4. H. Williams, and F. Nottebohm, *Science* **229**, 279 (1985); A. M. Liberman *et al.*, *Psychol. Rev.* **74**, 431 (1967).
5. J. Piaget, *The Origins of Intelligence in Children*, translated by M. Cook (International University Press, New York, 1952).
6. The degree of flatness of the random function has no bearing on the results. The range is 0 to 1.
7. This is formally shown in Eq. 6 and discussed there.
8. See M. Kuperstein [*Behav. Neurosci.* **102**, 148 (1988)] for adaptive neural architecture for eye foveation and first reference in (*2*) for eye movement generator.
9. E. S. Luschei and A. F. Fuchs, *J. Neurophysiol.* **35**, 445 (1972); D. A. Robinson, *ibid.* **33**, 393 (1972); P. H. Schiller, *Exp. Brain Res.* **10**, 347 (1970).
10. The recruitment function is the same as the position-threshold-slope function from the first reference in (*2*).
11. D. H. Hubel and T. N. Wiesel *J. Physiol. (London)* **195**, 215 (1968).
12. G. F. Poggio and B. Fischer, *J. Neurophysiol.* **40**, 1369 (1977).
13. D. Rose and V. G. Dobson, Eds., *Models of Visual Cortex* (Wiley, Chichester, U.K., 1985).
14. Normalized networks that have this property are similar to those used in S. Grossberg, *Studies of Mind and Brain* (Reidel, Dordrecht, 1982).
15. The present learning rule contrasts with a learning rule based on sensory errors [such as those used in (*8*)]. Unlike the former rule, the latter requires error directions to be calibrated a priori. Unlike the latter rule, the former requires exploratory motor signals to be generated. The use of each type of rule depends on the cost-benefit of their properties. They could also be used in parallel.
16. M. Kuperstein, in *Proc. IEEE Int. Conf. Automat. Robotics*, Raleigh, NC, 31 March to 3 April 1987 (Computer Society Press, Washington, DC, 1987), p. 1595.
17. I thank the National Science Foundation and the Air Force Office of Scientific Research for their financial support of this research (grant DMC-8521535).

2 November 1987; accepted 20 January 1988

Gene Encoding the β Subunit of S100 Protein Is on Chromosome 21: Implications for Down Syndrome

R. ALLORE, D. O'HANLON, R. PRICE, K. NEILSON, H. F. WILLARD, D. R. COX, A. MARKS, R. J. DUNN

S100 protein is a calcium-binding protein found predominantly in the vertebrate nervous system. Genomic and complementary DNA probes were used in conjunction with a panel of rodent-human somatic cell hybrids to assign the gene for the β subunit of S100 protein to the distal half of the long arm of human chromosome 21. This gene was identified as a candidate sequence which, when expressed in the trisomic state, may underlie the neurologic disturbances in Down syndrome.

DOWN SYNDROME (DS) IS THE most common genetic cause of human mental retardation, occurring with the frequency of about 1 per 800 live births (*1*). Individuals with this disorder have abnormalities in a number of different organ systems including the nervous system. Neuropathological changes consisting of neurofibrillary tangles, senile plaques, and neuronal loss are found in the brains of most DS individuals dying after the age of 35 years. These changes, which are qualitatively and quantitatively indistinguishable from those seen in Alzheimer's disease (AD), are often associated with the clinical features of presenile dementia (*2*). Cytogenetic studies have shown that a chromosome abnormality, trisomy of chromosome 21, is the primary cause of DS. This finding suggests that the neurologic abnormalities in DS are due to imbalance of one or more genes on chromosome 21. In order to understand the biochemical basis of the neurologic defects in DS it is necessary to identify these genes.

Two genes that may play a role in the neurologic abnormalities that characterize DS have recently been assigned to human chromosome 21 (*3, 4*). The first of these is the locus coding for amyloid β protein (APP), an important component of both cerebral vascular amyloid and amyloid plaques of AD and DS. The second gene, mutations of which result in early onset familial AD (which is autosomal-dominant), has been shown to be tightly linked to the chromosome 21 DNA marker, D21S16. Although both D21S16 and APP have been physically assigned to the proximal portion of the long arm of chromosome 21, crossovers between APP and the familial AD gene indicate that these are two separate loci (*5, 6*). We now report that a third gene, expressed primarily in the nervous system and encoding the β subunit of the S100 protein, maps to the distal half of the long arm of human chromosome 21 and is a candidate for the primary defect underlying the neurologic disturbances found in DS.

S100 protein is a calcium-binding protein widely distributed in the nervous system of vertebrates (*7*). It is structurally similar in the calcium-binding domains to calmodulin, an important transducer of calcium-mediated signals (*8*). S100 protein is composed of two subunits, α and β, which associate into αα, ββ, or αβ dimers (*9*). The highest levels of S100 protein are found in the brain. In particular, the β subunit of S100 protein is expressed in glial cells at levels at least tenfold higher than in most other cell types. The brain also contains small amounts of the α subunit at levels approximately one-tenth that of the β subunit (*10*). S100 protein accumulates during the maturation of the mammalian brain (*11*) and participates in several calcium-dependent interactions with neuroleptic drugs and brain proteins (*12*). Thus disturbances of S100 protein gene expression may play a fundamental role in the generation of neurologic defects associated with DS.

The human genomic and complementary DNA (cDNA) probes used to identify the chromosomal location of the S100 protein β subunit gene are shown in Fig. 1. The 742-

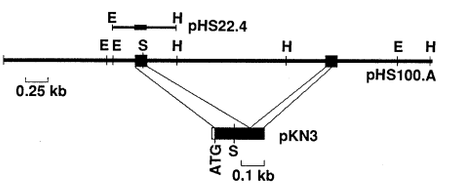

Fig. 1. Schematic representation of the genomic clone (pHS100.A), the genomic probe subclone (pHS22.4), and the cDNA clone probe (pKN3) used for Southern blot analysis. Protein coding region sequences are indicated by dark boxes. The heavy line indicates intron sequence in the genomic clone and the open box indicates the 5′ untranslated region sequence within the cDNA clone. Lines joining regions of cDNA and genomic clones bracket areas of identical sequence. The location of the ATG initiation codon is indicated. The DNA fragment used as a genomic hybridization probe (pHS22.4) is indicated above the genomic clone (pHS100.A) diagram. The cDNA clone was isolated by means of a previously characterized cDNA clone of the rat S100 protein β subunit (*17*), which was used as a hybridization probe. The Eco RI fragments from positive cDNA clones were subcloned into the Bluescript plasmid (Stratagene Cloning Systems, San Diego, CA). The genomic clone λHS100.1 was isolated by screening 3.6×10^5 recombinants from a λ Charon 4A human genomic library using the rat S100 protein cDNA probe (*18*). The 5.0-kb pHS100.A subclone of λHS100.1 contains the entire protein coding region from the S100 protein β subunit gene. The 0.746-kb genomic clone (pHS22.4) represents a 5′-coding region subclone from pHS100.A. Restriction enzyme sites: E, Eco RI; H, Hind III, and S, Sst I.

R. Allore, K. Neilson, H. F. Willard, R. J. Dunn, Department of Medical Genetics, University of Toronto, Toronto, Ontario, Canada M5S 1A8.
D. O'Hanlon and A. Marks, Banting and Best Department of Medical Research, University of Toronto, 112 College Street, Toronto, Ontario, Canada M5G 1L6.
R. Price and D. R. Cox, Department of Pediatrics, Biochemistry and Biophysics, M650, University of California, San Francisco, CA 94143.

bp genomic probe (pHS22.4) contains 138 nucleotides of exon sequence that code for the first 46 amino acids of the S100 protein β subunit. This exon is flanked by 262 and 352 nucleotides of 5′ and 3′ intron sequences respectively. The cDNA probe, pKN3, contains the coding region for the first 72 amino acids of the S100 protein β subunit flanked by 16 nucleotides of 5′ untranslated region.

Southern blot analysis with the pHS22.4 genomic probe of a Hind III digest of total human genomic DNA revealed the presence of a single 8.8-kb band (Fig. 2A, lane 1) suggesting the existence of only a single S100 β subunit gene in the human genome. No hybridizing bands were detected in mouse genomic DNA under the stringency conditions used (Fig. 2A, lane 2), although a 7-kb band was detected under lower stringency conditions (*13*).

To identify the chromosomal location of the human S100 protein gene, a total of 22 human-mouse hybrids was examined by Southern blot analysis (*14*). Hybridization patterns for Hind III–digested hybrid cell DNA with the genomic probe pHS22.4 are shown in Fig. 2A. As was the case for human DNA (lane 1), a single 8.8-kb band was observed for a human-mouse hybrid cell line which contained chromosome 21 as the sole human chromosome (lane 3). DNA from hybrid cell lines which contained multiple human chromosomes, but not chromosome 21, gave no hybridization signal (for example, lane 4). A summary of the results for the 22 human-mouse hybrid cell lines is shown in Table 1. The 8.8-kb Hind III band co-segregated with chromosome 21 in all hybrids tested. When the cDNA probe (pKN3) was used for hybridization, two Hind III fragments were detected, the 8.8-kb and an additional 1.6-kb fragment. The latter fragment contains the second exon of the gene (Fig. 1). Segregation analysis with the cDNA probe was identical to that obtained with the genomic probe (*13*). These data indicate a 100% concordant segregation between DNA sequences hybridizing with the S100 protein β subunit probes and chromosome 21, thus allowing assignment of the gene for the protein to this chromosome.

In order to localize the S100 protein β subunit gene to a specific region on chromosome 21, we compared Southern blot analysis of hybrids containing intact copies of chromosome 21 with those in which part of chromosome 21 had been removed by translocation (Fig. 2B). The human-mouse hybrid line AHVI-1, which contains an intact copy of human chromosome 21, showed, the presence of the 8.8-kb Hind III fragment hybridizing with the genomic probe pHS22.4 (lane 1). However, DNA from the hybrid AHVI-17, which contains all of human chromosome 21 with the exception of band 21q22, gave no hybridization signal (lane 2). Reprobing the DNA samples analyzed in lanes 1 and 2 with the chromosome 21 marker probe E9 (for locus D21S16 in 21q11-21), indicated that both lanes contained equal amounts of DNA (*13*).

The data presented in this report demonstrate that the human S100 protein β subunit gene is located in band 21q22 of human chromosome 21. As triplication of this region of chromosome 21 is sufficient to result in the facial features, mental retardation, and cardiac anomalies that are characteristic of DS, band 21q22 has been referred to as the "DS region" of chromosome 21. However, the brains of individuals trisomic for only band 21q22 have not been examined to determine whether or not they contain the neuropathological changes found in the brains of older DS individuals trisomic for all of chromosome 21. Furthermore, trisomy for only the proximal region of the long arm of chromosome 21 also causes mental retardation (*15*). These facts suggest that multiple loci on chromosome 21 may contribute to the neurologic defects associated with DS. Regional assignment of the S100 protein gene to band 21q22 excludes this gene as a candidate for the autosomal dominant AD gene assigned to band 21q21 (*4, 6*) but identifies it as a new candidate locus for neurologic defects in DS.

One of the rationales behind efforts to

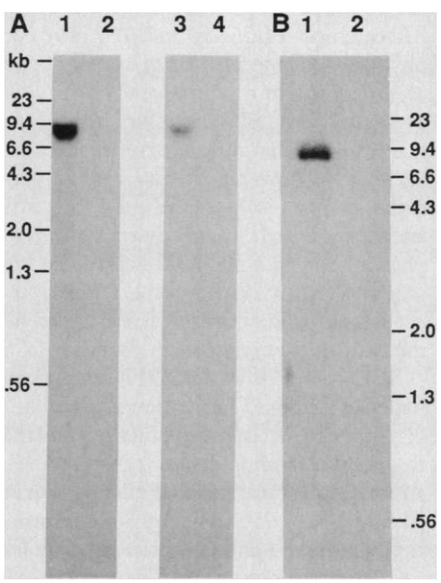

Fig. 2. (**A**) Representative samples from Southern blot analysis of human DNA (lane 1), mouse DNA (lane 2), and human-mouse hybrid cell DNA (lanes 3 and 4). Human-mouse hybrids used for the chromosome 21 subregion localization analysis of the S100 protein β subunit gene were isolated after fusing mouse A9 cells with human fibroblasts that were heterozygous for a balanced reciprocal translocation involving chromosome 1 and chromosome 21 [46 XY, t(1:21)(p31;q22)] (*19*). (**B**) Hybrids that contained intact copies of human chromosome 21 (AHVI-1) (lane 1) or copies of chromosome 21 that contained the proximal two-thirds of chromosome 21 but were missing the 21q22 region (AHVI-17) (lane 2) were analyzed. Samples (10 μg) of genomic DNA were digested with Hind III, fractionated on 0.9% agarose gels, and transferred to nitrocellulose membranes. All samples were hybridized with the genomic clone probe (pHS22.4). Hybridizations were carried out at 45°C overnight in 50% formamide, 5× SSC, 5× Denhart's solution, 0.1% SDS, 50 m*M* $NaHPO_4$, salmon sperm DNA (200 μg/ml), and yeast tRNA (1 mg/ml). DNA fragment probes were purified from agarose gels and labeled with [^{32}P]dCTP to a specific activity of 5×10^8 count/min per microgram of DNA, by means of the random primed DNA polymerase labeling method (*20*). Labeled probe was added to give a final concentration of approximately 3×10^6 count/min per milliliter. After hybridization, filters were washed three times in 2× SSC, 0.1% SDS at room temperature followed by three washes in 0.1% SDS, 0.1× SSC at 55°C.

Table 1. Correlation between the presence of specific human chromosomes and the human S100 protein β subunit gene in 22 human × mouse hybrids. The chromosome complement of each of the hybrids has been characterized previously (*14*). The numbers indicate the number of hybrid cell lines in which the particular human chromosome and the hybridizing band were both present (+/+) or both absent (−/−) (concordant) and the number of hybrid cell lines in which the hybridizing band was present but the human chromosome was not (+/−) or where the hybridizing band was absent while the human chromosome was present (−/+) (discordant).

Chromosome	Concordant		Discordant		Discordancy (%)
	+/+	−/−	+/−	−/+	
1	3	10	8	1	41
2	3	8	8	3	50
3	1	6	10	5	68
4	5	8	6	3	41
5	7	6	4	5	41
6	3	8	8	3	50
7	7	6	4	5	41
8	3	8	8	3	50
9	0	11	11	0	50
10	4	6	7	5	55
11	2	8	9	3	55
12	3	7	8	4	55
13	0	11	11	0	50
14	5	4	6	7	59
15	1	6	10	5	68
16	0	8	11	3	64
17	4	7	7	4	50
18	6	10	5	1	27
19	5	8	6	3	41
20	5	6	6	5	50
21	11	11	0	0	0
22	3	7	8	4	55
X	9	1	2	10	55
Y	0	11	11	0	50

completely map the human genome is that the map location of individual genes can often provide clues to the involvement of a gene in a particular genetic disorder. Indeed, as an illustration of this approach, the β subunit of S100 protein is one of the first genes assigned to chromosome 21 for which a large body of experimental evidence suggests a functional role in the central nervous system (*11, 12, 16*). Therefore, our results suggest that future studies on the role of S100 protein in the pathogenesis of the neurological features of DS are warranted.

REFERENCES AND NOTES

1. T. J. Hassold and P. S. Jacobs, *Annu. Rev. Genetics* **18**, 69 (1984).
2. C. J. Epstein, *New Perspectives on Down Syndrome*, S. M. Pueschel, Ed. (Paul Brookes, Baltimore, 1987), pp. 69–80.
3. R. E. Tanzi *et al.*, *Science* **235**, 880 (1987).
4. P. H. St George-Hyslop *et al.*, *ibid.*, p. 885.
5. C. Van Broeckhoven *et al.*, *Nature (London)* **329**, 153 (1987).
6. R. E. Tanzi *et al.*, *ibid.*, p. 156.
7. B. W. Moore, *Biochem. Biophys. Res. Commun.* **19**, 739 (1965); D. Kessler *et al.*, *Biochemistry* **7**, 758 (1968); P. Calissano *et al.*, *ibid.* **8**, 4318 (1969).
8. A. R. Means *et al.*, *Physiol. Rev.* **62**, 1 (1982).
9. T. Isobe and T. Okayama, *Eur. J. Biochem.* **89**, 379 (1978); *ibid.* **116**, 79 (1981).
10. K. Kato and S. Kimura, *Biochem. Biophys. Acta.* **842**, 146 (1985).
11. J. E. Zuckerman *et al.*, *J. Neurochem.* **17**, 247 (1970); T. J. Cicero *et al.*, *ibid.* **19**, 2119 (1972).
12. D. R. Marshak *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6793 (1981); J. Patel and P. J. Marangos, *Biochem. Biophys. Res. Commun.* **109**, 1089 (1982); R. Donato, *Cell Calcium* **6**, 343 (1985); D. B. Zimmer and L. J. Van Eldik, *J. Biol. Chem.* **261**, 11424 (1986).
13. R. Allore *et al.*, unpublished data.
14. H. F. Willard and M. T. Holmes, *Hum. Genet.* **66**, 272 (1984); H. F. Willard *et al.*, *Somatic Cell. Mol. Genet.* **11**, 511 (1985); H. F. Willard and J. Riordan, *Science* **230**, 960 (1985).
15. J. P. Park *et al.*, *Clin. Genet.* **32**, 342 (1987).
16. H. Hyden and P. W. Lange, *Proc. Natl. Acad. Sci. U.S.A.* **67**, 1959 (1970); D. Kligman and D. R. Marshak, *ibid.* **82**, 7136 (1985).
17. R. Dunn *et al.*, *J. Biol. Chem.* **262**, 3562 (1987).
18. R. J. Allore *et al.*, *Proc. Can. Fed. Biol. Soc.* **30**, 152 (1987).
19. D. R. Cox and C. J. Epstein, *Annu. Rev. N.Y. Acad. Sci.* **450**, 169 (1985).
20. A. P. Feinberg and B. Vogelstein, *Anal. Biochem.* **132**, 6 (1983).

21. Supported by grants from the MRC and NCI of Canada (H.F.W., A.M., and R.J.D.); NIH 140-17001 and Weingart Foundation (D.C.).

11 January 1988; accepted 4 February 1988

Technical Comments

ACTH Regulation and IL-1

Recently, three reports (*1, 2*) were published describing the release of adrenocorticotropin (ACTH) in response to interleukin-1 (IL-1) administration. A Perspective (*3*) describing, for the most part, conflicting results of the three reports was included in the same issue. We would like to point out that Uehara *et al.* (*4*) recently found that one preparation of IL-1α and two preparations of IL-1β were without effect on the release of five hormones, including ACTH, from cultured anterior pituitary cells, irrespective of whether pituitaries from male or female rats were used. Furthermore, IL-1β appears to be a potent stimulator for ACTH release in vivo, but IL-1α has very little such effect (*5*). Finally, in a recently published study, ACTH-releasing activity was blocked by immunoneutralization of endogenous corticotropin-releasing factor (*6*). In addition, we tested (*4*) the Cistron preparation of IL-1β [effective in (*1*)], which did not stimulate ACTH release in vitro.

AKIRA ARIMURA
U.S.–Japan Biomedical Research Laboratories, Tulane University Hebert Center, Belle Chasse, LA 70037

REFERENCES

1. E. W. Bernton *et al.*, *Science* **238**, 519 (1987).
2. R. Sapolsky, C. Rivier, G. Yamamoto, P. Plotsky, W. Vale, *ibid.*, p. 522, F. Berkenbosch, J. van Oers, A. del Rey, F. Tilders, H. Besedovsky, *ibid.*, p. 524.
3. M. D. Lumpkin, *ibid.*, p. 452.
4. A. Uehara, S. Gillis, A. Arimura, *Neuroendocrinology* **45**, 343 (1987).
5. A. Uehara, P. E. Gottschall, R. Dahl, A. Arimura, *Biochem. Biophys. Res. Comm.* **146**, 1286 (1987).
6. ———, *Endocrinology* **121**, 1580 (1987).

14 December 1987; accepted 26 January 1988