Mapping of the gene encoding the β -amyloid precursor protein and its relationship to the Down syndrome region of chromosome 21

DAVID PATTERSON^{*†}, KATHELEEN GARDINER^{*}, FA-TEN KAO^{*}, RUDOLPH TANZI[†], PAUL WATKINS[‡],
AND JAMES F. GUSELLA[†]

^{*}Eleanor Roosevelt Institute for Cancer Research, 1899 Gaylord Street, Denver, CO 80206; [†]Neurogenetics Laboratory, Department of Genetics, Program in Neuroscience, Harvard Medical School, Massachusetts General Hospital, Fruit Street, Research 3, Boston, MA 02114; and [‡]Integrated Genetics, 31 New York Avenue, Framingham, MA 01701

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ABSTRACT The gene encoding the β -amyloid precursor protein has been assigned to human chromosome 21, as has a gene responsible for at least some cases of familial Alzheimer disease. Linkage studies strongly suggest that the β -amyloid precursor protein and the product corresponding to familial Alzheimer disease are from two genes, or at least that several million base pairs of DNA separate the markers. The precise location of the β -amyloid precursor protein gene on chromosome 21 has not yet been determined. Here we show, by using a somatic-cell/hybrid-cell mapping panel, *in situ* hybridization, and transverse-alternating-field electrophoresis, that the β -amyloid precursor protein gene is located on chromosome 21 very near the 21q21/21q22 border and probably within the region of chromosome 21 that, when trisomic, results in Down syndrome.

The gene responsible for at least some cases of familial Alzheimer disease (FAD) has been mapped to human chromosome 21 (1). Simultaneously, the gene encoding the β -amyloid precursor protein (APP) was also mapped to chromosome 21 (2–4), perhaps in the 21q11.2–21q2105 region (5–7). Because a major constituent of the amyloid plaque, invariably associated with Alzheimer disease, is derived from the APP (8–10), these observations led naturally to the speculation that the APP gene and the FAD gene might be identical. However, observations of obligatory crossovers between FAD and APP seem to have ruled out this possibility, and it now appears that as much as 8 million base pairs of DNA may separate these two loci (11–13). This does not, however, rule out the possibility that altered expression of the APP gene or altered processing of its transcript, which has been reported by several groups (14–16), or even altered processing of its protein product may be critical to the development of the pathology seen in Alzheimer disease. In fact, it seems highly likely that one or more of these events must be related, either as a cause or a consequence, to this pathology.

Although the function of the APP is not yet clear, there is evidence that it may be a membrane protein and that it may be important in regulation of cell growth (17, 18). These observations are consistent with its demonstrated expression in a wide variety of tissues and animal species (2, 3).

The exact location of the APP gene on chromosome 21 is of considerable interest for several reasons. By the fourth decade of life, virtually all individuals with trisomy 21, Down syndrome, will develop amyloid plaques containing APP-derived material and a large fraction of these individuals will develop symptoms of Alzheimer disease (19–21). It is possible that APP accumulation in these individuals is due to the presence of an extra copy of the APP gene and resultant

overproduction of the APP. However, there is general agreement that trisomy of only band 21q22 is necessary and sufficient for development of the pathology associated with Down syndrome (22). Therefore, if the APP gene is not within 21q22, individuals with Down syndrome who are trisomic for only 21q22 would not be expected to accumulate APP and plaques and thus would not be expected to develop Alzheimer disease. It is also noteworthy that so far all genes regionally mapped on chromosome 21 appear to lie within the 21q22 band (with the possible exception of the FAD locus itself). If the APP gene does not lie within this region, then it represents the first known exception to this clustering of genes in the 21q22 region of the chromosome.

For these reasons we undertook a multifaceted approach to the high-resolution regional mapping of the APP gene on chromosome 21 by using a combination of somatic cell genetic, cytogenetic, and molecular techniques. These studies place the APP gene very close to the border between the 21q21 and the 21q22 bands and perhaps within 21q22. Furthermore, it appears that the gene is within 1000 kilobase pairs of the binding site for the unique anonymous DNA probe D21S12 (pPW267). D21S12 maps by *in situ* hybridization and by molecular analysis of somatic cell hybrids to the same region of chromosome 21 as APP. We interpret these results to mean that the APP gene may well reside within the region of chromosome 21 thought to be pathogenetic for Down syndrome. Therefore, overexpression of this gene due to gene dosage is a viable hypothesis to explain the appearance of the neuropathology and symptoms of Alzheimer disease in individuals with Down syndrome.

MATERIALS AND METHODS

Construction and Analysis of CHO–Human Somatic Cell Hybrids. Construction of the CHO–human hybrids and their analysis followed published procedures (23). Hybrids were analyzed routinely cytogenetically by G11 staining and G banding (24–26).

***In Situ* Hybridization.** The procedures for *in situ* hybridization to metaphase chromosomes were essentially as described (27, 28). Briefly, human chromosomes were prepared from phytohemagglutinin-stimulated peripheral blood lymphocytes synchronized with bromodeoxyuridine and thymidine and incubated with Colcemid (0.1 μ g/ml) for 10 min prior to harvest. The chromosome spreads were aged for 5 days, then treated with RNase (Sigma) (100 μ g/ml) for 1 hr at 37°C, rinsed in 2 $\times$ SSC (1 $\times$ SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0), and dehydrated in ethanol. The probes were labeled with [³H]dCTP, [³H]dTTP, and [³H]dATP (New England Nuclear) to a specific activity >10⁸

cpm/ μ g by using the random-primer-extension oligolabeling technique of Feinberg and Vogelstein (29). Hybridization was carried out at 37°C for 18 hr in 50% (vol/vol) formaldehyde/10% (wt/vol) dextran sulfate/2 $\times$ SSCP/denatured salmon sperm DNA (1 mg/ml)/1 $\times$ Denhardt's solution (1 $\times$ SSCP = 0.2 M sodium phosphate, pH 5.4/0.15 M sodium citrate/1.2 M NaCl; 1 $\times$ Denhardt's solution = 0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin). After hybridization, the slides were washed extensively in 50% formaldehyde/2 $\times$ SSC at 41°C and at room temperature, dehydrated in ethanol, and air-dried. Slides were coated with Kodak NTB2 nuclear track emulsion, stored at 4°C for 14 or 21 days and developed in Kodak Dektol. The slides were stained with Hoechst H33258 for 15 min, exposed to UV light, and restained with Giemsa (Fisher) in phosphate buffer at pH 6.8/0.07 M KH_2PO_4 /0.07 M Na_2HPO_4 .

Transverse-Alternating-Field Gel Electrophoresis (TAFE). Analysis of large [>50 kilobase (kb)] DNA fragments was carried out in the vertical-pulsed-field gel system (30), by TAFE. Modifications to the original design have been described (31) and it is this modified version that was used here. DNA from hybrid cell lines was isolated in low-melting-temperature agarose as described (30), except that 1/8-inch-diameter tubing (1 inch = 2.54×10^{-2} m) was used as a mold. This gives a final DNA concentration of $\approx 60 \mu\text{g/ml}$, or 1.2 μg per 0.5-cm plug. Total human DNA was prepared from 12 ml of fresh EDTA-anticoagulated blood as follows: 6 ml of whole blood was added to each of two Sepacell-MN colloidal silica columns (Sepratech, Oklahoma City, OK) and columns were centrifuged at 1200 rpm in a clinical centrifuge (VWR, GT-2) for 20 min. The layer of mononuclear cells was collected, diluted to 15 ml with isotonic phosphate-buffered saline (PBS), and counted. Cells were pelleted by centrifugation at 600 rpm for 10 min, resuspended to 12 million cells per ml in L buffer (100 mM EDTA/20 mM NaCl/10 mM Tris-HCl, pH 7.8) and processed as for tissue culture cells (30). For restriction fragment analysis, a 0.5-cm plug was digested in 150 μl of the manufacturer's (New England Biolabs) recommended buffer plus 12 units of enzyme (except *Nor* I, which required bovine serum albumin at 0.5 mg/ml and 30 units of enzyme) for 4 hr. Digestions were stopped with EDTA, and samples were equilibrated in TE (10 mM Tris-HCl/1 mM EDTA, pH 8.0) prior to loading on a gel. Gels were 0.8% agarose (Bethesda Research Laboratories; Ultrapur) in 1 $\times$ PF/EB buffer (10 mM Tris/0.5 mM EDTA, pH 8.5) and were electrophoresed in the same buffer for 18 hr, with a 60-sec pulse time at 150 mA (starting voltage, 260 V). A 0.5-cm plug was loaded per lane. *Saccharomyces cerevisiae* chromosomes, used as size markers, were prepared as described (30).

Standard Gel Electrophoresis and Southern Transfers. For *Eco*RI digestions, DNA was isolated in liquid by standard phenol/ CHCl_3 extraction procedures, and restriction fragments were separated on standard submarine gels of 0.75% agarose in 1 $\times$ Tris acetate buffer, pH 8 (40 mM Tris acetate/1 mM EDTA). For both standard and pulsed-field gels, DNA was transferred to Genatran nylon filters (Plasco, Woburn, MA) by established procedures (42). Prior to transfer, pulsed-field gels were incubated in 0.25 M HCl for 5 min to deplete the DNA.

DNA Probes and Hybridization. FB130 is a cDNA probe for the APP gene. D21S11 (pPW236B) and D21S12 (pPW267C) are anonymous genomic DNA probes described and regionally mapped on chromosome 21 (2, 23, 32, 33). Probes were labeled to specific activities of $>10^9$ cpm/ μg , by the random-primed oligonucleotide method (29). Hybridizations were by standard procedures (42).

RESULTS AND DISCUSSION

The work presented here demonstrates that the APP gene is located close to the boundary between chromosome bands 21q21 and 21q22. It could lie within the most centromere-distal region of 21q21 or the most centromere-proximal region of 21q22. This means that the gene could well lie within the region of chromosome 21 pathogenetic for Down syndrome. In fact, the definition of this region is limited presently by the resolution of cytogenetic techniques, and definition of the region of chromosome 21 important for Down syndrome as determined cytogenetically is controversial. We conclude that there is no basis for excluding the APP gene from the pathogenetic region. This conclusion is in contrast to that of Jenkins *et al.* (6, 7) who have presented data interpreted as suggesting that the APP gene must be located centromere-proximal to 21q2105 and, therefore, is not in the region of the chromosome commonly associated with the pathology seen in individuals with Down syndrome.

We have demonstrated that the APP gene is absent from the ACCEM chromosome 21 rearrangement thought by cytogenetic analysis to be missing a large portion of the long arm of the chromosome including at least a majority of band 21q21 (2, 40). This chromosome does possess the *PRGS* gene and the *SOD1* gene, both thought to be in the 21q22.1 sub-band. Molecular analysis of this chromosome (ref. 41 and D.P., J.F.G., R.T., P.W., M. L. Van Keuren, G. D. Stewart, C. M. Bradley, D. M. Kurnit, & R. L. Neve, unpublished results) reveals that it has lost perhaps all of band 21q21. So far, we cannot be certain whether any of band 21q22 is missing in the ACCEM chromosome. Here we confirm that this chromosome is indeed missing the APP gene.

To further aid in regional mapping of chromosome 21, a number of additional somatic cell hybrids have been used (refs. 23 and 41) (Fig. 1). One of these was constructed by fusion of Ade⁻C CHO cell mutants deficient in *PRGS* activity with GM1881 human fibroblast cells with the reported karyotype 46,XY,t(1;21)(1qter-1p31.2::21q22-21qter;21pter-21q22::1p31.2-1pter) (34). Hybrid 1881 C13C, isolated from this fusion, contains the 1p⁻ chromosome bearing the 21q22-21qter region of chromosome 21. This

FIG. 1. Mapping of the APP locus by using a somatic cell hybrid mapping panel. All hybrids except 1:21 have been described (23, 40, 41). The solid bars indicate the chromosome 21 fragments present in the hybrids identified above each bar. The horizontal dashed lines define the area within which the APP gene must lie. FB130 is a probe recognizing the APP gene. D21S11 and D21S12 are anonymous DNA sequences previously regionally mapped to chromosome 21.

hybrid contains no other cytogenetically detectable chromosome 21 material. Cytogenetic analysis of the derivative chromosomes in this cell line is not sufficient to rule out a breakpoint in 21q21 near the 21q21/21q22 boundary (refs. 35 and 41). As can be seen in Fig. 2A, this hybrid contains the human APP gene. This confines the location of the APP gene to the proximal centromeric region of 21q22, above both *PRGS* and *SOD1* on the basis of cytogenetic analysis. Genetic linkage analysis has also placed the APP gene proximal to *SOD1* by 4 centimorgans (cM) (R.T., unpublished data). Similarly, the anonymous DNA fragment D21S12 can be mapped to this region of chromosome 21 (Fig. 2B). This hybrid does not contain the sequence D21S11, which we have shown (2) is linked to the APP locus. These data are summarized in Fig. 1.

To confirm this localization, the APP probe and D21S12 were mapped by *in situ* chromosome hybridization. The results, depicted in Fig. 3, clearly demonstrate that both probes map very close to the 21q21/21q22 boundary and completely confirm the analysis with somatic cell hybrids.

We used TAFE to determine how close the APP gene and D21S12 actually are on chromosome 21. For this experiment, we digested DNA isolated from human cells with a variety of rare-cutting restriction endonucleases and separated the DNA by TAFE. Analysis of these DNAs by Southern blotting is shown in Fig. 4. APP and D21S12 recognize fragments of the same size after *Not* I (1300 kb) and *Bss*HII (1100 kb) digestions, but of different sizes after *Sac* II digestion (500 kb and 300 kb, respectively). In a similar analysis of DNA from 18 individuals, *Not* I digestion revealed an apparent polymorphism. In 10 of 18 samples, both APP and D21S12 recognized a 1300-kb fragment; in the remaining 8 of 18, both hybridized to material trapped in the wells—i.e., material was >2000 kb (data not shown). This is good evidence that these probes are indeed physically linked—i.e., they are actually contained within the same *Not* I and *Bss*HII fragments—and not merely on separate but comigrating fragments. This conclusion is in complete agreement with the somatic cell hybrid and *in situ* hybridization analyses.

Conclusions. These studies allow the mapping of the APP gene to the 21q22 region of chromosome 21 or, perhaps, to the most centromere-distal region of band 21q21 at the 21q21/

FIG. 3. Grain distribution on human chromosome 21 by *in situ* hybridization of ^3H -labeled probes to metaphase chromosomes from human lymphocytes. (Upper) APP probe. Thirty-one chromosome spreads with grains on chromosome 21 were analyzed. Among 109 grains (3.5 grains per spread), 39 (35%) were on chromosome 21. Twenty-one of the 39 grains (56%) on chromosome 21 were at the junction of bands q21.2 and q22.1. (Lower) Probe D21S12. Twenty-five chromosome spreads with grains on chromosome 21 were analyzed. Among 101 grains (4 grains per spread), 36 (36%) were on chromosome 21. Twenty of the 36 grains (56%) on chromosome 21 were at the junction of bands q21.2 and q22.1.

21q22 boundary, by two independent methods. Moreover, this gene lies within 1000 kilobase pairs of the anonymous unique DNA sequence D21S12 that has also been mapped to this same region. This regional mapping is consistent with results with irradiation reduction hybrids reported by Graw *et al.* (36). It is also consistent with the linkage of the APP gene to the *SOD1* gene (ref. 11 and R. T., unpublished data). It is in contrast to the conclusions reached by Jenkins *et al.* (6, 7). The work of Jenkins *et al.* (6, 7) to a large extent is

FIG. 2. Regional localization of APP and D21S12. (A) Ethidium bromide-stained gel of *Eco*RI-digested DNAs. (B) Same DNA bands, after transfer to nylon membrane, hybridized with the β -amyloid probe. (C) Same blot, after stripping, rehybridized with the anonymous DNA probe D21S12. Lanes: 1, wild-type Chinese hamster ovary cells (CHO K1); 2, ACEM; 3, 1;21; 4, 21q+; 5, 8q-; 6, 153E7b; 7, 2Fur-1. Hu, human-specific hybridization. The chromosome 21-specific content of the hybrids is shown in Fig. 1.

FIG. 4. Physical linkage of β -amyloid and D21S12. (A) Ethidium bromide-stained pulsed-field gel showing resolution of 50 kb to 1500 kb. Lanes: Yt, *S. cerevisiae* chromosomes (the smallest band visible is $\approx$ 200 kb; the largest band is $\approx$ 1500 kb); L, λ ladder; 1-3, total human (lymphocyte) DNA digested with *Not* I, *Bss*HII, and *Sac* II, respectively. (B) Lanes 1-3 from A, after transfer to nylon membrane, hybridized with the APP probe. (C) Same blot, after stripping, rehybridized with D21S12. (A is shown at a smaller magnification than B and C.)

dependent upon the cytogenetic interpretation of a single translocation involving chromosome 21 that contained in human cell strain GM693 and is reported to break chromosome 21 at 21q2105. It is our experience that cytogenetic analysis of chromosome 21 at this degree of resolution without independent corroborative data is problematic.

Our localization of the APP gene suggests that in most cases of translocation Down syndrome the APP gene will be in the trisomic region of chromosome 21. This raises the possibility that a defect in this gene, in these cases the presence of an extra copy of the gene, without a defect in the FAD gene, which appears to be located much more centromere-proximal on chromosome 21 (11, 12) and is almost certainly present in only two copies in at least some cases of translocation Down syndrome, could be sufficient to lead to Alzheimer disease. This would be consistent with the hypothesis that Alzheimer disease might arise through a variety of genetic mechanisms and that a common feature of all of these would be altered expression of the APP gene or at least some mechanism by which altered levels of APP or a degradation product would accumulate in the brains of affected individuals. This would also suggest that overproduction of the APP gene product by as little as 50% will be sufficient for the deleterious effects to be apparent. In fact, at least a 50% increase in the levels of APP mRNA has been reported in fetal brains of individuals with Down syndrome as compared to normal individuals (2).

With the assignment of the APP gene near or within the 21q22 Giemsa-light band of chromosome 21, of all the genes regionally mapped to this chromosome only the FAD gene can be confidently mapped outside this chromosomal region. This is consistent with the hypotheses of several workers that the Giemsa-light chromosome bands are gene-rich compared to Giemsa-dark bands (37, 38).

In this regard, the likely localization of the putative FAD gene to the 21cen-21q21 region is an anomaly. It may imply that this gene is a developmental gene as defined by Goldman *et al.* (39). One may also speculate, however, that the FAD gene may not even be a structural gene coding for a protein product but that it may be a DNA sequence that functions in some other way—for example, as a determiner of chromosomal pairing during meiosis or mitosis. It may also be that this sequence has a regulatory role that does not require the production of a protein product.

Further elucidation of these points will be aided greatly by determination of whether amyloid plaques accumulate in the brains of patients with translocation Down syndrome who are trisomic for APP but not FAD, by the determination of the function of the APP gene product, and by the isolation and characterization of the FAD locus.

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- 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. & Gusella, J. F. (1987) *Science* **235**, 885–889.
- Tanzi, R. E., Gusella, J. F., Watkins, P. C., Bruns, G. A. P., St George-Hyslop, P., Van Keuren, M. L., Patterson, D., Pagan, S., Kurnit, D. M. & Neve, R. L. (1987) *Science* **235**, 880–884.
- Goldgaber, D., Lerman, M. I., McBride, O. W., Saffiotti, U. & Gajdusek, D. C. (1987) *Science* **235**, 877–880.
- Robakis, N. K., Ramakrishna, N., Wolfe, G. & Wisniewski, H. M. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4190–4194.
- Robakis, N. K., Wisniewski, H. M., Jenkins, E. C., Devine-Gage, E. A., Houck, G. E., Yao, X. L., Ramakrishna, N., Wolfe, G., Silverman, W. P. & Brown, W. T. (1987) *Lancet* **i**, 384–385.
- Jenkins, E. C., Devine-Gage, E. A., Yao, X.-L., Houck, G. E., Brown, W. T., Robakis, N. K. & Wisniewski, H. M. (1987) *Am. J. Hum. Genet.* **41**, A125 (abstr.).
- Jenkins, E. C., Devine-Gage, E. A., Robakis, N. K., Yao, X.-L., Brown, W. T., Houck, G. E., Wolfe, G., Ramakrishna, N., Silverman, W. P. & Wisniewski, H. M. (1988) *Biochem. Biophys. Res. Commun.* **151**, 1–8.
- Glennier, G. G. & Wong, C. W. (1984) *Biochem. Biophys. Res. Commun.* **120**, 885–890.
- Masters, C. L., Multhaup, G., Simms, G., Pottgiesser, J., Martins, R. N. & Beyreuther, K. (1985) *EMBO J.* **4**, 2757–2763.
- Masters, C. L., Simms, G., Weinman, N. A., Multhaup, G., McDonald, B. L. & Beyreuther, K. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4245–4249.
- Van Broeckhoven, C., Genthe, A. M., Vandenberghhe, A., Horsthemke, B., Backhovens, H., Raeymaekers, P., Van Hul, W., Wehnert, A., Gheuens, J., Cras, P., Bruylant, M., Martin, J. J., Salbaum, M., Multhaup, G., Masters, C. L., Beyreuther, K., Gurling, H. M. D., Mullan, M. J., Holland, A., Barton, A., Irving, N., Williamson, R., Richards, S. J. & Hardy, J. A. (1987) *Nature (London)* **329**, 153–155.
- Tanzi, R. E., St George-Hyslop, P. H., Haines, J. L., Polinsky, R. J., Nee, L., Foncin, J. F., Neve, R. L., McClatchey, A. I., Conneally, P. M. & Gusella, J. F. (1987) *Nature (London)* **329**, 156–157.
- Marx, J. L. (1987) *Science* **238**, 1352–1353.
- Ponte, P., Gonzalez-DeWhitt, P., Schilling, J., Miller, J., Hsu, D., Greenberg, B., Davis, K., Wallace, W., Lieberburg, I., Fuller, F. & Cordell, B. (1988) *Nature (London)* **331**, 525–527.
- Tanzi, R. E., McClatchey, A. I., Lamperti, E. D., Villa-Komaroff, L., Gusella, J. F. & Neve, R. L. (1988) *Nature (London)* **331**, 528–530.
- Kitaguchi, N., Takahashi, Y., Tokushima, Y., Shiojiri, S. & Ito, H. (1988) *Nature (London)* **331**, 530–532.
- Kang, J., Lemaire, H. G., Unterbeck, A., Salbaum, J. M., Masters, C. L., Grzeschik, K. H., Multhaup, G., Beyreuther, K. & Müller-Hill, B. (1987) *Nature (London)* **325**, 733–736.
- Zimmermann, K., Herget, T., Salbaum, J. M., Schubert, W., Hilbich, C., Cramer, M., Masters, C. L., Multhaup, G., Kang, J., Lemaire, H. G., Beyreuther, K. & Starzinski-Powitz, A. (1988) *EMBO J.* **7**, 367–372.
- Oliver, C. & Holland, A. J. (1986) *Psychol. Med.* **16**, 307–322.
- Burger, P. C. & Vogel, F. S. (1973) *Am. J. Pathol.* **73**, 457–476.
- Heyman, A., Wilkinson, W. E., Hurwitz, B. J., Schmechel, D., Sigmon, A. H., Weinberg, T., Helms, M. J. & Swift, M. (1983) *Ann. Neurol.* **14**, 507–515.
- Niebuhr, E. (1974) *Hum. Genet.* **21**, 99–101.
- Van Keuren, M. L., Watkins, P. C., Drabkin, H. A., Jabs, E. W., Gusella, J. F. & Patterson, D. (1986) *Am. J. Hum. Genet.* **38**, 793–804.
- Friend, K. K., Chen, S. & Ruddle, F. H. (1976) *Somat. Cell Genet.* **2**, 183–188.
- Alhadeff, B., Velivasakis, M. & Siniscalco, M. (1977) *Cytogenet. Cell Genet.* **19**, 236–239.
- Seabright, M. (1971) *Lancet* **ii**, 971–972.
- Law, M. L., Cai, G. Y., Lin, F. K., Wei, Q., Huang, S. Z., Hartz, J. H., Morse, H., Lin, C. H., Jones, C. & Kao, F. T. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 6920–6924.
- Hawkins, J. W., Van Keuren, M. L., Piatigorsky, J., Law, M. L., Patterson, D. & Kao, F. T. (1987) *Hum. Genet.* **76**, 375–380.
- Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132**, 6–13.
- Gardiner, K., Laas, W. & Patterson, D. (1986) *Somat. Cell Mol. Genet.* **12**, 185–195.
- Gardiner, K. & Patterson, D. (1988) *Nature (London)* **331**, 371–372.
- Watkins, P., Tanzi, R., Gibbons, K., Tricoli, J. V., Landes, G., Eddy, R., Shows, T. B. & Gusella, J. F. (1986) *Nucleic Acids Res.* **13**, 6075–6080.
- Watkins, P. C., Tanzi, R. E., Cheng, S. V. & Gusella, J. F. (1987) *J. Med. Genet.* **24**, 257–270.
- Rodewald, A., Cleve, H., Stengel-Rutkowski, S., Wirtz, A.,

- Murken, J. D., DiValerio, M., Mulivor, R. A., Greene, A. E. & Coriell, L. L. (1980) *Cytogenet. Cell Genet.* **27**, 267.
35. Korenberg, J. R., Croyle, M. L. & Cox, D. R. (1987) *Am. J. Hum. Genet.* **41**, 963–978.
36. Graw, S., Davidson, J., Gusella, J., Watkins, P., Tanzi, R., Neve, R. & Patterson, D. (1988) *Somat. Cell Mol. Genet.* **14**, 233–242.
37. Hoehn, H. (1975) *Am. J. Hum. Genet.* **27**, 676–686.
38. Holmquist, G., Gray, M., Porter, T. & Jordan, J. (1982) *Cell* **31**, 121–129.
39. Goldman, M. A., Holmquist, G. P., Gray, M. C., Caston, L. A. & Nag, A. (1984) *Science* **224**, 686–692.
40. Bradley, C. M., Patterson, D. & Robinson, A. (1983) *Trisomy* **21**, 41–52.
41. Gardiner, K., Watkins, P., Muenke, M., Drabkin, H., Jones, C. & Patterson, D. (1988) *Somat. Cell Mol. Genet.*, in press.
42. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).