Vol. 150, No. 1, 1988 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 15, 1988 Pages 75-81

AMYLOID ß-PROTEIN GENE DUPLICATION IS NOT COMMON IN ALZHEIMER'S DISEASE: ANALYSIS BY POLYMORPHIC RESTRICTION FRAGMENTS

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Received November 19, 1987

The amyloid ß-protein(BP) is an important component of amyloid fibrils of both Alzheimer's disease(AD) and adult Down syndrome(DS). It has been hypothesized that sporadic AD may involve the duplication of a subregion of chromosome 21 containing the BP locus. However, an improved method for detection of the BP gene duplication using polymorphic Hind III fragments led us to a conclusion that BP gene duplication is rare, if any, in (Japanese) sporadic AD patients, indicating that the duplication of the BP gene itself is not the common underlying genetic defect in AD

Alzheimer's disease(AD) is an age-related condition characterized by a widespread functional disturbance of the human brain. A major component of amyloid fibrils of neurofibrillary tangles within neurons, the extracellular amyloid plaque cores, and the cerebrovascular amyloid deposits in AD is called amyloid ß-protein(BP)(1,2) or A4 protein(3,4). A complementary DNA(cDNA) encoding the BP was recently cloned and its corresponding gene was assigned to chromosome 21 using a panel of somatic cell hybrids and by means of in situ hybridization(5-9). Since BP deposition is also found in the brain of older individuals with Down syndrome(DS, congenital trisomy 21)(2,4), the presence of the BP gene on chromosome 21 raised an presumption that there may be a causal relation between AD neuropathology and the triple gene dosage effect seen in DS. In fact, Delabar et al. recently reported that leukocyte DNA from three patients with sporadic AD contained three copies of this gene(10). However, based on the method using polymorphic restriction fragments, we show here evidence that BP duplication is rare, if any, in (Japanese) AD patients.

0006-291X/88 $1.50

MATERIALS AND METHODS

Subjects: We analyzed 14 healthy control subjects(ranging in age from 25 to 62 years), five patients with trisomy 21 DS(from 6 to 31 years, mean 22 years) and 16 patients with clinically diagnosed AD(Japanese, sporadic cases; from 62 to 91 years, mean 80 years). Its clinical diagnosis were based on medical history, neurological examination and CT scanning to permit the exclusion of other neurological disorders. All AD patients fell into the PROBABLE diagnostic category by the NINCDS-ADRDA workgroup(11).

Preparation of High Molecular Weight DNA: Peripheral blood samples (10-20ml) were obtained, and high molecular weight DNA was prepared from white blood cells by the procedure of Ryan et al.(12) with minor modifications. Isolation of tissue DNA was performed as described by Grosschedl et al.(13).

DNA Blotting Analysis Genomic DNA(7μg) digested with Hind III, were loaded on to agarose gel for electrophoresis. The gel containing DNA fragments of high molecular weight (more than 9kb) was exposed to ultraviolet (UV, 254nm) irradiation (84kJ/m^2) to allow efficient transfer of DNA to filters. After UV irradiation, DNA was blotted onto nitrocellulose filters as described method(14), and hybridized with [^{32}P] labeled BP cDNA (Nucleotide position 849-1,795)(5) and transthyretin (TTR, also called prealbumin) gene fragment(Nucleotide position 2-2212)(15) as probes. Isotopic labelling was achieved with Multiprime DNA Labelling System (Amersham). Hybridization was carried out in 50% formamide / 5 X SSC / 2 X Denhardt's solution / 0.5% SDS / 100μg/ml denatured herring sperm DNA / 20mM phosphate buffer (pH7.0) at 42°C for 24 hours. Final washing was carried out in 0.1 X SSC / 0.1% SDS at 65°C. Autoradiography was done at -80°C with an intensifying screen.

Estimation of Gene Dosage: Quantitative analysis of the DNA sequence recognized by the probed was carried out with two different methods by transmission densitometry of autoradiograms with a laser scanner(Zeineh). In "conventional analysis", for each lane of the bands, the ratio of intensity of the 3.7kb BP band versus reference 4.7kb band (Fig. 1) was calculated. Ratio values were standardized by assuming a gene dosage number of two in the DNA from control. In "polymorphic band analysis", ratio of the intensity of the 15kb to 11kb Hind III polymorphic band (Fig. 1) was obtained from the autoradiogram shown in Fig. 1.

RESULTS AND DISCUSSION

Genomic DNA was prepared from peripheral bloods, digested by Hind III and subjected to Southern blotting using human amyloid ß-protein(BP) cDNA and transthyretin (TTR) DNA as probes(Fig. 1). At first, we estimated the dosage of the BP gene by the method similar to that of Delabar et al.(10) (conventional analysis, Fig. 2), in which the gene dosage was estimated from the ratio of the intensities of the BP DNA bands in autoradiogram to that of a reference DNA band (proα2(I) collagen probe(COL1A2) on chromosome 7 in the case of Delabar et al.(10) and

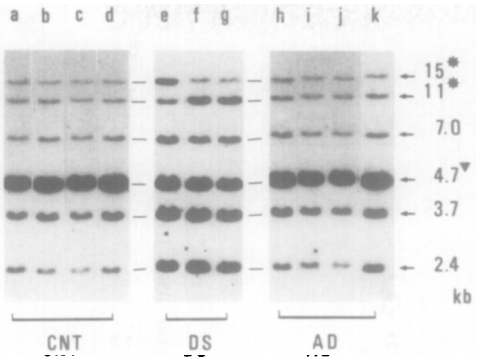

Figure 1. Autoradiogram of Southern blotting analysis of DNA from normal control subjects (CNT, lane a-d), DS patients (21 trisomy)(DS, lane e-g) and AD patients(AD, lane h-k). Two BP bands of 15kb and 11kb are polymorphic(*) and others(7.0, 3.7 and 2.4kb) are invariant. The 4.7kb TTR band(▼) was used as a reference in conventional analysis(see text).

TTR gene on chromosome 18 in our case(16,17)). The intensities of each band in the autoradiograms were measured by densitometric method and we calculated the BP gene dosage number from the ratio of the intensities of the invariant BP bands (7.0kb, 3.7kb and 2.4kb) to the TTR reference band (4.7kb) by assuming that the DNA from the control subjects had a gene dosage of two for both TTR gene and BP gene. The results are summarized in Fig. 3A. Most of AD patients gave the gene dosage number similar to that of the control subjects, indicating that

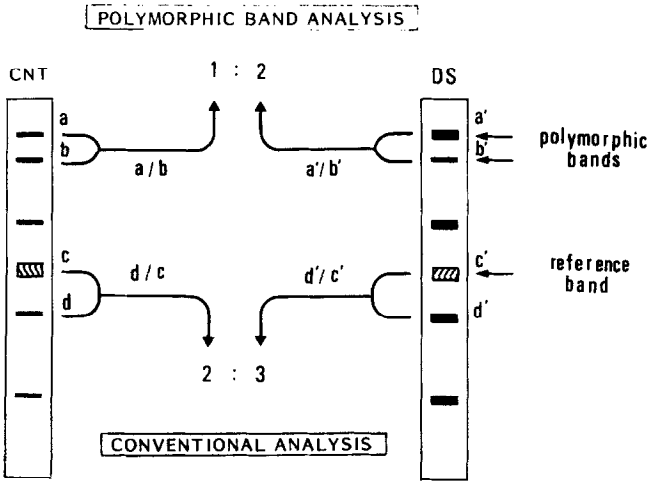

Figure 2. Schematic presentation of polymorphic band analysis and conventional analysis. Examples of Southern blotting profiles of normal control(CNT) and Down syndrome(DS) are shown.

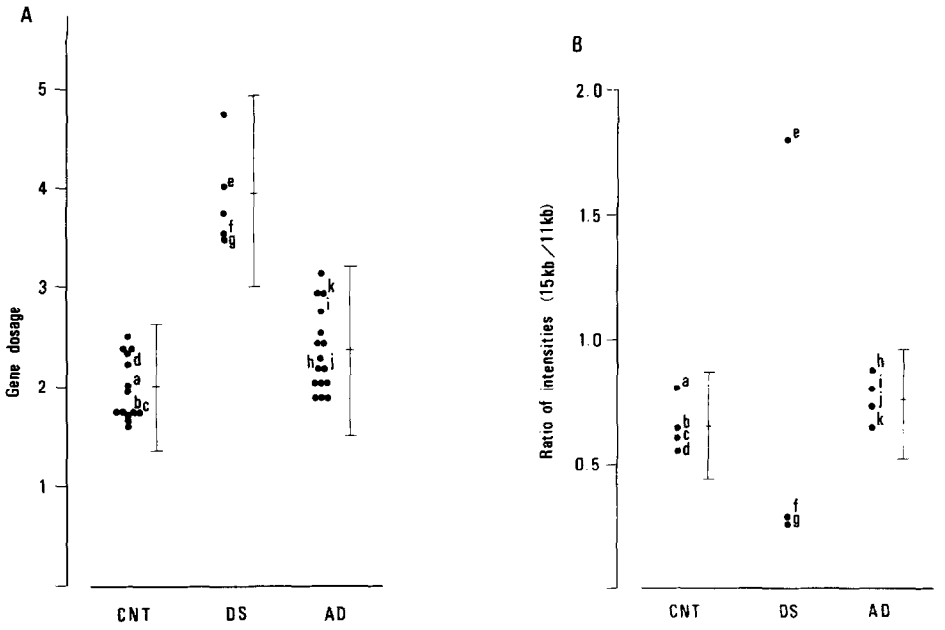

Figure 3. Estimation of BP gene dosage.
(A) BP gene dosage number of normal controls(CNT), DS patients(DS) and AD patients(AD) calculated by the conventional analysis. The overall data for the ratio of the other invariant bands (7.0 and 2.4kb) to reference band also support this result (data not shown). Subjects 'a-k' were all heterozygous for the Hind III site and subjected to further analysis shown in (B).
(B) Ratio of the intensity of the 15kb to 11kb Hind III polymorphic band was obtained from the autoradiogram shown in Fig. 1. Vertical bar indicates mean±2 SD.

they have the BP gene dosage number of two. However, the results appeared not to be conclusive. For example, the ranges of mean ± 2SD overlapped between AD and DS in our analysis and the possibility remained that some of AD patients may have the gene dosage of three.

We previously identified an allele restriction fragment length polymorphism(RFLP) (11kb and 15kb bands, Fig. 1) in the BP locus with restriction endonuclease Hind III(18). If the subject to be tested is heterozygous for the polymorphic Hind III site, the ratio of the intensities of the allelic band will allow us to detect the duplication of the BP gene with high reliability as illustrated in Fig. 2 (polymorphic band analysis). If one of the BP gene is duplicated, the ratio of the intensities of the allelic bands should be either double or half of that of non-duplicated control and consequently the ratio of values to be compared should increase from 2:3 (in conventional analysis) to 1:2 (in polymorphic band analysis).

Using this method, we analyzed four control subjects, four AD patients and three DS patients. All of them were heterozygous for the polymorphic site (Fig. 1). As summarized in Fig. 3B, all of the AD patients tested showed approximately the same values as the control subjects, indicating that these AD patients have a BP gene dosage number of two. It should be emphasized that the AD subject 'K', the gene dosage of which remained ambiguous in the conventional method, clearly showed the gene dosage number of two in this method.

One may argue that the BP gene might be duplicated in brain of AD patients even if it is not duplicated in leukocytes. Because of limitation of fresh brain samples, we could not carry out precise calculation but our preliminary study (by the conventional analysis) suggested that the BP gene dosage is two in three AD brains (one Japanese and two Caucasian, DEFINITE AD(11)) and three in two DS patients(Caucasian)(data not shown).

These results indicate that the BP duplication observed by Delabar et al.(10) is rare, if any, in (Japanese) patients with sporadic AD. Although there may be a racial difference between Japanese and Caucasian, a simple explanation that a dosage-related increase in the concentration of the BP causes amyloid fibril formation is unlikely in sporadic AD. Recent linkage analysis of familial AD revealed that a genetical factor(s) other than BP is involved in AD pathogenesis(19,20). Further investigations of this chromosomal region, as well as studies on other genetic and environmental factors, are required to elucidate the etiology of AD.

The method described here (polymorphic band analysis) may be generally useful for detection of regional duplication of chromosomal DNA when RFLPs are available.

ACKNOWLEDGEMENT

We thank Drs. Rumiko Shibata, Masahiro Okayama, Isao Takamatsu and Yasuo Nishihara for allowing us to study patients under their care and supplying blood samples of patients. Brain tissue samples of Japanese AD patient and control were kindly supplied by Prof. J. Tateishi, Department of Neuropathology, Neurological Institute, Kyushu University, Japan.

REFERENCES

1. Glenner, G.G., and Wong, C.W. (1984) Biochem. Biophys. Res. Commun. 120, 885-890.

2. Glenner, G.G., and Wong, C.W. (1984) Biochem. Biophys. Res. Commun. 122, 1131-1135.

3. Masters, C.L., Multhaup, G., Simms G., Pottgiesser J., Martins, R.N. and Beyreuther, K. (1985) EMBO J. 4, 2757-2763.

4. Masters, C.L., Simms, G., Weinman, N.A., Multhaup, G., McDonald, B.L. and Beyreuther, K. (1985) Proc. Natl. Acad. Sci. U.S.A 82, 4245-4249.

5. Kang, J., Lemaire, H.-G., Unterbeck, A., Salbaum, J.M., Masters, C.L., Grzescjol, K.-H., Multhaup, G., Beyreuther, K. and Muller-Hill, B. (1987) Nature(London) 325, 733-736.

6. Goldgaber, D., Lerman, M.I., McBride, O.W., Saffiotti, U. and Gajdusek, D.C. (1987) Science 235, 877-880.

7. 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. and Neve, R.L. (1987) Science 235, 880-884.

8. Robakis, N.K., Ramakrishna, N., Wolfe, G. and Wisniewski, H.M. (1987) Proc. Natl. Acad. Sci. U.S.A. 84, 4190-4194.

9. 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. and Brown, W.T. (1987) Lancet 1987-I, 384.

10. Delabar, J.M., Goldgaber, D., Lamour, Y., Nicole, A., Huret, J.-L., Grouchy, H.J., Brown, P., Gajdusek, D.C., Sinet, P.-M. (1987) Science 235, 1390-1392.

11. McKhann, G., Drachman, D., Folstein, M., Katzman, R. Price, D. and Stadlan, M. (1984) Neurol. 34, 939-945

12. Ryan, J., Barker, P.E., Shimizu, K., Wigler, M. and Ruddle, F.H. (1983) Proc. Natl. Acad. Sci. U.S.A. 80, 4460-4463.

13. Grosschedl, R., Weaver, D., Baltimore, D. and Constrantini, F. (1984) Cell 38, 647-658.

14. Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular cloning. A Laboratory Manual. p382-389. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

15. Sasaki, H., Yoshioka, N., Takagi, Y. and Sakaki, Y. (1985) Gene(Amst.) 37, 191-197.

16. Wallace, M.R., Naylor, S.L., Kluve-Beckerman, B., Long, G.L., McDonald, L., Shows, T.B. and Benson, M.D. (1985) Biochem. Biophys. Res. Commun. 129, 753-758.

17. Sparkes, R.S., Sasaki, H., Mohandas, T., Yoshioka, K., Klisak, I., Sakaki, Y., Heinzmann, C.,and Simon, M.I. (1987) Hum. Genet. 75, 151-154.

18. Sasaki, H., Oishi, N., Furuya, H., Yoshioka, K., Yamada, T., Ogawa, S., Wong, C.W., Glenner, G.G. and Sakaki, Y. (1987) Nucleic Acids Res. 15, 6309.

19. Broeckhoven, C.V., Genthe, A.M., Vandenberghe, A., Horsthemke, B., Backhovens, H., Raeymaekers, P., Hul, W.V., Wehnert, A., Gheuens, J., Cras, P., Bruyland, 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. and Hardy, J.A. (1987) Nature(London) 329, 153-155.

20. Tanzi, R.E., St. George-Hyslop, Haines, J.L., Polinsky, R.J., Nee, L., Foncin, J.-F., Neve, R.L., McClatchey, A.I., Conneally, P.M. and Gusella, J.F. (1987) Nature(London) 329, 156-157.