Regional Mapping of the Human MP70 (Cx50; Connexin 50) Gene by Fluorescence In Situ Hybridization to 1q21.1

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Purpose: Gap junctions play a critical role in the metabolic homeostasis and maintenance of transparency of fibers within the ocular lens. As part of a long-term effort to establish the relationship between lens gap junction proteins, normal lens development, and cataractogenesis, we report here the regional localization of the human MP70 (Connexin 50) gene.

Methods: Fluorescence in situ hybridization (FISH) was used to regionally map the human MP70 gene. The DNA probe contained the entire MP70 coding region within a clone isolated from a human genomic DNA library.

Results: The human gene encoding the lens intrinsic membrane protein MP70 was regionally mapped to q21.1 on the long arm of chromosome 1.

Conclusions: This study confirms the previous provisional assignment of MP70 to human chromosome 1 and regionally localizes the gene to 1q21.1. When combined with previous mapping information, these data are consistent with the hypothesis that a genetic lesion in the gene encoding the lens intrinsic membrane protein MP70 may be the underlying molecular defect for zonular pulverulent (Coppock) cataract. Furthermore, these combined data support the hypothesis that other forms of human hereditary cataract may be the result of a mutation in one or more of the genes encoding gap junction proteins found in the ocular lens.

METHODS

Oligonucleotide primers were selected from the 5′ and 3′ ends of the coding region of a previously published mouse Cx50 gene (4). These were used to amplify the coding region from mouse genomic DNA by PCR. A 1337 base pair (bp) amplified product was used as a probe to screen a human genomic DNA library generated from the human lung fibroblast cell line WI38. Four positive clones were isolated (5). The clone HCx50/SstI-EcoRV, which contained a 4.0 kb fragment encompassing the entire Cx50 coding region, was used as a probe for FISH.

Human metaphase chromosomes were prepared from peripheral blood drawn in heparin collection tubes. Lymphocytes were cultured in RPMI-1640 medium (Gibco/BRL Life Technologies, Inc., Gaithersburg, MD) supplemented with 20% fetal calf serum (FCS) (Gibco/BRL Life Technologies, Inc.), 3% phytohemagglutinin (Gibco/BRL Life Technologies, Inc.) and 100 U/ml penicillin-streptomycin (P/
DNA isolated from the FixII clone, Hcx50/SstI-EcoRV, was labeled with biotin-14-dATP using the BioNick™ Labeling system (Gibco/BRL Life Technologies, Inc.). FISH to human metaphase chromosomes was performed by adding 100 ng of biotinylated probe suspended in 10 µL of hybridization buffer (Hybrisol VII, Oncor, Inc., Gaithersburg, MD) to each slide. Slides were sealed with glass coverslips and incubated for 48 hr in a humidified 37°C chamber. Slides were first washed in 50% formamide/2X SSC (0.3 M NaCl/0.03 M sodium citrate, pH 7.0) and then in 2X SSC, both for 2 min at 37°C. Sequence-specific signal was detected with an avidin-FITC conjugate (Oncor, Inc.). Chromosomes were counter stained with propidium iodide (Oncor, Inc.) and the chromosomal identity was confirmed by subsequent trypsin-Giemsa banding. The fluorescent signal was visualized on a Leitz Orthoplan 2 Epifluorescence microscope (Leitz Wetzlar, Germany) and photographed using Kodak Gold ASA 400 film.

Figure 1. Localization of the Cx50 gene to human chromosome 1 by FISH. Accumulation of sequence specific signal detected with an avidin-FITC conjugate (yellow dots) after hybridization of human metaphase chromosomes with a DNA probe containing the entire MP70 coding region.
RESULTS & DISCUSSION

Accumulation of fluorescent signal was observed on the long arm of chromosome 1 (Figure 1). At least 10 separate spreads were observed, none of which contained fluorescent signal in any area other than on chromosome 1. Essentially all of the signal was localized at band q21.1 on chromosome 1 (Figure 2 and Figure 3). We report the regional mapping of human MP70 to this location and confirm the previous provisional assignment by Church et al. (5) to chromosome 1. The q21.1 region of human chromosome 1 shows conserved synteny with mouse chromosome 3, where the murine homologue of MP70 has been regionally mapped (8). More interestingly, this region of human chromosome 1 is the same location to which zonular pulverulent (or Coppock) cataract was previously mapped (9). We therefore hypothesize that the Coppock cataract may be the result of a mutation in the gene encoding MP70, based upon these mapping data. Furthermore, there may be other forms of hereditary cataract that are the result of a genetic lesion in one or more of the gap junction proteins present in the ocular lens.

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