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ASSIGNMENT OF THE HUMAN CONNEXIN43 GENE, *GJA1*, TO CHROMOSOME 6q22.3

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Summary Connexin43 is one of connexin proteins which make up the intercellular gap junctions. Targeted null mutation of the mouse connexin43 gene has been reported to result in a cardiac malformation. Moreover, single-base mutations of the human homolog (*GJA1*) were identified in patients with laterality defects of the chest and abdominal organs, suggesting that connexin43 contributes to the determination of laterality during organogenesis. We mapped *GJA1* to 6q22.3 by fluorescence *in situ* hybridization, using a bacterial artificial chromosome (BAC) clone that covered almost the entire *GJA1*-cDNA, as a probe.

Key Words connexin43 gene, *GJA1*, 6q22.3, FISH, BAC clone

INTRODUCTION

The connexin proteins constitute intercellular channels of gap junction that permit transfer of small molecules from cell to cell (Beyer *et al.*, 1988). Connexin43, a member of this gene family, is abundantly expressed in the mammalian heart and plays a role in synchronizing the heart beat rhythm (Goshima, 1971). A connexin43-gene knockout mouse has been reported to be neonatally lethal due to right ventricular outlet stenosis (Reaume *et al.*, 1995). Single-base substitutions in the human homolog (*GJA1*) were identified in patients with heart malformations and laterality defects (Britz-Cunningham *et al.*, 1995), suggesting that connexin43 is participating in embryogenesis, cellular differentiation, and development of the heart. *GJA1* has been mapped to chromosome 6q21-q23.2 (Corcos *et al.*, 1993). Here we isolated a bacterial artificial chromosome (BAC) clone containing *GJA1* and narrowed the assignment of the gene to 6q22.3 by fluorescence *in situ* hybridization.

MATERIALS AND METHODS

Isolation of BAC clone containing the human GJA1. A human BAC

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library (Research Genetics, USA) was screened by means of polymerase chain reaction (PCR) according to the manufacturer's protocol. Two sets of primers were designed from the partial sequence of the human *GJA1* (Fishman *et al.*, 1991). GJA primer sequences (sense, GCTCTGTGCTCCAAGTTACA; antisense, AGTAGTGAAGTCACGCCAAG) were designed to amplify the most part of exon 1 and a small portion of the 5′ untranslated region of the gene, excluding the sequence of a processed pseudogene. CXD primer sequences (sense, AGCTGCTG-GACATGAATTAC; antisense, CTAGATCTCCAGGTCATCAG) were chosen from the 3′ portion of the *GJA1*-cDNA. Using GJA as a primer, PCR was performed for 30 cycles under the following conditions: denaturation at 94°C for 30 sec, annealing at 58°C for 30 sec and extension at 72°C for 30 sec. The GJA-PCR products were subjected to the second PCR using CXD to evaluate whether these clones covered the entire cDNA. The PCR products using isolated BAC clones as templates were cloned with pGEM T-vector (Promega, USA) and sequenced by the dideoxy chain-termination method.

Fluorescence in situ hybridization (FISH). Chromosome preparations for FISH were made according to the method of Dutrillaux and Viegas-Pequignot (1981). In brief, cultures of peripheral blood lymphocytes were synchronized overnight by adding 0.3 μg/ml BrdU, and then by replacing with 200 μg/ml thymidine 6.5 hr prior to harvest. Isolated BAC clones were labeled with biotin-16-dUTP (Boehringer-Mannheim, Germany) by nick-translation. FISH was performed using human Cot-I DNA (GIBCO BRL, USA) as a competitor, and fluorescence signals were detected as described previously (Ohta *et al.*, 1993). After observation of FISH signals, the slides were rinsed in water to remove the antifading agent and washed in 4×SSC for 10 min, and then chromosomes were re-stained with 1 μg/ml Hoechst 33258 in 4×SSC for 10 min. The slides mounted in phosphate buffer were exposed to UV at 75°C for 3 min, and subsequently stained with 4% Giemsa.

RESULTS AND DISCUSSION

By a PCR-based screening of a human BAC library using the GJA-primer set, two positive clones, 342M16 and 326J20, were isolated. Another PCR with the CXD primers and the sequence data confirmed that both clones contained the entire *GJA1*-cDNA sequences and of the 5′-UTR sequence of the true gene (data not shown). FISH with the isolated BAC showed FITC-signals on the long arm of chromosomes 6 in almost all the 50 metaphase cells observed (Fig. 1a). There were no signals on other chromosomes, especially on chromosomes 5 at which the locus for processed pseudogene of connexin43 exists (Fishman *et al.*, 1991). Comparison of PI-stained chromosomes 6 with G-bands on the same metaphase cells revealed that the signals were consistently located to 6q22.3 (Fig. 1b). Thus, we successfully assigned the locus of expressed connexin43 gene, *GJA1*, at 6q22.3.

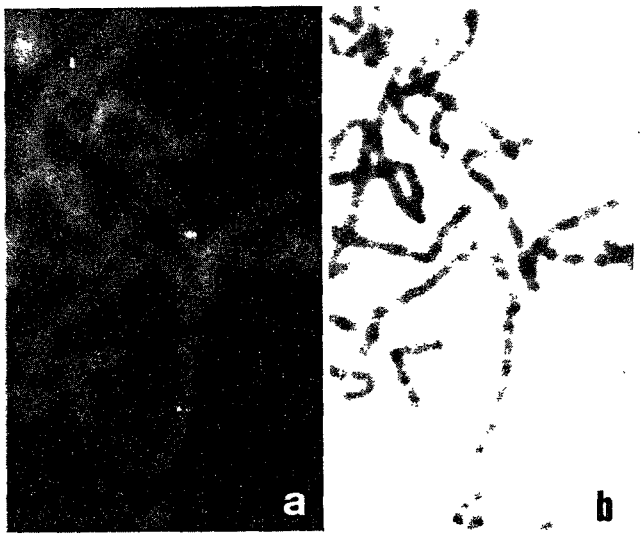

Fig. 1. FISH on human prometaphase chromosomes using a BAC clone containing the human connexin43 gene (*GJA1*) as a probe. FITC-signals appear on PI-stained chromosomes (a) and location of the signals corresponds to 6q22.3 on G-banded chromosomes (b).

The expressed *GJA1* has been mapped to chromosome 6 (Willecke *et al.*, 1990; Fishman *et al.*, 1991; Hsieh *et al.*, 1991). By a further study with a human/rodent somatic cell hybrid panel, *GJA1* has been sublocalized to band 6q21-q23.2 (Corcos *et al.*, 1993). Our data narrowed the *GJA1* localization to 6q22.3 and provide useful information for the further study of connexin43.

Contribution of connexin43 to the determination of left-right asymmetry was recently discussed. Britz-Cunningham *et al.* (1995) reported single-base substitutions in the cytoplasmic tail of *GJA1* in 6 patients with complex heart malformations and laterality defects. However, similar studies by two other groups were unsuccessful to identify any mutations in a total of 40 patients with laterality defects and/or cardiac anomalies (one patient without it) (Casey and Ballabio, 1995; Penman Splitt *et al.*, 1995). We recently reported a patient with heterotaxia associated with a *de novo* balanced translocation (6;18)(q21 or q22;q21.3 or q22) (Kato *et al.*, 1996). The breakpoint of the translocated chromosome 6 was near to the *GJA1* localization, although they seem different at the chromosome band level. It remains to be seen that *GJA1* is disrupted in the heterotaxia patient.

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