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Fine Mapping of an Alzheimer Disease-Associated Gene Encoding Beta-Amyloid Protein

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SUMMARY: We have sublocalized an Alzheimer Disease-associated gene, which encodes for cerebrovascular beta-amyloid protein, to the region from the centromere through the proximal half of band 21q21 using both somatic cell and in situ mapping techniques. In addition we found repeatedly significant but weaker hybridization of the beta-amyloid protein probe to the short arm of chromosome 20. 794 cells were analyzed from whole blood, lymphoblastoid and skin cultures. The latter two types of cultures had parts of the 21st chromosome translocated to other chromosomes facilitating sublocalization.

INTRODUCTION: Alzheimer disease is the most frequent cause of dementia which presently afflicts an estimated two million individuals in the United States. Cerebral lesions used in the postmortem diagnosis of AD include the extracellular deposition of amyloid fibrils both in the neuritic (senile) plaques and on the walls of the cerebral vessels. Similar deposits are found in the brains of Down syndrome individuals over the age of 40 (1). The amyloid deposits consist of a low molecular weight (4-5 KDa) peptide variably termed beta protein as A4 peptide (2,3,4). Recently, we (5,6,7) and others (8,9,10) reported that beta protein derives from the proteolytic cleavage of a large precursor encoded by a chromosome 21 gene. Using in situ hybridization we were able to sublocalize the beta protein gene to 21q21 (5).

Using Southern blot analysis of somatic cell hybrids containing parts or all of chromosome 21 and using in situ chromosome hybridzation to translocations involving parts of chromosome 21, we have demonstrated that the gene encoding the beta-amyloid protein is localized within the region delmited by bands 21q11.2 to 21q2105. In addition hybridization to chromosome 20 was observed when a single stranded probe was used.

Abbreviatons: AD, Alzheimer Disease; BAP, beta-amyloid protein; CHO, Chinese hamster ovary; WB_1, whole blood culture series 1 from a normal male; WB_2, whole blood culture series 2 from a normal female; SSP, single-stranded probe; DSP, double-stranded probe.

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MATERIALS AND METHODS: The cDNA clone that we used as a probe for in situ chromosome hybridization was isolated as described elsewhere (6,7). cDNA fragment B2.3 encoding the BAP (6) was subcloned into vector M13mp18. The single-stranded plasmid was labelled with H^3 by means of an oligonucleotide primer (New England Biolabs), so that the cDNA probe remained single-stranded. Double-stranded B2.3 insert was labelled by nick translation and heat denatured.

Somatic cell hybrid lines carrying rearrangements involving human chromosome 21 as well as multiple copies of the 21st chromosome are labelled here as lines A-D. Human translocation marker chromosomes on a CHO background for lines A-C are karyotypically described: A = 21pter to 21q22.2::8q22.1 to 8qter. B = 8pter to 8q22.1::21q22.2 to 21qter. C or $2Fu^r1$ (12) contains the long arm of chromosome 21 that was translocated to a CHO chromosome. DNA from lines A-C was kindly provided by Dr. Antonarakis. Line D consisted of a mouse-human hybrid whose only human chromosomes are multiple copies of chromosome 21 (13).

The procedure used for in situ chromosome hybridization has been published previously (14,15). Short-term whole blood cultures from a normal male and female as well as lymphoblastoid and skin fibroblast cultures (GM6135, GM693, respectively; Mutant Cell Repository, Camden, N.J.) were used. GM6135 and GM693 contained balanced reciprocal translocations which involved different parts of chromosome 21. The karyotypic description for GM6135 is 46,XX,t(10;21)(p11.2;q22.3) and for GM693 it is 46,XX,t(2;21) (q37;q2105). The concentrations of the beta-amyloid DNA ranged from 5-25 ng/ul with autoradiographic exposure times of 5-12 days. GM6135 chromosomes were hybridized to the double-stranded form of the BAP probe while the GM693 study used the single stranded form.

RESULTS: Initial mapping of the beta-amyloid protein probe to chromosome 21 was effected by using a series of somatic cell hybrids, each carrying all or part of chromosome 21. Southern blot analysis of hybridization results indicated the gene mapping on chromosome 21 from 21qcen to 21q22.1, shown in Fig. 1.

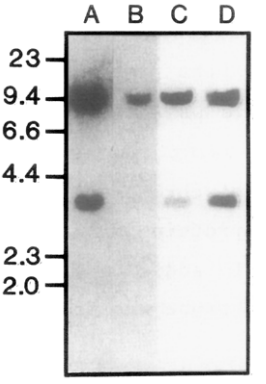

Fig. 1 - Southern blot autoradiograph showing hybridization of the B-amyloid cDNA probe to EcoRI digested DNA from somatic cell hybrid lines carrying different portions of chromosome 21. A - DNA from somatic cell hybrid which contained all of chromosome 21 except for 21q22.3; B - only band 21q22.3 was present; C - contains 21q; D - contains multiple copies of chromosome 21.

TABLE I
X^2 ANALYSIS OF BETA-AMYLOID PEPTIDE PROBE IN SITU HYBRIDIZATION TO CHROMOSOMES FROM WHOLE BLOOD

CHR.	NO. OF GRAINS EXPECTED		NO. OF GRAINS OBSERVED		$\frac{(OBS-EXP)^2}{EXPECTED}$	
	SINGLE	DOUBLE	SINGLE	DOUBLE	SINGLE	DOUBLE
1	126.84	105.04	113	94	1.51	1.16
2	120.63	99.90	111	92	0.77	0.62
3	100.58	83.29	99	69	0.02	2.45
4	94.37	78.15	84	64	1.14	2.45
5	92.78	76.84	83	63	1.03	2.56
6	85.14	70.51	82	72	0.12	0.03
7	78.93	65.37	60	61	4.54	2.97
8	72.73	60.23	53	45	4.93	3.85
9	69.55	57.60	69	68	0.004	1.88
10	68.11	56.40	65	54	0.14	0.10
11	68.11	56.40	55	42	2.52	3.68
12	66.52	55.09	51	51	3.62	0.30
13	54.11	44.81	61	35	0.88	2.15
14	49.49	40.99	50	36	0.01	0.61
15	47.91	39.67	45	48	0.18	1.75
16	46.46	38.48	39	43	1.20	0.53
17	43.29	35.85	54	39	2.65	0.28
18	40.26	33.34	53	33	4.03	0.004
19	32.47	26.89	42	39	2.80	5.45
20	34.05	28.20	72	39	42.30	4.14
21	24.82	20.55	70	68	82.24	109.56
22	26.26	21.75	32	40	1.25	15.31
	1443.41	1195.35	1443	1195	157.88	161.74

A total of 600 metaphases from the two different series of whole blood cultures, WB_1 and WB_2, were exposed to either the single- or double-stranded form of the BAP probe. Observed and expected silver grain distributions for both single- and double-stranded probes are shown in Table I. The X^2 values shown in Table I, 157.88 and 161.74 with 21 degrees of freedom, were significant at $p<.001$. Of the 2,638 grains counted, 138 or 5.23% were localized to the 21st chromosome versus an expected 1.72%.

When the SSP silver grain distribution for chromosome 21 was compared to that for all other chromosomes, a X^2 value of 81.86 with one degree of freedom and resultant probability of <.001 was observed. Chromosome 20 also exhibited a significantly increased grain distribution of 4.99% compared to an expected 2.36% ($X^2=81.86$; df=1; $p<.001$). For DSP, chromosomes 21, 22, 19 and 20 exhibited increased silver grain distributions: Chr. 21: $X^2=109.17$; df=1; $p<.001$. Chr. 22: $X^2=14.76$; df=1; $p<.001$. Chr. 19: $X^2=5.13$; df=1; $p<.05$. Chr. 20: $X^2=3.86$; df=1; $p<.05$. However, when WB_1 and WB_2 were considered separately (150 cells exposed to DSP in WB_1 and analyzed; 150 cells exposed to DSP in WB2 and analyzed), the only chromosome with consistently increased *in situ* hybridization was chromosome 21. For WB_1/Chr. 21: $X^2=58.20$; df=1; $p<.001$. For WB_2/Chr. 21: $X_2=51.40$; df=1; $p<.001$. In contrast, when WB_1 and WB_2 were

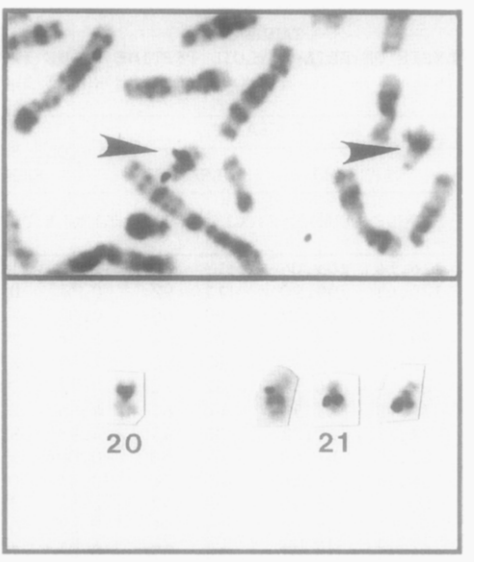

Fig. 2a - partial metaphase from whole blood cultures showing hybridization of the beta-amyloid protein probe to both 21st chromosomes on band 21q21 (arrows).

Fig. 2b - an example of BAP probe hybridization to the short arm of chromosome 20 and additional examples of hybridization to chromosome 21 on band 21q21.

considered separately after SSP in situ hybridization, both chromosomes 21 and 20 exhibited consistently, increased silver grain distributions. For WB_1/Chr. 21: X^2=35.88; df=1; p<.001. For Chr. 20: X_2=5.38; df=1; p<.05. For WB2/Chr. 21: X^2=46.15; df=1; p<.001. For Chr. 20: X^2=52.53; df=1; p<.001. Examples of in situ hybridization of the BAP probe to chromosomes 21 and 20 are given in Fig. 2.

When the silver grain distribution was determined for der(21), the short arm of der(10) and the normal 21 in 40 cells from GM6135 lymphoblastoid cultures, 23 grains were present on chromosome 21 material in der(21) versus 19 on the normal 21. Only one grain was observed on chromosome 21 material of der(10p). This indicates that the gene for BAP is localized proximal to band 21q22.3 since no significant hybridization was observed on der(10p). An idiogram and partial autoradiographic karyotype of chromosomes 10, der (10), 21 and der(21) are shown in Fig. 3. Note that the der(21) chromosome is labelled within band 21q21. We previously suggested this localization from a statistical analysis of grain distribution on the intact 21st chromosome in short-term whole blood cultures (5).

Further sublocalization was possible when an analysis of silver grain distributions on derivative chromosomes 2 and 21 as well as on the normal 21 was carried out in 54 cells from GM693 skin cultures. Der(21) exhibited 46 grains as compared to 43 on the normal 21 indicating that the BAP gene is located between the centromere and band 21q2105 which was the portion of

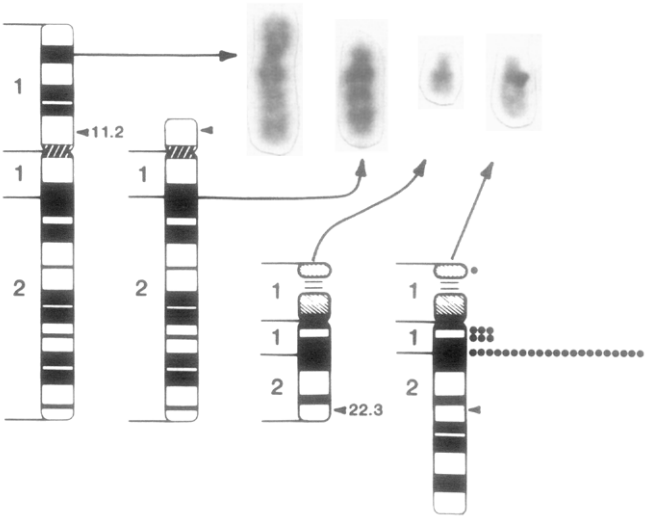

Fig. 3 - breakpoints are identified (arrows) on both normal and derivative (der) chromosomes for GM6135 cells. BAP probe hybridization occurred on der(21) indicating that the location of the BAP gene was proximal to 21q22.3. Note that the actual der(21) hybridized with BAP probe on band 21q21.

chromosome 21 present on the long arm of der(21). No significant hybridization was observed on the chromosome 21 material of der(2). Fig. 4 is an idiogram and partial autoradiographic karyotype of the normal and derivative chromosomes 2 and 21. Note that the silver grain on der(21) is on band 21q21.

An analysis of 400 $_{W}B_{1}$ and WB_{2} cells which were hybridized with the single-stranded form of the BAP probe revealed a total of 100 grains on chromosome 20. 58 grains hybridized to the short arm of No. 20 while 42 were observed on the long arm indicating probable sublocalization of the BAP probe to 20p (X^2=5.47; df=1; p<.05).

DISCUSSION: Recently, using in situ chromosome hybridization on 145 cells, we reported that the gene encoding BAP is located on 21q21 (5). Using a panel of somatic cell hybrids and Southern blot analyses, Tanzi et al (10) suggested that the BAP gene is located within the region from 21q112 to 21q21. The only other report on in situ chromosome hybridization used the whole chromosome 21 only to localize the BAP probe between bands 21q21 and 21q22 (16). In the present study we used normal cells as well as a panel of somatic cell hybrid lines and lymphoblastoid and skin fibroblast lines carrying translocations involving part of chromosome 21, to map the position of BAP gene more precisely. A total of 794 cells were analyzed. Both Southern blot and in situ hybridization techniques were used to precisely define the region

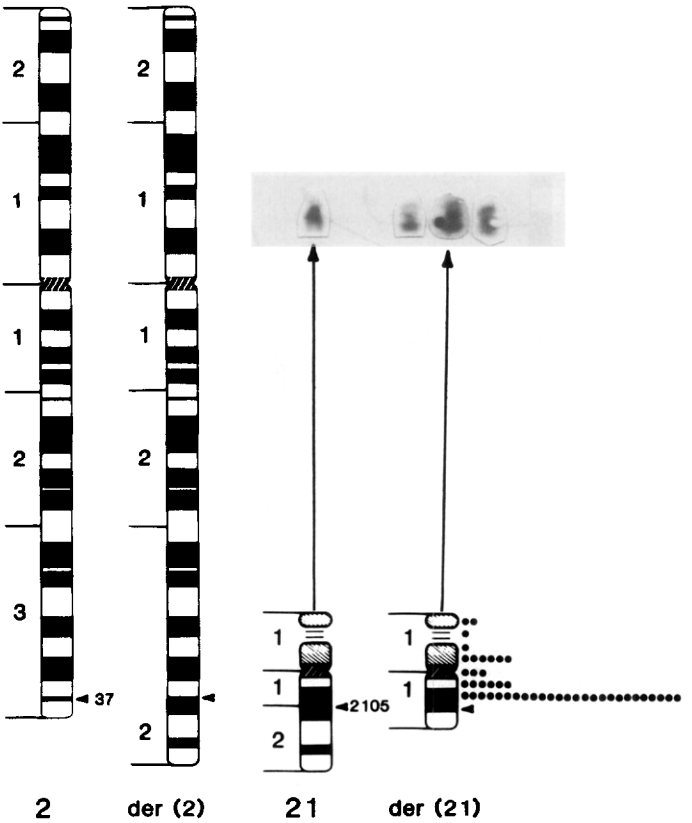

Fig. 4 - breakpoints are identified (arrows) on idiograms of the normal chromosomes 2 and 21 and they are also indicated on the derivative chromosome from GM693. Hybridization of the BAP probe is shown on an idiogram as well as a photomicrograph of der(21) indicating that the physical location of the BAP gene is proximal to band 21q2105. Silver grain distribution is illustrated on the der(21) idiogram and shown on the autoradiograph of the actual chromosome.

encoding for the BAP locus. With the previous reports reviewed above coupled to the new information presented here, it can be concluded that this gene is included within the region delimited by bands 21q11.2 to 21q2105.

Genetic linkage studies have demonstrated recently that the FAD locus is separate from the BAP gene. Tanzi et al (17) suggested that the FAD locus is proximal to the BAP gene and Van Broeckhoven et al. (18) indicated that the BAP gene may be linked to the locus for SOD. Since our data suggest that the BAP locus is no more distally located than 21q2105, it is likely that the FAD locus is proximal to 21q2105 and distal to or at 21q11.2 (19).

We have confirmed our original suggestion (5) that additional but weaker hybridization was observed on the short arm of chromosome 20 when human chromosomes were exposed to the single-stranded form of the beta-amyloid probe. This suggests that sequences related to the BAP gene are present on this chromosome, in agreement with our previous results obtained by Southern transfers. They demonstrated the presence of additional sequences related to

the BAP gene (6). It appears that the single-stranded form of the beta-amyloid protein probe is more sensitive than the double-stranded form since it consistently hybridized to both chromosomes 21 and 20. We have mapped a gene that encodes for another amyloidogenic protein, PrP 27-30, to the same area on the short arm of chromosomes 20 (20).

In conclusion, we have confirmed earlier reports (5,16) and added new information further sublocalizing the BAP gene proximal to the region, 21q22 to 21qter, that is commonly associated with the Down syndrome phenotype (21,22). However, this region is present in triple dose in trisomy 21 and may be pathogenically related to the neuropathological features of Alzheimer disease which is commonly seen in Down syndrome individuals.

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