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The Human Chromogranin A Gene: Chromosome Assignment and RFLP Analysis

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Summary

Chromogranin A/secretory protein I (CgA) is a glycoprotein that is stored and released along with peptide hormones and neurotransmitters from several tissues, although its exact function is not known. A cDNA (gene symbol CHGA) clone was used as a probe in Southern blot analyses of human-rodent somatic cell hybrid DNAs. Discordancy analysis allowed confirmation of the assignment of the gene to chromosome 14. These results were extended using in situ chromosome hybridization, and a signal was found at 14q32. *Bgl*II digestion of genomic DNA from 28 unrelated Caucasian individuals probed with CHGA detected a two-allele RFLP with allelic frequencies of .34 and .66.

Introduction

Chromogranin A (CgA) is a highly acidic glycoprotein that was originally isolated as the principle component of the adrenal medulla chromaffin granules (Blashko et al. 1967; Sage et al. 1967). This protein is stored and released along with peptide hormones and neurotransmitters in tissues of endocrine and neuroendocrine origin (Winkler 1976), although its exact function remains unclear. This protein was originally thought to be unique to the adrenal gland; however, parathyroid gland secretory protein-I (SP-I) has been shown to be biochemically similar to CgA (Cohn et al. 1982; Iacangelo et al. 1986).

Earlier studies based on cell-free translation of SP-I mRNA have suggested that several different proteins are apparent and that they must be encoded by more than one gene (Majzoub et al. 1979; Serck-Hanssen and O'Connor 1984). However, more recent blot and S1 nuclease analyses of RNA from normal bovine pituitary, adrenal, and parathyroid tissues and from normal and malignant human tissues suggest that a single gene encodes the CgA/SP-I protein and that the protein is present in different tissues of the diffuse neuroendocrine system (Ahn et al. 1987; Helman et al. 1988).

Murray et al. (1987) previously localized this gene to chromosome 14 by using flow-sorted chromosome libraries. In the present study the chromosome map position of the chromogranin A gene (gene symbol CHGA) was confirmed using Southern blotting analyses of somatic cell hybrids and in situ chromosome hybridization. Further, in order to develop this gene as a marker for inherited disorders, it was used to identify an RFLP by screening genomic DNA from unrelated individuals by using Southern blot analysis.

Material and Methods

Hybrid cell lines were obtained by PEG-mediated fusion of fresh human lymphocytes and mutant ($HPRT^-$) rodent cells (mouse RAG or Chinese hamster E36) and were propagated in HAT medium. Karyotypic and allozymic characterization of each cell line were carried out on the same passage from which high-molecular-weight genomic DNA was extracted. The entire hybrid panel contains 44 cell lines (O'Brien et al. 1983). Genomic DNA was isolated from parental and all hybrid cell lines and used in Southern blotting analyses.

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The procedures used in DNA transfer, hybridization, and washing have been described elsewhere (Modi et al. 1988). The probe used in both Southern blotting and in situ hybridization analyses was a construct called pHCGA and contains a 1.8-kb CHGA insert that was isolated from a human pheochromocytoma cDNA library (Helman et al. 1988).

Hybridization of the tritiated probe to metaphase chromosomes derived from peripheral lymphocytes was done following the method of Modi et al. (1987) with the following modifications: Prior to denaturation the slides were treated with 0.5% acetic anhydride in 0.1 M triethanolamine buffer (pH 8.0) at room temperature for 10 min (Hayashi et al. 1978). Probe concentrations in the hybridization solution ranged from 0.10 to 0.30 μg/ml.

To identify RFLPs, DNA from eight unrelated Caucasians was digested with 10 restriction enzymes (*Ava*II, *Bam*HI, *Bgl*II, *Eco*RI, *Hin*dIII, *Msp*I, *Pst*I, *Pvu*II, *Rsa*I, and *Taq*I) and was analyzed by Southern blotting. DNA was blotted and hybridized, by a method described elsewhere (Dean et al. 1987), to the CHGA probe. Potential RFLPs were typed in an additional 20 unrelated Caucasians, including individuals from a 3-generation pedigree (K-1333) obtained from the Camden Cell Repository, and in seven unrelated blacks.

Results and Discussion

The Southern blot analyses of human-rodent somatic cell hybrid DNAs indicates that *Bgl*II digestion yields a constant human fragment of 6.0 kb and two allelic fragments of 24.0 and 8.0 kb (fig. 1*A*). A discordancy analysis of the entire panel of 44 hybrid cell lines indicates that the lowest discordancy values are obtained with respect to chromosome 14 (two discordant hybrids out of 42, or 4.8% discordancy) and with respect to its associated isozyme-marker nucleoside phosphorylase, NP (three discordant hybrids out of 44, or 6.8% discordancy). The additional discordancy with respect to nucleoside phosphorylase is presumably due to a translocation. All other chromosomes and isozyme markers have substantially higher discordancies (22%–55%) (fig. 1*B*).

Hybridization of the radiolabeled CHGA cDNA clone to metaphase chromosome preparations yielded the following results: In an examination of 120 cells, 40 (9.8%) of 410 autoradiographic silver grains were found in the region 14q32.2-q32.3 (fig. 2). This peak was significantly above background levels, confirms the

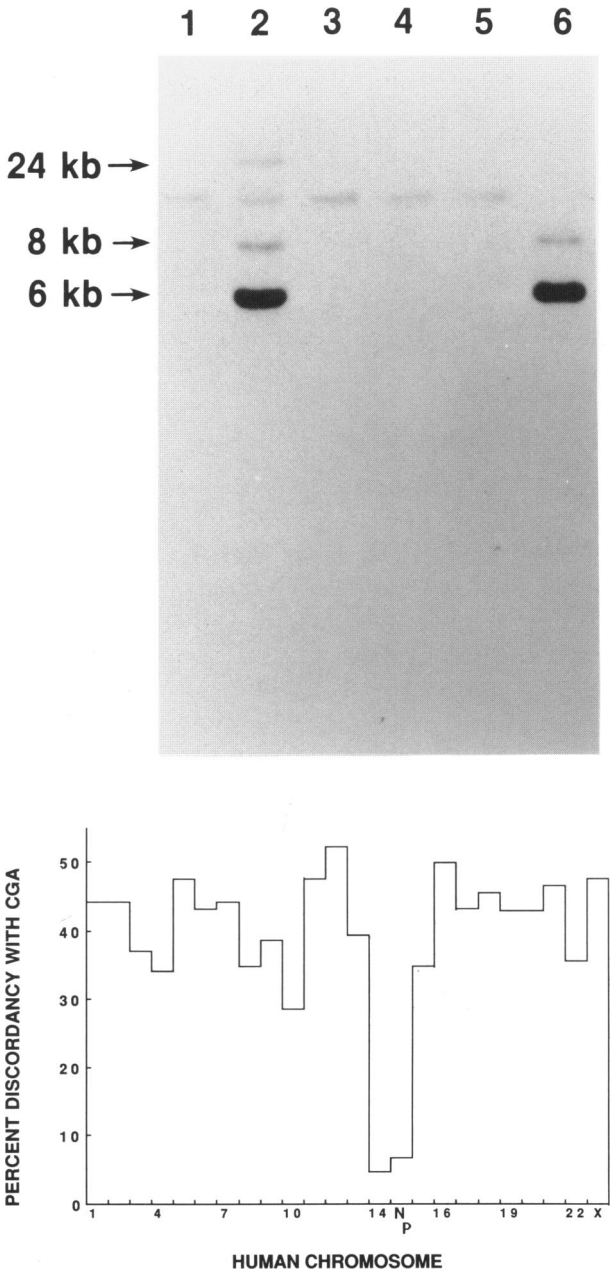

Figure 1 *A*, Southern blot analysis of *Bgl*II-digested somatic cell hybrid and parental DNAs after probing with the CHGA-cDNA clone. Lanes 1–4, human-hamster hybrid DNAs; lane 5, hamster (H84) DNA; lane 6, human (Q220) DNA. Diagnostic human fragments of 6, 8, and 24 kb can be seen in one hybrid DNA (lane 2), and fragments of 6 and 8 kb can be seen in the human control (lane 6). A 14-kb hamster band was present in all hybrids. *B*, Chromosome discordancy profile (isozyme markers not included, except for chromosome 14) resulting from the Southern blot analysis of the human-rodent somatic cell hybrid DNAs when probed with the CHGA cDNA clone. Chromosome 14 and its isozyme marker NP (nucleoside phosphorylase) are shown to have the lowest discordancy values—4.8% and 6.8%, respectively.

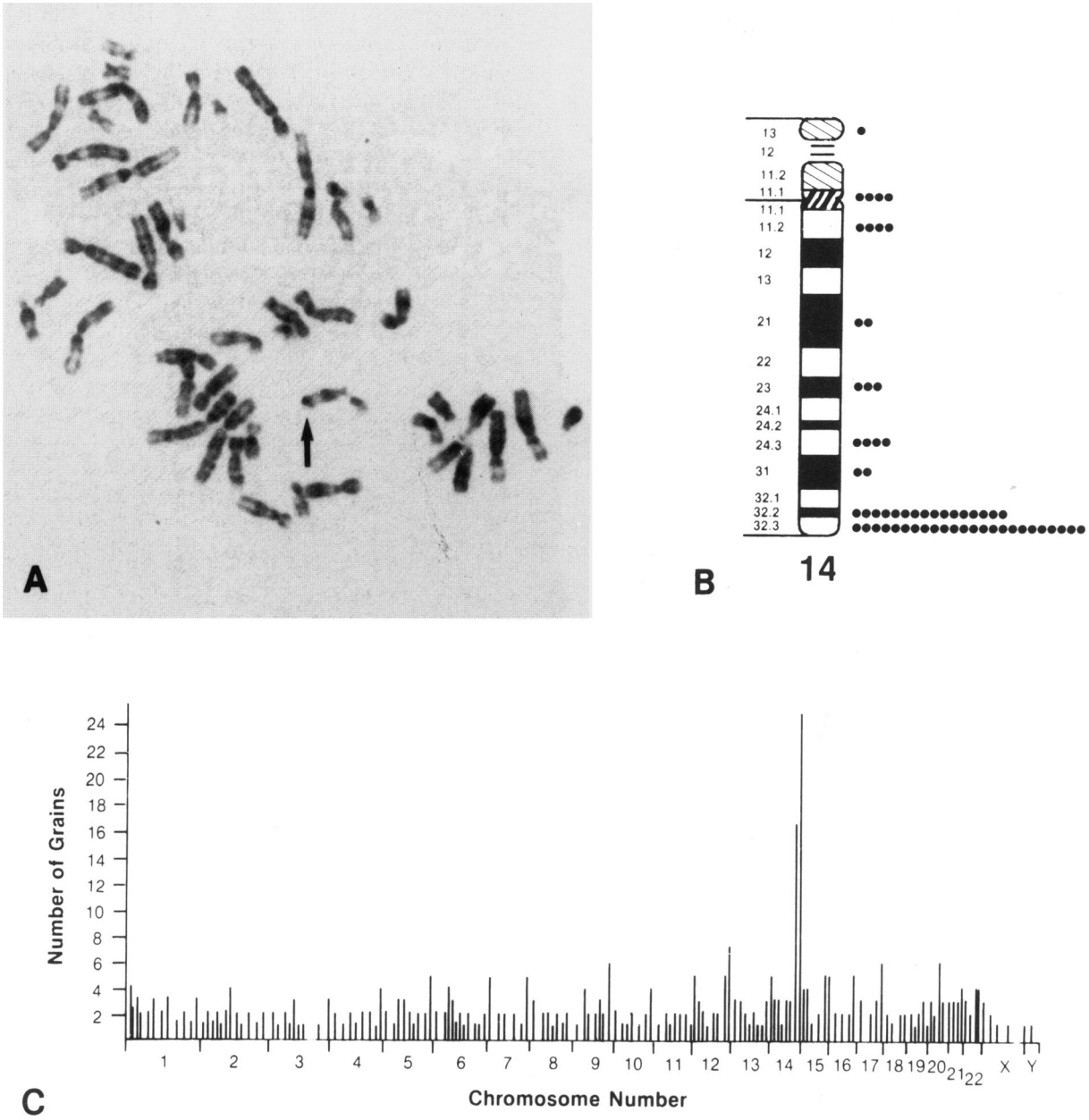

Figure 2 Results of the in situ hybridization analysis. *A*, G-banded metaphase chromosomes with an autoradiographic silver grain on the long arm of chromosome 14 (arrow). *B*, Idiogram of chromosome 14, illustrating the distribution of 60 autoradiographic silver grains. *C*, Frequency histogram displaying the distribution of 410 silver grains over the entire karyotype recorded from 120 metaphase cells.

results of the somatic cell hybrid analyses, and permits assignment of CHGA to 14q32.

In a set of Southern blots containing DNA from eight Caucasian individuals that was digested with 10 restriction enzymes, nine of the 10 enzymes did not identify an RFLP; however, *Bgl*II detects both a two-allele RFLP having alleles of 24.0 and 8.0 kb and a constant band of 6 kb (fig. 3). This polymorphism was found to segregate in the 3-generation pedigree K-1333 shown in figure 3. In a total of 56 chromosomes from unrelated Caucasian individuals, the two alleles have frequencies of .34 (24.0 kb) and .66 (8.0 kb) and the RFLP was

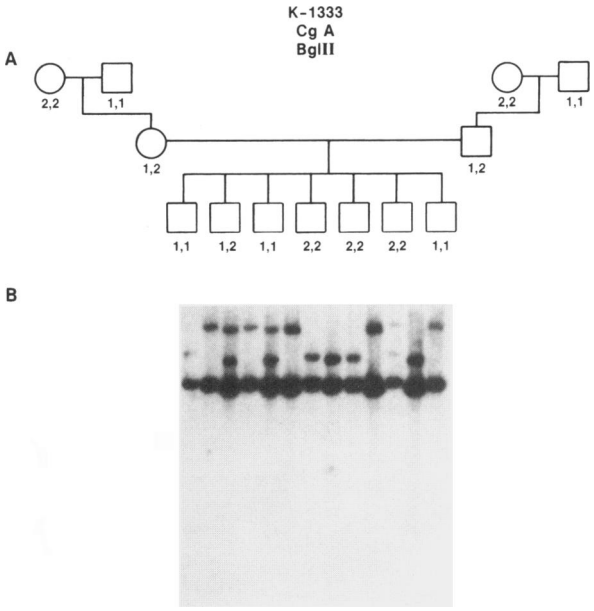

Figure 3 Segregation of *Bgl*II RFLP detected by CHGA. *A*, Pedigree for kindred K-1333, showing the genotypes of the CHGA RFLP under the symbol for each individual. *B*, Southern blot of the individuals of K-1333, in the same order as shown in panel *A*.

heterozygous in nine (32%) of the 28 individuals tested. Further, in a total of 14 chromosomes from unrelated blacks, the two alleles have frequencies of .21 and .79, respectively, with three (43%) of the seven individuals heterozygous.

Murray et al. (1987) assigned the CHGA gene to chromosome 14 by hybridizing a CHGA cDNA probe to flow-sorted chromosome-specific libraries. Our results, in studies using both cell hybrids and in situ hybridization, confirm and extend their findings by regionally localizing this gene to 14q32. Other genes that reside in this general chromosomal region include the immunoglobulin heavy-chain cluster, the alpha-1-antichymotrypsin, the brain form of creatine kinase, the beta-spectrin, and the AKT-1 cellular protooncogene (Ropers et al. 1987). The genes encoding two peptides associated with CgA, i.e., calcitonin and parathyroid hormone, are both found on the short arm of chromosome 11 (Naylor et al. 1984; Przepiorka et al. 1984).

With the recent ability of recombinant DNA technology to identify extensive polymorphisms in the human genome, it has become possible, by using a linkage approach, to isolate and characterize individual genes that in a mutated form are responsible for various inherited disorders (Botstein et al. 1980). The CHGA clone was found to detect an RFLP on *Bgl*II digestion. The location of the CHGA gene on chromosome 14 at band q32 makes this RFLP potentially useful for linkage mapping of other gene loci on chromosome 14. Skolnick and White (1982) have concluded that for a RFLP to be generally useful as a genetic marker the probability that an offspring is informative (i.e., the PIC value) must be at least .15. The *Bgl*II RFLP at CHGA satisfies this criterion; the allelic frequency of the 20-kb fragment is .34, corresponding to a PIC value of .35. The probability of missing the polymorphism in a nine-member screening panel is <.02, making this a very useful RFLP.

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