THE GENE FOR CILIARY NEUROTROPHIC FACTOR MAPS DISTAL TO CD5 AND LY-10 ON MURINE CHROMOSOME 19

Jeffrey T. Henderson, Nadine Seniuk, and John C. Roder
Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Division of Neurobiology and Molecular Immunology 600 University Ave., Toronto, Ontario M5G-1X5

Introduction

Ciliary neurotrophic factor (CNTF) is a 23 kD cytoplasmic protein which is produced in astrocytes in the central nervous system and myelinating Schwann cells in the peripheral nervous system. This factor has been shown to promote the survival of sensory, sympathetic, parasympathetic and motor neurons *in vitro* (1). *In vivo*, CNTF has been shown to prevent programmed motoneuron death; as well as the axotomy-induced death of motoneurons in the facial nucleus and cholinergic neurons in the medial septum (1-3). In order to more precisely map Cntf with respect to other neurological loci, we have analyzed a series of C57BL/6J X DBA/2J (BXD) recombinant inbred strains, by restriction fragment length polymorphism (RFLP). The strain distribution pattern (SDP) for Cntf demonstrates tight linkage to Osbp on murine chromosome 19.

Materials and Methods

Genomic DNA samples were digested overnight and separated on a 0.7% agarose gel. Gels were then stained with ethidium bromide to determine the position of the size standards and transferred onto Hybond N$^+$ nylon membranes under standard conditions (4). Hybridizations were performed at 65°C, using the 600 bp full-length cDNA for rat CNTF. Membranes were washed sequentially for 30 min. in 2x SSC and 2x SSC, 0.1% SDS at 65°, followed by autoradiography. The resulting BXD strain distribution patterns obtained were analyzed using the statistical approaches outlined by Silver and Buckler, 1986 (5). Genomic DNA samples of existing BXD R.I. strains were obtained from The Jackson Laboratories, Bar Harbor Maine. Current SDP data for the BXD R.I. lines was kindly provided by Dr. B. A. Taylor, of The Jackson Laboratory.

Results

A series of restriction digests were performed on C57BL/6J and DBA/2J genomic DNA. Southern analysis of these digests revealed several informative RFLP's between the two lines. Among these, Pvu II digestion of C57BL/6J and DBA/2J genomic DNA gave rise to hybridizing bands of 5.5 kb and 11 kb respectively, whereas Taq 1 digestion generated bands of 2.6 and 4.7 kb respectively. These enzymes were then used to analyze a set of twenty-six BXD recombinant inbred strains for their CNTF RFLP patterns (6). The full strain distribution pattern obtained for Cntf with respect to nearby markers is shown in Table 1. As expected, both Pvu II and Taq I gave identical Cntf SDP's. Lines 3, 4, 7, 10, 17, 26 were extinct at the time of these analyses, and thus the SDP's could not be determined. As can be seen from these data, Cntf demonstrates closest linkage to the oxysterol binding protein (Osbp), with an identical segregation pattern for all twenty-six available inbred strains. Previous work, based on the segregation of an interspecific *Mus spretus/ Mus m. domesticus* backcross has demonstrated linkage (0/60 recombinants) between Cntf and Ly-1(now CD5) (7); suggesting that Cntf lies 0-1.7 cM on either side of Ly-1. Among the BXD R.I. strains however, there is a discordancy of 1/26, 2/26, 3/26, and 3/26 for CD5, D19Byu2, Ly-10 and D19Rp19e respectively. The recombination frequency, linkage probability, linkage distance of Cntf with respect to these loci/markers is shown in Table II.

Table I: Strain distribution pattern of CNTF and nearby loci

BXD Strain:	1	5	10	15	20	25	30
Loci/Marker:							
D19Byu1	D B U U B D U D B U **B** B D B B B U B B **B** B D D B U D B B **B** D B						
D19Rp19e	D B U U B D U D B U **B** B D B B B U B B **D** B B D D B U D **3** B **B** D B						
Ly-10	D B U U B D U D B U **B** B D B B B U B B **D** B B D D B U D B B **B** D B						
D19Byu2	D B U U B D U D B U **B** B D B B B U B B **D** B B D D B U D B B D D B						
CD5	B B B D B D U D B U **B** B D B B B U B B **D** B B D D B U D B B D D B						
Osbp	B B B D B D U D B U D B D B B B U B B **D** B B D D B U D B B D D B						
Cntf	B B U U B D U D B U D B D B B B U B B **D** B B D D B U D B B D D B						
Gsl-5	B B U U B D U D B U D B D B B **D** U B B U B B **B** B B U D B B D **B** B						
D19Byu3	B B U U B D U D B U D B D B B **D** U B B D B B **B** B B U D B B D **B** B						
D19J1	D B U U B D U D B U D B **B** B B **D** U B B D U B **B** B B U D B B D **B** B						

B - C57BL/6J distribution pattern, D - DBA/2J distribution pattern
U - undefined/extinct at time of determination, **bold** deviation from CNTF SDP

Table II: Linkage of CNTF to nearby loci

Locus/marker:	R	rf	$\pm\ ^{m}_{95}$	P(L/l)
Osbp	0/26	0	3.6	1.00
CD5	1/26	1.0	5.5	1.00
D19Byu2	2/26	2.2	7.8	0.999
Ly-10	3/26	3.3	11.1	0.993
D19Rp19e	3/26	3.3	11.1	0.993

R - proportion of discordant BXD strains; rf - recombination frequency based upon rf = R/(4-6R)
$^{m}_{95}$ - distance in centimorgans which has a 95% chance of containing the indicated locus and CNTF (1)
P(L/l) - probability of linkage between Cntf and locus (1); (1) - as described by Silver and Buckler, 1986

Although one cannot be certain that an entire region of an R.I. strain has been inherited intact, the cumulative linkage information derived from a number of different methods support the gene/marker order given in Figure 1(8, 9), which are in good agreement with the current BXD R.I. data. From these data we postulate that Cntf is distinct from CD5, mapping distal to both the CD5 and Ly-10 loci. The maximum physical distance of Cntf from Osbp for the BXD R.I. data is defined by the C57Bl/6J x DBA/2J recombination breakpoint between CD5 and Osbp (R.I. line 11), and the most proximal Osbp/Gsl-5, D19Byu-3 breakpoint within the lines 16, 23, 24 or 31, as shown in figure 1. Since the position of these breakpoints are presently unknown, the distal limit can be inferred from the recombination frequency of Cntf with respect to Osbp; which is similar to the present map position for Gsl-5/D19Byu-3 (3.6 cM , see table II). However, previous work by Kaupmann suggests that Cntf lies no greater than 1.7 centimorgans distal to CD5. Taken together, these data suggest that the gene for CNTF lies distal to CD5, ± 0.7 cM from Osbp.

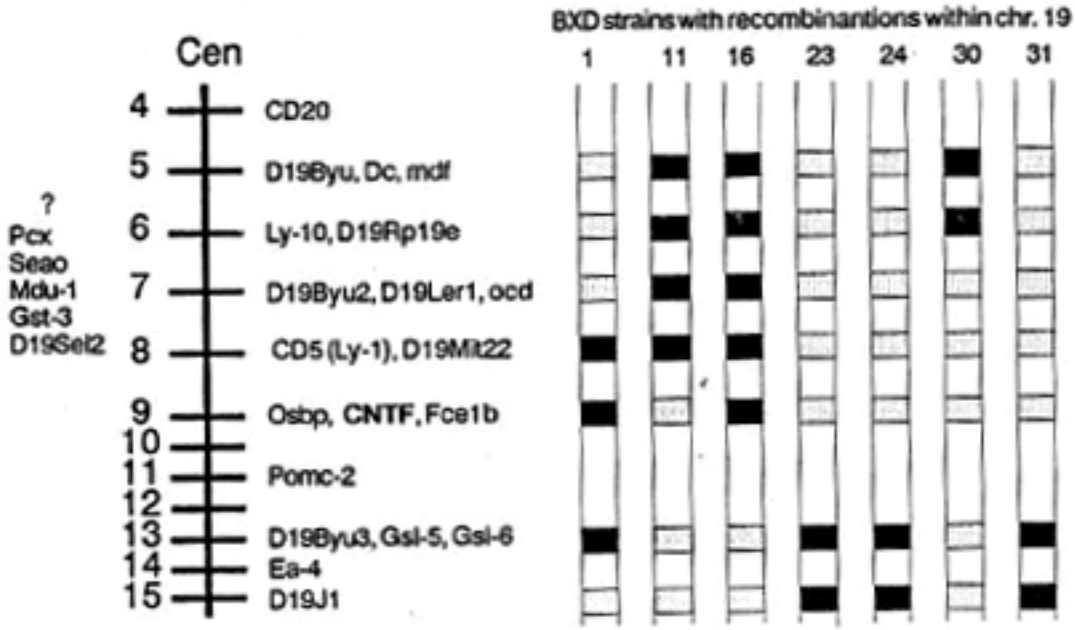

Fig. 1. Model of the positional map for Cntf with respect to associated loci in the proximal region of murine chromosome 19. The spatial order of the loci indicated are based upon the cumulative GBASE linkage information derived from a variety of mapping methods. Locus designations conform to those listed by Guenet et al., 1992 (8). A schematic model of those BXD lines which provide linkage information in this region of the chromosome are shown to the right. These BXD strain representations are drawn based upon the known SDP's for these loci and markers, and are shown such that the number of double crossovers are minimized (solid - C57BL/6J, stippled - DBA/2J). Gaps represent positions for which BXD SDP's are not currently known, and are thus presently undefined for either parental genotype. Other loci which map in the general region of Cntf, but which have not yet been definitively placed, are shown to the left.

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