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Localization of Two Human Autoantigen Genes by PCR Screening and *in Situ* Hybridization—Glycyl-tRNA Synthetase Locates to 7p15 and Alanyl-tRNA Synthetase Locates to 16q22

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Aminoacyl-tRNA synthetases (aminoacyl-RS) catalyze the attachment of an amino acid to its cognate tRNA. Five of 20 human aminoacyl-RS (histidyl-RS, threonyl-RS, isoleucyl-RS, glycyl-RS, and alanyl-RS) have been identified as targets of autoantibodies in the autoimmune disease polymyositis/dermatomyositis (PM/DM; 9). A sixth autoantigenic aminoacyl-RS, lysyl-RS, was recently reported (10). The genes for histidyl-RS and threonyl-RS have been assigned to chromosome 5, as have the genes for leucyl-RS and arginyl-RS (references in 3). Six other aminoacyl-RS (glutamyl-prolyl-RS, valyl-RS, cysteinyl-RS, methionyl-RS, tryptophanyl-RS, and asparaginyl-RS) were assigned to chromosomes 1, 6, 11, 12, 14, and 18, respectively (3). The reason for a preponderance of aminoacyl-RS genes on chromosome 5 is unknown, but it has been suggested that regulatory relatedness might be a factor (3). Recently the entire or partial cDNA sequences for two autoantigenic aminoacyl-RS genes, glycyl-RS (gene symbol GARS; 4) and alanyl-RS (gene symbol AARS; 1), were reported. To understand further the genesis of autoimmune responses to aminoacyl-RS and to determine whether genes for autoantigenic aminoacyl-RS colocalize to chromosome 5, we have determined the chromosomal site of the GARS and AARS genes by PCR-based screening of somatic cell hybrid panels and by fluorescence *in situ* hybridization (FISH) analysis.

A Coriell rodent/human somatic cell hybrid panel (Mapping Panel 2, Coriell Cell Repositories, Camden, NJ) was used to localize the genes encoding GARS and AARS. PCR primer pairs (GARS: sense 5′-cccagcatagtccaagacctagccaat; antisense 5′-tcaggattctttcaactttaattgtggggg; AARS: sense 5′-aggcaagaacgttggctgcctgcaggagg; antisense 5′-ggttctagaccgatggcactgacgtggagg) were synthesized using 3′-UT nucleotide sequences of the respective genes, and PCR was performed under stringent conditions ((1) 94°C, 120 s; (2) 94°C, 30 s, 60–70°C, 30 s, 72°C, 60 s for 35 cycles; (3) 72°C, 300 s). A PCR product of the expected size (280 nt) was generated using GARS primers when the chromosome 7 hybrid was used as template. With AARS primers the expected product (288 nt) was generated using the chromosome 16 hybrid. The identity of PCR products was confirmed by Southern hybridization with ^{32}P-radiolabeled internal oligonucleotide probes (GARS, 5′-gtccactttacaaaagaaaacagcattgtga; AARS, 5′-atttgaatcttgggacctttaaagagcccc). Control experiments using total human genomic DNA were positive, and mouse and hamster genomic DNA controls were negative by PCR screening and by Southern analysis. Chromosomal assignments were confirmed by PCR analysis using a BIOS rodent/human somatic cell hybrid panel (polychromosomal PCRable DNA somatic cell hybrid panel, BIOS Laboratories, New Haven, CT; data not shown).

DNA purified from P1 clones was used to localize aminoacyl-RS by FISH. P1 clones for GARS and AARS were obtained from Genome Systems (St. Louis, MO) by screening P1 libraries using established methods (8) with PCR products generated with primer pairs specific for the 3′-UT regions (see above for sequences). FISH analysis was performed by Genomes Systems as described in their literature. Briefly, biotin-labeled DNA was hybridized to normal metaphase chromosomes from PHA-stimulated peripheral blood lymphocytes, incubated with fluorescein-conjugated avidin, and counterstained with propidium iodide. Results were confirmed by cohybridizing with chromosome-specific centromere probes, and the specific location of each locus was measured as the average percentage distance from the telomere on 10 chromosomes. The results of FISH analyses using DNA from the GARS- and AARS-specific P1 clones are shown in Fig. 1. In each case centromere-specific labeling is evident. The GARS locus was measured at 7p15. However, assignment of AARS was less clear due to the apparent staining of chromosome-specific repetitive elements on chromosome 16. Tentative assignment was made to 16q22. To verify this assignment we obtained a chromosome 16-specific panel of mouse/human somatic cell hybrids from Dr. D. F. Callen and Dr. M. J. Siciliano (2, 6). Partial chromosome 16 hybrids that contain 16q22 (CY3, CY6, CY7, CY12, and CY165) were positive by PCR screening. Hybrid controls (CY2, CY5, I-E, and 92-2) specific for chromosome 16 but not containing 16q22 were negative. These results confirm the FISH analysis and demonstrate that the locus of AARS is 16q22.

The etiology of autoimmune PM/DM is not known. The role of genetic factors relating to the histocompatibility complex

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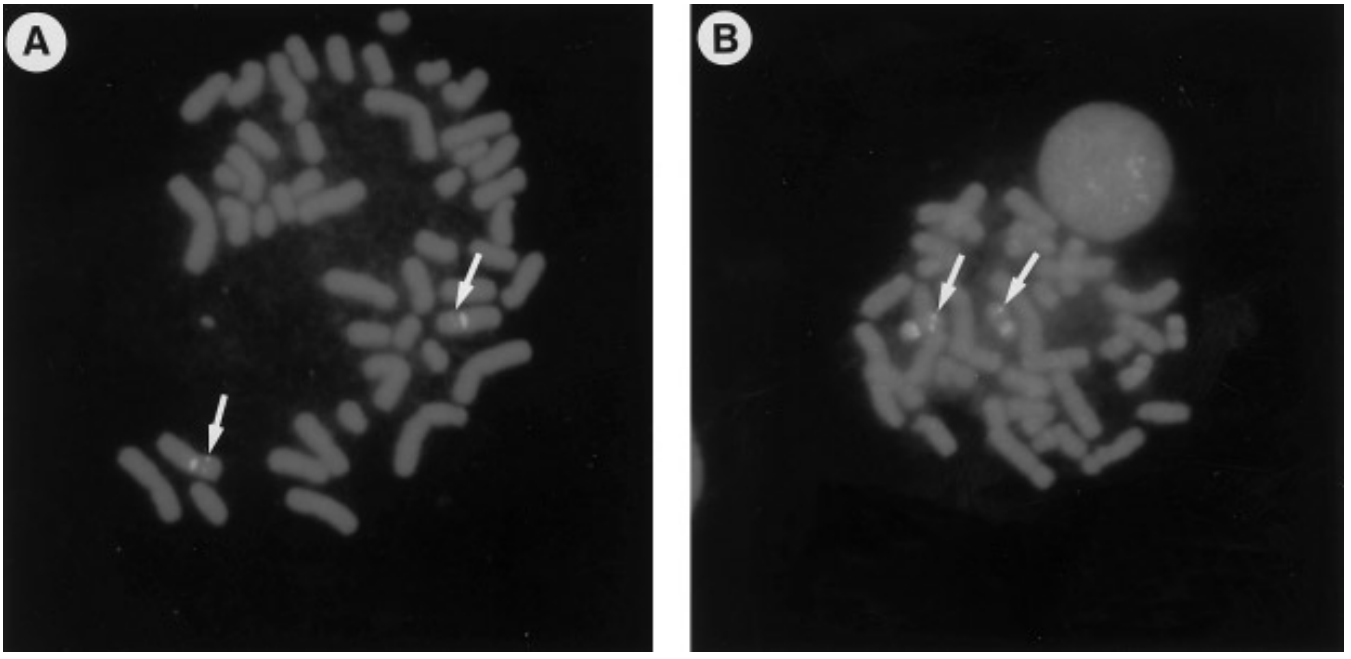

FIG. 1. *In situ* hybridization of human metaphase chromosomes. P1 DNA from aminoacyl-RS-specific clones was biotinylated and cohybridized with centromeric probes. For GARS (**A**) and AARS (**B**) fluorescence detection shows the aminoacyl-RS probe (arrows) and centromere probe (large bands) located on the same chromosomes.

has been reported (5), but because the aminoacyl-RS autoantigens have been shown to drive the immune response and because the autoantibodies antedate clinical disease (7), it is probable that the gene products are directly related to disease pathogenesis. It has been proposed, for example, that a viral agent initiates the disease, perhaps by specific association between aminoacyl-RS and viral RNA (9). For this reason and because genes for two autoantigenic aminoacyl-RS (histidyl-RS and threonyl-RS) have previously been localized to chromosome 5, we decided to determine the chromosomal assignment of genes for two autoantigenic aminoacyl-RS that had not yet been localized. Using PCR-based screening and Southern analysis of somatic cell hybrid panels followed by FISH analysis, we have assigned the genes for GARS and AARS to 7p15 and 16q22, respectively. The finding that we report here, that genes for autoantigenic aminoacyl-RS do not localize to the same chromosome, indicates that *cis* coregulation of these genes is an unlikely factor in the generation of autoimmune responses to these proteins. Furthermore, the assignment of the gene for AARS demonstrates the value of chromosome-specific hybrid panels for mapping genes.

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