Genet. Res., Camb. (1995), **66**, *pp.* 175–178 *With 2 text-figures*

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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(*Received 9 June 1995 and in revised form 30 June 1995*)

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 ClC-0 from the electric organ of *Torpedo* (Jentsch *et al.* 1990), a new family of chloride channels ClC-n has been discovered (review: Jentsch, 1994). The first member of this family to be characterized in mammals was ClC-1, a chloride channel specific for mature skeletal muscle (Steinmeyer *et al.* 1991*b*). Subsequently, six more members of this family, the ubiquitously expressed ClC-2 (Thiemann *et al.* 1992), CIC−3 (Kawasaki *et al.* 1994), ClC-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 ClC-1 cDNA probe (Steinmeyer *et al.* 1991*a*) 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, 1990*b*).

So far, only for ClC-1 and ClC-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*/+ × SEG/1 +/+) F_1 *wr*/+ × C57BL/6J *wr*/+), originally set up to map the *wobbler* gene (Kaupmann *et al.* 1992). This interspecific backcross panel has been typed for over 150 loci distributed over all autosomes and the X chromosome. A total of 129 F_2R 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 2·8 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). *Hin*d III digested DNAs yielded fragments of 6·6 (B) and 8·0 (S), respectively and three nonpolymorphic fragments of 2·4, 1·6 and 1·1 Kb (Fig. 1). The segregation of the 8·0 Kb *M. spretus* fragment was scored in the F_2R backcross progeny. Segregation

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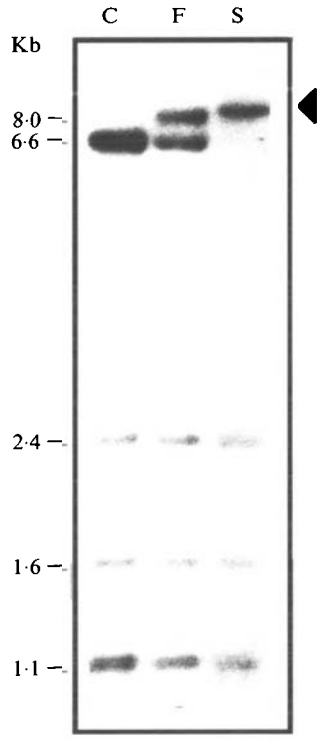

Fig. 1. *Clc2* restriction fragment variants used for segregation analysis. Southern hybridization of *Hind* III digested DNAs from *Mus musculus* C57BL/6J (C), *Mus spretus* (S) and F_1 = C57BL/6J × SEG/1 (F) with the CIC-2 probe. Arrow: SEG/1 fragment that was scored for segregation analysis. lengths of the restriction fragments (in Kb) were estimated on the basis of a lambda DNA *Hind* III fragment ladder.

data were processed as reported by Kaupmann and coworkers (1992).

3. Results

In a preliminary screen, linkage of *Clc2* to *Igl1* on Chr 16 was found. To verify *Clc2* localization on Chr 16, we used *Smst*, the gene for somatostatin, and three informative microsatellites, *D16Mit87*, *D16Mit102*, and *D16Mit63*, as reference markers. The probe for *Smst* was a 600 bp *Hind* III/*Pvu* II human genomic fragment including exon 2 of the gene (Shen & Rutter, 1984) that detected *Xba*I fragments of 3·2 (B) and 2·3 (S) Kb (from ATCC, Rockville, MD, USA). Nucleotide sequences for the microsatellite primers were obtained from the MIT mouse genome database, and primers were purchased from TIB Molbiol (Berlin, FRG). PCR was performed with 100 ng genomic DNA, 50 pM of each primer, 200 μM each dNTP and 1 unit *Taq* polymerase with 1 × buffer as recommended by the manufacturer (Promega Corporation, Madison, WI, USA). Amplifications were carried out with an initial denaturation step at 94 °C for 4 min, followed by 35 cycles of denaturation (90 °C for 1 min), annealing (56 °C, 1 min for the *D16Mit102*, 60 °C, 1 min for *D16Mit87* and *D16Mit63* locus) and extension (72 °C for 1 min 30 s) in a TRIO-Thermoblock (Biometra, Göttingen, FRG). PCR products from the *D16Mit102* and *D16Mit63* locus were

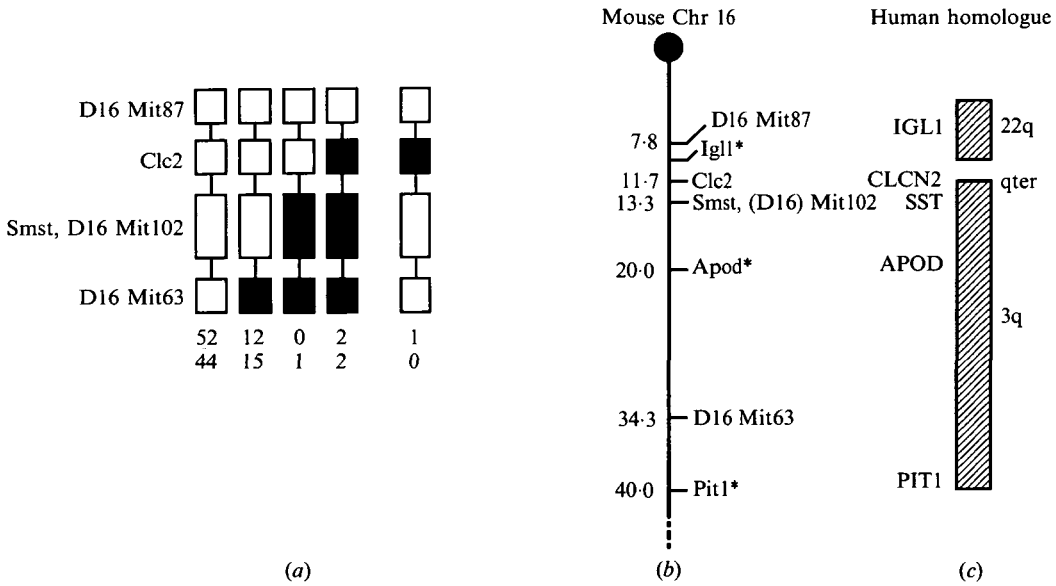

Fig. 2. Mapping of the *Clc2* gene. (*a*) Haplotypes for *Clc2* and flanking markers. Each column represents two haplotypes of Chr 16 with the number of BC individuals given beneath (upper number: filled squares/rectangles, SEG/1 allele; empty squares/rectangles, C57BL/6J allele; lower number, the reverse). (*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 (*Igl1*) or wholly from the literature (Pearson *et al.* 1994; Lyon & Kirby, 1995; MIT databank, 1995). Gene symbols: *Igl1*/*IGL1*, immunoglobulin lambda chain 1; *Clc2*/*CLCN2*, chloride channel 2; *Smst*/*SST*, somatostatin; *Apod*/*APOD*, apolipoprotein D; *Pit1*/*PIT1*, pituitary hormone.

https://doi.org/10.1017/S0016672300034522 Published online by Cambridge University Press

separated on 2% Tris-borate/EDTA (TBE-)agarose gels (Life Technologies Inc., Eggenstein, FRG) and on a nondenaturing 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*$-3{\cdot}9 \pm 1{\cdot}7$ cM$-$*Clc2*$-1{\cdot}6 \pm 1{\cdot}0$ cM$-$*Smst*, *D16Mit102*$-20{\cdot}9 \pm 3{\cdot}6$ cM$-$*D16Mit63* (Fig. 2*a*, *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 *Pit1*, 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. 2*c*).

Available data on mouse Chr 16 (Lyon & Kirby, 1995) do not indicate heredity diseases that might be connected to ClC-2's presumed function. The role of the *Clc1* gene had been elucidated in a straightforward manner (Steinmeyer *et al.* 1991*a*) because a functional loss of the gene product, ClC-1, is not 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, 1990*b*). In contrast, as a result of the ubiquitous expression of ClC-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.

We thank R. Klocke for typing the manuscript. Supported by the Deutsche Forschungsgemeinschaft, SFB 223 (Bielefeld).

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https://doi.org/10.1017/S0016672300034522 Published online by Cambridge University Press