Am. J. Hum. Genet. 44:20–24, 1989

Precise Localization of NF1 to 17q11.2 by Balanced Translocation

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Summary

A female patient is described with von Recklinghausen neurofibromatosis (NF1) in association with a balanced translocation between chromosome 17 and 22 [46,XX,t(17;22)(q11.2;q11.2)]. The breakpoint in chromosome 17 is cytogenetically identical to a previously reported case of NF1 associated with a 1;17 balanced translocation and suggests that the translocation events disrupt the NF1 gene. This precisely maps the NF1 gene to 17q11.2 and provides a physical reference point for strategies to clone the breakpoint and therefore the NF1 gene. A human-mouse somatic cell hybrid was constructed from patient lymphoblasts which retained the derivative chromosome 22 (22pter→22q11.2::17q11.2→17qter) but not the derivative 17 or normal 17. Southern blot analysis with genes and anonymous probes known to be in proximal 17q showed ErbA1, ErbB2, and granulocyte colony–stimulating factor (CSF3) to be present in the hybrid and therefore distal to the breakpoint, while pHHH202 (D17S33) and beta crystallin (CRYB1) were absent in the hybrid and therefore proximal to the breakpoint. The gene cluster including ErbA1 is known to be flanked by the constitutional 15;17 translocation breakpoint in hybrid SP3 and by the acute promyelocytic leukemia (APL) breakpoint, which provides the following gene and breakpoint order: cen–SP3–(D17S33,CRYB1)–NF1–(CSF3,ERBA1,ERBB2)–APL–tel. The flanking breakpoints of SP3 and APL are therefore useful for rapidly localizing new markers to the neurofibromatosis critical region, while the breakpoints of the two translocation patients provide unique opportunities for reverse genetic strategies to clone the NF1 gene.

Introduction

Initial mapping of von Recklinghausen neurofibromatosis (NF1) placed the gene on chromosome 17 in the pericentromeric region by virtue of (*a*) its close linkage to pA10-41 (D17S71, which maps to p11.2) and p3-6 (D17Z1, corresponding to the alpha satellite sequences of the pericentromeric region) and (*b*) its loose linkage to the long-arm marker nerve-growth-factor receptor (NGFR, which maps to q21-22) (Barker et al. 1987; Seizinger et al. 1987). More recently, probe pHHH202 (D17S33) was found to be tightly linked to NF1, but multilocus analysis could not definitively order NF1 relative to pA10-41, the centromere, and pHHH202 (White et al. 1987). Therefore, it has been difficult to determine by linkage methods alone whether NF1 is on the proximal short arm or proximal long arm of chromosome 17 (Skolnick et al. 1987).

Cytogenetic evidence from patients with NF1 provided the first evidence that the gene was on the proximal long arm. Schmidt et al. (1987) reported two unrelated cases with NF1 showing constitutional or acquired rearrangements of chromosome 17 with a breakpoint in 17q11.2. In the first, a mother and her two children were each affected with NF1 and had a balanced translocation between chromosomes 1 and 17, t(1;17)(p34.3;q11.2). Although other family members could not be studied, it is a reasonable hypothesis that the translocation in this family actually disrupts the NF1 gene at 17q11.2, analagous to the X;autosome translocations found in females with Duchenne muscular dystrophy (Boyd et al. 1986, 1987; Bodrug et al. 1987). In the second case in the report of Schmidt et al. (1987), a patient had both NF1 and a presumed secondary myelodysplastic syndrome with an unbalanced transloca-

Received July 21, 1988; revision received September 19, 1988.

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0002-9297/89/4401-0004$02.00

tion involving a breakpoint at 17q11.2 observed in bone marrow only. The relationship of this rearrangement and NF1 is much less certain.

Assignment of NF1 to 17q11.2 is supported by regional mapping studies of closely linked markers by somatic cell hybrid analysis. Use of a detailed regional mapping panel has allowed relatively high-resolution physical mapping for genes and anonymous probes on chromosome 17 (vanTuinen et al. 1987). Several breakpoints in this panel are particularly relevant to the mapping of NF1. A centromere breakpoint, defined by hybrid LS-1, distinguishes probes on the short and long arms. SP3 is a hybrid constructed from a fibroblast cell line of a patient from Spain with a constitutional 15;17 translocation (Ferro and San Roman 1981). Although this breakpoint originally was reported as being in 17q21, subsequent high-resolution studies have reassigned it to 17q11.2 (LeBeau et al. 1986; Mitelman et al. 1986). P12.3B is a hybrid constructed from bone marrow of a patient with the 15;17 translocation characteristic of acute promyelocytic leukemia. Although ambiguity still exists regarding the exact cytogenetic placement of this breakpoint, the most recent consensus places it in 17q11.2 or q12 (Bloomfield et al. 1987). The breakpoints of SP3 and APL, although cytogenetically similar, are known to be different on a molecular level on the basis of the mapping of a cluster of genes between the two (Sheer et al. 1985; vanTuinen et al. 1987). These genes include ErbA1, ErbB2, granulocyte colony–stimulating factor (CSF3), and beta crystallin (CRYB1). We previously mapped pHHH202 to this same region between the SP3 and APL breakpoints, supporting the localization of NF1 on the long arm within this region (vanTuinen et al. 1987).

We now report a second patient with NF1 associated with a balanced translocation involving chromosome 17, whose breakpoint characterization further refines the localization of the gene. Hybrids constructed from this patient's cell line allow rapid mapping of probes proximal and distal to NF1 and also allow physical strategies to clone the translocation breakpoint, and presumably, the NF1 gene.

Clinical Report

The patient was referred for evaluation at the age of 33 years because of a history of "von Recklinghausen neurofibromatosis" and a balanced reciprocal translocation involving chromosome 17. She first noticed her dermal neurofibromas in early adulthood. The diagnosis of NF1 was initially made by a dermatologist who also recognized axillary freckling and then applied this label. No biopsy was ever taken of the cutaneous masses. The proband's medical history is otherwise uncomplicated, and, in particular, there is no history of internal tumors, scoliosis, or learning disabilities. The patient has above-average intelligence and holds two postgraduate degrees. She had also noted the presence of café-au-lait spots prior to her evaluation by us.

Family history shows no one else with any signs or symptoms of NF1 on either side of the proband's family. The patient had one pregnancy that resulted in the 1984 birth of an infant with multiple congenital malformations. This baby had a Dandy-Walker malformation with an occipital cervical myelomeningocele, ventricular septal defect, and postnatal linear growth deficiency. The child died at 10 mo of age after surgery to repair the ventricular septal defect. Chromosomes were obtained during the early months of life and showed an apparently balanced translocation involving chromosomes 17 and 22. This particular study has not been available for review by the authors. In this child café-au-lait spots were neither recorded in her medical history nor noted by her parents. It was because of the balanced translocation in her child that our patient had her chromosomes analyzed, a study indicating that she also had a balanced 17;22 translocation. Her chromosome analysis was repeated by the authors, and the results are summarized below.

On examination at 33 years of age, height was 160 cm (just below the 50th percentile) and head circumference was 58 cm (97th percentile). Craniofacial features were normal, with the exception of three (3–5-mm) dermal neurofibromas on the face. Examination of the skin showed four café-au-lait spots >1.5 cm. in diameter and marked freckling in the axillary and inguinal regions. In addition, there were many dermal neurofibromas on the skin, especially on the trunk. There were three plexiform neurofibromas, one of the abdomen, another on the left elbow region, and one on the posterior thigh. This latter plexiform neurofibroma had hyperpigmentation overlying the skin. All of these plexiform neurofibromas were soft and fluctuant and measured about 4 cm. The remainder of the physical examination was within normal variation, and, in particular, there were no cranial defects, scoliosis, or limb abnormalities.

Slit-lamp examination of the irises was recommended but had not yet been accomplished at the time of the writing of the present report. The combination of multiple dermal neurofibromas, multiple plexiform neuro-

fibromas, and axillary and groin freckling make the diagnosis of NF1.

Methods and Results

Cell Lines and Cytogenetic Analysis

A lymphoblastoid cell line was established from the patient by Epstein-Barr virus transformation and maintained in RPMI 1640 medium. Cytogenetic analysis was performed on the lymphoblastoid line after synchronization with bromodeoxyuridine (BrdU). In brief, BrdU was added at 100 μg/ml for 16 h. After this time, cells were washed once with Hanks's balanced salt solution and resuspended in medium with 10^{-5} M thymidine. Approximately 6 h later, colcemid (0.10 μg/ml) was added for 15 min. Slides were G-banded by standard trypsin treatment.

Analysis of metaphase and early-metaphase cells showed a balanced translocation between chromosome 17 and 22. The breakpoint was quite close to the centromere in each of the two chromosomes but was determined to be in the proximal long arm of each at 17q11.2 and 22q11.2 (fig. 1). The patient's karyotype is thus designated 46,XX,t(17;22)(q11.2;q11.2).

Somatic Cell Hybrid Construction

The lymphoblast cell line was fused by conventional polyethylene glycol techniques to clone 1D, a mouse thymidine kinase $(TK)^-$ cell line, and selected in HAT (hypoxanthine-aminopterin-thymidine) media. Standard G-band analysis was performed on resulting clones to determine their human chromosome complement, and G-11 differential staining was performed on selected clones.

Two hybrid clones were selected for detailed characterization. NF-13 retained the patient's derivative chromosome 22 (containing the segment 17q11.2-qter which includes the selectable marker TK) in all cells examined but did not retain the normal 17 or derivative 17. In addition, it contained human chromosomes 3, 7, and the normal 22 in a significant percentage (>25%) of cells. This clone was therefore used for mapping of probes to either side of the translocation breakpoint.

NF-9 retained the derivative chromosome 22 in all cells but also contained the derivative 17 in the majority (75%) of cells. Other human chromosomes present in >25% of cells were 1, 19, and 21. Since both derivative chromosomes were present but neither the normal 17 nor the normal 22 was present, this hybrid is useful for pulsed-field gel analysis to detect the translocation breakpoint by using proximal or distal probes (see Fountain et al. 1989).

Mapping Clones Relative to the NF-13 Breakpoint

All probes used in this analysis have been described elsewhere (vanTuinen et al. 1987) and include S12-30 (D17Z1), pHHH202 (D17S33), pUC14A1 (CRYB1), pG-CSF6 (CSF3), pHerbA1 (ERBA1), and pKX044 (ERBB2). DNA preparations of somatic cell hybrids were made at the same passage as was cytogenetic analysis. DNA isolation, digestions, agarose-gel electrophoresis, and Southern hybridization were by routine methods.

Probe S12-30 (D17Z1), which detects the chromosome 17 alpha-satellite sequence, was negative when hybridized to NF-13, confirming that the breakpoint in the translocation was below the centromere of 17, as had been determined cytogenetically. Probe pHHH202 (D17S33) and CRYB1 were also negative, indicating they are both proximal to the NF-13 breakpoint. ERBA1, ERBB2, and CSF3 were all positive when hybridized to NF-13, indicating they are distal to the NF-13 breakpoint.

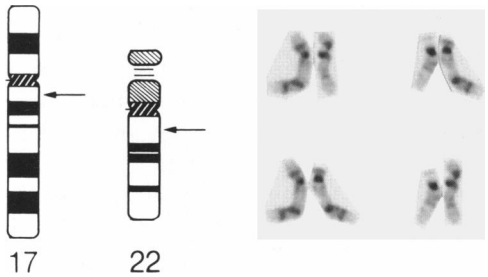

Figure 1 G-banded cytogenetic analysis of the patient's 17;22 translocation. To the left are idiograms of chromosome 17 and 22 at approximately the 550-band stage of resolution (Harnden and Klinger 1985). The arrows indicate the breakpoints in 17q11.2 and 22q11.2. To the right is a partial karyotype of the patient, from a single cell cut out twice for breakpoint comparisons. From left, the top row pairs the normal 17 with the derivative 17 and the normal 22 with the derivative 22. The bottom row pairs the normal 17 with the derivative 22 and the normal 22 with the derivative 17.

Discussion

A summary of the breakpoint positions and mapping data is presented in figure 2. As can be seen, the SP3 breakpoint must be quite close to the centromere on the long arm, although other investigators have mapped numerous anonymous clones to the region between the LS-1 and SP3 breakpoints (O'Connell et al.

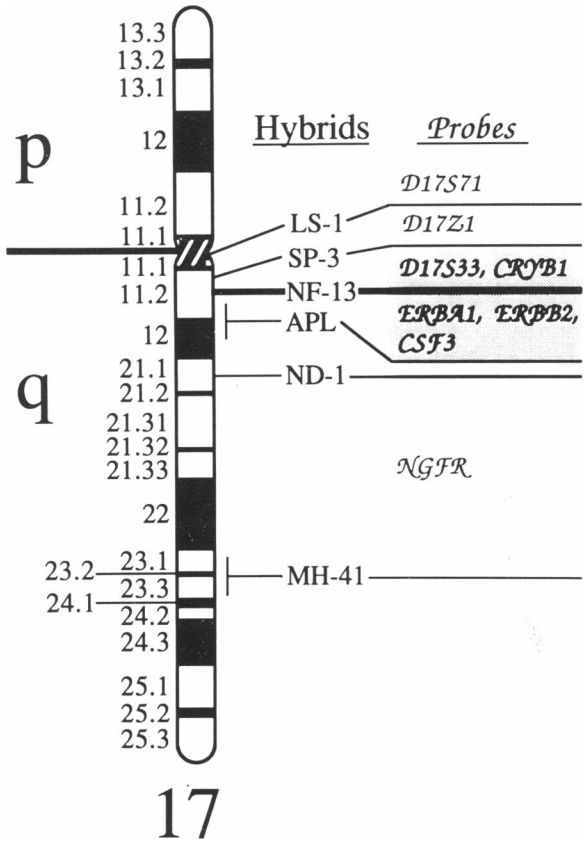

Figure 2 Refined map of the NF1 region. An idiogram of chromosome 17 is shown at approximately the 850-band stage of resolution (Harnden and Klinger 1985). To right are breakpoint localizations of hybrids LS-1, SP-3, P12.3B (APL), ND-1, and MH-41 (vanTuinen et al. 1987). Hybrid NF-13 has been placed between SP-3 and APL by the present mapping data for D17S33, CRYB1, CSF3, ERBA1, and ERBB2. The NF1 region (shaded area) can therefore be precisely defined between SP-3 and APL, an area presumably corresponding to the NF-13 breakpoint. D17S71 (pABL10-41), previously mapped to the proximal short arm (vanTuinen et al. 1987), and NGFR, previously mapped to the region q21.1-q23 (vanTuinen et al. 1987), are shown here for reference.

1989). Probe HHH202 and CRYB1 map distal to SP3 but proximal to NF-13, which unambiguously orders these two breakpoints. The gene cluster including CSF3, ERBA1, and ERBB2 maps distal to NF-13 but proximal to APL. These data place the NF-13 breakpoint—and, presumably, the NF1 gene—between the flanking breakpoints of SP3 and APL.

Although it is not certain that our patient's translocation is the cause of her NF1 by interruption of the gene itself, this is a reasonable conjecture. Duchenne muscular dystrophy was first mapped to Xp21 by the observation of several affected females with balanced X;autosome translocations with a consistent breakpoint at p21 (reviewed in Boyd et al. 1986). Molecular characterization of several of these translocation breakpoints has confirmed that they occur at various positions within the DMD gene, validating the hypothesis of causal relationship (Bodrug et al. 1987; Boyd et al. 1987). The high mutation rate of NF1 suggests the possibility that, like DMD, it may represent a large gene. Translocation within this target sequence could represent one mechanism of mutation, as suggested by the occurrence of two unrelated cases of NF1 associated with translocation (case 1 of Schmidt et al. 1987; present report).

The hybrid mapping panel is useful for rapidly assigning cloned DNA sequences to the NF1 critical region defined by the flanking breakpoints of hybrids SP3 and P12.3B (APL), proximal or distal to the NF-13 breakpoint. A large number of anonymous cosmid clones within this region have been efficiently identified in this manner (O'Connell et al. 1989). In addition, the patient's lymphoblast and hybrid cell lines will be useful in strategies to clone the NF1 gene itself. Pulsed-field gel electrophoresis with probes that map to this region should allow both detection of the breakpoint and reverse genetic strategies to isolate the gene itself (see Fountain et al. 1989).

Acknowledgments

The authors express their appreciation to our patient for the time and energy that she expended in participating in this study. The authors also thank Beth Ann Bloom, M.S., for patient referral and Mercedes Garcia-Heras for expert technical assistance. We thank Drs. G. Rovera, D. J. Tweardy, and F. S. Collins for the gift of cloned genes used in this study. This work was supported by National Institutes of Health grant HD20619 to D.H.L.

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