gelsolins are encoded by a single gene and contain a duplicated actin-binding domain. *Nature* **323:** 455–458.

5. Kwiatkowski, D. J., Mehl, R., and Yin, H. L. (1988). Genomic organization and biosynthesis of secreted and cytoplasmic forms of gelsolin. *J. Cell. Biol.* **106:** 375–384.
6. Levy, E., Haltia, M., Fernandez-Madrid, I., Koivunen, O., Ghiso, J., Prelli, F., and Frangione, B. (1990). Mutation in gelsolin gene in Finnish hereditary amyloidosis. *J. Exp. Med.* **172:** 1865–1867.
7. Maury, C. P. J., Alli, K., and Baumann, M. (1990). Finnish hereditary amyloidosis. Amino acid sequence homology between the amyloid fibril protein and human plasma gelsoline. *FEBS Lett.* **260:** 85–87.
8. Maury, C. P. J., Kere, J., Tolvanen, R., and de la Chapelle, A. (1990). Finnish hereditary amyloidosis is caused by a single nucleotide substitution in the gelsolin gene. *FEBS Lett.* **276:** 75–77.
9. Meretoja, J. (1969). Familial systemic paramyloidosis with lattice dystrophy of the cornea, progressive cranial neuropathy, skin changes and various internal symptoms. A previously unrecognized heritable syndrome. *Ann. Clin. Res.* **1:** 314–324.
10. Meretoja, J. (1972). Comparative histopathological and clinical findings in eyes with lattice corneal dystrophy of two different types. *Opthalmologica* **165:** 15–37.
11. Meretoja, J. (1973). Genetic aspects of familial amyloidosis with corneal lattice dystrophy and cranial neuropathy. *Clin. Genet.* **4:** 173–185.
12. Syvänen, A.-C., Aalto-Setälä, K., Harju, L., Kontula, K., and Söderlund, H. (1990). A primer-guided nucleotide incorporation assay in the genotyping of apolipoprotein E. *Genomics* **8:** 684–692.
13. Valle, D., Mitchell, G. A., Brody, L. C., Martin, L. S., Steel, G., and Sipilä, I. (1989). Mutation detection and structure–function studies at the ornithine δ-aminotransferase locus. *In* "Polymerase Chain Reaction" (H. A. Erlich, R. A. Gibbs, and H. H. Kazazian, Jr., Eds.), pp. 63–68, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Genetic Linkage Mapping of the m4 Human Muscarinic Receptor (CHRM4)

Raji P. Grewal,* Maria Martinez, Margret Hoehe, Tom I. Bonner,† Elliot S. Gershon, and Sevilla Detera-Wadleigh[1]

*Clinical Neurogenetics Branch and †Laboratory of Cell Biology, National Institute of Mental Health; and *Developmental and Metabolic Neurology Branch, National Institute of Neurological Disorders and Stroke, Bethesda, Maryland 20892*

Received June 25, 1991; revised November 26, 1991

The actions of the acetylcholine neurotransmitter are mediated by two classes of receptors, the nicotinic, which are ligand-gated ion channels, and the muscarinic, which are G-protein-coupled receptors. The m4 receptor is one of five different muscarinic receptors that have been cloned (1) and found to be widely expressed in the brain (2). The m4 gene has been mapped by *in situ* hybridization to the short arm of chromosome 11 (11p11.2–p12; Bonner *et al.*, in preparation). We sought to confirm and extend this observation by genetic linkage mapping.

An *Sst*I polymorphism for the m4 gene creating two alleles, A1 (3.2 kb) and A2 (3.5 kb), has been previously described (4). Southern blots were prepared using *Sac*I-digested genomic DNA from 40 matings in the pedigree collection of the Centre d'Etude du Polymorphisme Humain (CEPH). These blots were hybridized with the m4 probe (4) to identify potentially informative pedigrees. Five families comprising 72 individuals were found to be informative. Genotyping revealed a Mendelian codominant inheritance (data not shown). Two-point and multipoint analyses were performed using the MLINK and ILINK programs of the LINKAGE (V5.03) package (9). Pairwise analysis detected significant evidence for linkage between m4 and five loci, D11S149 (6, 7), D11S436 (12), D11S141 (5), p5BE1.2 (3), and D11S97 (11), as shown in Table 1. These results indicate that the m4 gene is closely linked to both 11q and 11p markers that are located near the centromere.

Since there is no existing primary genetic linkage map that includes all six chromosome 11 loci, we performed a multipoint analysis using the ILINK program to determine the maximum likelihood order between m4 and the other marker loci. Differences in recombination fraction between males and females were sought under three different models: no sex difference, variable sex difference, and constant sex difference within each interval. The analysis revealed that m4 is in a cluster with D11S149 and D11S436, but the precise gene order of the six chromosome 11 loci was not unambiguously established. The odds ratios, however, strongly suggest that p5BE1.2 is proximal to the D11S149/m4/D11S436 (odds = 1.30×10^7:1), while D11S97 and D11S141 lie distal to this cluster (odds = 1.28×10^8:1 and 1.15×10^5:1, respectively). The map generated showed significant sex differences, with a female/male genetic distance constant ratio equal to 3.3 ($P < 0.001$). We performed additional multipoint analysis using the CAT gene (10) and other markers known to reside on 11p, but the same maximum likelihood order was found with D11S149 and D11S436 flanking m4. Both m4 and D11S436 have been mapped to 11p11–p12 (Bonner *et al.*, in preparation; 12), while D11S149 has been mapped to 11q12 (7). Although this discrepancy remains to be resolved, the genetic linkage mapping data presented here confirm that the CHRM4 locus is located near the centromere of chromosome 11.

[1] To whom correspondence and reprint requests should be addressed at DIRP, NIMH, 9000 Rockville Pike, Bldg. 10, Rm 3N218, Bethesda, MD 20892.

TABLE 1

Probe (locus)	Maximum lod score (Z)	Recombination fraction (θ)	Location (Ref.)
pTHH26 (D11S149)	4.21	0.00	11q12 (7)
CI11-30 (D11S436)	4.17	0.00	11p11–p12 (12)
CRI-L762 (D11S141)	3.91	0.00	11q13 (7)
p5BE1.2	3.61	0.00	11p (3)
pMS51 (D11S97)	3.49	0.10	11q13 (11)
pINT-800 (CAT)	2.96	0.00	11p13 (10)

ACKNOWLEDGMENT

We thank Dr. R. O. Brady for his support and encouragement.

REFERENCES

1. Bonner, T. (1989). The molecular basis of muscarinic receptor diversity. *Trends Neurosci.* **12:** 148–151.
2. Buckley, N. J., Bonner, T. I., and Brann, M. R. (1988). Localization of a family of muscarinic receptor mRNAs in rat brain. *J. Neurosci.* **8:** 4646–4652.
3. Compton, D. A., Weil, M. M., Jones, C. A., Riccardi, V. M., Strong, L. C., and Saunders, G. F. (1988). Long range physical map of the Wilms' tumor–aniridia region on human chromosome 11. *Cell* **55:** 827–836.
4. Detera-Wadleigh, S., Wiesch, D., and Bonner, T. (1989). An SstI polymorphism for the human muscarinic acetylcholine receptor gene, m4 (CHRM 4). *Nucleic Acids Res.* **17:** 6431.
5. Donis-Keller, H., Green, P., Helms, C., Cartinhour, S., Weiffenbach, B., Stephens, K., Keith, T. P., Bowden, D., Smith, D. R., Lander, E. S., Botstein, D., *et al.* (1987). A genetic linkage map of the human genome. *Cell* **51:** 319.
6. Holm, T., Nakamura, Y., Ballard, L., O'Connell, P., Leppert, M., Lathrop, G. M., Lalouel, J.-M., and White, R. (1988). Isolation and mapping of a polymorphic DNA sequence (pTHH26) on chromosome 11 (D11S149). *Nucleic Acids Res.* **16:** 4746.
7. Julier, C., Nakamura, Y., Lathrop, M., O'Connell, P., Leppert, M., Litt, M., Mohandas, T., Lalouel, J-M., and White, R. (1990). A detailed genetic map of the long arm of chromosome 11. *Genomics* **7:** 335–345.
8. Klinger, H. P. (Ed.) (1989). HGM10. *Cytogenet. Cell Genet.* **51:** 239.
9. Lathrop, G. M., Lalouel, J.-M., Julier, C., and Ott, J. (1985). Multilocus linkage analysis in humans: Detection of linkage and estimation of recombination. *Am. J. Hum. Genet.* **37:** 482–498.
10. Rose, E. A., Glaser, T., Jones, C., Smith, C., Lewis, W. H., Call, K. M., Minden, M., Champagne, E., Bonettta, L., Yeger, H., and Houseman, D. E. (1990). Complete physical map of the WAGR region of 11p13 localizes a candidate Wilms' tumor gene. *Cell* **60:** 495–508.
11. Royle, N. J., Irwin, D. M., Koschinsky, M. L., MacGillivray, R. T. A., and Hamerton, J. L. (1987). Human genes encoding prothrombin and ceruloplasmin map to 11p11–q12 and 3q21–24, respectively. *Somatic Cell Mol. Genet.* **13:** 285–292.
12. Tokino, T., Takahashi, E., Mori, M., Tangami, A., Glaser, T., Park, W., Jones, C., Hori, T., and Nakamura, Y. (1991). Isolation and mapping of 62 new RFLP markers on human chromosome 11. *Am. J. Hum. Genet.* **48:** 258–268.

Localization of the Human Eosinophil Charcot–Leyden Crystal Protein (Lysophospholipase) Gene (CLC) to Chromosome 19 and the Human Ribonuclease 2 (Eosinophil-Derived Neurotoxin) and Ribonuclease 3 (Eosinophil Cationic Protein) Genes (RNS2 and RNS3) to Chromosome 14

D. M. Mastrianni,* R. L. Eddy,† H. F. Rosenberg,‡ S. E. Corrette,‡ T. B. Shows,† D. G. Tenen,* and S. J. Ackerman‡

**Divisions of Hematology/Oncology and ‡Infectious Diseases, Beth Israel Hospital and Department of Medicine, Harvard Medical School, Boston, Massachusetts; and †Department of Human Genetics, Roswell Park Memorial Institute, Buffalo, New York*

Received July 18, 1991; revised December 5, 1991

The human Charcot–Leyden crystal protein (CLC) comprises approximately 7–10% of eosinophil cellular protein and forms the distinctive hexagonal bipyramidal Charcot–Leyden crystals described in a variety of eosinophil and basophil-related inflammatory states and some myeloid leukemias (1). The CLC protein is a hydrophobic polypeptide with a mass of 17,400 Da and exhibits lysophospholipase activity (lysolecithin acylhydrolase, E.C.3.1.1.5) of uncertain physiologic significance (15). A full-length cDNA clone for eosinophil CLC protein has been isolated and sequenced (2); the predicted amino acid sequence has similarities to members of the S-type β-galactoside binding animal lectin superfamily (3). CLC protein has been assigned Mendelian Inheritance in Man (MIM) number 153310. We report that the gene for CLC protein is found on human chromosome 19.

A human CLC cDNA probe was used in these studies and consisted of a 529-bp cDNA originally obtained from an HL-60 3c5I λ gt11 library. The probe contains the entire coding region of CLC protein (2). All probes were radiolabeled with [α-^{32}P]dCTP using the random oligomer priming method (4) and hybridized to *Kpn*I-digested human DNA, mouse DNA,

GENOMICS **13,** 240–242 (1992)
0888-7543/92 $3.00