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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.

The lens is an avascular organ consisting of a single layer of epithelial cells which elongate and lose their organelles and nuclei upon differentiation into mature fiber cells. Due to these unique anatomical and physiological characteristics, these cells depend upon extensive metabolic and electrochemical coupling via gap junctions in order to remain vital and transparent. These cell junctions also serve the important role of maintaining a very narrow intercellular space, critical for lens transparency. It has been suggested that cataractogenesis may be in part due to the disruption of the unique cytoarchitecture of mature fiber cells resulting in changes in this intercellular space.

Lens intrinsic membrane protein MP70 is one of four proteins that make up the majority of the total transmembrane protein found in fiber cells of the vertebrate ocular lens (1,2). MP70 was first isolated from ovine lens (3), where its presence was noted to be coincident with fiber gap junctions. Subsequently, connexin 50 (Cx50) was cloned from the mouse and demonstrated to be both a classical gap junction protein and the murine homologue of MP70 (4). The isolation and characterization of a human clone containing the complete coding region of MP70 (Cx50) has been reported (5).

Direct evidence that mutations in lens fiber cell transmembrane proteins MP26 (MIP) and MP19 (also referred to as MP20) can lead to cataract development in the mouse has been reported (6,7). To date, however, there has been no report of a cataract caused by a mutation in a lens gap junction protein. The critical role of gap junctions in maintaining lens transparency, however, makes these proteins very good candidates.

As part of our long-term efforts to determine the relationship between lens gap junction proteins and cataractogenesis, we report here the regional localization of the MP70 (Cx50) gene to human chromosome 1q21.1 by fluorescence in situ hybridization (FISH).

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/

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S) (Gibco/BRL Life Technologies, Inc.) at 37° C for 72 hr in the presence of CO_2. Cell division was synchronized with 10^{-7} M methotrexate (Sigma Chemical Co., St.Louis, MO) for 17 hr at 37° C. The cells were washed twice in unsupplemented RPMI-1640 medium before being treated with RPMI-1640 medium supplemented with FCS, P/S, and 30 mg/ml bromo-deoxyuridine (Sigma Chemical Co.) for 7 hr at 37° C. The cell cycle was arrested by a 20 min incubation in 0.5 mg/ml Colcemid (Gibco/BRL Life Technologies, Inc.) at 37° C. The cells were subjected to hypotonic treatment in 0.075 M KCl for 15 min at 37° C, followed by fixation in Carnoy's fixative (3:1 methanol:glacial acetic acid). Metaphase preparations were dropped onto glass slides which were then aged for 5-7 days at room temperature.

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.

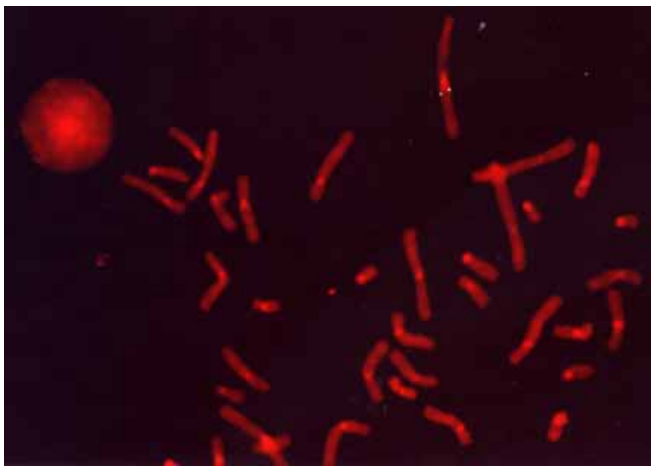


Figure 1. Localization of the Cx50 gene to human chromosome 1 by FISH. Accumulation of sequence specific signal detected with an avidin-FITC conjugate (yellow dots) after hybridization of human metaphase chromosomes with a DNA probe containing the entire MP70 coding region.

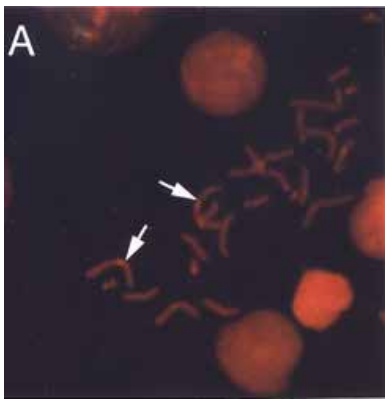
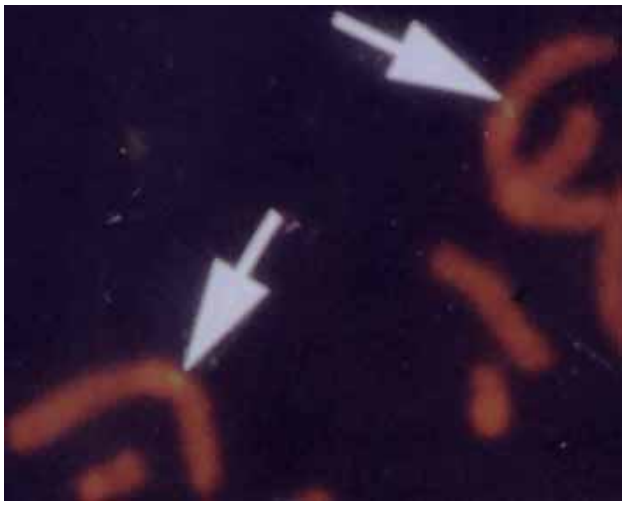


Figure 2. Localization of FISH signal to chromosome 1q21.1. (a) FISH of human metaphase chromosomes with a DNA probe containing the entire MP70 coding region. Note the accumulation of fluorescent signal (arrows).



A magnification of the central portion of the image better illustrates the fluorescent signal.

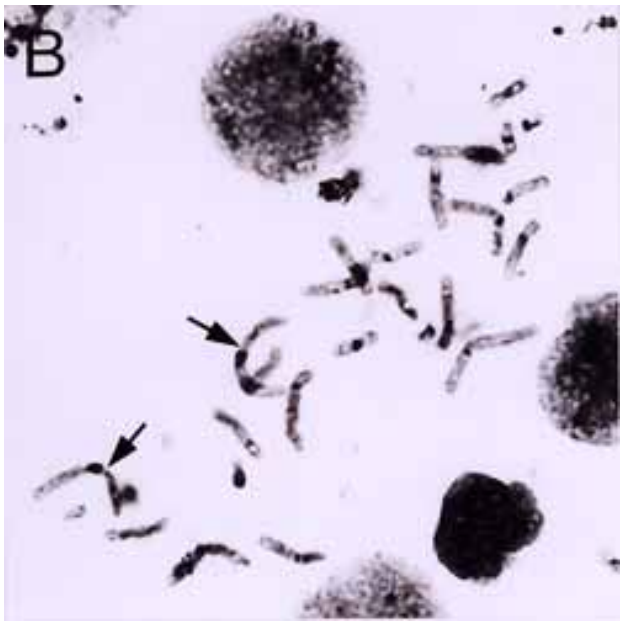


Figure 2. (b) Subsequent trypsin-Giemsa banding identifying FISH localization to chromosome 1q21.1.

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.

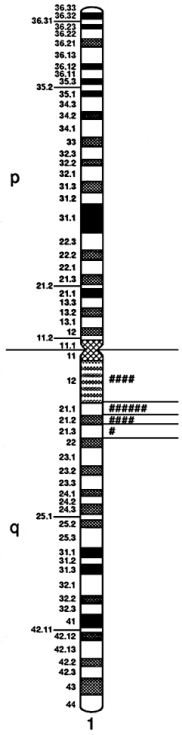


Figure 3. Diagram of human G-banded chromosome 1. The distribution of labeled sites for the Cx50 probe are marked with "#". A large majority of the fluorescent signal is associated with band q21.1.

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