SHORT PAPER

Chloride channel 2 gene (Clc2) maps to chromosome 16 of the mouse, extending a region of conserved synteny with human chromosome 3q

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Summary

The Clc2 gene of the mouse codes for the ubiquitously expressed chloride channel ClC-2, a member of a family of at least seven voltage gated chloride channels, some of which are implicated in hereditary diseases. Using a mouse interspecies back-cross panel, we have mapped Clc2 to Chr 16, proximal to the somatostatin gene Smst, extending a region of documented conserved synteny to human Chr 3q.

1. Introduction

Based on the expression cloning of the voltage gated chloride channel CIC-0 from the electric organ of Torpedo (Jentsch et al. 1990), a new family of chloride channels CIC-n has been discovered (review: Jentsch, 1994). The first member of this family to be characterized in mammals was CIC-1, a chloride channel specific for mature skeletal muscle (Steinmeyer et al. 1991b). Subsequently, six more members of this family, the ubiquitously expressed ClC-2 (Thiemann et al. 1992), CIC-3 (Kawasaki et al. 1994), CIC-4 (van Slegtenhorst et al. 1994) and three kidney specific channels (Fisher et al. 1994; Kieferle et al. 1994) have been described. The chromosomal mapping to the same locus on Chr 6 of the murine disease gene adr (Mehrke et al. 1988; Jockusch, 1990a,b) and the structural gene defined by the CIC-1 cDNA probe (Steinmeyer et al. 1991a) has been instrumental in elucidating the molecular cause of the muscle disease myotonia. Furthermore, the localization of the corresponding human (Thomsen/Becker) myotonia gene (Abdalla et al. 1992; Koch et al. 1992) has been predicted on the basis of a stretch of conserved synteny between mouse Chr 6 and human Chr 7 (Jockusch, 1990b).

So far, only for CIC-1 and CIC-5, are clinically relevant mutations known. The chloride channel ClC-2 is thought to play an important role in the regulation of osmolarity and volume control in a great variety of cells (Thiemann et al. 1992). In order to elucidate possible relationships of the Clc2 gene to hereditary abnormalities, we have chromosomally mapped the Clc2 gene and found it to be localized on Chr 16 of the mouse, extending a region of conserved synteny with human Chr 3q.

2. Materials and methods

Segregation studies were based on an interspecific mouse backcross (C57BL/6J wr/+ x SEG/1 +/+ ) F2R progeny. A total of 129 F2R mice were used to map the Clc2 locus. Mus musculus C57BL/6J (B) and Mus spretus SEG/1 (S) DNAs were digested with several restriction enzymes and analysed by Southern blot hybridization for informative restriction fragment length variants (RFLVs), using a 28 Kb Xho I/Xba I fragment of rat ClC-2 cDNA (Thiemann et al. 1992) as a probe. Southern blots, probe labelling and hybridization were done by standard procedures (Feinberg & Vogelstein, 1983; Sambrook et al. 1989). Hind III digested DNAs yielded fragments of 6-6 (B) and 8-0 (S), respectively and three nonpolymorphic fragments of 2-4, 1-6 and 11 Kb (Fig. 1). The segregation of the 8-0 Kb M. spretus fragment was scored in the F2R backcross progeny. Segregation

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Kb

8.0 ~
6.6 ~
2.4 ~
1.6 ~
1.1 ~

Fig. 1. Clc2 restriction fragment variants used for segregation analysis. Southern hybridization of Hind III digested DNAs from Mus musculus C57BL/6J (C), Mus spretus (S) and F1 = C57BL/6J x SEG/1 (F) with the Clc2 probe. Arrow: SEG/1 fragment that was scored for segregation analysis. lengths of the restriction fragments (in Kb) were estimated on the basis of a lambda DNA Hind III fragment ladder.

Fig. 2. Mapping of the Clc2 gene. (a) Haplotypes for Clc2 and flanking markers. Each column represents two haplotypes of Chr 16 with the number of BC individuals given beneath (upper number: filled squares/rectangles, SEG/1 allele; empty squares/rectangles, C57BL/6J allele; lower number, the reverse). (b) Position of the Clc2 locus on mouse Chr 16 as derived from data shown in (a); distances from the centromere (as based on the distance of D16Mit87 from the centromere, Ref.: Dietrich et al. 1994) in cM on the left. (c) Homologous human chromosome segment with localization of CLCN2 on terminal 3q. * Linkage data taken in part (Igll) or wholly from the literature (Pearson et al. 1994; Lyon & Kirby, 1995; MIT databank, 1995). Gene symbols: Igll/IGL1, immunoglobulin lambda chain 1; Clc2/CLCN2, chloride channel 2; Smst/SST, somatostatin; Apod/APOD, apolipoprotein D; Pitl/PIT1, pituitary hormone.
separated on 2% Tris-borate/EDTA (TBE-)agarose gels (Life Technologies Inc., Eggenstein, FRG) and on a non-denaturing 12% polyacrylamide gel for the D16Mit87 products and documented after staining with ethidium bromide.

The Clc2 gene was found to be localized between D16Mit87 and D16Mit102 with lod scores > 25, tightly linked to Smst (with one recombinant in 129 tested individuals). The most likely gene order and distances were Cen-D16Mit87 - 3-9 ± 1-7 cM - Clc2 - 1-6 ± 1-0 cM - Smst, D16Mit102 - 20-9 ± 3-6 cM - D16Mit63 (Fig. 2a, b). These results are in good agreement with published data for mouse Chr 16 (Dietrich et al. 1994; Lyon & Kirby, 1995). Clc2 was thereby localized in close proximal neighbourhood of a 24 cM stretch of mouse Chr 16, extending from Smst to Pitl, with well-documented homology to human Chr 3q.

4. Discussion

Recently, human CLCN2 has been localized on Chr 3q26-qter (Cid et al. 1995). Thus, the homology of mouse Chr 16 to human Chr 3q extends proximally by at least 2 cM and comprises the terminal third of the long arm of human Chr 3 (Fig. 2c).

Available data on mouse Chr 16 (Lyon & Kirby, 1995) do not indicate hereditary diseases that might be connected to CIC-2’s presumed function. The role of the Clc1 gene had been elucidated in a straightforward manner (Steinmeyer et al. 1991a) because a functional loss of the gene product, CIC-1, is lethal and affects specifically mature skeletal muscle. Furthermore, electrophysiological analysis of the resulting disease, myotonia, had already pointed to a muscular chloride channel as a candidate gene, long before the relevant gene had been cloned (cf. Mehrke et al. 1988; Jockusch, 1990b). In contrast, as a result of the ubiquitous expression of CIC-2, possible disease symptoms are not easy to predict, and functional loss of this ion channel may lead to embryonic lethality.

In that case, only mild or conditional alleles, causing altered regulation or stability of the channel, or a disease based on partial dominant negative effects, would be expected.

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