SHORT COMMUNICATION

Genomic Organization and Expression of the Human β-Synuclein Gene (SNCB)

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The β-synuclein protein is highly homologous to the α-synuclein protein for which two mutations were reported in some familial cases of Parkinson disease. It has been shown that both α- and β-synucleins may be able to inhibit phospholipase D2 selectively. We have observed that the β-synuclein gene (HGMW-approved symbol, SNCB) is highly expressed in brain including the substantia nigra, the main region of neuronal degeneration in patients with Parkinson disease. We have determined the intron–exon structure of the β-synuclein gene and established sequencing assays that will facilitate the search for mutations in the β-synuclein gene in patients with Parkinson disease or other neurodegenerative disorders.

Parkinson disease is the second most common neurodegenerative disorder after Alzheimer disease (10). The clinical symptoms, which include bradykinesia, muscular rigidity, resting tremor, and impaired postural reflexes (8), are due to the loss of dopaminergic neurons in the substantia nigra. Two missense mutations in the α-synuclein gene have been described in families with early onset autosomal dominant Parkinson disease (4, 9). It has been shown that both α- and β-synucleins are concentrated in presynaptic nerve terminals (1, 6, 7) and that they are apparently involved in the regulation of phospholipase D2 (2). In the search for additional potential genetic factors that could account for other familial or some sporadic cases of PD, we characterized the human β-synuclein gene, known to be highly homologous to the α-synuclein gene (1).

The β-synuclein gene is predicted to be transcribed as an ~1.1-kb messenger RNA, not including the poly(A) tail. We studied the expression of the human β-synuclein gene[2] by Northern blot analysis as previously described (5), using as a probe an oligonucleotide (β40) from the 3′ untranslated region that is not homologous to α- or γ-synuclein. We observed a strong signal at ~1.5 kb in the brain, with no apparent splice variant; the β-synuclein gene was in fact expressed at high levels in various areas of the brain, including the substantia nigra, which is the main area of neuronal degeneration in Parkinson disease patients, the thalamus, the hippocampus, and the amygdala and at a lower level in the caudate nucleus; no signal was detected in the corpus callosum or the subthalamic nucleus (Fig. 1). The pattern of expression of β-synuclein is therefore somewhat different than that of α-synuclein, which is expressed not only in the substantia nigra, the thalamus, the hippocampus, and the amygdala, but also at about the same level in the corpus callosum and the caudate nucleus (data not

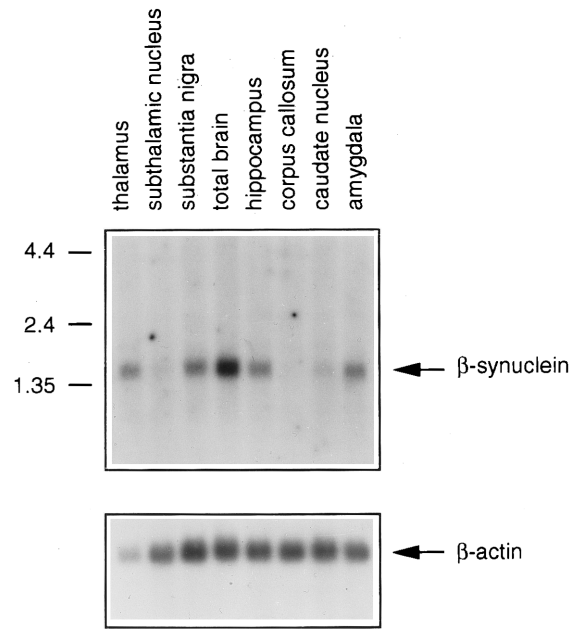

FIG. 1. Expression of β-synuclein in various areas of the human brain. Each lane contains 2 μg of poly(A)$^+$ RNA from human tissues (Clontech). The sizes in kilobase pairs of a molecular weight standard RNA are indicated to the left. A β-actin cDNA fragment (Clontech) was used as a control.

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[2] The HGMW-approved symbol for the gene described in this paper is SNCB.

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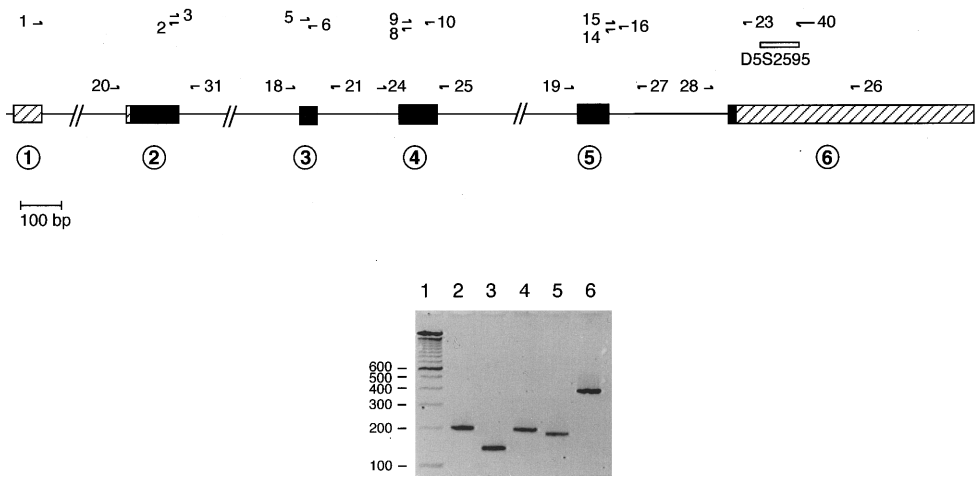

FIG. 2. (**Top**) Human β-synuclein gene structure. Exons are numbered and are represented by boxes (black for the coding region). Introns are represented by an horizontal line, interrupted when the entire intron is not shown. Marker D5S2595 is represented by an open rectangle. The β oligonucleotides used are shown above the gene, and their 5′ to 3′ sequences were as follows: β1, tatatatatacagccccggcccccgcatcca; β2, acgccctccttggtcttctc; β3, gagaagaccaaggagggcgt; β5, gcaagacccgagaaggtgtg; β6, ctgaagccacaccttgtacc; β8, ccagatgtgaggcctgttcc; β9, ggaacaggcctcacatctgg; β10, tagggaattcctccctcttc; β14, cttctggctccatcaggggc; β15, gcccctgatggagccagaag; β16, ggtgggtcctcataactctc; β18, cagcgcagagtccttaaatg; β19, tcctcacgagtcctgacctt; β20, agtgcaccggtgcccgtgtatc; β21, cggccagatcatccgcctaa; β23, ggctcatactcctgatattc; β24, tttccccctggctccccaaac; β25, ctgcatgtgcgggtcagaag; β26, cgtcgctcggatcttcgtt; β27, agctagggacggcagcaatca; β28, aaactcctcctccttttgc; β31, agccctgcagccccagaaac; β40, ccgaggggggttgcctcagagcggaaggaagatctcgtgat. PCRs were carried out as previously described (5) with the exception of amplicon β20–β31, which was obtained as follows: 98°C for 10 min; 94°C for 1 min, 65°C for 30 s, 72°C for 1 min, for 30 cycles; 72°C for 10 min, then a step at 4°C, in 50 mM KCl, 10 mM Tris–HCl, pH 8.3, 2 mM $MgCl_2$, 0.01% gelatin, 250 mM dNTP, 5% formamide, 10% glycerol, with 80 ng of each primer, and 5 U of AmpliTaq polymerase added after the 10-min denaturation step. The annealing temperature was 55°C for amplicons β18–β21 and β26–β28 and 60°C for β24–β25 and β19–β27. (**Bottom**) Specific PCR amplifications of the five coding exons of the human β-synuclein gene. Samples were run in 6% Visigel as recommended by the manufacturer (Stratagene). Lane **1,** 250 ng of a molecular weight standard DNA (Gibco BRL), with sizes in basepairs shown to the left. Lanes **2–6,** 5 μl of PCR amplification of exons 2–6, 198 bp (β20–β31), 139 bp (β18–β21), 197 bp (β24–β25), 186 bp (β19–β27), 403 bp (β26–β28), respectively.

shown). The fact that the largest amount of β-synuclein mRNA was seen in the total brain RNA sample indicated that the region of the brain most abundantly expressing β-synuclein was not one of the regions examined in our Northern blot. As previously reported (1), no signal was observed in any of the other adult tissues tested: heart, placenta, lung, liver, skeletal muscle, kidney, or pancreas (data not shown).

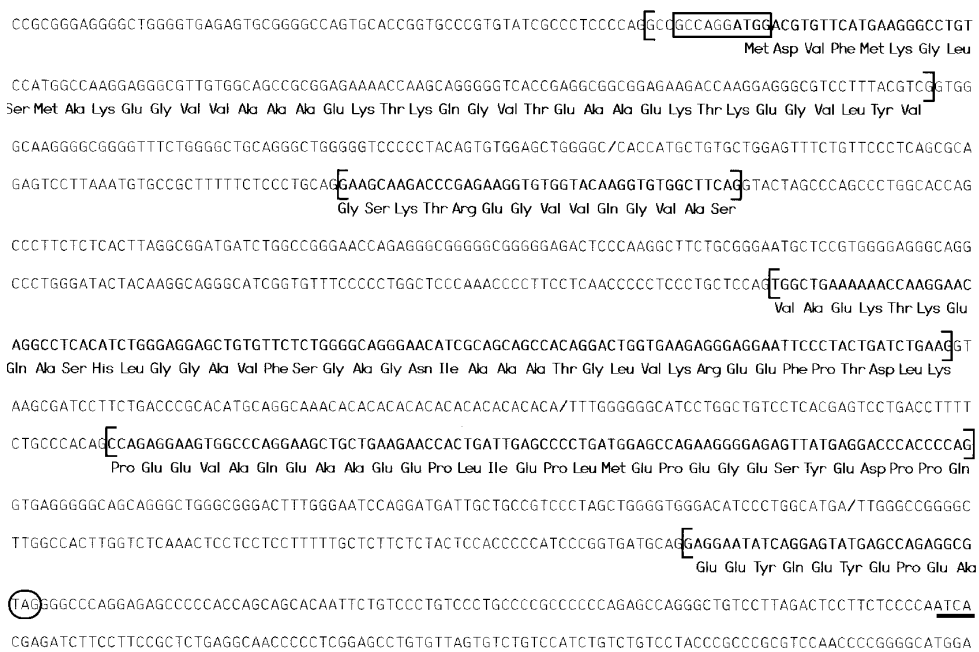

FIG. 3. Partial genomic sequence of the human β-synuclein gene. The protein sequence is shown below the coding nucleotide sequence (boldface). Exons are delimited by brackets, and interruptions in introns are indicated by (/). The Kozak sequence is boxed, and the stop codon is circled. The sequence corresponding to oligoprobe β40 is underlined. Additional sequence is available in GenBank under Accession Nos. AF053134–AF053136.

Using marker D5S2595 (11) located in the 3′ untranslated region of the β-synuclein gene, we isolated a genomic clone from a BAC library (Genome Systems, St. Louis, MO). This BAC clone, 139A20, gave a unique signal on 5q35 in metaphase FISH experiments (data not shown), thus confirming the chromosomal localization of the β-synuclein gene previously reported (11). To determine the intron–exon structure of the β-synuclein gene, we performed PCR experiments, using DNA of BAC clone 139A20 as a template, as previously described (5). Because of the high homology between α- and β-synuclein cDNAs (GenBank Accession Nos. L08850 and S69965, respectively), we assumed that the overall genomic organization of the β-synuclein gene would be similar to that of the α-synuclein gene (GenBank Accession Nos. U46896–46901). We used this assumption to design oligonucleotide pairs that would amplify introns (Fig. 2, top). PCR on the BAC 139A20 clone DNA resulted in the following amplicons: β1–β2 (~800 bp), β3–β6 (~5 kb), β5–β8 (~280 bp), β5–β10 (~350 bp), β9–β14 (~7.8 kb), β9–β16 (~7.8 kb), and β15–β23 (440) bp. Alignment of the sequence of these PCR fragments with the β-synuclein cDNA (GenBank Accession No. S69965) allowed us to determine that the β-synuclein gene was composed of five coding exons and at least one 5′ untranslated exon (Fig. 2), with intron–exon junctions similar to that of α-synuclein. The ATG start codon was identified based on the sequence homology with the α-synuclein protein and the presence of a good Kozak sequence: GCCAGGATGG (3) (Fig. 3).

Based on the intron–exon structure of the β-synuclein gene, we have developed intronic oligonucleotides and PCR conditions to amplify specifically each of the five coding exons from genomic DNA (Fig. 2, bottom). Sequencing confirmed the specificity of the assays, which should allow rapid screening for mutations in this gene and facilitate the study of the β-synuclein gene and its potential role in Parkinson disease or other neurodegenerative disorders.

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