SHORT COMMUNICATION

Refined Localization of Human Connexin32 Gene Locus, GJB1, to Xq13.1

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Received October 10, 1991

Connexins are the peptide subunits of gap junctions that interconnect cells to allow the direct, intercellular transfer of small molecules. Recently, the human connexin32 gene (locus designation GJB1) has been regionally mapped by three other laboratories to Xp11–q13, Xcen–q22, and Xp11–q22. The smallest region of overlap from these studies is Xcen–q13. By using a series of somatic cell hybrid mapping panels and a rat connexin32 cDNA probe, we have localized the human GJB1 locus to a much smaller region in proximal Xq13.1, in interval 8, as described by Lafrenière *et al.* (8).

Connexins are the peptide subunits of the channel-forming gap junction. These junctions interconnect cells to allow the direct, intercellular transfer of small molecules. Because gap junctional communication has been hypothesized to play an important role in various processes such as cell growth regulation (12) and development (1), there has been recent interest in mapping various connexin genes in both human and mouse. In particular, the human connexin32 (locus designation GJB1) has been regionally mapped by three other laboratories to Xp11–q13 (14), Xcen–q22 (6), and Xp11–q22 (3). By using a series of somatic cell hybrid mapping panels and a rat connexin32 cDNA probe (10), we have refined the localization of the human GJB1 gene to a much smaller region, the proximal region of Xq13.1.

We mapped the human GJB1 gene in three steps. First, we determined the chromosomal assignment of GJB1 by performing Southern analysis of DNA from the NIGMS Human/Rodent Somatic Cell Hybrid Mapping Panel 1 (NIGMS Human Genetic Mutant Cell Repository, Camden, NJ). Hybond N^+ filters (Amersham, Arlington Heights, IL) containing 5 μg each of *Eco*RI-digested DNA from each cell line were hybridized with 25 ng of random-primed ^{32}P-labeled rat connexin32 probe. Our results showed that GJB1 mapped to the human X chromosome (data not shown).

To regionally sublocalize the locus on the X chromosome, an X-chromosome mapping panel was assembled. The probe was hybridized to filters containing 5 μg each of *Eco*RI-digested DNA from each of the human × rodent X chromosome hybrid cell lines. Results of Southern blot hybridization of the rat connexin32 probe to the X chromosome mapping panel are shown in Table 1. A human-specific 5.0-kb band was detected in total human DNA and hybrids GM10063, HPP1, GM10501, B13.3, A481Fa, GM09191, and FUdR1-6, indicated by a +. Hybrids indicated with a −, t(X;3), HC10, and X3000, showed only rodent-specific bands. Our results showed that GJB1 mapped to band Xq13 of the X chromosome between the breakpoints of hybrid GM09191 at Xq13 and HC10 at Xq13.3. These results mapped the locus more finely than previous reports and allowed us to define further the location of GJB1 within Xq13, proximal to the Menkes disease locus.

Next, we used a recently described X-chromosome mapping panel designed to map finely the region Xp21.1–q21.3. Details of the construction, cytogenetics, and preparation of this panel have been described elsewhere (8). Briefly, this panel consists of human × mouse somatic cell hybrids containing fragments of the human X chromosome with breakpoints between Xp21.1 and q21.3. Approximately 10 μg of *Hin*dIII-digested genomic DNA was Southern blotted and probed with the rat connexin32 probe, revealing an approximately 9-kb human-specific band, clearly separated from the approximately 30-kb mouse-specific band (Fig. 1A). The human-specific band was present in hybrids A63-1A, t11PP-5A,

TABLE 1

Sublocalization of GJB1 on the X Chromosome

Hybrid	Portion of X present	References	GJB1
t(X;3)	Xp22.1–Xpter	(2)	−
GM10063	Xp21.2–Xqter	[a]	+
HPP1	Xp11.21–Xqter	Glover, unpublished results	+
GM10501	Xp11–Xqter	[a]	+
B13.3	Xp11.1–Xqter	(5)	+
A481Fa	Xcen–Xqter	(8)	+
GM09191	Xq13–Xqter	[a]	+
HC10	Xq13.3–Xqter	(13)	−
X3000	Xq24–Xqter	(9)	−
FUdR1-6	Xpter–Xq27	(4)	+

[a] NIGMS Human Genetic Mutant Cell Repository, Camden, NJ.

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GENOMICS 13, 479–480 (1992)
0888-7543/92 $5.00

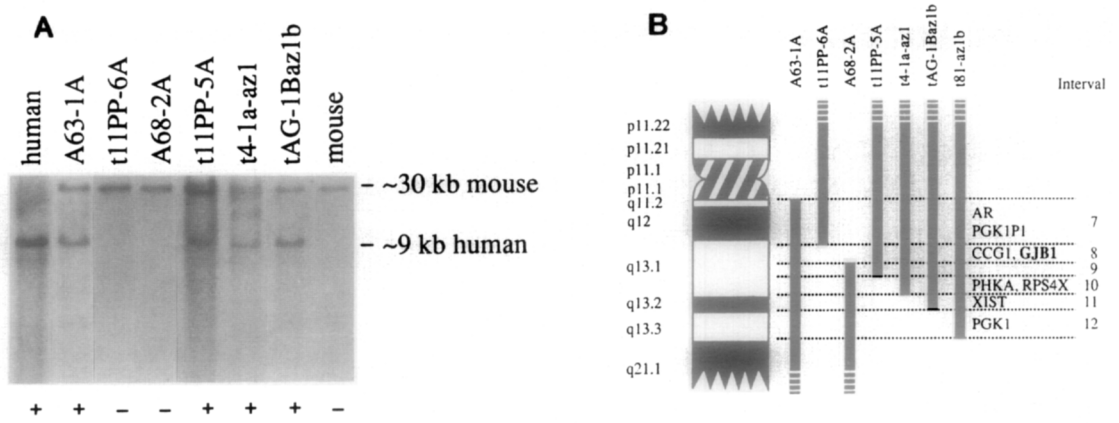

FIG. 1. (**A**) Southern blot analysis of human × mouse somatic cell hybrids using the rat connexin32 cDNA probe. Genomic DNA was digested with *Hin*dIII, electrophoresed on a 1% agarose gel, and blotted overnight onto Hybond-N$^+$ (Amersham, Arlington Heights, IL) nylon membrane. Sizes for the mouse and human bands are specified to the right of the autoradiogram. Presence (+) or absence (−) of a human-specific signal is indicated at the bottom of each lane. (**B**) Schematic map of the cloned genes localized to the human X proximal long arm. The hybrid t81-az1b, containing the PGK1 locus, is shown for reference. Human X chromosomal content of hybrids used in the panel is shown as dark bars. Intervals are numbered according to those defined by Lafrenière *et al.* (8).

t4-1a-az1, tAG-1Baz1b, and t81-az1b, but absent from t11PP-6A and A68-2A. Hybrid t81-az1b, not shown in Fig. 1A, was positive for the presence of GJB1 in a separate experiment; the results are included in Fig. 1B for reference. We localized GJB1 to the proximal side of band Xq13.1, in interval 8, as defined by Lafrenière *et al.* (8) (see Fig. 1B).

In the context of other cloned X-linked proximal long arm genes and pseudogenes, GJB1 maps in the same physical interval as a gene that complements a hamster cell cycle mutation (CCG1), distal to the androgen receptor (AR) gene and a phosphoglycerate kinase pseudogene (PGK1P1), and proximal to the phosphorylase kinase A (PHKA), X-linked ribosomal protein S4 (RPS4X), inactive X-specific transcripts (XIST), and phosphoglycerate kinase (PGK1) genes. GJB1 was previously assigned to Xp11–q22 (3), Xp11–q13 (14), and Xcen–q22 (6). The smallest region of overlap from these studies is Xcen–q13. Our results are in agreement with these data and allow sublocalization of GJB1 to a very narrow region in proximal Xq13.1.

ACKNOWLEDGMENTS

We thank Dr. C. K. Stein for her advice and Dr. J. L. Gorski for hybrid B13.3. This work was supported by research grants from the Office of the Vice President for Research, University of Michigan and NIH ES04424 to R.L-C., the Molecular Biology Core of the Center for the Study of Reproduction (NIH HD18258), and NIH HG00013 to H.F.W.

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