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The Order of Loci in the Pericentric Region of Chromosome 17, Based on Evidence from Physical and Genetic Breakpoints

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Summary

Previous genetic analyses of chromosome 17 markers and NF1 (Fain et al. 1987) were extended in an attempt to order marker loci that map physically to 17cen→17q12. Three additional markers (HHH202, CRI-L581, and CRI-L946) were included in the analyses. Recombinants within the cluster of seven unordered marker loci were identified by pairwise analyses for each family and by examining the within-sibship segregation patterns for different markers. Changes in the segregation pattern for different loci define genetic breakpoints. Given that interference is complete in the region, markers with the same segregation pattern lie on one side of the breakpoint, while markers with different segregation patterns lie on opposite sides of the breakpoint. If the order of boundary markers is known, markers on each side of a breakpoint can be oriented in relation to the centromere. The order cen–(HHH202/NF1)–(EW207)–(EW203/CRI-L581)–(CRI-L946)–(HOX-2/NGFR)–qter was inferred by combining information from physical breakpoints in a panel of mouse/human hybrids and information from genetic breakpoints found in 16 NF1 families.

Introduction

In previous studies gene linkage between the NF1 locus and the centromere of chromosome 17 was demonstrated (Barker et al. 1987*b*). Probes were isolated from chromosome 17 libraries, screened for polymorphism, and localized physically using human/mouse hybrids with rearranged or deleted forms of 17 (Barker et al. 1987*a*, 1988). Results of genetic analyses of marker data in NF1 families refined the region flanking NF1 to 10–15 cM (Fain et al. 1987). Subsequently, additional testing of the centromere marker revealed a single recombinant with NF1. The multilocus data favored a position for NF1 on the long arm, a result that further delimited the flanking region (Fain et al., in press).

Localization of NF1 on 17q is supported by data for two additional centromere recombinants that were identified by other laboratories (Pericak-Vance et al. 1987. Upadhyaya et al. 1987). A series of markers were physically mapped to the region 17cen→17q12, which includes about one-sixth of the chromosome, and at least one of these markers was shown to be distal to NF1 (Fain et al. 1987). Another marker in the region showed no recombination with NF1 in previous studies (vanTuinen et al. 1987; White et al. 1987). To date, no other information on the order of marker loci within this region has been reported.

The ability to order loci in relation to breakpoints provided by human/mouse hybrid panels has greatly improved the efficiency of genetic testing of chromosome 17 markers for linkage with NF1. Given certain assumptions, we can interpret recombinants between markers as individual crossover events or as meiotic breakpoints, and inferences about order can be made using a direct approach that is analogous to the approach used to interpret physical mapping data. In this study, we present results of ordering markers in the 17cen→17q12 region in relation to a series of genetic breakpoints that were identified in NF1 families.

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Methods

Gametic Sample

Recombinants were isolated from among offspring

of 16 families who were ascertained for NF1 linkage studies. The Utah resource of 14 NF1 families (designated UTK) has been described elsewhere (Fain et al. 1987). In that study, apparent contradictions in ordering the NF1 locus in relation to the markers were resolved by excluding family history of NF1 as a criterion for diagnosis. This could be justified by the fact that all relatives included in a linkage analysis necessarily satisfy this criterion. Two families who were uninformative for linkage with NF1 but who were informative for marker-marker linkages were included in the present analysis, which focused on ordering the markers in relation to each other. Gametes from both affected and unaffected parents were considered in the analysis.

Chromosome 17 Markers

Chromosome 17 markers used in the analysis included the most informative subset of those previously reported (Fain et al. 1987): HHH202, also reported previously for this sample of families (White et al. 1987); CRI-L581; and CRI-L946 (obtained from Collaborative Research, Inc.). Additional typing results for the centromere marker p3–6 (Willard et al. 1987) and for NGFR (obtained from M. Chao) were also available. Table 1 lists the markers and their Human Gene Mapping (HGM) designations.

Physical Mapping

A panel consisting of four mouse/human hybrid lines was used to assign probes to five different physical regions of chromosome 17, as described elsewhere (Barker et al. 1987*a;* 1988; Fain et al. 1987; Willard et al. 1988, and in press). All markers in series EW200 mapped to a region on the long arm just below the centromere (17cen→17q12). HHH202 has also been mapped to this region (vanTuinen et al. 1987). As part of the present study, probes CRI-L581 and CRI-L946 were localized to the same region.

Table 1

Chromosome 17 Polymorphic DNA Markers Used in the Present Study

Probe	HGM Designation
pHHH202	D17S33
CRI-L946	D17S36
CRI-L581	D17S37
EW203	D17S54
EW204	D17S55
EW206	D17S57
EW207	D17S73
EW301	D17S58
C3	NGFR
pBS-3	HOX2
pA10-41	D17S71
p3-6	D17Z1

Genetic Analyses

Assumptions.—The interpretation of recombinants as genetic breakpoints requires two assumptions: (1) that there is a negligible frequency of multiple crossovers in the region bounded by markers; and (2) that the order of the boundary markers in relation to unordered markers is known.

On the basis of crossover distributions derived from assuming chiasma distributions observed at diakinesis in the human male (Hulten 1974) and on the basis of a four-strand model of crossing-over, double crossovers on the long arm of chromosome 17 are expected in only about 5% of gametes. In general, a single chiasma tends to occur at distal ends of chromosome arms while two chiasmata are usually distant from each other—i.e., one occurs near the centromere and the other occurs more distally than is generally found for single chiasma. The recombination fraction between the centromere and nerve growth factor receptor (NGFR) is about 10% in males, although NGFR has been localized to 17q22 (Huebner et al. 1986), more than half the physical distance to the telomere. Although no cytological data are available for females, and although significant sex differences in the pericentric region have been observed (Fain et al. 1987), the data for males, as well as the small physical size of the chromosome, make it reasonable to assume that one—or, rarely, two—chiasma(ta) occur(s) on the entire chromosome arm at meiosis in males and that the occurrence of more than two may be rare in females. Under a four-strand model, the expected frequency of double crossovers is considerably less than the frequency of multiple chiasmata. Although cytological and genetic data justify the assumption of complete interference within the region bounded by the centromere and NGFR, the assumption may be less tenable when the centromere marker is uninformative, particularly in females.

Identification of breakpoints.—A computer program (BREAKPT) was written to assign the origin of alleles to informative offspring, and to identify recombinants in the marker string. For each marker locus, offspring were classified into two groups, depending on the allele that was inherited from a given parent. When all offspring in a sibship are nonrecombinant, these groups maintain their integrity for all informative markers.

Recombinants became apparent when the group membership of one or more children differed for different markers. The marker loci could then be classified into two or more groups according to the specific among-sib segregation pattern observed for marker alleles. Given the assumptions outlined above, it can be inferred that markers with different segregation patterns are on opposite sides of a genetic breakpoint that occurred in at least one sibling while markers with identical segregation patterns are on the same side of the breakpoint. The region of a breakpoint is defined in this way, although it is not always possible to infer in whom—or in how many siblings—a crossover has occurred. After consolidating the evidence from genetic breakpoints into a map of ordered loci, genetic distances were obtained through pairwise analyses between adjacent markers.

Results

Pairwise recombination fractions for markers that were not included in previous studies (Fain et al. 1987, and in press) are given in table 2. Sex differences in recombination between NF1 and CRI-L581 were statistically significant ($P < .05$), which is consistent with previous observations (Fain et al. 1987).

Results of ordering loci in relation to six genetic breakpoints that were found in the region are given in figure 1. The combination of breakpoints places HHH202 proximal to five of the other six markers that map to the same physical region. No recombination was observed between HHH202 and EW206, and their order could not be inferred indirectly from other breakpoints. Similarly, EW204 could not be ordered in relation to EW207, EW206, EW203, and CRI-L581, but genetic breakpoints place it distal to HHH202 and proximal to CRI-L946. No recombination has been observed between EW203 and CRI-L581 ($\hat{\theta} = .0, \hat{z} = 21.07$), but both could be localized distal to EW207 and proximal to CRI-L946.

All recombinants that were identified from pairwise analyses could be interpreted as genetic breakpoints. In addition, crossovers that, because of uninformative intercrosses, were not apparent from pairwise analyses could also be identified through the analysis of sibship patterns. Within the sample of breakpoints, there was no contradictory evidence to suggest that the assumption of complete interference be reevaluated. A summary of results of ordering marker loci and NF1 in relation to physical and genetic breakpoints is given in figure 2. The NF1 locus was previously localized to 17q in a position proximal to probe EW207, both through the analyses of genetic breakpoints and through formal multilocus analysis. No recombination between HHH202 and NF1 was observed in our sample of families.

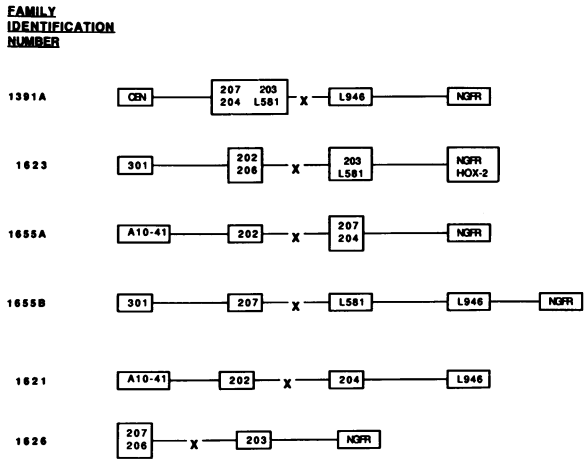

Figure 1 Genetic breakpoints identified by an analysis of segregation patterns within sibships. Markers on one side of a breakpoint have identical segregation patterns among siblings, while markers on opposite sides of a breakpoint have different segregation patterns. Marker prefixes EW (from EW203, EW204, EW206, and EW207), HHH (from HHH202), and CRI- (from CRI-L581 and CRI-L946) have been eliminated for brevity (see table 1).

Discussion

Throughout the fine mapping phase of genetic analyses of NF1, the most likely order of loci has been inferred initially by examining multilocus data from individual recombinants. In all cases, the inferred order was validated by applying statistical models of multilocus recombination. As the density of the regional map increases, the number of separate analyses that must be performed to consider each possible order becomes unmanageable. In addition, results of a given analysis

Table 2

Lod Scores Obtained from Pairwise Analysis of NF1 and Two Additional Chromosome 17 Markers

	$\hat{\theta}_m$	$\hat{\theta}_f$	$z\hat{\theta}_m,\hat{\theta}_f$	$\hat{\theta}_m = \hat{\theta}_f$	$z\hat{\theta}_m = \hat{\theta}_f$
CRI-L581	.00	.13	8.159	.04	7.187
CRI-L946	.08	.09	3.935	.08	3.930

NOTE.—Also see Fain et al. (1987, and in press).

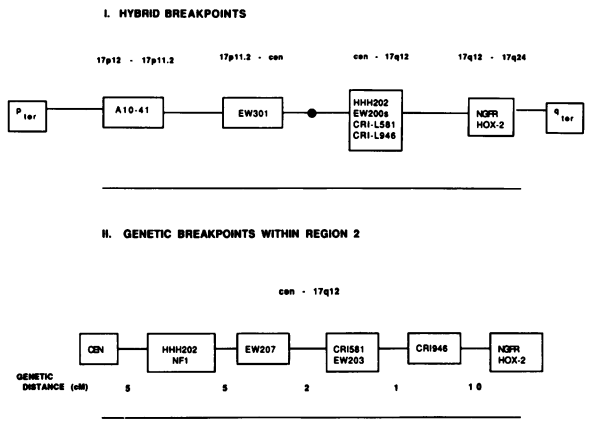

Figure 2 Map of markers in the pericentric region of chromosome 17, based on physical breakpoints (panel I), and refinements in the map of the region 17cen→17q12, obtained by ordering loci in relation to genetic breakpoints (panel II). EW200s designates a set of six arbitrary DNA segments that map physically to the region cen→17q12. Pairwise estimates of recombination fractions between adjacent intervals were used as estimates of genetic distance (in cM), which requires the assumption of complete interference.

are not easily combined with data for new families and/or with data for additional marker loci for the same families.

Statistical models that are commonly used for multilocus analyses typically assume that the occurrence of multiple exchanges between homologues is random and that an unlimited number of exchanges occur. Although the distributions of crossover frequency and position for different human chromosomes have not been determined with precision, there is substantial evidence that both are nonrandom in the majority of diploid organisms, including man. The consequences of assuming randomness are unfortunate when an attempt is made to order loci between which crossing-over is rare, since much of the information for determining relative odds for different orders may be contained in data for a single recombinant, combined with the frequency assumed for multiple crossovers in a region. Although more studies are needed to validate the assumption that the frequency of multiple chiasmata is negligible in the region of interest, the order inferred from interpretation of genetic breakpoints is invariably the order favored by multilocus analysis. Until more is learned about the distribution of crossing-over on different chromosomes, neither approach can be justified for quantifying the evidence.

Under our assumptions, the positions of genetic breakpoints are usually obvious in a minimum family structure consisting of two parents and two children. By digesting and blotting DNA of a minimum number of individuals from key recombinant families, a set of ordered breakpoints for orienting new marker loci, which we continue to identify, is provided. The direct interpretation of crossover events in this way emphasizes the analogy between (*a*) orienting loci around stable chromosome breaks, through the analysis of banding patterns from a panel of hybrid cultures, and (*b*) orienting loci around transient chromosome breaks that occur at meiosis, through the analysis of patterns of allelic inheritance among siblings.

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References

Barker, D., E. Wright, P. Fain, D. Goldgar, M. Skolnick, S. Latt, and H. Willard. 1988. 30 new chromosome 17 DNA markers (HGM9). Cytogenet. Cell Genet. **46**:576.

Barker, D. F., E. Wright, K. Nguyen, L. Cannon, P. Fain, D. Goldgar, D. T. Bishop, J. Carey, B. Baty, J. Kivlin, H. Willard, J. S. Waye, G. Greig, L. Leinwand, Y. Nakamura, P. O'Connell, M. Leppert, J.-M. Lalouel, R. White, and M. Skolnick. 1987*a*. The gene for von Recklinghausen neurofibromatosis is on chromosome 17 near the centromere. Am. J. Hum. Genet. [Suppl.] **41**:A157.

Barker, D., E. Wright, K. Nguyen, L. Cannon, P. Fain, D. Goldgar, D. Bishop, J. Carey, H. Willard, J. S. Waye, G. Greig, L. Leinwand, Y. Nakamura, P. O'Connell, M. Leppert, J. M. Lalouel, R. White, and M. Skolnick. 1987*b*. Gene for von Recklinghausen neurofibromatosis is in the pericentromeric region of chromosome 17. Science **236**: 1100–1102.

Fain, P., D. Barker, D. Goldgar, E. Wright, K. Nguyen, J. Carey, J. Johnson, J. Kivlin, H. Willard, C. Mathew, B. Ponder, and M. Skolnick. 1987. Genetic analysis of NF1: identification of close flanking markers on chromosome 17. Genomics **1**:340–345.

Fain, P., D. Barker, D. Goldgar, E. Wright, K. Nguyen, H. Willard, P. vanTuinen, D. Ledbetter, C. Mathew, B. Ponder, and M. Skolnick. Genetic analysis of NF1: results of genetic fine mapping with pericentromeric markers. *In* Molecular probes: technology and medical applications. Raven, New York (in press).

Huebner, K., M. Isobe, M. Chao, M. Bothwell, A. Ross, J. Finan, J. Hoxie, A. Sehgal, C. Buck, A. Lanahan, P. Nowell, H. Koprowski, and C. Croce. 1986. The nerve growth factor receptor gene is at human chromosome region 17q12–17q22, distal to the chromosome 17 breakpoint in acute leukemias. Proc. Natl. Acad. Sci. USA **83**:1403–1407.

Hulten, M. 1974. Chiasma distribution at diakinesis in the normal human male. Hereditas **76**:55–78.

Pericak-Vance, M., L. Yamaoka, J. Vance, K. Small, G. Rosenwasser, P. Gaskell, W. Hung, M. Alberts, C. Haynes, M. Speer, J. Gilbert, M. Herbstreith, A. Aylsworth, and A. Roses. 1987. Genetic linkage studies of chromosome 17 RFLPs in von Recklinghausen neurofibromatosis (NF1). Genomics **1**:349–352.

Upadhyaya, M., M. Sarfarzi, S. Huson, K. Stephens, W. Broadhead, and P. Harper. 1987. Chromosome 17 markers and von Recklinghausen neurofibromatosis: a genetic linkage study in a British population. Genomics **1**:358–360.

vanTuinen, P., D. Rich, K. Summers, and D. Ledbetter. 1987. Regional mapping panel for human chromosome 17: application to neurofibromatosis type 1. Genomics **1**:374–381.

White, R., Y. Nakamura, P. O'Connell, M. Leppert, J. Lalouel, D. Barker, D. Goldgar, M. Skolnick, J. Carey, C. Wallis, C. Slater, C. Mathew, and B. Ponder. 1987. Tightly linked markers for the neurofibromatosis type I gene. Genomics **1**: 364–367.

Willard, H. F., S. J. Durfy, M. Mahtani, H. Dorkins, K. E. Davies, and B. R. G. Williams. Regional localization of the TIMP gene on the human X chromosome: extension of a conserved synteny and linkage group on proximal Xp. Hum. Genet. (in press).

Willard, H. F., G. M. Greig, V. E. Powers, and J. S. Waye. 1987. Molecular organization and haplotype analysis of centromeric DNA from human chromosome 17: implications for linkage in neurofibromatosis. Genomics **1**: 368–373.

Willard, H. F., R. Wevrick, W. C. Earnshaw, P. N. Howard-Peebles, and V. E. Powers. 1988. Origin of two functional centromeres from a single block of alpha satellite DNA in a constitutional rearrangement of chromosome 17. Am. J. Hum. Genet. [Suppl.] **44**:A207.