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Physical Mapping of the von Recklinghausen Neurofibromatosis Region on Chromosome 17

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Summary

The von Recklinghausen neurofibromatosis (NF1) locus has been linked to chromosome 17, and recent linkage analyses (see accompanying papers in this issue) place the gene on the proximal long arm. NF1 probably resides in 17q11.2, since two unrelated NF1 patients have been identified who possess constitutional reciprocal translocations involving 17q11.2 with chromosomes 1 and 22. We have used a somatic-cell hybrid from the t(17;22) individual, along with other hybrid cell lines, to order probes around the NF1 locus. An additional probe, 17L1, has been isolated from a *NotI* linking library made from flow-sorted chromosome 17 material and has been mapped to a region immediately proximal to the translocation breakpoint. While neither NF1 translocation breakpoint has yet been identified by pulsed-field gel analysis, an overlap between two probes, EW206 and EW207, has been detected. Furthermore, we have identified the breakpoint in a non-NF1 translocation, SP-3, on the proximal side of the NF1 locus. This breakpoint has been helpful in creating a 1,000-kb pulsed-field map, which includes the closely linked NF1 probes HHH202 and TH17.19. The combined somatic-cell hybrid and pulsed-field gel analysis we report here favors the probe order D17Z1-HHH202-TH17.19-CRYB1–17L1-NF1-(EW206, EW207, EW203, L581, L946)-(ERBB2, ERBA1). The agreement in probe ordering between linkage analysis and physical mapping is excellent, and the availability of translocation breakpoints in NF1 should now greatly assist the cloning of this locus.

Introduction

Von Recklinghausen neurofibromatosis (NF1) is an autosomal dominant disorder characterized by neurofibromas, cafe-au-lait spots, and Lisch nodules (Crowe et al. 1956; Riccardi 1981). Affected individuals are also at a higher risk for certain types of malignancy, especially neurofibrosarcoma (Riccardi and Eichner 1986). Recently, the locus for NF1 was mapped to chromosome 17 (Barker et al. 1987; Seizinger et al. 1987), and subsequent linkage analyses now assign the gene to the proximal long arm (see accompanying papers in this issue). In addition, two unrelated NF1 patients have been identified in whom constitutional reciprocal translocations have occurred involving 17q11.2, in one instance with chromosome 1 (Schmidt et al. 1987) and in the other with chromosome 22 (Ledbetter et al. 1989). The locus for NF1 is therefore thought to reside at or near these translocation breakpoints.

The mutation rate for NF1 is unusually high, about 10^{-4}/generation, and approximately 30%–50% of affected individuals represent new mutations (Riccardi and Eichner 1986). This high mutation rate could be explained by locus heterogeneity or could indicate that the NF1 gene is very large or easily mutated. To date,

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no evidence of locus heterogeneity exists; a large number of families have been analyzed in linkage studies, and no evidence of nonlinkage to 17q has been found. The identification of two unrelated affected individuals with balanced translocations breaking within the NF1 gene would tend to support the notion of a large or unusually fragile locus.

Pulsed-field gel electrophoresis (PFGE) is useful in the identification of gross DNA rearrangements by permitting the visualization of up to 10 Mb of continuous DNA (Smith and Cantor 1987). For example, PFGE has been used to identify large chromosomal rearrangements involving the Duchenne muscular dystrophy (DMD) and retinoblastoma (Rb) genes (den Dunnen et al. 1987; Goddard et al. 1988). In DMD, X-autosomal translocations in females with DMD and a male with a deletion of Xp21 played an instrumental role in cloning the gene (Monaco et al. 1986), and it is now clear that many affected males have large deletions of the DMD locus. Similarly, somatic and germ-line rearrangements found in Rb-affected individuals were instrumental in the identification of this gene. PFGE has also been used to identify the breakpoint in somatic-cell chromosomal rearrangements such as Philadelphia chromosome–positive acute lymphoblastic leukemia (ALL) (Rubin et al. 1988). Therefore, it is highly appropriate to apply PFGE analysis to the identification of the two NF1 translocation breakpoints and to the cloning of the NF1 gene.

Somatic-cell hybrids have been helpful in localization of probes that map to the general NF1 region on chromosome 17. A large number of somatic-cell hybrids have been constructed by using naturally occurring translocations, deletions, or other aberrations involving chromosome 17 (Fain et al. 1987; vanTuinen et al. 1987). These hybrids are thought to contain simple translocations or deletions. In addition to the panel of hybrids already published, two new somatic-cell hybrids have been produced from the t(17;22) NF1 cell line (Ledbetter et al. 1989): NF13 contains the der(22) translocation chromosome, and NF9 contains both the der(17) and der(22) chromosomes. The t(17;22) translocation breakpoint can thus be visualized by PFGE from both the proximal and distal sides by using NF9 and NF13, respectively. Furthermore, the NF13 hybrid provides a convenient means of ordering probes relative to the t(17;22) breakpoint assumed to be the site of the NF1 locus.

An additional panel of reduced chromosome 17 hybrids has been produced by Leach and Fournier using microcell fusion techniques (O'Connell et al. 1989). These hybrids contain differing amounts of the centromeric region of chromosome 17 in addition to a retrovirally *neo*-tagged distal segment of 17q around the thymidine kinase (TK) locus (17q21–25), with no other human chromosomal material. We have determined that at least one of these lines, 7AE-11, contains probes that map just proximal and just distal to the NF1 gene and should, therefore, also contain the NF1 locus.

Material and Methods

DNA Probes

DNA clones used in this study are listed in table 1. Single-copy DNA fragments were isolated in 1.5% LMT agarose (SeaPlaque;® FMC) before use as probes on Southern blots. 17L1 is a clone isolated in this laboratory from a flow-sorted chromosome 17 *Not*I linking library (Wallace et al., submitted).

Somatic-Cell Hybrids and Cell Lines

A panel of cell hybrids is listed in table 2, and several of these are depicted in figure 1. P12.3B, MH-74, LS-1, and SP-3 were described previously by vanTuinen et al. (1987). P12.3B contains the der(17) chromosome from a t(15;17)(q22;q11.2–12) cell line isolated from a patient with acute promyelocytic leukemia (APL), MH-74 contains a ring(17)(p13.3q25.3), LS-1 contains an isochromosome 17q, and SP-3 contains the der(15) from a t(15;17)(q22;q11.2) which originally arose in an individual referred to as Spain. The Spain parental fibroblast strain was also used in these studies.

Additional reduced chromosome 17 hybrids (the "7A" series) have been made using microcell fusion techniques and are described by O'Connell et al. (1989). Most of these rat-human hybrids retain varying amounts of the centromeric region of human chromosome 17 along with a *neo*-tagged region near the TK locus (17q21–25). Of nine such lines studied, 7AE-11, 7AE-23, 7AD-8, and 7AD-11 are the hybrids of interest for mapping the NF1 region on chromosome 17.

Other cell lines and hybrids used in the present study are Gus5824B, 11405, NF13, and NF9. Gus5824B is an Epstein-Barr virus (EBV)—lymphoblastoid cell line derived from the t(1;17)(p34.3;q11.2) NF1 patient, originally described by Schmidt et al. (1987). 11405 is an EBV-transformed lymphoblastoid cell line which harbors the t(17;22)(q11.2;q11.2) NF1 translocation (Ledbetter et al. 1989). Two somatic-cell hybrids, NF13 and NF9, have been constructed from this parental cell line (Ledbetter et al. 1989). NF13 contains the der(22) trans-

Table 1

Single-Copy[a] DNA Fragments Used as Probes

Clone	Locus	Fragment(s)	Reference
A10-41	D17S71	Uncut	Barker et al. 1987
17H8	D17Z1	2.7-kb *Eco*RI	Willard et al. 1986
HHH202	D17S33	1.4-kb *Bam*HI/*Pst*I	Nakamura et al. 1987
TH17.19		1.3-kb *Hin*dIII	O'Connell et al. 1989
pUC14A1	CRYB1	2.35-kb *Hin*dIII	Law et al. 1986
17L1(A)[b]		.8-kb *Xho*I/*Not*I	Wallace et al., submitted
17L1(B)[b]		.7-kb *Not*I/*Pvu*II	Wallace et al., submitted
EW206	D17S57	5.0-kb *Eco*RI	Fain et al. 1987
EW207	D17S73	3.2-kb *Eco*RI	Fain et al. 1987
EW203	D17S54	1.8-kb *Eco*RI/*Xba*I	Fain et al. 1987
CRI-L581	D17S36	5.0, 6.8-kb *Eco*RI	Stephens et al. 1987
CRI-L946	D17S37	5.5, 6.8-kb *Eco*RI	Stephens et al. 1987
CER204	NGL (ERBB2)	1.4-kb *Eco*RI/*Pvu*II	Yamamoto et al. 1986
he-A1	ERBA1	2.9-kb *Eco*RI/*Hin*dIII	Rider et al. 1987
BS3	HOX2	5.6-kb *Eco*RI	Murphy et al. 1987
pGEM1-PP	PPY	.4-kb *Bam*HI/*Hin*dIII	Takeuchi and Yamada 1985
9TAU	MTBT1 (tau)	2.9-kb *Eco*RI	Neve et al. 1986

NOTE.—Locus identifications are those of Human Gene Mapping 9.

[a] Do not require competitive hybridizations using human placental DNA.

[b] 17L1(A) and 17L1(B) were used as two independent probes of the *Not*I linking clone 17L1; they are physically separated from each other by a *Not*I restriction site.

Table 2

Probe Locations in Somatic Cell Hybrids

	Hybrid									
Probes	P12.3B	MH-74	LS-1	SP-3	7AE-11	7AE-23	7AD-8	7AD-11	NF13	NF9
A10-41	ND	+	ND	ND	−	−	−	−	ND	ND
D17Z1	+	+	+	−	+	+	+	−	ND	ND
HHH202	+	+	+	+	+	+	+	−	−	+
TH17.19	+	+	+	+	+	+	+	−	−	+
CRYB1	+	ND	+	+	+	+	−	−	−	+
17L1	+	+	+	+	+	−	−	+	−	+
EW206	+	+	+	+	+	−	−	−	+	+
EW207	+	+	+	+	+	−	−	−	+	+
EW203	+	+	+	+	+	−	−	−	+	ND
CRI-L581	+	+	+	+	+	−	−	−	+	ND
CRI-L946	+	+	+	+	+	−	−	−	+	+
ERBB2	+	+	+	+	−	−	−	−	+	ND
ERBA1	+	ND	+	+	−	−	−	−	+	ND
HOX2	−	+	+	+	−	−	−	−	+	ND
PPY	−	+	+	+	−	ND	ND	ND	ND	ND
TAU	−	+	+	+	−	+	−	−	+	ND

NOTE.—The order of probes as listed is from 17p to 17q (with D17Z1 at the centromere) and was determined using physical mapping data reported in this work. Linkage information (Goldgar et al. 1989) was used, however, to order the probes EW206, EW207, EW203, CRI-L581, and CRI-L946. The order of ERBA1 and ERBB2 is not established here, nor is the order of the last three probes. A plus sign (+) denotes presence of probe in hybrid; a minus sign (−) denotes absence of probe in hybrid. ND = not determined.

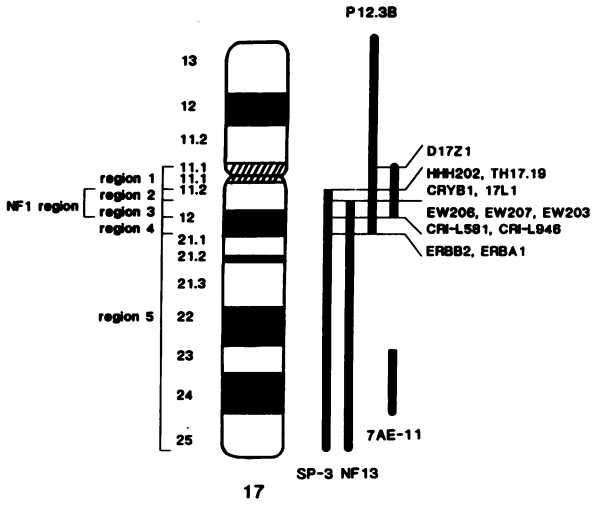

Figure 1 The segments of human chromosome 17 present in four somatic-cell hybrids are shown in this schematic. SP-3 is the Spain translocation hybrid, NF13 is the der(22) hybrid derived from the t(17;22) NF1 patient, P12.3B is the APL translocation hybrid, and 7AE-11 is a reduced chromosome 17 hybrid. The endpoints of these segments allow 17q to be divided into five regions, with the NF1 gene assumed to be within the span of regions 2 and 3, on the basis of the t(17;22) breakpoint. Probes were localized to these regions by their presence or absence in each hybrid. Those probes found to be in the vicinity of the NF1 locus are shown in their assigned regions.

location chromosome, and NF9 contains both translocation chromosomes.

Twenty EBV-transformed lymphoblastoid cell lines were established from unrelated NF1 patients and used in these studies. Several control lymphoblastoid cell lines and skin fibroblast strains were also analyzed by PFGE for comparison.

All lymphoblastoid cell lines were maintained in RPMI medium (Hazelton) supplemented with 15% FBS (Gibco). Reduced chromosome 17 hybrid cell lines (the "7A" series) were maintained in 50% nutrient mixture F12 (HAM; Gibco) and 50% Dulbecco's modified Eagle medium (DMEM; Gibco) supplemented with 10% FBS and 400 μg active geneticin/ml (G418; Gibco). All other hybrid cell lines and fibroblast strains were maintained in either minimal essential medium (MEM; Gibco) or DMEM supplemented with 15% FBS. Hypoxanthine aminopterin thymidine (HAT; Hazelton) was added to the medium where needed in order to maintain human chromosome 17 in the hybrid lines.

PFGE

DNA blocks were made as described elsewhere (Drumm et al. 1988). In brief, cells were centrifuged at 800 rpm for 5 min, rinsed once with PBS, and resuspended in PBS at a concentration of 3×10^7 cells/ml. An equal volume of 2% LMT agarose (SeaPlaque;® FMC) was added to the cell suspension at 50 C. This mixture was then pipetted into a mold, and the resultant 0.1-ml cell blocks were suspended in a lysis solution consisting of 0.5 M EDTA, pH 8.0, with 1% *N*-lauroylsarcosine (Sigma). Proteinase K (BRL) was added at a final concentration of 1 mg/ml, and incubation took place for 2 h at 50 C. An additional aliquot of proteinase K was added to the suspension, and digestion was continued overnight at 50 C. Lysis solution was decanted, and proteinase K was inactivated by two washes of 1 mM phenylmethyl sulfonylfluoride (PMSF) in TE (10 mM Tris, pH 8.0, 1 mM EDTA) at room temperature, each for 2 h. Three additional TE washes were performed before the blocks were stored at 4 C.

Half blocks (equivalent to 5 μg of DNA) were digested for 3–5 h, at 37 C in 0.3 ml of the appropriate buffer by using 30 U of the appropriate enzyme for complete digests and 5 U or less for partials. Blocks were loaded into 1% agarose gels made with 0.5 × TBE (Tris-borate-EDTA; Maniatis et al. 1982). Field-inversion gel electrophoresis (Carle et al. 1986) was performed in 0.5 × TBE for 65 h using a programmable time ramp (DNAstar, Madison, WI). The range of forward pulse times was 3–210 s, and that of reverse pulse times was 1–70 s, with a constant ratio of 3:1 for forward to reverse pulse times. Gels were stained with ethidium bromide and UV-nicked on a 254-nm light box for 2.5 min.

Conventional Electrophoresis

Liquid genomic DNA samples from the hybrid cell lines and appropriate controls were prepared by isolation and lysis of the nuclei, followed by a proteinase treatment and precipitation (Thompson and Neiman 1987). Ten micrograms of each DNA sample were digested to completion, and the digested DNAs were electrophoresed through a 1% agarose gel in 1 × TBE.

Southern Blotting and Hybridization Techniques

Electrophoresed gels were denatured in 0.25 M NaOH, 1.5 M NaCl and then were neutralized with 1 M Tris, 1.5 M NaCl, pH 7.5. Overnight transfer to Hybond® (Amersham) was done in 10 × SSC. Hybond® filters were briefly rinsed in 2 × SSC and air-dried overnight. DNA was UV-cross-linked to filters by using a 330-nm light box for 1.5 min.

Prehybridizations and hybridizations were performed in 1 M NaCl, 10% dextran sulfate, 1% SDS, and 1 × Denhardt's (Maniatis et al. 1982). Prehybridizations

were routinely performed overnight. Probes were labeled overnight using the random priming method of Feinberg and Vogelstein (1983). Probes were run over a Sephadex® G50 (Sigma) column and boiled with 10 mg of sheared salmon DNA before being added to filters in hybridization solution. After 16–24 h hybridization, filters were washed to a final stringency of 0.1 × SSC, 1% SDS at 65 C for 15 min. Blots were routinely exposed on XAR-5 film (Kodak) for 1–3 days.

Results

Sublocalization of Chromosome 17 Probes by Using Somatic-Cell Hybrids

Figure 1 depicts the locations of probes, using four hybrids that divide 17q into five definable regions: (1) the first region extends from the centromere to the SP-3 ("Spain") breakpoint; (2) the second region begins at the SP-3 breakpoint and ends at the NF13 breakpoint; (3) the third region extends from the NF13 breakpoint to the distal end of the 7AE-11 centromeric fragment; (4) the fourth region begins at the end of this 7AE-11 segment and ends at the APL translocation in P12.3B; and (5) the fifth region, which is quite large, includes the rest of 17q distal to the APL breakpoint. The placement of the distal end of the 7AE-11 segment is based upon the observation that it contains some probes distal to the NF13 breakpoint (EW206, etc.) but not all of those present in P12.3B (it lacks ERBB2 and ERBA1). The area denoted as the NF1 region includes the second and third intervals which flank the t(17;22) NF1 breakpoint on chromosome 17. Probes are listed in a tentative order from proximal 17p to distal 17q (table 2), though a precise order cannot always be determined for probes that map within the same region. In some instances, however, linkage analysis allows us to infer their order (such as in region 3; see accompanying papers in this issue). In region 2, the placement of β-crystallin (CRYB1) between TH17.19 and 17L1 is based on hybrids 7AD-8 and 7AE-23. If either of these hybrids contains a rearranged segment of DNA around the centromere of 17, the ordering of these probes could be incorrect. ERBA1 and ERBB2 map distal to the end of 7AE-11, and, therefore, distal to the NF1 region. The probes HOX2, PPY, and TAU map telomeric to the APL breakpoint; their presence in some of the "7A" hybrids must therefore be due to the additional distal 17q segment.

PFGE Analysis of NF1 Patients by Using Chromosome 17 Probes

PFGE analysis was performed using DNAs obtained from 20 unrelated individuals affected with NF1, including the t(1;17) and t(17;22) patients. DNAs from unaffected individuals were used as normal controls. Care was taken to compare DNA from similar cell types, since methylation differences between cell lines can influence digest patterns. A summary of pulsed-field fragment sizes and enzymes used for this analysis is shown in figure 2. The probes D17Z1, HHH202, TH17.19, CRI-L946, and ERBB2 were used extensively in this study, and no reproducible differences were found between NF1 individuals and normal controls. In one instance, two NF1 patients showed a novel restriction fragment with the CRI-L946 probe, but this difference was not apparent in peripheral blood leukocytes from the same patients, suggesting that this was either a clonal methylation difference in the EBV-transformed lymphoblasts or an EBV-induced mutation. None of these probes detected either one of the NF1 translocations.

Two sets of probes could be overlapped using PFGE analysis. TH17.19 and HHH202 hybridized to the same pulsed-field fragments for all enzymes tested except *Sfi*I and *Xho*I; HHH202 detected a 50-kb *Sfi*I fragment

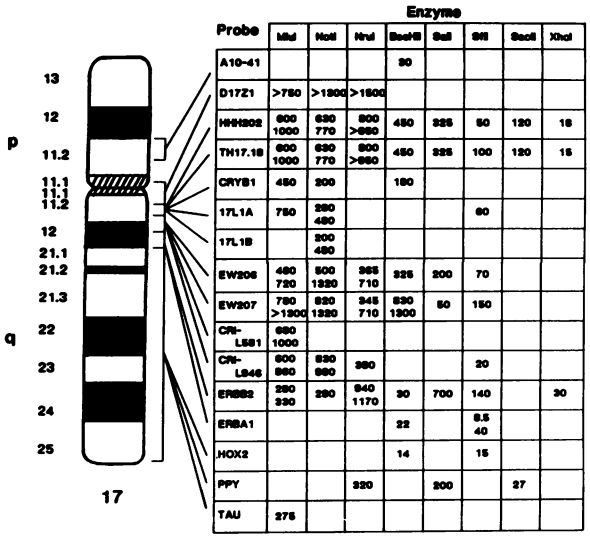

Figure 2 Chart of pulsed-field fragments detected by chromosome 17 probes. Sizes of fragments (in kb) were determined by yeast chromosome markers. A greater-than sign (>) indicates that the fragment was difficult to size accurately using field-inversion techniques or that the fragment size varied owing to RFLP differences between individuals (as with the probe D17Z1); in the latter case, the smallest fragment size is indicated. Boxes with two entries show sizes of primary and of most commonly seen partial digest fragments. The probes are listed (top to bottom) in tentative order from pter to qter with their approximate locations indicated on chromosome 17.

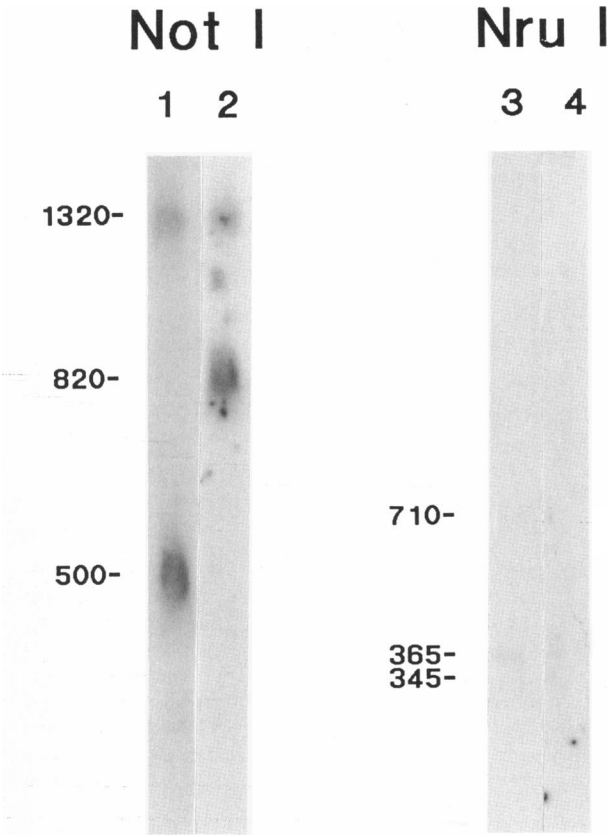

Figure 3 Southern blot analysis of representative DNAs (5 μg each) digested with *Not*I and *Nru*I and separated by PFGE. The filter was probed with EW206 (lanes 1 and 3), stripped, and then reprobed with EW207 (lanes 2 and 4). While both probes detect different primary pulsed-field fragments, they hybridize to the same partial digest fragments of 1,320 kb for *Not*I and 710 kb for *Nru*I.

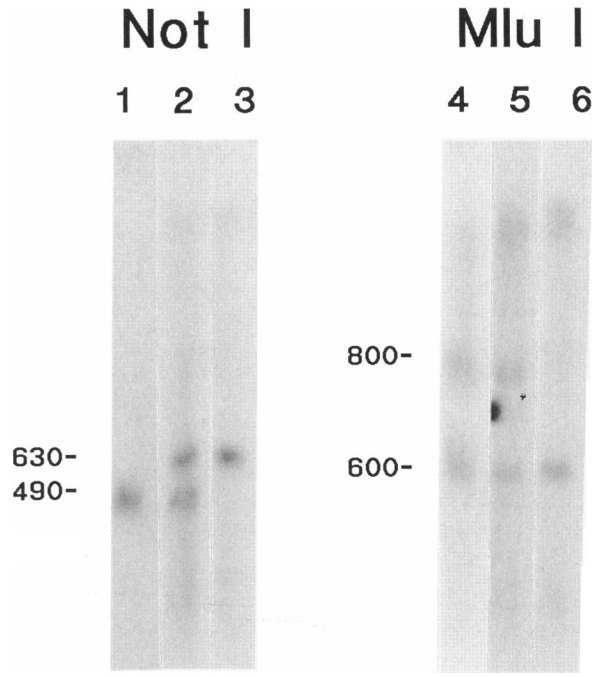

Figure 4 Southern blot analysis of *Not*I and *Mlu*I digests of SP-3 (lanes 1 and 4), Spain parental fibroblast (lanes 2 and 5), and control fibroblast (lanes 3 and 6) DNAs probed with HHH202. DNA (5 μg) was separated by PFGE, and fragment sizes (in kb) are indicated. A novel *Not*I fragment of 490 kb and a novel partial *Mlu*I fragment of 800 kb arise from the Spain translocation chromosome.

while TH17.19 hybridized to a larger fragment of 100 kb. This finding was not unexpected, since TH17.19 was isolated from a library made from the 630-kb *Not*I fragment that hybridizes with HHH202 (O'Connell et al. 1989). An additional physical overlap was seen with EW206 and EW207. These two probes hybridized to the same partial *Not*I and *Nru*I fragments of 1,320 kb and 710 kb, respectively (fig. 3).

Detection of the Spain Translocation Breakpoint

The Spain breakpoint was identified using PFGE analysis and the probe HHH202 (fig. 4). We detected a novel *Not*I fragment of 490 kb in both SP-3 and Spain parental digests (fig. 4, lanes 1 and 2) but not in a control *Not*I digest (fig. 4, lane 3). The *Not*I fragment seen with HHH202 in all other DNAs tested was approximately 630 kb. In addition, the partial digest *Mlu*I fragment of 800 kb seen in the SP-3 and Spain parental digests was also unique (fig. 4, lanes 4 and 5). When this same blot was stripped and reprobed with EW206, identically sized fragments were seen with the Spain parental DNA and the control DNA (data not shown), indicating that the *Not*I doublet seen with HHH202 in lane 2 of figure 4 was not produced by an artifact in gel migration. Definite fragment size differences were also seen between SP-3 and control DNAs in *Bss*HII and *Sal*I digests probed with HHH202 (data not shown).

PFGE Map of the HHH202/TH17.19 Region on Chromosome 17

A map of the proximal side of the NF13 breakpoint was constructed using the probes HHH202 and TH17.19 (fig. 5). Partial digest fragments were oriented with respect to the Spain breakpoint by comparing SP-3 and control DNA digests (data not shown). Pulsed-field fragments shared by both these DNAs must reside distal to the breakpoint, whereas those that were not shared must reside proximal to the Spain translocation.

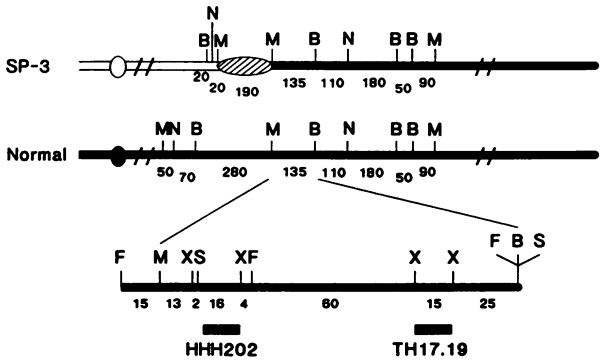

Figure 5 Pulsed-field map of the centromeric side of the NF1 region, showing the Spain translocation breakpoint. The t(15;17) chromosome from SP-3 and a normal chromosome 17 are portrayed. The black regions represent chromosome 17 DNA while the white region depicts DNA from chromosome 15, with centromeres shown as circles and the p arm to the left. The striped oval area on the SP-3 chromosome designates the zone where the t(15;17) breakpoint resides. Enzyme sites are marked by vertical lines, and designations are as follows: B = *Bss*HII; M = *Mlu*I; N = *Not*I; F = *Sfi*I; X=*Xho*I; and S = *Sac*II. Restriction-fragment sizes (in kb) are indicated beneath the chromosomes.

The probe HHH202 is no more than 220 kb distal to the Spain breakpoint and can be used to visualize 530 kb of distal 17q11.2 in the direction of the NF13 breakpoint. The TH17.19 probe is found to be located 60–90 kb distal to HHH202, on the basis of the results of single- and double-digest PFGE blots (data not shown).

Discussion

The emergence of "reverse genetics" (Ruddle 1984, Orkin 1986) as a viable approach to the cloning of human disease genes whose normal functions and protein products are unknown has added a new dimension to human molecular genetics. Indeed, the DMD (Monaco et al. 1986), chronic granulomatous disease (Royer-Pokora et al. 1986), and Rb (Friend et al. 1986) genes have already been cloned by this approach, and the identification of the cystic fibrosis gene appears to be close at hand (Estivill et al. 1987; Drumm et al. 1988; Rommens et al. 1988). Thus, it is now appropriate to approach the cloning of the NF1 gene in a similar fashion. In this regard, the identification of two NF1 patients with cytogenetic abnormalities of 17q11.2 is of crucial importance, since these breakpoints should provide discrete signposts indicating the location of the NF1 gene.

In this report we have utilized a panel of somatic-cell hybrids which divide 17q into five distinct intervals (fig. 1), and we have placed DNA probes known to be closely linked to NF1 (see accompanying papers in this issue) within these intervals. Region 2 can be additionally subdivided into three intervals by using hybrids 7AD-8 and 7AE-23. The agreement between the order obtained by hybrid panel mapping and the order obtained by linkage analysis is excellent and adds credibility to the validity of both approaches. In some instances linkage analysis is better able to determine the order of probes that map to a single region (e.g., region 3 in figure 1), whereas in other situations hybrid analysis can successfully order probes that are currently poorly polymorphic (such as ERBA1) or not polymorphic at all (such as CRYB1, ERBB2, and 17L1); thus, the physical and genetic approaches are truly complementary. A particularly useful hybrid is NF13, since it allows any probe to be placed centromeric or telomeric to the NF1 gene, if it is assumed that the NF13 breakpoint actually falls within the disease locus. This cell line can allow NF1 to be placed on any multipoint genetic map, even if that map is derived solely from reference pedigrees.

The data presented here argue for exclusion of the following three possible NF1 candidate genes: (1) The oncogene ERBB2 (NGL), also denoted *neu* or HER-2, was first cloned from a rat glioblastoma by using an NIH3T3 cell transfection assay (Shih et al. 1981) and thus would seem an attractive candidate gene for a disease characterized by neuronal neoplasms. The hybrid mapping data clearly shows, however, that ERBB2 is too far distal if we assume that the 7AE-11 proximal 17q segment is continuous. Furthermore, no rearrangements of this locus have been identified by PFGE analysis of NF1 patient DNAs (J. W. Fountain and P. O'Connell, unpublished data). ERBB2 does not detect any RFLPs and has, therefore, never been used in linkage studies. (2) Another oncogene, ERBA1, is apparently ruled out for similar reasons, since it maps to the same region as ERBB2. A previous report (Seizinger et al. 1987) had also excluded linkage of NF1 and ERBA1 at a distance less than about 4 cM, suggesting that ERBA1 is not the NF1 gene. (3) The possibility that NF1 might be a disease of the centromere itself (Mulvihill and Parry 1987) is also apparently excluded. There is still a possibility, however, that the NF1 locus is extremely large. Determination of the translocation breakpoint from the as yet poorly characterized t(1;17) cell line should help to clarify this issue.

We have also presented here initial segments of a PFGE map of the NF1 region. The amount of DNA in this region of 17q11.2-q12 is estimated to be about

8–15 Mb, on the basis of both cytogenetic analysis and adding up of the pulsed-field fragments visualized on probing 7AE-11 DNA with human repetitive sequences (J. W. Fountain, unpublished data). With current probes, about 4–6 Mb of this region can be visualized on PFGE blots (fig. 2), which means that only a few more probes may be needed to build a complete map. For this purpose we are constructing *Not*I linking libraries from the 7AE-11 cell line and from flow-sorted chromosome 17 material. *Not*I linking clones, such as 17L1, are contiguous fragments of DNA containing a *Not*I restriction site (Poustka and Lehrach 1986; Smith et al. 1987). These have the advantage of detecting two adjacent *Not*I fragments on a PFGE blot, which greatly assists construction of a map. *Not*I linking clones may also prove useful in identifying the NF1 locus, since they usually reside in hypomethylated CG-rich regions which often mark the location of expressed genes (Bird 1986).

Though the number of probes is currently insufficient to build a complete map, we have determined that HHH202 and TH17.19 lie less than 100 kb apart (not surprising in view of the cloning strategy for TH17.19; O'Connell et al. 1989), with TH17.19 slightly closer to NF1. We have also deduced that EW206 and EW207 lie within 710 kb of each other (fig. 3). This latter result is in agreement with linkage analysis results, which have uncovered only rare crossovers between EW206 and EW207 in a large number of informative meioses (Goldgar et al. 1989).

Finally, we have also succeeded in identifying the Spain translocation breakpoint (not associated with NF1) and have shown that it lies within 220 kb of the HHH202 probe. If such a conclusion is to be drawn from PFGE analysis, it is crucial to be certain that a novel fragment arising from the translocation chromosome is not due to an RFLP or a methylation difference. The fact that, with three different enzymes, abnormal fragments are seen in both the parental diploid and the SP-3 hybrid line adds considerable strength to the conclusion. The placement of the Spain breakpoint on the map serves an additional useful purpose, as it allows partial digest fragments to be assigned proximal or distal to this point (fig. 5).

The next immediate goal of the physical mapping approach is to identify the translocation breakpoints in the t(1;17) and t(17;22) NF1 patients. From the mapping efforts around HHH202 (fig. 5), we can deduce that the NF13 breakpoint must lie at least 530 kb distal to this probe. The generation of additional probes in the NF1 region, together with judicious application of complete and partial digest PFGE analysis, should define the precise location of these breakpoints and, by direct implication, the location of the NF1 gene itself.

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