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BRIEF REPORT—GENE MAPPING

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The human regulator of G-protein signaling protein 6 gene *(RGS6)* maps between markers WI-5202 and D14S277 on chromosome 14q24.3

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Abstract The recently discovered regulators of G-protein signaling proteins, termed the RGS family, have been shown to modulate the functioning of G-proteins by activating the intrinsic guanosine triphosphatase (GTPase) activity of the α subunits. Here, we report the chromosomal location and tissue expression of the human regulator of *RGS6* gene. The messenger RNA was ubiquitously expressed in various tissues. Polymerase chain reaction (PCR)-based analysis with a human/rodent monochromosomal hybrid panel and a radiation hybrid panel indicated that the gene was mapped between genetic markers WI-5202 and D14S277 on chromosome 14q24.3 region.

Key words Regulators of G-protein signaling proteins · RGS6 · Chromosome mapping · Chromosome 14q24.3

Introduction

G-proteins are heterotrimers composed of single α, β, and γ subunits. The α subunits are guanine nucleotide-binding proteins that are regulated through cycles of guanosine triphosphate GTP and guanosine diphosphate (GDP) binding. In the inactive state, the α subunits are bound to GDP (for reviews, see Neer 1995; 1997). The recently discovered regulators of G-protein signaling proteins, termed the RGS family, have been shown to modulate the functioning of G-proteins by activating the intrinsic GTPase activity of the α subunits (for reviews, see Dohlman and Thorner 1997; Koelle 1997; Neer 1997).

At present, full or partial sequences of the mRNAs for numerous putative RGS proteins have been identified in mammalian species (De Vries et al. 1995; Chen et al. 1996; Druey et al. 1996; Koelle and Horvitz, 1996; Siderovski et al. 1996; Gold et al. 1997; Snow et al. 1997; 1998; Seki et al. 1998a). The cDNA sequence of human *RGS6* has recently been registered with the public database (accession number AF073920). We are attempting to carry out systematic chromosome mapping of signaling molecules including RGS5 (G-protein signaling regulator protein 5), ZIP kinase (serin/threonine kinase gene), and KIP (kinase interacting protein) to contribute to positional candidate approaches (Seki et al. 1998a,b; Saito et al. 1998). We report here the expression profile and chromosomal localization of the human *RGS6* gene.

Expression profile of *RGS6* gene

We examined the tissue distribution of the transcript in various human tissues by reverse transcription-coupled polymerase chain reaction (RT-PCR). Primers used for RT-PCR were to amplify the 533-bp cDNA of the transcript. Three amplified products, of 533-bp, 660-bp, and 765-bp-long were finally detected, although it is yet to be determined whether they reflected alternatively spliced multiple messengers or distinct mRNAs derived from related genes. The products were not amplified in liver and were slightly detected in thymus and spleen, indicating that the expression of *RGS6* may be suppressed or weakly promoted in such blood and lymphatic organs. In both fetal and adult brain, all three amplified products were obtained, suggesting the possible transcription of a brain-specific longer form. The shortest product was predominant in brains and heart, whereas the middle-sized product was abundant in the other tissues examined (Fig. 1).

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Chromosome mapping of *RGS6* gene

Chromosomal assignment of RGS6 was done by PCR analysis of a human/rodent somatic cell hybrid panel and a radiation hybrid panel. The PCR primer set was designed for the 3′ untranslated region of the gene (5′-TAGGCAAGCTGGCGTGTGGAC-3′, 5-CTATGCGCTCTCGTCCCCGTC-3′, PCR product size, 110 bp). DNA of the human-rodent somatic cell hybrid panel was purchased from the National Institute of General Medicine Service, Coriell Cell Repositories (Camden, NJ, USA). The specific amplified PCR product for human was detected only from the hybrid containing human chromosome 14 (data not shown). PCR was carried out as described previously (Saito et al. 1997; Seki et al. 1997). We performed further mapping analysis using a PCR-based radiation hybrid panel (Genebridge 4; Research Genetics, Huntsville, AL, USA) with the same primers as those used in the assay for the human/rodent somatic cell hybrid panel. Statistical analysis of the radiation hybrid data was performed using the RHMAPPER software package (http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl). The data vector for the *RGS6* gene was 0010100100 0000021010 0002100000 0211000100 0011100000 0001000001 0000010100 0011000000 1011111000 000 and the consequent report indicated that the gene was mapped between markers WI-5202 and D14S277, both of which have been cytogenetically mapped to 14q24.3 (Fig. 2). The position of the gene is 7.80 cR distal from WI-5202 (lod > 3.0).

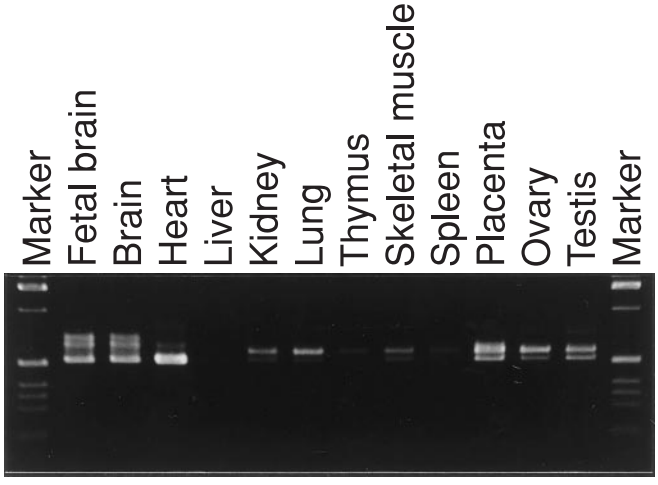

Fig. 1 Primers used for reverse transcription-coupled polymerase chain reaction (RT-PCR) were to amplify the 533-bp cDNA of the transcript. RT-PCR amplification primers were (5′-GGGGCGGGACCAGTTTCTACG-3′) and (5′-CCCCACCATCGCCCTTCATTG-3′). It was confirmed that these primers gave no visible PCR product from genomic DNA. The templates of the human tissues of poly(A)+ RNAs were purchased from Clontech (Palo Alto, CA, USA). The cDNA templates for RT-PCR were synthesized from 2 μg of poly (A)+, using excess amounts of Superscript II reverse transcriptase (GIBCO BRL, Gaithersburg, MD, USA) and random hexamer primers. PCR was carried out in a final volume of 10 μl containing 1 × LA-PCR buffer (Takara, Kyoto, Japan), 2 μM each primer, 200 μM each dNTP, 50 ng template DNA, and 0.01 units of LA-Taq DNA polymerase (Takara). Temperature and time schedules were: 30 cycles at 95°C for 20 s and 64°C for 1 min. PCR products were separated on 2% GTG gel (Takara) with a 1-kb ladder DNA marker (BRL, Gaithersburg, MD, USA)

The region of chromosome 14q24.3 has been of particular interest, as it is known to contain one of the early-onset Alzheimer disease genes (*AD3*) (Sherrington et al. 1995). In the 14q24 region, several groups have reported the physical and transcription maps as a step toward identifying the Alzheimer disease gene or cosegregating with an anterior polar cataract (Cruts et al. 1995; Clark et al. 1995; Roux et al. 1997; Sharma et al. 1998). A goal of the Human Genome

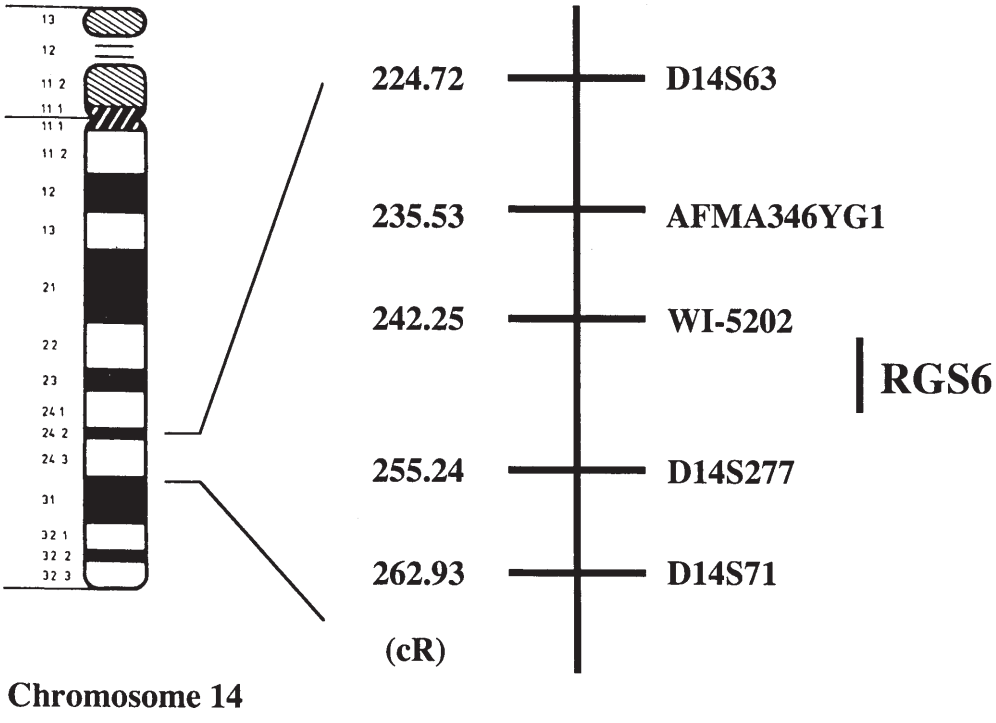

Fig. 2 Chromosomal placement of the human *RGS6* gene at a relative distance to framework markers on the WICGR radiation hybrid map of the human genome. The approximate corresponding cytogenetic location of the gene on chromosome 14q24.3 region is indicated. Distances are in centirays (cR) from the top of the chromosome 14 linkage group

Project is the construction of a physical and transcription map of the human genome (Collins and Galas 1993). Our precise chromosomal positioning data for *RGS6* the gene could contribute toward ongoing positional candidate approaches for the above-mentioned disease genes linked to this genomic locus.

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