
Brief Communication

Chromosomal Localization of 15 Ion Channel Genes

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Received 23 September 1996—Final 23 September 1996

Abstract—Several human Mendelian diseases, including the long-QT syndrome, malignant hyperthermia, and episodic ataxia/myokymia syndrome, have recently been demonstrated to be due to mutations in ion channel genes. Systematic mapping of ion channel genes may therefore reveal candidates for other heritable disorders. In this study, the GenBank and dbEST databases were used to identify members of several ion channel families (voltage-gated calcium and sodium, cardiac chloride, and all classes of potassium channels). Genes and ESTs without prior map localization were identified based on GDB and OWL database information and 15 genes and ESTs were selected for mapping. Of these 15, only the serotonin receptor 5HT3R had been previously mapped to a chromosome. A somatic cell hybrid panel (SCH) was screened with an STS from each gene and, if necessary, the results verified by a second SCH panel. For three ESTs, rodent derived PCR products of the same size as the human STS precluded SCH mapping. For these three, human P1 clones were isolated and the genomic location was determined by metaphase FISH. These genes and ESTs can now be further evaluated as candidate genes for inherited cardiac, neuromuscular, and psychiatric disorders mapped to these chromosomes. Furthermore, the ESTs developed in this study can be used to isolate genomic clones, enabling the determination of each transcript's genomic structure and physical map location. This approach may also be applicable to other gene families and may aid in the identification of candidate genes for groups of related heritable disorders.

INTRODUCTION

Ion channels are integral components of intra- and intercellular signaling, electrolyte homeostasis, and osmotic regulation. Recently, several heritable human diseases, including the long-QT syndrome (1-3), malignant hyperthermia (4), and episodic ataxia/myokymia syndrome (5) have been determined to be a consequence of ion channel defects. In each case, the disease gene was initially determined to be a candidate based upon its physical proximity to the disease locus as mapped by

linkage analysis [the "positional candidate" approach, (6)]. A vast number of human ion channels have been identified using a variety of different strategies: functional cloning, random sequencing of cDNAs, and identification of the human homologues and relatives of known *Drosophila* (7), rodent (8), and human genes (9). Due to their potential role in human diseases, a systematic mapping of these ion channels and their related expressed sequence tags (ESTs) may be beneficial to positional cloning projects involving inherited cardiovascular, neuromuscular, or psychiatric disorders.

In this study we sought to localize members of several ion channel families using the following algorithm. Previously unmapped members of several ion channel families—voltage-gated sodium and calcium channels, cardiac chloride channels, and several potassium channel families—were identified. Sequence from a representative member of each family [voltage-gated sodium (embl:M91556); voltage-gated calcium (L06110, embl:M59786); chloride (P35526); potassium (embl:M26685, JU0271, P25122, embl:M55515, embl:M61157, embl:X16476, A38074, U12507) channels] was used to search the non-redundant GenBank database [BLAST programs, (10)]. All members of each gene family represented in the public databases were identified, including previously-cloned genes and ESTs. GenBank accession numbers for each gene or EST were used to retrieve mapping information from the GDB (11) and OWL (12) databases. All highly homologous genes and ESTs which had not been mapped as of June 1, 1995 were identified for further evaluation. Each of the 20 previously-unmapped entries was then used to search the non-redundant GenBank database. Five of the ESTs were removed from further consideration due to nearly identical nucleotide sequence matches (except for single base pair insertions or deletions in the coding sequence) with other genes or ESTs. These five ESTs were assumed to be identical to the previously-mapped genes that they matched.

METHODS

Chromosomal Localization. The somatic cell hybrid panel DNA was purchased from Coriell Institute (Camden, New Jersey, NIGMS monochromosomal panel) or Bios Laboratories Inc. (New Haven, Connecticut, polychromosomal panel). P1 DNA was prepared using the Qiagen Maxi prep kit (Qiagen Inc., Chatsworth, California). Human genomic P1 genomic library clones (DMPC-HFF #1; 13) were isolated by PCR screening. The chromosomal location of these P1s were determined by FISH using

standard methods (14). DAPI was used as a DNA counterstain. Images were acquired using IPLab Spectrum (Signal Analytics Corporation, Virginia).

Database Searching, and STS Generation. Sequences for ion channel related genes were extracted from GenBank by serial BLAST searches (using the default parameters) through June 1995. STS primer sequences are shown in Table 1. PCR reactions were performed under standard conditions in a Perkin-Elmer (Branchburg, New Jersey) 9600 thermal cycler. After 4 minutes of denaturation at 94°C, thirty-five cycles were performed as follows: 1 minute at 94°C, 45 seconds at annealing temperature, 2 minutes at 72°C. The products were resolved on a 3% agarose/2% Nusieve agarose (FMC Products, Rockland, Maine).

RESULTS

Primers to amplify sequence tagged sites (STSs) were designed from the 3' end of the available sequence for each of the remaining 15 genes and used to screen a monochromosomal somatic cell hybrid (SCH) panel (Fig. 1). For those genes that could not be unambiguously mapped using this panel, a second, polychromosomal SCH panel (Bios Laboratories Inc., New Haven, Connecticut) was screened to verify the results. Nine genes were mapped using only the monochromosomal panel (SCH), and three mapped using both panels [SCH ($\times 2$)] (Table 1). Three genes could not be mapped using SCH panels due to the presence of an identically-sized PCR product from mouse or hamster DNA. For these three genes, a human genomic P1 clone was isolated by PCR screening. The chromosomal location of these P1s were determined by FISH. Each P1 gave an unambiguous signal (Fig. 2).

Since, prior to this study, two loci for the inherited cardiac repolarization defect, long-QT syndrome, had been determined to be secondary to ion channel defects (1, 2) and given our interest in the long QT syndrome locus that maps to 11p15.5 (LQT1) (15, 16), the two ion

Gene family	Accession number	Primer sequence	Annealing Temperature	Amplified product size	Mapping method	Chromosomal location
Potassium channels						
<i>hSlo</i> (Ca-dep K ⁺)	U13913	5'-JTTCCTGTGTTTCCCTGTAGTCC 5'-GGTCCGTCTGCTTATTTGCTGTG	53	136	SCH	10
K ⁺ channel	L02840	5'-CAGCGCAAACCCCTACTCAAATG 5'-TCCTCCCTCCAACCCATAAACAATG	60	201	SCH	20
C-ATP (K ⁺ channel)	X83582	5'-GGGGCCACCGATTCACACCACT 5'-AGCCGGCCCTTCCCTCTTCATTCT	64	142	FISH	11q25
HH-IRK	U12507	5'-CGAAGTCCCAACACTCCCTT 5'-GGCGTGTCCGTACTAGTGCITTTCT	62	165	SCH	17
HERG-like K ⁺ channel	F02206	5'-GTGATTCGGGCTTTTATTCCATT 5'-TGCTGCGAGTAAAAGAGITTCATT	54	194	SCH	2
ROM-K ⁺	U12542	5'-GTAACAGTGCCCTTTTCAACAGGAGTA 5'-GCGGGGCTCAGGGGTCTT	56	146	SCH (+J1)	11q21-25
KChC (K ⁺ channel)	L02752	5'-ACCATTTGGGGGAAAGATAGTG 5'-CTCCCGGTGGTGAAGAAGTGTG	53	111	SCH	1
cardiac beta subunit	L39833	5'-TGCTGCTGTCGCTACCACCTGT 5'-GCCCCATATTTCTCCCAATTG	53	142	SCH	3
Chloride channels						
CL ⁻ channel	T17495	5'-CTTCTGCTGCCCGACGCAA 5'-GCCCCATCACTGCCAGCCTTC	64	77	SCH (X2)	17
CL ⁻ channel (P64 homologue)	T11650	5'-AGCTCGTCGCCATCCAAAACT 5'-CCCCCTCCAGAGAAGTGTG	60	82	SCH (X2)	6
CL ⁻ channel	T92523	5'-CCCTCTGCTGATGAAATAGATGC 5'-CTCGTCCCCACCCAGAAACTT	58	79	FISH	21q22.1
CL ⁻ channel	T24611	5'-CATCCCCCTCCAGAGAAGTGTG 5'-GGGTGAGCTCGTTGCCATCCA	64	89	FISH	6p21.3
CL ⁻ channel regulatory protein	F00895	5'-GAAGATGGGATGGAGGTGGATA 5'-CTGGTTCAGGTTGGGTTTTGG	58	193	SCH	6
Ligand-gated channel						
5HT ₃ R (serotonin receptor)	D49394	5'-ACTGCAGCCACATGGGAGGACC 5'-CATTGGAAACAGGGCACGGGACTT	62	427	SCH (+J1)	11q21-25
Putative channel						
MYASTH A ₂ B	S60415	5'-GCACCGAGTGACTGAATGGTTGAGA 5'-ATACAGAGCGAGGTGGGCAGAATG	58	98	SCH (X2)	10

channel genes that mapped to chromosome 11 were further localized using somatic cell hybrids that were derived from the J1 series (17) and contain subfragments of human chromosome 11. One of these genes, *5HT₃R*, a serotonin receptor that functions as a ligand-gated cation channel, had previously been mapped to chromosome 11 (18) but had not been further localized. Both *5HT₃R* and the *ROM-K* potassium channel were localized to 11q21-25 using this panel. During the course of this work the gene responsible for chromosome 11-linked long QT syndrome (*LQT1*) was identified by positional cloning (3). This novel potassium gene, *KVLQT1*, was not represented in the dbEST database.

Two genes in this study map to chromosomes known to contain a related transcript. Recently, *hSlo*, a novel relative of the slowpoke calcium-dependent potassium channel, was cloned and characterized (19). In this study, we mapped *hSlo* to chromosome 10.

The slowpoke gene had previously been mapped to chromosome 10 (20), and shares nucleotide identity with *hSlo* over 70% of the coding sequence. However, the 5' coding sequence and the 3' untranslated sequence (from which the STSs were generated) are markedly different between the two genes. Therefore, they could represent two different loci on chromosome 10, however, we can not rule out that they do not share multiple exons in common. This mapping information, combined with the extensive differences in the translated portion of the mouse *mSlo* gene (21), suggest that *hSlo* and slowpoke could also be alternatively-spliced forms of the same gene.

Secondly, two ESTs with homology to the same sequence of the bovine P64 chloride channel mapped to chromosome 6, and shared a nucleotide sequence identity of approximately 90%. However, the nucleotide mismatches in the putative coding sequence were randomly

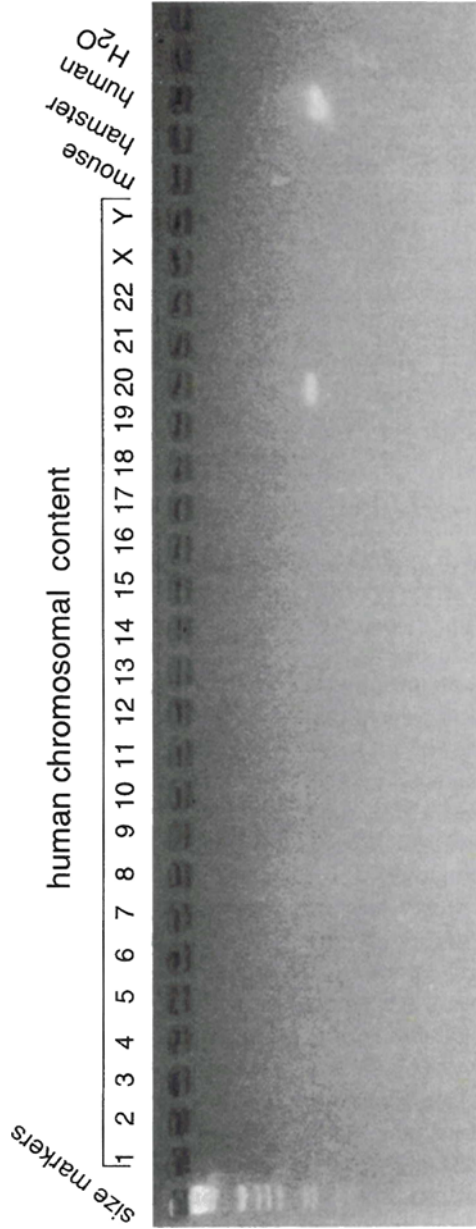


Fig. 1. SCH mapping of the potassium channel gene, L02840. PCR assay of the monochromosomal somatic cell hybrid panel, ethidium bromide stained agarose gel. First lane: 1 kb ladder, the human chromosome complement of the hybrid DNA used as the template is shown above each subsequent lane. The remaining lanes contain mouse, hamster and human genomic DNA and water respectively. Note the band corresponding to the monochromosome 20 SCH.

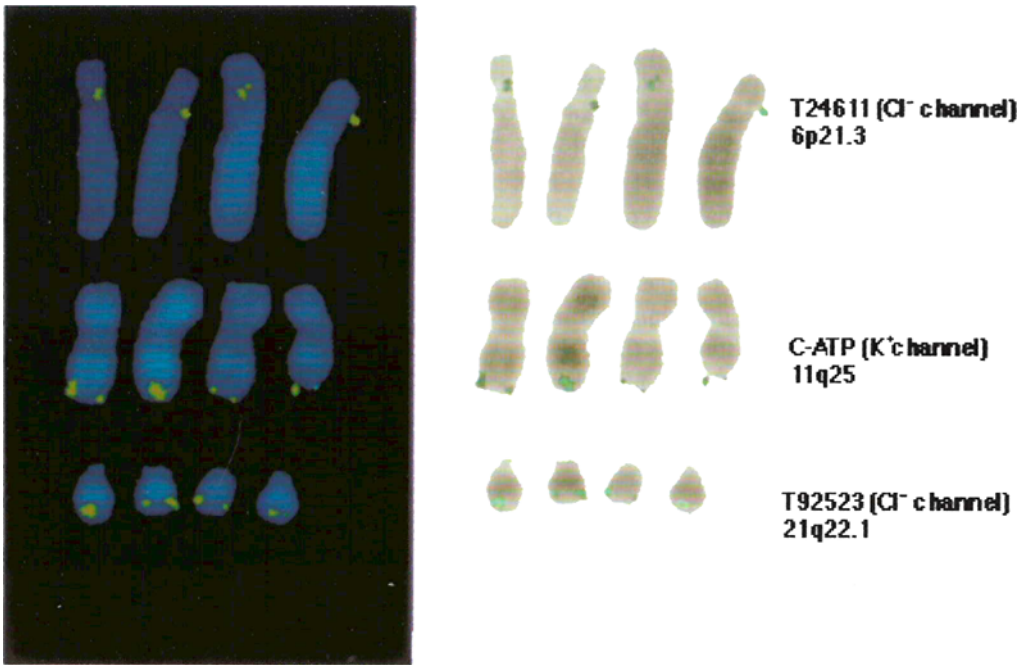


Fig. 2. FISH of the c-ATP potassium channel, and two putative chloride channels. Results from metaphase chromosome spreads from a human lymphocyte cell line. Left panel. DAPI stained chromosomes, probe signal is shown in green (FITC). Right panel, hybridization signals are superimposed on video inverted DAPI image to facilitate band identification. Top, FISH analysis using a P1 (70F9) probe for a chloride channel homologue gene (T24611) demonstrates a single hybridization signal on the short arm of chromosome 6. Middle, hybridization with P1 (57F7) containing the C-ATP (K^+ channel) gene at the end of the long arm of chromosome 11. Bottom, P1 (6A11) probe containing a putative chloride channel (T92523) localized to chromosome 21q22.1.

distributed rather than primarily third base pair changes and often were single base pair insertions or deletions. Therefore, these two ESTs may represent 1) a single gene with sequencing errors of the ESTs, 2) a gene and an expressed processed pseudogene, or 3) two different genes.

Since the completion of this study, the chromosomal localization of four of these genes has been independently confirmed by either the Cooperative Human Linkage Center (CHLC) (U13913, S60415), the Whitehead Institute (L02840) or the Stanford (U12542) EST mapping projects. For the potassium channel L02840, our somatic cell hybrid mapping result strengthens its localization on a chromosome 20 YAC contig (GenBank) which was based on STS content mapping of two chimeric YACs.

DISCUSSION

With the rapid development of a "transcript map" of the human genome, the chromosomal and eventually subchromosomal localization of nearly all publicly available ESTs may be performed by the transcript mapping projects at the Whitehead Institute, Stanford and other centers. In the near future, it may be possible to perform candidate cloning of human disease genes using a fully-developed transcript map to identify all the candidate genes in the region of interest as defined by linkage analysis. However, directed efforts to map gene families, as was performed in this study, may currently still be beneficial in mapping and identifying candidate genes for certain types of human diseases.

In conjunction with linkage studies, the data presented here will now allow these 15

genes to be considered as candidates for human diseases that have been mapped to specific chromosomes. For example, one of the human diseases that is likely secondary to an ion channel dysfunction, rippling muscle disease (*RMD1*), maps to chromosome 1q41 (22). This disorder is characterized by muscle cramps, pain and stiffness, particularly following exercise and is secondary to electrically silent muscle contractions that are elicited by mechanical stimuli. One of the genes in this study, *KCHC*, is an inward rectifying potassium channel that is expressed in nervous tissue and maps to chromosome 1. *KCHC* has three potential calcium-calmodulin-dependant (CaM II) protein kinase phosphorylation sites (7) which might make it responsive to mechanical stimuli.

Two of the other mapped genes (the HH-IRK potassium channel and a putative chloride channel) map to chromosome 17, which contains a locus for malignant hyperthermia (*MHS2*; 23). Other diseases which may be secondary to ion channel defects and map to one of these chromosomes include juvenile myoclonic epilepsy I (6p; 24) and an EEG variant disorder (*EEGVI*; 25) on chromosome 20. These mapped genes can now be evaluated as candidates for other neurologic, cardiovascular, neuromuscular, or psychiatric disorders. Furthermore, they may be considered as candidates for as yet unmapped disorders or for disorders with genetic heterogeneity where not all of the loci have been identified, such as the long-QT syndrome and RMD. The STSs described in this paper can be used to place these genes on the physical maps for that chromosome and aid in their evaluation as candidates for human diseases.

NOTE ADDED IN PROOF

The following genes have now been mapped by radiation hybrid analysis: F02206, V12542, L39833, D49394 and S60415. Please see *Science* **274**:540–546.

ACKNOWLEDGMENTS

We would like to thank Christiano Cummings for help with the isolation of P1 clones. This work was supported by a National Research Service Award (M.W.W.R.).

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