA Markers. Probes and their sources are as follows: BCL-1, Y. Tsujimoto (19); HSTF1, M. Terada (21); CI-i cDNA, S. Bock (24); CD20, T. F. Tedder (25); INT-2 (SS6 fragment), N. Spurr (20); clone 8/D11S751, T. Rabbitts (16); and PPP1A, P. Cohen (26). pMS51/D11S97 came from ICI (27). p3C7/D11S288, phpep14-21/PGA, pHBI59/D11S146, pMCMP1/PYGM, and pTHH26/D11S149 were obtained from the ATCC (28–33). cCI11-297/D11S471, cCI11-288/D11S469, cCI11-319/D11S480, and cCI11-4/D11S427 have been published (34). cCL15 is a cosmid clone isolated using a unique fragment of PYGM. cCLGW4/D11S750 and cCLGW39/D11S807 are clones isolated from a cosmid library made from the radiation-reduced hybrid cell lines R184-3A1 and R184-7C1 (35). U2/7 was a gift from K. Hammarström (Karo-BIO Novum, Huddinge, Sweden) (36).

Cell Lines. Human–hamster cell hybrid cell lines were used to confirm the localization of the DNA markers; MC-1 contains human chromosome 11pter-11q23, J1-44 carries a human chromosome 11 with an interstitial deletion involving 11q12-q22, and R28-4E carries a translocation chromosome, Xqter-p21::11q13.2-qter. Goss–Harris radiation-reduced hybrids derived from a hybrid cell line, J1-9, that contains all of 11q and a small part of 11p (37) on a hamster background, were used for more detailed mapping of the DNA probes. The hybrid cells were irradiated with 8000 rads (1 rad = 0.01 Gy)

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MEN1, multiple endocrine neoplasia type 1; PFGE, pulsed-field gel electrophoresis; cM, centimorgan(s).

†To whom reprint requests should be addressed.

of γ-irradiation and fused with recipient Chinese hamster cells. We selected radiation-reduced hybrids with monoclonal antibody 4F2 that express the MDU1 human cell surface antigen, which is encoded by a gene that maps to the 11q13 region using methods similar to those previously described (38). The radiation-reduced hybrid cell lines R184-1A2, R184-3A1, R184-7C1, R184-5D1, R184-4C2, and R131-33B1 were used in the mapping of DNA markers.

PFGE. Preparation of DNA in agarose blocks and subsequent restriction endonuclease digests were modified from protocols from D. Barlow and H. Lehrach (39). Very high molecular weight DNA was prepared from either peripheral blood or cultured Epstein–Barr virus-transformed lymphocytes from randomly chosen, nonrelated individuals. Each 80-μl agarose plug containing DNA from 0.5×10^6 cells was digested to completion with 20–40 units of restriction endonuclease (New England Biolabs). DNA from radiation-reduced hybrid cell lines was prepared and digested in the same way. Using the hexagonal electrode array, 1% agarose gels were run in Pharmacia-LKB Pulsaphor units in 0.5× TBE buffer (0.04 M Tris borate/0.001 M EDTA, pH 8.0). Switching times as well as run times were varied to achieve best possible separation over the various size regions and to avoid compression zones for fragments to be sized. Yeast chromosomes of *Hansenula wingei* and *Saccharomyces cerevisiae*, strain YP148 were used as size markers (40). YP148 contains pBR sequences in two chromosomes of 90 kb and 1025 kb, respectively, making these visible as bands on the autoradiogram when a pBR-containing probe is used.

DNA was transferred onto GeneScreen*Plus* filters (New England Nuclear) in 0.5 M NaOH/1.5 M NaCl; this was followed by neutralization and immobilization by baking at 80°C. Probes were labeled by random priming with random oligomers (41). Different hybridization protocols were used, though the Church–Gilbert protocol (0.5 M sodium phosphate, pH 7.2/7% SDS/1 mM EDTA) gives very reliable results and is convenient to use (42). Filters were washed in 40 mM sodium phosphate/1% SDS at 65°C. Probes containing repetitive elements were allowed to compete with nonradioactive sonicated single-stranded total human DNA (43). Before rehybridization, the probe was removed from the filter in 0.4 M NaOH for 10–20 min at room temperature; this was followed by neutralization.

The bending of lanes run at the outer edges of the gel and the fact that the migration of DNA in PFGE varies with sample concentration (3) make for errors in the sizing of fragments. We estimate this to be of the order of 10–15%. For these reasons, fragments detected by different probes were compared after successive hybridizations to the same filters and found to be identical if reproducible on more than one filter. The presence of more than one fragment in common for two probes was taken as a strong indicator that they are physically linked. When two markers only have one band in common, probes were considered to be possibly linked if this was in agreement with data obtained from the radiation-reduced cell hybrid panel. Bands in the region of the limited mobility of the gel were not regarded as specific fragments.

RESULTS

Mapping of DNA Markers on Radiation-Reduced Somatic Cell Hybrids. All probes collected for this study were first tested on a panel of known cell hybrids containing large fragments of chromosome 11, MC-1, J1-44, and R28-4D (data not shown). On this basis probes were selected for hybridization to a panel of radiation-reduced somatic cell hybrids containing fragments of and around 11q13, which served the purpose of sublocalizing and differentiating them into subgroups. The probes were ordered along a line assuming the human DNA to be contiguous in the hybrid genome (Fig. 1). These data were also in agreement with the linkage data available for some of the markers (23). This primary sorting into groups facilitated the subsequent hybridization to PFGE filters, so that possible link-up probes were hybridized to the same filter. This was also helpful in the assessment of the probability of two probes hybridizing to the same restriction fragment or different fragments of same size.

The grouping of the DNA markers based on the hybridization to the radiation-reduced hybrid panel was only used as a general guideline, since the human DNA fragments could be in rearranged form or the recipient cell could contain more than one noncontinuous fragment of human DNA. To resolve this further, high molecular weight DNA prepared from the radiation-reduced cell hybrids was digested with a rare-cutting enzyme, *Not* I, separated on PFGE, and hybridized to a probe, U2/7, that contains several human-specific *Alu* repeats (36). This resulted in several distinct bands, ranging in size from 2 megabases (Mb) to 50 kb (data not shown). When comparing these bands obtained for the various cell lines, it was evident that the numbers of bands shared correlated roughly with the degree of DNA markers shared among the cell lines according to the hybrid panel. When some of the DNA markers that were used in this study (BCL-1, pMS51/D11S97, PGA, cCL15) were hybridized to the *Not* I-digested cell line DNA, all cell lines gave identical bands for each probe when positive. These markers, except cCL15, also give rise to the same-sized *Not* I fragment

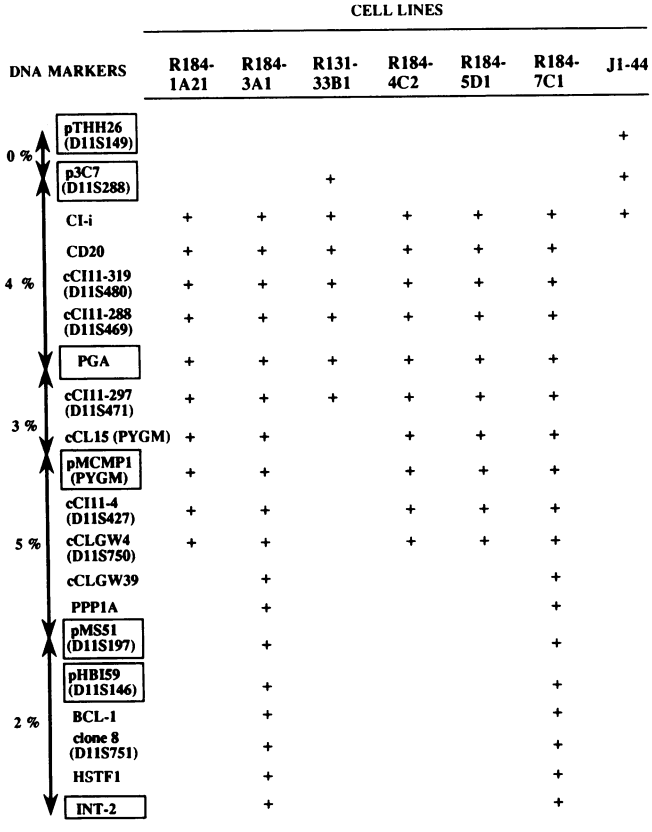

FIG. 1. Hybridization pattern obtained for DNA markers on the radiation-reduced hybrid cell panel. The cell lines are listed horizontally along the top, and the markers are listed vertically to the left. +, Human-specific restriction fragments observed for the hybrid cell DNA and genomic human DNA but not in the hamster genome. The markers have been ordered assuming continuous human DNA in the hybrids and according to linkage data when available. DNA probes within squares denote anchor markers that have been mapped previously by linkage in reference families (23). The numbers on the left indicate the published map distances in percent meiotic recombination between these markers.

whether hybridized to human genomic DNA or human DNA in cell hybrids. cCL15, on the other hand, hybridizes to a much larger fragment, 850 kb in all hybrid cell lines compared to 400 kb in human genomic DNA, possibly due to a variation in methylation status. Taken together, these PFGE analyses give no evidence for any major rearrangements in the human DNA contained in the radiation-reduced somatic cell hybrids.

When mapped onto the panel of somatic cell hybrids, several markers—for example, in the cluster of cCLGW39, PPP1A, pMS51/D11S97, pHBI59/D11S146, BCL-1, clone 8/D11S751, HSTF1, and INT-2—show identical hybridization patterns, indicating a close localization (Fig. 1). This made it impossible to order them in relation to each other by relying solely on the available somatic cell hybrids. We therefore proceeded to analyze and map the markers by PFGE.

Mapping of DNA Markers on PFGE. Seven DNA probes are marked as "anchor markers" in Figs. 1 and 2 since their gene order and map distances have been determined through linkage mapping on reference families (23). The most telomeric of these anchor markers is INT-2, located at a recombination distance of 2% telomeric to pMS51/D11S97. INT-2 is known to coamplify with BCL-1 and HSTF1 in the 11q13 amplicon found in some breast carcinoma cell lines (44), supporting the close localization of these markers. Furthermore, INT-2 and HSTF1 have been shown to be located at a distance of 35 kb apart (45). Despite this, we found INT-2 difficult to link to other markers using PFGE, due to the clustering of sites for rare-cutting restriction enzymes around this locus, giving rise mainly to small-sized bands. However, a consistent double band of 1200 kb and 1300 kb for *Nru* I joins it to a group of closely linked probes, HSTF1, BCL-1, pHBI59/D11S146, and pMS51/D11S97 (Fig. 3). Clone 8/D11S751 is a weakly hybridizing probe that hybridizes to the same fragments as HSTF1 and BCL-1 but gives a weak signal for fragments already giving a less intense signal for the more strongly hybridizing probes, HSTF1, BCL-1, pHBI59/D11S146, and pMS51/D11S97. These five markers also share a common 650-kb *Bss*HII fragment but fall into two subgroups, pMS51/D11S97 and pHBI59/D11S146 on one side, within 70 kb from each other (*Nae* I), and BCL-1, clone 8, and HSTF1 on the other side, within 150 kb from each other (*Nae* I) (Fig. 2). HSTF1, BCL-1, and pHBI59/D11S146 also share a doublet for *Mlu* I, where the larger fragment of 700 kb sometimes gives rise to an equally strong signal as seen for the shorter fragment of 625 kb, but usually it is substantially weaker (Fig. 3). This phenomenon could be explained by a variation in methylation status.

pMS51/D11S97 is a *Sau*3A–*Eco*RI DNA fragment cloned from a tandem repeat minisatellite region, highly variable but specific for 11q13 (27). The minisatellite nature of this probe may explain the presence of multiple bands of very varying intensity observed for this marker (Fig. 3), but only sizes corresponding to consistent bands are shown in the figures. pMS51/D11S97 is well linked through several fragments to pHBI59/D11S146 but is not linked with anything centromeric, except possibly to PPP1A with two weakly hybridizing fragments observed in one individual, a 250-kb *Mlu* I band and a 450-kb *Nru* I band (data not shown). Since this link has not been established in other individuals, these data are not included in the figures.

Two cosmid clones, cCLGW4/D11S750 and cCLGW39, both isolated from a library constructed from two of the radiation-reduced hybrid cell lines, R184-3A1 and R184-7C1, are linked through a common 525-kb *Mlu* I fragment. It is possible to order them by their hybridization pattern on the hybrid cell panel; cCLGW4/D11S750 has the same hybridization pattern as pMCMP1/PYGM, suggesting it is centromeric to cCLGW39, which has the same hybridization pattern as PPP1A. cCLGW4/D11S750 seems to be situated near CpG-islands since it mainly hybridizes to very small fragments for most rare-cutting enzymes. CLGW/39 is also linked to PPP1A through two *Nru* I fragments of 350 kb and 600 kb, respectively.

There is a gap between cCLGW4/D11S750 and the next group of linked probes on its centromeric side, cCI11-4/D11S427, pMCMP1/PYGM, cCL15, cCI11-297/D11S471, and PGA. Family studies have shown that markers for the *PYGM* locus, pMCMP1 and cCL15, are tightly linked to

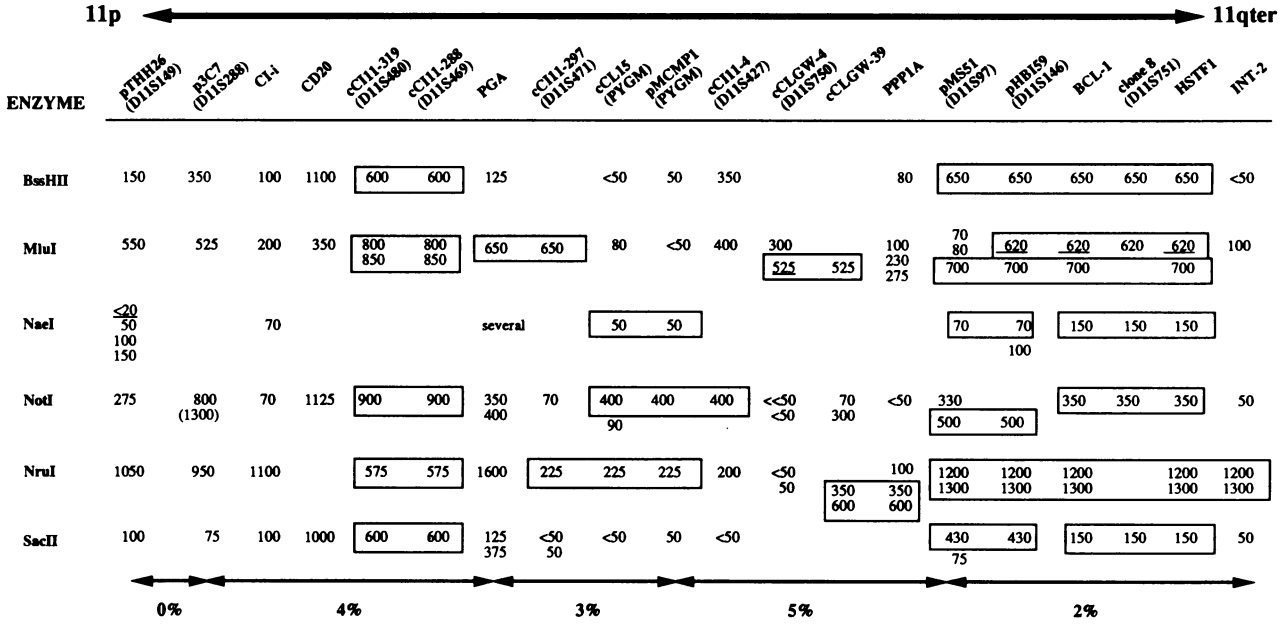

FIG. 2. Restriction fragment sizes determined by PFGE. Estimates of fragment sizes from the PFGE analyses of markers are given in kb. The markers are listed horizontally along the top, and the restriction endonucleases used are given on the left. When a probe has constantly given rise to several fragments, the more intense band has been underlined. In general, bands that have only been visible on some but not all filters have not been included. The genetic recombination frequencies between anchor markers according to Julier *et al.* (23) are shown along the bottom (see Fig. 1).

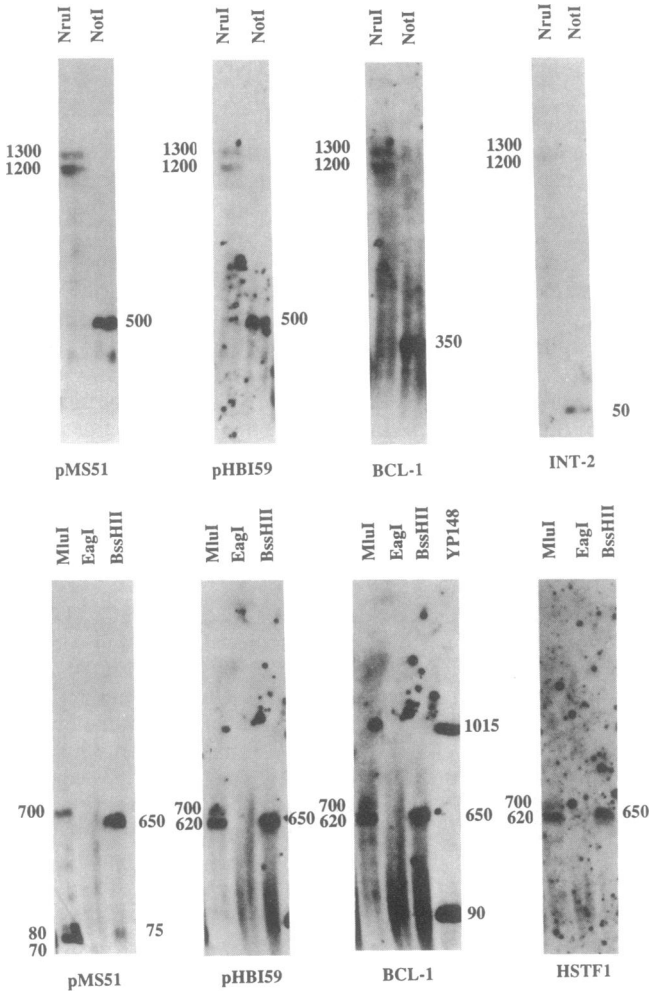

FIG. 3. Physical linkage on PFGE for DNA markers PPP1A, pMS51/D11S97, pHBI59/D11S146, BCL-1, and INT-2 or HSTF1 hybridized to the same filter. Each lane contains DNA prepared from Epstein–Barr virus-transformed lymphocytes from two unrelated individuals that has been digested to completion with the restriction endonucleases used indicated above each lane; the hybridizing probe is given below the lanes. The sizes of fragments are in kb. YP148 indicates undigested yeast DNA from the strain YP148 as size marker, with marker bands of 1025 kb and 90 kb indicated.

MEN1 with no meiotic recombinations reported (8, 35, 46), and pMCMP1/PYGM and PGA are genetically linked at a recombination distance of 3% from each other (23). cCI11-297/D11S471, with the same hybridization pattern on the cell hybrid panel as PGA, is linked to PGA also on PFGE through one 650-kb *Mlu* I fragment and to pMCMP1/PYGM and cCL15 through a 225-kb *Nru* I fragment (Fig. 4), thereby joining pMCMP1/PYGM physically with PGA, within a maximum distance of 875 kb.

The markers centromeric of PGA are ordered on the radiation-reduced hybrid panel, but few can be linked so far on PFGE, despite the relative abundance of large restriction fragments and the tight genetic linkage between these markers. There are only cCI11-288/D11S469 and cCI11319/D11S480, which are well linked on PFGE by identical fragments for most enzymes used. However, they give rise to separate polymorphisms (34). CD20 is placed on the centromeric side of these two markers, based on linkage information (35, 46). No definitely shared fragment can be identified between pTHH26/D11S149 and p3C7/D11S288. This is somewhat surprising since no recombination was found between these loci in Julier (23). However, they can be separated on the hybrid panel where pTHH26/D11S149 hybridizes only to the cell line J1-44, indicating that it is situated at the centromeric end of the spectrum, whereas p3C7/D11S288 hybridizes to cell line R185-33B1 as well (Fig. 1). The difficulties of discriminating for the centromeric region on somatic cell hybrids and the radiation-reduced hybrids make us reluctant to definitely rule on which side of the centromere these most proximal markers, pTHH26/D11S149 and p3C7/D11D288, are located.

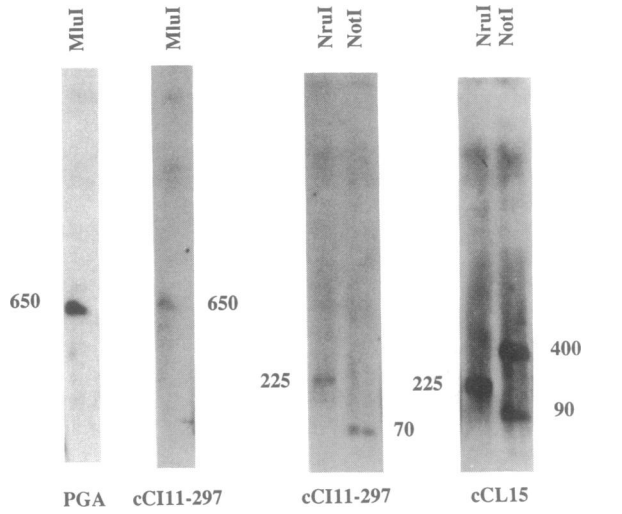

FIG. 4. Physical linkage on PFGE for DNA markers PGA, cCI11-297/D11S471, and pMCMP1/PYGM hybridized to the same filter. Restriction endonucleases used are given above each lane and the DNA markers are indicated below the lanes. The sizes of fragments are in kb.

DISCUSSION

The aim of this work has been to provide a tentative physical map of the region of chromosome 11q12-13, spanning a total genetic distance of ≈14% of meiotic recombination between pTHH26/D11S149 and INT-2 and including the *MEN1* locus. Through the use of radiation-reduced cell hybrids we have sublocalized and attempted to order 20 genetic markers within this region. Using PFGE technology, a detailed map has also been constructed, covering a total of 8 Mb. Parts of this map are noncontiguous and contain gaps of unknown size between some of the markers. Hence, we have not reached a definite estimate of the physical length of the region between THH26/D11S149 and INT-2.

However, a comparison of the genetic distance and the physical distance over the region covered by this map shows some interesting features. For the most centromeric region of the map we have pTHH26/D11S149 and p3C7/D11S288. It has not been possible to separate these markers by linkage analysis in reference families, though we have found no obvious physical linking fragments, despite some very large restriction fragments for both markers. Hence, the ratio of cM over Mb is probably low—i.e., 1 cM corresponds to >1 Mb in this region. This relationship also seems to apply to the region extending from p3C7/D11S288 toward PGA, which corresponds to a recombination distance of 4% and where most markers hybridize to several large restriction fragments, close to 1 Mb in size, but still cannot be linked on PFGE. This is in contrast to other parts of the map. PGA and pMCMP1/PYGM are at a maximum physical distance of 875 kb as mapped on PFGE, whereas the meiotic crossovers occur at a rate of 3% between them (23), which seems to suggest a relatively high recombination rate over a short physical distance, ≈300 kb/cM, a figure far from the generally assumed relationship of 1 cM = 1 Mb.

The same trend also carries through to the most telomeric part of this map. pMS51/D11S97 and INT-2 are at a recombination distance of 2% from each other (23). However, according to our PFGE data, they are at a maximum of 1200 kb apart, though considering the close physical proximity of HSTF1 with INT-2 this distance could be as little as 700 kb (45). These data suggest that, between this pair of markers, a genetic distance of 1 cM corresponds to ≈350 kb, or at the most 600 kb; the former figure is very similar to the one obtained for the distance between PGA and pMCMP1/PYGM.

We have not been able to continuously link the interval between pMCMP1/PYGM and pMS51/D11S97 on PFGE. However, assuming that the relationship of cM to Mb obtained for the flanking markers is constant over the region between these, this would correspond to a physical distance of less than half the expected 5 Mb. We can account for ≈1500 kb by adding up the sizes for the enzymes covering the longest distance, *Mlu* I, between pMCMP1/PYGM and pMS51/D11S97. Taking into consideration that the genetic location for the *MEN1* locus has been further narrowed down to be centromeric of pMS51/D11S97 in linkage studies (unpublished work, C.L., B.W., Y.N., and M.N.) and that the area of minimal deletion falls between pMCMP1/PYGM and pHBI59/D11S146 (10), our data suggest an upper limit of this tentative *MEN1* region of about 2.5 Mb, of which a greater part has already been accounted for in our physical map. This size of genomic DNA is within the limits for cloning and constructing a contig in cosmids or yeast artificial chromosomes, which would then contain the *MEN1* gene.

This work was supported by Axel and Margaret Ax:son Johnson's Fund, the Swedish Medical Research Council, the Swedish Cancer Society and Torsten och Ragnar Söderberg's Fund, the Magnus Bergvalls Fund, Barncancerfonden, National Cancer Institute Grant CA18734, and the United Kingdom Human Genome Project.

1. Schwartz, D. C. & Cantor, C. R. (1984) *Cell* **48,** 813–825.
2. Bird, A. (1986) *Nature (London)* **321,** 209–213.
3. Burmeister, M., Monaco, A. P., Gillard, E. F., van Ommen, G. J., Affara, N. A., Ferguson-Smith, M. A., Kunkel, L. M. & Lehrach, H. (1988) *Genomics* **2,** 189–202.
4. Gessler, M. & Bruns, G. A. P. (1989) *Genomics* **5,** 43–55.
5. Rose, E. A., Glaser, T., Jones, C., Smith, C. L., Lewis, W. H., Call, K. M., Minden, M., Champagne, E., Bonetta, L., Yeger, H. & Housman, D. E. (1990) *Cell* **60,** 509–520.
6. Viskochil, D., Burchberg, A. M., Xu, G., Cawthon, R. M., Stevens, J., Wolff, R. K., Culver, M., Carey, J. C., Copeland, N. G., Jenkins, N. A., White, R. & O'Connell, P. (1990) *Cell* **62,** 187–192.
7. Wermer, P. (1954) *Am. J. Med.* **16,** 363–371.
8. Larsson, C., Skogseid, B., Öberg, K., Nakamura, Y. & Nordenskjöld, M. (1988) *Nature (London)* **332,** 85–87.
9. Nakamura, Y., Larsson, C., Julier, C., Byström, C., Skogseid, B., Wells, S., Öberg, K., Carlson, M., Taggart, T., O'Connell, P., Leppert, M., Lalouel, J.-M., Nordenskjöld, M. & White, R. (1989) *Am. J. Hum. Genet.* **44,** 751–755.
10. Byström, C., Larsson, C., Blomberg, C., Sandelin, K., Falkmer, U., Skogseid, B., Öberg, K., Werner, S. & Nordenskjöld, M. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 1968–1972.
11. Friedman, E., Sakaguchi, K., Bale, A. E., Falchetti, A., Streeten, E., Zimering, M. B., Weinstein, L. S., McBride, W. E., Nakamura, Y., Brandi, M.-L., Norton, J. A., Auerbach, G. D., Spiegel, A. M. & Marx, S. J. (1989) *N. Engl. J. Med.* **321,** 213–218.
12. Thakker, R. V., Bouloux, P., Wooding, C., Chotai, K., Broad, P., Spurr, N. K., Besser, G. M. & O'Riordan, J. L. H. (1989) *N. Engl. J. Med.* **321,** 218–224.
13. Knudson, A. G. (1971) *Proc. Natl. Acad. Sci. USA* **68,** 820–823.
14. Yunis, J. J. (1983) *Science* **221,** 227–236.
15. Melo, J. V., Brito-Babapulle, V., Foroni, L., Robinson, D. S. F., Luzzatto, L. & Catovsky, D. (1986) *Int. J. Cancer.* **38,** 531–538.
16. Rabbitts, P. H., Douglas, J., Fischer, P., Nacheva, E., Karpas, A., Catovsky, D., Melo, J. V., Baer, R., Stinson, M. A. & Rabbitts, T. H. (1988) *Oncogene* **3,** 99–103.
17. Yunis, J. J. & Soreng, A. L. (1984) *Science* **226,** 1199–1204.
18. van den Berghe, H., Vermaelen, K., Louwagie, A., Criel, A., Mecucci, C. & Vaerman, J.-P. (1984) *Cancer Genet. Cytogenet.* **11,** 381–387.
19. Tsujimoto, Y., Jaffe, E., Cossman, J., Gorham, J., Nowell, P. C. & Croce, C. M. (1985) *Nature (London)* **315,** 340–343.
20. Casey, G., Smith, R., McGillivray, D., Peters, G. & Dickson, C. (1986) *Mol. Cell. Biol.* **6,** 502–510.
21. Taira, M., Yoshida, T., Miyagawa, K., Sakamoto, H., Terada, M. & Sugimura, T. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 2980–2992.
22. Cookson, W. O. C., Sharp, P. A., Faux, J. A. & Hopkin, J. M. (1989) *Lancet* **i,** 1292–1294.
23. Julier, C., Nakamura, Y., Lathrop, M., O'Connell, P., Leppert, M., Litt, M., Mohandas, T., Lalouel, J.-M. & White, R. (1990) *Genomics* **7,** 335–345.
24. Bock, S. C., Skriver, K., Nielsen, E., Thøgersen, H.-C., Wiman, B., Donaldson, V. H., Eddy, R. L., Marrinan, J., Radziejewska, E., Huber, R., Shows, T. B. & Magnusson, S. (1986) *Biochemistry* **25,** 4292–4301.
25. Tedder, T. F., Streuli, M., Schlossman, S. F. & Saito, H. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 208–212.
26. Barker, H. M., Jones, T. A., da Cruz e Silva, E. F., Spurr, N. K., Sheer, D. & Cohen, P. T. W. (1990) *Genomics* **7,** 159–166.
27. Jeffreys, A. J., Wilson, V., Neumann, R. & Keyte, J. (1988) *Nucleic Acids Res.* **16,** 10953–10971.
28. Leach, R. & White, R. (1984) *Cytogenet. Cell Genet.* **37,** 521.
29. Taggart, R. T., Mohandas, T. K., Shows, T. B. & Bell, G. I. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 6240–6244.
30. Nakamura, Y., Gillian, S., O'Connell, P., Leppert, M., Lathrop, G. M., Lalouel, J.-M. & White, R. (1988) *Nucleic Acids Res.* **16,** 376.
31. Burke, J., Hwang, P., Anderson, L., Lebo, R., Gorin, F. & Fletterick, R. (1987) *Proteins* **2,** 177–187.
32. Carlson, M., Nakamura, Y., Gillian, S., O'Connell, P., Lebo, R., Gorin, F., Lathrop, G. M., Lalouel, J.-M. & White, R. (1988) *Nucleic Acids Res.* **16,** 10403.
33. Holm, T., Nakamura, Y., Ballard, L., O'Connell, P., Leppert, M., Lathrop, G. M., Lalouel, J.-M. & White, R. (1988) *Nucleic Acids Res.* **16,** 4746.
34. Tokino, T., Takahashi, E., Mori, M., Tanigami, A., Glaser, T., Park, J. W., Jones, C., Hori, T. & Nakamura, Y. (1991) *Am. J. Hum. Genet.* **48,** 258–268.
35. Larsson, C., Weber, G., Kvanta, E., Lewis, K., Janson, M., Jones, C., Glaser, T., Evans, G. & Nordenskjöld, M. (1991) *Hum. Genet.*, in press..
36. Hammarström, K., Westin, G., Bark, C., Zabielski, J. & Petterson, U. (1984) *J. Mol. Biol.* **179,** 157–169.
37. Glaser, T., Housman, D., Lewis, W. H., Gerhard, D. & Jones, C. (1990) *Somatic Cell Mol. Genet.* **15,** 477–501.
38. Glaser, T., Rose, E., Morse, H., Housman, D. & Jones, C. (1990) *Genomics* **6,** 48–64.
39. Herrmann, B. G., Barlow, D. P. & Lehrach, H. (1987) *Cell* **48,** 813–825.
40. Jones, C. P., Janson, M. & Nordenskjöld, M. (1989) *Techniques* **1,** 90–95.
41. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
42. Church, G. M. & Gilbert, W. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1991–1995.
43. Litt, M. & White, R. L. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 6206–6210.
44. Lafage, M., Nguyen, C., Szepetowski, P., Pébusque, M.-J., Simonetti, J., Courtois, G., Gaudray, P., deLapeyriere, O., Jordan, B. & Birnbaum, D. (1990) *Genes Chrom. Cancer* **2,** 171–181.
45. Nguyen, C., Roux, D., Mattei, M. G., deLapeyiere, O., Goldfarb, M., Birnbaum, D. & Jordan, B. (1988) *Oncogene* **3,** 703–708.