Cell, Vol. 49, 589-594, June 5, 1987, Copyright © 1987 by Cell Press

Genetic Linkage of von Recklinghausen Neurofibromatosis to the Nerve Growth Factor Receptor Gene

B. R. Seizinger,[1] G. A. Rouleau,[1] L. J. Ozelius,[1,2] A. H. Lane,[1,3] A. G. Faryniarz,[1] M. V. Chao,[4] S. Huson,[5] B. R. Korf,[6] D. M. Parry,[7] M. A. Pericak-Vance,[8] F. S. Collins,[9] W. J. Hobbs,[1] B. G. Falcone,[1] J. A. Iannazzi,[1] J. C. Roy,[1] P. H. St George-Hyslop,[1] R. E. Tanzi,[1] M. A. Bothwell,[10] M. Upadhyaya,[5] P. Harper,[5] A. E. Goldstein,[6] D. L. Hoover,[6] J. L. Bader[7], M. A. Spence,[11] J. J. Mulvihill,[7] A. S. Aylsworth,[12] J. M. Vance,[8] G. O. D. Rossenwasser,[8] P. C. Gaskell,[8] A. D. Roses,[8] R. L. Martuza,[3] X. O. Breakefield,[2] and J. F. Gusella[1]

Summary

von Recklinghausen neurofibromatosis (VRNF) is one of the most common inherited disorders affecting the human nervous system. VRNF is transmitted as an autosomal dominant defect with high penetrance but variable expressivity. The disorder is characterized clinically by hyperpigmented patches of skin (café au lait macules, axillary freckles) and by multiple tumors of peripheral nerve, spinal nerve roots, and brain (neurofibromas, optic gliomas). These tumors can cause disfigurement, paralysis, blindness, and death. We have determined the chromosomal location of the VRNF gene by genetic linkage analysis using DNA markers. The VRNF gene is genetically linked to the locus encoding nerve growth factor receptor, located on the long arm of chromosome 17 in the region 17q12→17q22. However, crossovers with the VRNF locus suggest that a mutation in the nerve growth factor receptor gene itself is unlikely to be the fundamental defect responsible for the VRNF phenotype.

[1] Neurogenetics Laboratory, Massachusetts General Hospital, and Department of Genetics, Harvard Medical School, Boston, Massachusetts 02114.
[2] Department of Molecular Neurogenetics, E. K. Shriver Center, Waltham, Massachusetts 02254.
[3] Neurosurgical Service, Department of Surgery, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114.
[4] Department of Cell Biology and Anatomy, Cornell University Medical College, New York, New York 10021.
[5] Section of Medical Genetics, University of Wales College of Medicine, Heath Park, Cardiff, United Kingdom.
[6] Departments of Genetics, Neurology and Ophthalmology, Children's Hospital and Harvard Medical School, Boston, Massachusetts 02115.
[7] Clinical Epidemiology Branch and Radiation Oncology Branch, National Cancer Institute, Bethesda, Maryland 20205.
[8] Division of Neurology, Department of Medicine, Duke University, Durham, North Carolina 27710.
[9] Department of Medicine, University of Michigan, Ann Arbor, Michigan 48109.
[10] Departments of Physiology and Biophysics, University of Washington, Seattle, Washington 98185.
[11] Departments of Psychiatry and Biomathematics, UCLA School of Medicine, Los Angeles, California 90024.
[12] Department of Pediatrics, University of North Carolina, Chapel Hill, North Carolina 27514.

Introduction

von Recklinghausen neurofibromatosis (VRNF) is one of the most frequent and clinically important Mendelian disorders in man, with an incidence of 1 in 3000 (Crowe et al., 1956). The disease displays autosomal dominant inheritance and affects all races, with half of all cases thought to result from new mutations (Sergeyev, 1975). The penetrance of the defect is high, but expression of the disease phenotype is extremely variable, even within families (Riccardi, 1981). VRNF has numerous manifestations, including mental retardation, learning disabilities, macrocephaly, and bone abnormalities, as well as the formation of multiple tumors affecting various organ systems. While different cell types can be affected, the most common abnormalities are in cells of neural crest origin (Riccardi, 1981; Sorensen et al., 1986). The disorder is diagnosed by the presence of multiple café au lait macules and axillary freckles (dysregulated growth or metabolism of melanocytes), iris hamartomas (Lisch nodules), and neurofibromas (disordered growth of Schwann cells) which can be painful, disfiguring, and disabling (Riccardi, 1981). Other nervous system tumors, such as spinal nerve root neurofibromas and optic gliomas, can lead to serious neurological complications. Moreover, patients with VRNF have an increased incidence of malignant neurofibrosarcomas, which are usually fatal (Sorensen et al., 1986; Storm et al., 1980). The primary biochemical defect in VRNF is not known, but may relate to fundamental mechanisms controlling growth and differentiation during development of the nervous system.

With the recent use of recombinant DNA techniques to generate large numbers of polymorphic markers in the human genome, genetic linkage analysis has become a powerful method for approaching inherited diseases where no defective protein has been identified (Botstein et al., 1980; Gusella, 1986). The defects causing Huntington's disease (Gusella et al., 1983), polycystic kidney disease (Reeders et al., 1985), cystic fibrosis (Tsui et al., 1985; Knowlton et al., 1985; White et al., 1985; Wainwright et al. 1985), and familial Alzheimer's disease (St George-Hyslop et al., 1987) have all been localized to specific autosomal regions by demonstrating cosegregation of the disease with particular DNA markers displaying restriction fragment length polymorphism (RFLP). We have used this strategy to determine the chromosomal location of the gene causing VRNF as a first step in applying chromosome specific cloning techniques to isolation and characterization of the defect. In this paper we provide conclusive evidence that the VRNF gene is genetically linked to the locus encoding the receptor for nerve growth factor (NGF) on the long arm of chromosome 17 in region 17q12→17q22. The detection of recombination events separating the two loci suggests however, that if VRNF is not heterozygous, the NGF receptor gene is not the site of the primary defect in this disease.

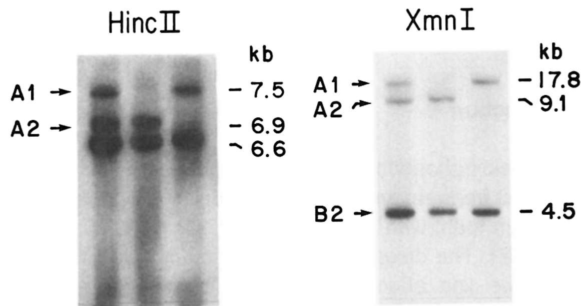

Figure 1. RFLPs at the NGF Receptor Locus

Genomic DNA from members of the VRNF families was digested with the restriction enzymes HincII and XmnI, respectively. The DNA fragments were separated by electrophoresis on 0.8% agarose gels at 90V for 16 hr. Southern blots were hybridized to a repeat-free fragment from genomic clone pE51, representing the NGF receptor locus (Chao et al., 1986). A1 and A2 represent allelic fragments for the respective RFLPs. The fragment labeled B2 in the XmnI digests represents the most frequent allele of a second XmnI RFLP whose infrequent alternate allele (B1) was not seen in this study (Breakefield et al., 1986).

Results

VRNF Pedigrees

For the efficient pursuit of genetic linkage investigations in VRNF, we have formed an international collaboration to identify and analyze large disease pedigrees from widely differing geographic locations. As a permanent source of DNA for RFLP investigations, we have established permanent lymphoblastoid cell lines from 239 members of 13 independent kindreds with typical VRNF, including 120 samples from affected individuals. Twelve of the thirteen pedigrees represent three generation families. Diagnosis of VRNF was based on criteria in agreement with that recently established by the National Institutes of Health (Mulvihill, 1986). von Recklinghausen neurofibromatosis is considered to be present in an individual with two or more of the following: (1) at least five café au lait macules over 5 mm in diameter (prepubertal) or six café au lait macules over 15 mm in diameter (postpubertal); (2) two or more neurofibromas of any type, or one plexiform neurofibroma; (3) multiple axillary or inguinal freckles; (4) sphenoid wing dysplasia, congenital bowing, or thinning of long bone cortex; (5) bilateral optic nerve gliomas; (6) two or more iris Lisch nodules on slit lamp examination; and (7) a first-degree relative with VRNF.

Strategy of Linkage Analysis

In the absence of any direct clue to the chromosomal position of a particular disease gene, the use of DNA markers chosen from many different chromosomal regions can provide a reasonable probability of finding positive genetic linkage (Gusella, 1986). This approach can employ either anonymous DNA sequences or cloned genes that detect RFLP. If, however, the markers chosen represent cloned genes that are reasonable candidates for the site of the primary defect, positive linkage with no recombination may signify direct identification of the defective locus. Since VRNF is associated with inherited susceptibility to tumor formation, oncogenes, growth factors, and their receptors are candidate genes where mutations might cause the disease phenotype. We have previously tested a battery of probes for such candidate genes for linkage to VRNF with negative results (Seizinger et al., 1987b).

NGF and its receptor are thought to play an important role in the development of sympathetic and sensory neurons and probably other neural-crest-derived cell types, such as Schwann cells (Levi-Montalcini and Angeletti, 1968; Thoenen and Barde, 1980; Taniuchi et al., 1986). The β NGF gene has previously been tested and eliminated as a potential site of the primary defect in VRNF (Darby et al., 1985; Seizinger et al., 1987b). Recently, genetic linkage data from a number of laboratories was compiled at the European Symposium on Neurofibromatosis (Egham, Surrey, UK, February 5–7, 1987). A number of papers summarizing the data from several groups along with a tentative exclusion map for VRNF will be published in the Journal of Medical Genetics (Sarfarazi et al., 1987). This exclusion map revealed that the region on the long arm of chromosome 17 containing the structural gene for the NGF receptor (Huebner et al., 1986) remained a possible location of the VRNF defect. In view of recent studies reporting the expression of NGF receptor in neurofibromas (Ross et al., 1984; Sonnenfeld et al., 1986), we concentrated our efforts on testing this locus as a candidate for the site of the primary defect in VRNF.

RFLP at the NGF Receptor Locus

We have shown that a 4.1 kb genomic EcoRI–BamHI fragment of clone pE51 from the NGF receptor locus detects RFLP with the enzymes HincII and XmnI, as displayed in Figure 1 (Breakefield et al., 1986). A second XmnI RFLP seen previously was not detected in the current study. The HincII RFLP has allelic fragments of 7.5 kb and 6.9 kb with frequencies of 0.26 and 0.74, respectively (N=152). The 17.8 kb and 9.1 kb allelic fragments of the XmnI RFLP have frequencies of 0.89 and 0.11, respectively (N=152). The two RFLPs display no apparent linkage disequilibrium with each other. Typing results from both sites can be combined to generate four haplotypes (as described in Figure 2): A, B, C, and D, with observed frequencies (based on 144 chromosomes) of 0.20, 0.03, 0.65, and 0.12, respectively.

Genetic Linkage of NGF Receptor to VRNF

Members of the 13 VRNF kindreds were typed for both RFLPs at the NGF receptor locus. Figure 2 shows a representative example of segregation of the marker locus in a single family, pedigree 13. Typing data for all families were analyzed for genetic linkage to VRNF using the computer program LIPED (Ott, 1974, 1976). The results are expressed in Table 1 as a lod score (z) representing the $\log_{10}$ of the ratio of the likelihood of linkage to VRNF at the specified recombination fractions (θ) relative to the likelihood of nonlinkage (θ = 0.50). Generally, a lod score $>+3$ is considered proof of linkage, while a lod score <-2 excludes linkage at the specified θ value.

Nine of the 13 families yielded non-zero lod scores, indi-

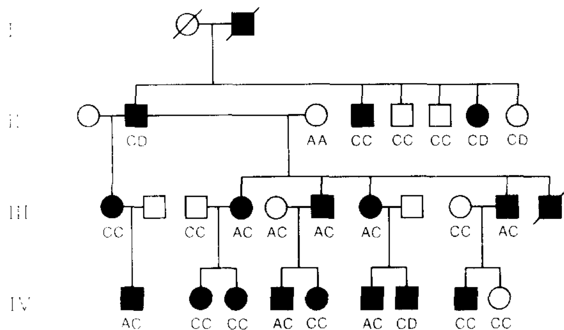

Figure 2. Segregation of NGF Receptor Haplotypes in a VRNF Family

Circles and squares represent females and males, respectively. A black symbol indicates that the individual is affected with VRNF. A slashed symbol indicates that the individual is deceased. The sexes have been changed in a few instances to preserve confidentiality. Members of pedigree 13 were typed for both the HincII and XmnI RFLPs at the NGF receptor locus and the individual alleles for each RFLP were combined to generate four haplotypes: A, A1 for both RFLPs; B, A1 for HincII, A2 for XmnI; C, A2 for HincII, A1 for XmnI; D, A2 for both RFLPs.

cating they contained some genetic information concerning linkage of the disease to the marker, although pedigree 9 contributed minimally. Eight of the pedigrees yielded positive lod scores, with five of these containing no detectable crosses between the two loci. One family, pedigree 10, produced mildly negative scores. Under the assumption of genetic homogeneity, lod scores can be summed across families. For this data set, the lod score exceeded +3 for a wide range of θ values, constituting significant linkage of VRNF to the NGF receptor gene.

The maximum likelihood estimate of the true frequency of recombination between the two loci ($\hat{\theta}$) corresponds to the value of θ with the maximum lod score ($\hat{z}$). The maximum lod score for linkage of VRNF to the NGF receptor gene, $\hat{z}$ = 4.41, occurred at $\hat{\theta}$ = 0.14, indicating a significant genetic separation between the two loci. The 1-lod unit confidence interval on this estimate of θ (approximating a 95% confidence interval) extends from 0.05 to 0.28.

Discussion

The discovery of a genetic marker linked to VRNF has profound implications both for fundamental research and clinical practice, and dramatically enhances the prospect of identifying the nature of the primary defect in this disorder. The observation in some families of recombination events between the two loci makes it unlikely that the underlying cause of VRNF is a mutation directly affecting expression of the NGF receptor gene in the pedigrees. It remains possible that VRNF is a heterozygous disorder and that the NGF receptor locus is the site of the defect in those families showing no crossovers. However, if there is only one locus for VRNF, it is most probably at some distance away from the NGF receptor locus, in a neighboring gene on the long arm or proximal short arm of chromosome 17.

A second gene from chromosome 17q that could be considered a candidate for causing VRNF is *NGL*, the human homolog of the *neu* oncogene associated with the formation of neuroblastomas and glioblastomas in the rat (Schechter et al., 1984; Schechter et al. 1985). The isolation of additional polymorphic DNA markers from this region, including RFLPs at the *NGL* locus, and their analysis in VRNF pedigrees along with construction of a general linkage map for chromosome 17, should clarify the precise genetic position of the VRNF gene.

Additional DNA markers more tightly linked to, and flanking, the disease gene will also be needed to deliver accurate prenatal and presymptomatic diagnosis for VRNF. Before such testing can begin, however, it is essential to explore the possibility of nonallelic heterogeneity in VRNF. With one exception, all of the families in Table 1 gave positive lod scores for linkage with the NGF receptor gene. The negative lod score from family 10 reflects the presence of an obligate crossover between the marker and the disease in a relatively small pedigree, and does not constitute strong evidence in favor of nonallelic heterogeneity. However, it is not advisable to proceed with predictive testing until markers closer to the disease gene have been found and many more VRNF pedigrees have been typed to confirm the absence of heterogeneity.

A remarkable feature of VRNF that has yet to be explained is the very high apparent rate of new mutations which has been used as an argument in favor of nonallelic heterogeneity (Sergeyev, 1975; Riccardi, 1982). An alternative explanation, by analogy to the Duchenne muscular

Table 1. Lod Scores for Linkage of VRNF to the NGF Receptor Gene

VRNF	Recombination Fraction (θ)							Peak Lod Score	
Pedigree[a]	0.00	0.01	0.05	0.10	0.20	0.30	0.40	($\hat{z}$) at	$\hat{\theta}$
4	0.80	0.78	0.72	0.63	0.44	0.24	0.07	0.80	0.00
5	0.37	0.36	0.32	0.27	0.17	0.09	0.02	0.37	0.00
6	0.28	0.27	0.24	0.20	0.12	0.06	0.02	0.28	0.00
7	0.86	0.84	0.78	0.69	0.51	0.34	0.17	0.86	0.00
9	0.07	0.06	0.05	0.04	0.02	0.01	0.00	0.07	0.00
10	$-\infty$	−1.22	−0.55	−0.29	−0.09	−0.02	0.00		
11	$-\infty$	0.29	0.87	1.02	0.98	0.77	0.44	1.04	0.13
12	−6.38	−2.23	−0.52	0.26	0.80	0.78	0.39	0.83	0.24
13	1.35	1.39	1.46	1.44	1.23	0.86	0.41	1.46	0.06
All Families	$-\infty$	0.54	3.37	4.26	4.18	3.13	1.52	4.41	0.14

[a] Pedigrees 1, 2, 3, and 8 were uninformative for the NGF receptor RFLPs, giving lod scores of 0.00 for all values of θ.

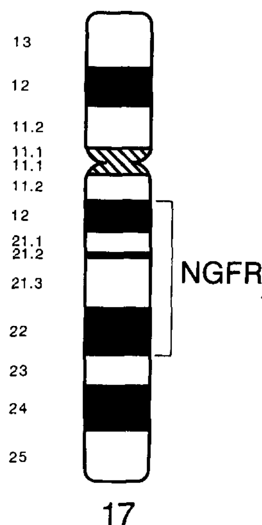

Figure 3. Chromosomal Location of the NGF Receptor Gene
The location of the NGF receptor gene (NGFR) to chromosome 17 in the region 17q12→17q22 is based on the combined somatic cell genetic and in situ hybridization analyses of Huebner et al., 1986.

dystrophy locus (Monaco et al., 1986), is that the VRNF gene is relatively large, increasing the effective target size for mutation. A third possibility is that VRNF is actually caused by two very tightly linked mutations (perhaps within the same gene) that must be present on the same chromosome (in *cis*) in order to produce the disease phenotype. Both mutations could then exist independently in the population without causing the disease, but occasional recombination events in doubly heterozygous individuals could bring them together on the same chromosome, giving the appearance of new mutations occurring at relatively high frequency. The availability of RFLP markers linked to VRNF should permit testing of this latter hypothesis even before the disease gene is cloned.

Another question that can now be approached directly with a linked marker for VRNF relates to the mechanism of tumorigenesis in this disorder. The germ-line mutation transmitted in VRNF might produce an altered protein whose activity predominates over its normal counterpart and causes tumor formation. Alternatively, the VRNF mutation could involve inactivation of a "tumor suppressor" gene, similar to that postulated in retinoblastoma (Cavenee, 1986). Tumor formation would only occur when a second somatic mutation event destroyed the activity of the remaining normal homolog. We have presented evidence that the latter mechanism is operative in bilateral acoustic neurofibromatosis (BANF), an autosomal dominant disorder genetically and clinically distinct from VRNF, but also involving formation of Schwann cell tumors (Seizinger et al., 1986; Seizinger et al., 1987a; Rouleau et al., unpubished data). In BANF, specific loss of alleles from chromosome 22 is associated with several different tumor types (Seizinger et al., 1987a). While we have not observed loss of alleles from chromosome 22 in VRNF tumors (Seizinger et al., unpublished observations), it is possible that a similar mechanism involving chromosome 17 occurs in this disorder. If so, the search for small deletions in tumors associated with VRNF may provide a means to narrow the location of the defective gene and to facilitate its isolation.

Even if chromosome 17 deletions or rearrangements do not play a role in tumorigenesis in VRNF, cloning and the characterization of the disease gene should be possible from the knowledge of its map location provided by linked DNA markers. Numerous methods have been developed in recent years to implement this strategy, which is currently being pursued in several other chromosomally localized disorders, and has already been successful in certain instances (Royer-Pokora et al, 1986; Friend et al., 1986; Monaco et al., 1986). The isolation of the VRNF gene would probably permit generation of a true animal model for the disorder. An understanding of the nature of the VRNF defect might also provide the basis for designing rational therapies to interfere with expression of the disease phenotype. Ultimately, it is likely that efforts targeted at elucidating the function of both defective and normal alleles at the VRNF locus will yield significant insights into mechanisms controlling differentiation of neural crest cells, and development of the nervous system in man.

Experimental Procedures

Pedigree DNAs and Cell Lines

Approximately 40 ml of blood was obtained from each individual from the 13 VRNF families. Twenty milliliters of each sample was used to establish Epstein–Barr virus-transformed lymphoblastoid cell lines as a permanent source of DNA (Anderson and Gusella, 1984). The remaining portion of each sample was used for direct DNA extraction (Seizinger et al., 1986). DNA was also prepared from lymphoblastoid cell lines as previously described (Gusella et al., 1979).

RFLP Typing

For RFLP typing, 5 μg of each DNA was digested to completion with the indicated restriction enzyme as outlined by the supplier (Boehringer Mannheim and New England Biolabs). The resultant DNA fragments were resolved according to size by horizontal agarose gel electrophoresis in TBE buffer, and, after staining with ethidium bromide for direct visual inspection, the DNA fragments were denatured in situ in the gel with 1 N NaOH. The denatured DNA fragments were transferred to a nylon filter support (Zetapor, AMF/Cuno, Meriden, CT) by Southern blotting, and fixed to the filter by baking. DNA probes were labeled with [^{32}P]-ATP (Amersham) by random oligonucleotide priming (Feinberg and Vogelstein, 1984), and then hybridized to the filter for 48 hr at 65°C in 6× SSC, 1× Denhardt's solution, 0.3% SDS, and 100 μg/ml of salmon testis DNA. The filters were washed in 0.5 × SSC at 65°C, and exposed to X-ray film (Kodak XAR-5) with a Dupont Cronex intensifying screen at −80°C for 48 hr. Alleles present in each individual were deduced from the sizes of bands on the developed film.

Linkage Analysis

Linkage analysis was carried out using the LIPED computer program (Ott, 1974, 1976). For the calculations in Table 1, the mutation rate was set at 0.00015, the frequency of the defective allele at 0.0003 and penetrance at 95% based on the infrequent observation of obligate gene carriers showing no signs of the disease (Spence et al., 1983; Carey et al., 1979). Altering these assumptions to a zero mutation rate, 0.0001 frequency for the disease gene or to each of 100%, 90% and 85% penetrance did not significantly alter the overall lod scores.

Acknowledgments

B. R. S. is supported by a grant and fellowship from the National Neurofibromatosis Foundation. G. A. R. is supported in part by the Fonds

de Recherche en Sante du Quebec, and, along with P. St G.-H., is a fellow of the Medical Research Council of Canada. R. L. M. is the recipient of a National Institute of Neurological and Communicative Disorders and Stroke Teacher-Investigator Career Development Award (NS00654). J. F. G. is a Searle Scholar of the Chicago Community Trust. Funds for this research were provided by National Institute for Neurological and Communicative Disorders and Stroke grants NS22224, NS20012, NS23008, and NS24279, and by grants from the McKnight Foundation, the Dystonia Medical Research Foundation, and the Julieanne Dorn Fund for Neurological Research.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *"advertisement"* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received May 4, 1987.

References

Anderson, M. A., and Gusella, J. F. (1984). The use of cyclosporin A in establishing human EBV-transformed lymphoblastoid cell lines. In Vitro *11*, 856–858.

Botstein, D., White, R. L., Skolnick, M., and Davis, R. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. *32*, 314–331.

Breakefield, X. O., Ozelius, L., Bothwell, M. A., Chao, M. V., Axelrod, F., Kramer, P. L., Kidd, K. K., Lanahan, A. A., Johnson, D. E., Ross, A. H., and Gusella, J. F. (1986). DNA polymorphism for the nerve growth factor receptor gene excludes its role in familial dysautonomia. Mol. Biol. and Med. *3*, 483–494.

Carey, J. C., Laub, J. M., and Hall, B. D. (1979). Penetrance and variability in neurofibromatosis: a genetic study of 60 families. Birth Defects *15*(5B), 271–281.

Cavenee, W. (1986). The genetic basis of neoplasia: the retinoblastoma paradigm. Trends Genet. *2*, 299–300.

Chao, M. V., Bothwell, M. A., Ross, A. H., Koprowski, H., Lanahan, A. A., Buck, C. R., and Sehgal, A. (1986). Gene transfer and molecular cloning of the human NGF receptor. Science *232*, 518–521.

Crowe, F. W., Schull, W. J., and Neel, J. V. (1956). A Clinical, Pathological, and Genetic Study of Multiple Neurofibromas (Springfield, IL: Charles C. Thomas).

Darby, J. K., Feder, J., Selby, M., Riccardi, V., Ferrell, R., Siao, D., Goslin, K., Rutter, W., Shooter, E. M., and Cavalli-Sforza, L. L. (1985). A discordant sibship analysis between β-NGF and neurofibromatosis. Am. J. Hum. Genet. *37*, 52–59.

Feinberg, A. P., and Vogelstein, B. (1984). A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity: addendum. Anal. Biochem. *137*, 266–267.

Friend, S. H., Bernards, R., Rogelj, S., Weinberg, R. A., Rapaport, J. M., Albert, D. M., and Dryja, T. P. (1986). A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. Nature *323*, 643–646.

Gusella, J. F., Varsanyi-Breiner, A., Kao, F. T., Jones, C., Puck, T. T., Keys, C., Orkin, S., and Housman, D. E. (1979). Precise localization of the human β globin gene complex on chromosome 11. Proc. Natl. Acad. Sci. USA *76*, 5239–5243.

Gusella, J. F., Wexler, N. S., Conneally, P. M., Naylor, S. L., Anderson, M. A., Tanzi, R. E., Watkins, P. C., Ottina, K., Wallace, M. R., Sakaguchi, A. Y., Young, A. B., Shoulson, I., Bonilla, E., and Martin, J. B. (1983). A polymorphic DNA marker genetically linked to Huntington's disease. Nature *306*, 234–238.

Gusella, J. F. (1986). DNA polymorphism and human disease. Ann. Rev. Biochem. *55*, 831–54.

Huebner, K., Isobe, M., Chao, M., Bothwell, M., Ross, A. H., Finan, J., Hoxie, J. A., Sehgal, A., Buck, C. R., Lanahan, A., Nowell, P. C., Koprowski, H., and Croce, C. (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.

Knowlton, R. G., Cohen-Hagenauer, O., Van Cong, N., Frezal, J., Brown, V. A., Barker, D., Braman, J. C., Schumm, J. W., Tsui, L. C., Buchwald, M., and Donis-Keller, H. (1985). A polymorphic DNA marker linked to cystic fibrosis is located on chromosome 7. Nature *318*, 380–382.

Levi-Montalcini, R., and Angeletti, P. U. (1968). Nerve growth factor. Physiol. Rev. *48*, 534–569.

Monaco, A. P., Neve, R. L., Colletti-Feener, C., Bertelson, C. J., Kurnit, D. M., and Kunkel, L. M. (1986). Isolation of candidate cDNAs for portions of the Duchenne muscular dystrophy gene. Nature *323*, 646–650.

Mulvihill, J. J. (1986). Tentative definitions of neurofibromatosis. Neurofibromatosis Res. Newsletter *2*, 1.

Ott, J. (1974). Estimation of the recombination fraction in human pedigrees: efficient computation of the likelihood for human linkage studies. Am. J. Hum. Genet. *26*, 588–597.

Ott, J. (1976). A computer program for linkage analysis of general human pedigrees. Am. J. Hum. Genet. *28*, 528–529.

Reeders, S. T., Breuning, M. H., Davies, K. E., Nicholls, R. D., Jarman, A. P., Higgs, D. R., Pearson, P. L., and Weatherall, D. J. (1985). A highly polymorphic DNA marker linked to adult polycystic kidney disease on chromosome 16. Nature *317*, 542–544.

Riccardi, V. M. (1981). von Recklinghausen neurofibromatosis. N. Engl. J. Med. *305*, 1617–1627.

Riccardi, V. M. (1982). Neurofibromatosis: clinical heterogeneity. Curr. Prob. Cancer *7*, 1–34.

Ross, A. H., Grob, P., Bothwell, M., Elder, D. E., Ernst, C. S., Marano, N., Ghrist, B. F. D., Slemp, C. C., Herlyn, M., Atkinson, B., and Koprowski, H. (1984). Characterization of nerve growth factor receptor in neural crest tumors using monoclonal antibodies. Proc. Natl. Acad. Sci. USA *81*, 6681–6685.

Royer-Pokora, B., Kunkel, L. M., Monaco, A. P., Goff, S. C., Newburger, P. E., Baehner, R. L., Cole, F. S., Curnutte, J. T., and Orkin, S. H. (1986). Cloning the gene for an inherited human disorder—chronic granulomatous disease—on the basis of its chromosomal location. Nature *322*, 32–38.

Sarfarazi, M., Huson, S. M., and Edwards, J. H. (1987). An exclusion map for von Recklinghausen neurofibromatosis. J. Med. Genet., in press.

St George-Hyslop, P. H., Tanzi, R. E., Polinsky, R. J., Haines, J. L., Nee, L., Watkins, P. C., Myers, R. H., Feldman, R. G., Pollen, D., Drachman, D., Growdon, J., Bruni, A., Foncin, J. F., Salmon, D., Frommelt, P., Amaducci, L., Sorbi, S., Piacentini, S., Stewart, G. D., Hobbs, W. J., Conneally, P. M., and Gusella, J. F. (1987). The genetic defect causing familial Alzheimer's disease maps on chromosome 21. Science *235*, 885–890.

Schechter, A. L., Stern, D. F., Vaidyanathan, L., Decker, S. J., Drebin, J. A., Greene, M. I., and Weinberg, R. A. (1984). The neu oncogene: an erb-B-related gene encoding a 185,000-Mr tumour antigen. Nature *312*, 513–516.

Schechter, A. L., Hung, M. C. Vaidyanathan, L., Weinberg, R. A., Yang-Feng, T. L., Francke, U., Ullrich, A., and Coussens, L. (1985). The neu gene: an erbB-homologous gene distinct from and unlinked to the gene encoding the EGF receptor. Science *229*, 976–978.

Seizinger, B. R., Martuza, R. L., and Gusella, J. F. (1986). Loss of genes on chromosome 22 in tumorigenesis of human acoustic neuroma. Nature *322*, 664–667.

Seizinger, B. R., Rouleau, G. A., Ozelius, L. J., Lane, A. H., St George-Hyslop, P., Huson, S., Gusella, J. F., and Martuza, R. L. (1987a). Common pathogenetic mechanism for three tumor types in bilateral acoustic neurofibromatosis. Science *236*, 317–319.

Seizinger, B. R., Rouleau, G., Lane, A. H., Ozelius, L. J., Faryniarz, A. G., Iannazzi, J., Hobbs, W., Roy, J. C., Falcone, B., Huson, S., Harper, P., Parry, D. M., Bader, J. L., Spence, M. A., and Gusella, J. F. (1987b). DNA linkage analysis in von Recklinghausen's neurofibromatosis. J. Med. Genet., in press.

Sergeyev, A. S. (1975). On the mutation rate of neurofibromatosis. Hum. Genet. *28*, 129–138.

Sonnenfeld, K. H., Bernd, P., Sobue, G., Lebwohl, M., and Rubenstein, A. E. (1986). Nerve growth factor receptors on dissociated neurofibroma Schwann-like cells. Cancer Res. *46*, 1446–1452.

Sorensen, S. A., Mulvihill, J. J., and Nielsen, A. (1986). Long-term follow-up of von Recklinghausen neurofibromatosis: survival and malignant neoplasms. New Engl. J. Med. *314*, 1010–1015.

Spence, M. A., Bader, J. L., Parry, D. M., Field, L. L,, Funderburk, S. J., Rubenstein, A. E., Gilman, P. A., and Sparkes, R. S. (1983). Linkage analysis of neurofibromatosis (von Recklinghausen disease). J. Med. Genet. *20*, 334–337.

Storm, E. K., Eilber, F. R., Mirra, J., and Morton, D. L. (1980). Neurofibrosarcoma. Cancer *45*, 126–129.

Taniuchi, M., Clark, H. B., and Johnson, E., Jr. (1986). Induction of nerve growth factor receptor in Schwann cells after axotomy. Proc. Natl. Acad. Sci. USA *83*, 4094–4098.

Thoenen, H., and Barde, Y. A. (1980). Physiology of nerve growth factor. Physiol. Rev. *60*, 1284–1335.

Tsui, L., Buchwald, M., Barker, D., Braman, J., Knowlton, R., Schumm, J., Eiberg, H., Mohr, J., Kennedy, D., Plavsic, N., Zsiga, M., Markiewicz, D., Akots, G., Helms, C., Gravius, T., Parker, C., Rediker, K., and Donis-Keller, H. (1985). Cystic fibrosis locus defined by a genetically linked polymorphic DNA marker. Science *230*, 1054–1057.

Wainwright, B., Scambler, P. J., Schmidtke, J., Watson, E. A., Law, H. Y., Farrall, M., Cooke, H. J., Eiberg, H., and Williamson, R. (1985). Localization of cystic fibrosis locus to human chromosome 7cen-q22. Nature *318*, 384–386.

White, R. L., Woodward, S., Leppert, M., O'Connell, P., Hoff, M., Herbst, J., Lalouel, J. M., Dean, M., and Van de Woude, G. (1985). A closely linked genetic marker for cystic fibrosis. Nature *318*, 382–384.

Note Added in Proof

In agreement with the findings reported in this paper, Barker et al. independently found genetic linkage of von Recklinghausen neurofibromatosis to a probe that maps to the centromeric region of chromosome 17 (Science, in press).