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Evidence for genetic homogeneity in autosomal recessive generalised myotonia (Becker)

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Abstract

Generalised myotonia Becker (GM) is an autosomal recessively inherited muscle disorder. Affected subjects exhibit myotonic muscle stiffness in all skeletal muscles with marked hypertrophy in the legs. A transient muscle weakness is particularly pronounced in the arms and hands and is a typical symptom of the disorder. Recently, we showed complete linkage of the disorder GM to the gene (CLCN1) coding for the skeletal muscle chloride channel CLC-1 and the TCRB gene on chromosome 7 in German families. In the study presented here we performed linkage analysis on 14 new GM families. The GM locus was again completely linked to both the CLCN1 and the TCRB gene in all families with a combined lod score of $Z = 9{\cdot}26$ at a recombination fraction of $\theta = 0{\cdot}00$. This confirms our previous data and supports the hypothesis that GM is a genetically homogeneous disorder. The previously detected T to G missense mutation is found on 15% of the 66 GM chromosomes counted so far.

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Following the description by Thomsen[1] of an autosomal dominant myotonic disorder in 1876, subsequent reports classified all patients with non-dystrophic human myotonia as having Thomsen disease. In 1948 Thomasen[2] discussed the possibility of a non-dystrophic myotonic disorder inherited in an autosomal recessive manner. It was Becker, however, who, using pedigree analysis in 104 German families, showed convincingly that inheritance of a human myotonia was compatible with autosomal recessive transmission.[3 4] Becker named the disorder generalised myotonia (MIM 255700) to distinguish it from the well recognised autosomal dominant myotonia congenita (Thomsen disease, MIM 160800).

Generalised myotonia (GM) is a non-dystrophic myotonic disorder of the skeletal muscle with an estimated prevalence of 1:50 000 and a heterozygote frequency of about 1% in the German population.[4] Data from other populations are sparse and are limited to single cases and family reports.[5–9]

Symptoms of GM may appear as early as 2 years of age or as late as the beginning of the third decade. Most patients notice symptoms primarily in the leg muscles. Within a few years the stiffness is also experienced in the arms, the neck, and the facial muscles. Many patients exhibit marked hypertrophy of the thigh, gluteal, and calf muscles. In a number of patients myotonic stiffness is followed by a transient weakness, which is particularly pronounced in the arms and hands.[10] Electromyographic investigation shows abundant myotonic discharges in all skeletal muscles. There are no pathognomonic structural abnormalities in the muscle fibres and muscle biopsy is therefore not useful to aid diagnosis.

The basis of the myotonic symptoms is a hyperexcitability of the muscle fibre membrane. In GM this hyperexcitability is thought to be based on a reduced sarcolemmal chloride conductance.[11 12] Studies of the mouse ADR phenotype and genotype, a recessively inherited myotonic mutant, which is considered to be a realistic animal model for human myotonia with an autosomal recessive mode of inheritance, gave the first clues to the molecular identification of the human GM gene.[13–17] In a second step the major mammalian skeletal muscle chloride channel CLC-1 was cloned.[18] A transposon, which destroys the coding sequence for several membrane spanning domains of the skeletal muscle chloride channel in ADR mice, was identified.[19] In addition this gene was mapped to mouse chromosome 6 between marker genes Tcrb and Hox1.1, both of which are located in human chromosome 7. Subsequently, a partial human CLC-1 cDNA clone (pL7) derived from the skeletal muscle chloride channel gene (CLCN1) was localised to human chromosome 7q32-qter and was shown to be tightly linked to TCRB.[20] Tight linkage of these two loci to the GM gene was found in seven German families. A mutation in the CLCN1 gene was also identified, a phenylalanine to cysteine substitution in the putative transmembrane domain D8 of the channel protein.

The present study was undertaken to collect further data in additional well defined GM families in order to determine whether the disease shows allelic or non-allelic genetic heterogeneity.

Materials and methods

FAMILY STUDIES

Clinical and genetic studies were performed in 14 well defined German GM families (78 subjects) comprising 25 affected (aged 3 to 70 years) and 21 unaffected offspring. All family members were personally seen by two of the authors (MCK, KR). An EMG in at least one affected family member was performed. In addition 12 isolated patients with the diagnosis GM were screened for the rare pL7/*Nsi*I allele A3, representing the T to G missense muta-

Table 1 Probes and polymorphisms used in the analysis.

Locus/probe	Polymorphism	Allele sizes	Allele frequencies	PIC
CLCN1/pL7	*Nsi*I	A1 > 30 kb	0·6	
		A2 21 kb, 17 kb	0·4	
		A3 21 kb, 10 kb, 7 kb	T > G	0·37[20]
	*Ava*II	A1 15 kb	0·2	
		A2 12 kb	0·8	0·27[20]
	*Sau*96I	A1 22 kb	0·18	
		A2 18 kb	0·82	0·25[26]
TCRB	PCR type	7 alleles*		
		240–280 bp		
		A1–A4	0·165	
		A5	0·01	
		A6	0·32	
		A7	0·01	0·76[23]

* Data from this study.

tion. Altogether 37 patients (n = 25 + 12) were investigated.

DNA ANALYSIS

Genomic DNA was isolated from blood samples and aliquots were digested with restriction enzymes (table 1), subjected to electrophoresis on 0·8% agarose gels, and transferred to nylon membranes by standard methods.[21] Filters were hybridised to the radiolabelled partial CLC-1 cDNA insert (2.5 kb *Eco*RI) of the probe pL7 (extending from bp 510 to the poly-A tail), corresponding to parts of the CLCN1 gene.[19 20]

PCR BASED ANALYSIS

PCR analysis was set up using standard conditions with minor modifications.[22] Genomic DNA was subjected to amplification by using primers for a multiallelic dinucleotide repeat polymorphism in the TCRB gene described by Abdalla *et al.*[33] Each reaction was performed in a 25 to 50 µl volume containing 500 ng genomic DNA template, 200 ng of each primer, 200 µmol/l each dGTP, dATP, dTTP, 20 µmol/l dCTP, and 1·0 µCi [α-^{32}P]dCTP at 3000 Ci/mmol, 5 µl 10 × reaction buffer (15 mmol/l $MgCl_2$) plus 1 U *Taq* polymerase.

The cycling conditions (30 ×) were 94°C, 60°C, and 72°C for one minute, respectively. PCR products were electrophoresed on 6% denaturing polyacrylamide gels and dried gels were exposed on radiographic film. Polymorphism in the size of PCR products was determined in 90 unrelated German controls. Seven alleles were found, the most common allele being allele A6 of approximately 244 bp (table 1).

Table 2 Summary of the lod score results between chromosome 7q markers and generalised myotonia Becker (GM).

Linkage comparison	Recombination fraction (θ) 0·00	0·001	0·01	0·05	0·1	0·2	0·3
A This study (n = 14 families)							
GM *v* TCRB	8·28	8·26	8·05	7·12	5·97	3·80	1·93
GM *v* CLCN1	6·01	5·99	5·84	5·20	4·40	2·87	1·49
GM *v* TCRB/CLCN1	9·26	9·24	9·02	8·06	6·86	4·50	2·35
B Data from Koch *et al*[20] (n = 7 families)							
GM *v* TCRB	2·53	2·52	2·45	2·16	1·81	1·13	0·56
GM *v* CLCN1	4·69	4·67	4·57	4·10	3·50	2·29	1·15
GM *v* TCRB/CLCN1	5·79	5·77	5·61	5·00	4·23	2·72	1·35
C Combined multipoint lod score for both studies (n = 21 families)	15·05	15·01	14·63	13·06	11·09	7·22	3·7

LINKAGE ANALYSIS

Two point and multipoint lod scores were calculated by the method of maximum likelihood and the computer program LINKAGE 5·04, updated by Ott.[24] Autosomal recessive inheritance with complete penetrance was assumed for GM.

HETEROGENEITY TEST

There are three alternative hypotheses in the HOMOG programs: H_0, H_1, and H_2.[24 25] The hypothesis in favour of heterogeneity (H_2) assumes two family sets in the study, one that does show linkage to the marker locus tested and one that does not. The homogeneity hypothesis (H_1) assumes that the relationship between the disease locus and marker locus is the same in all families studied. The null hypothesis (H_0) assumes both homogeneity and absence of linkage. The related program HOMOG2 divides the families under study into two categories which both show linkage but to different markers on the chromosome.

Results

CLINICAL FINDINGS

All subjects classified as affected fulfilled the diagnostic criteria outlined in the introduction. Four families came from the same remote rural area and parental consanguinity could not be excluded. The remaining 10 families were not related. In 12 families both parents were included in the linkage analysis and in two families only one parent was available.

An EMG was performed on 11 obligate heterozygote parental couples. In one family both parents and in a second family the mother exhibited discrete myotonic discharges. Of the 21 unaffected sibs, 16 were shown to be heterozygous gene carriers by haplotype information. Of the 37 affected subjects 27 showed transient weakness, in five patients the examination was equivocal, and in five patients no weakness was detected.

LINKAGE ANALYSIS

The lod scores from the two point and multipoint analyses at a recombination fraction of θ = 0·00–0·30 are summarised in table 2. All parents were typed with both gene probes and the informative polymorphisms were used to type the offspring. No recombination was found between GM and the markers applied. Each gene probe separately, and the combined haplotype of the two, were tightly linked to GM with a maximum lod score of Z = + 9·26 at θ = 0·00 (support interval 0·00–0·04). The combined multipoint lod score of 21 GM families, comprising 14 GM families from this study and seven GM families from our previous study, resulted in an overall score of Z = + 15·05 at θ = 0·00 (support interval 0·00–0·02). No preferential haplotype segregated with the disease in the different families. Pedigrees and linkage data of two representative German GM families are shown in the figure.

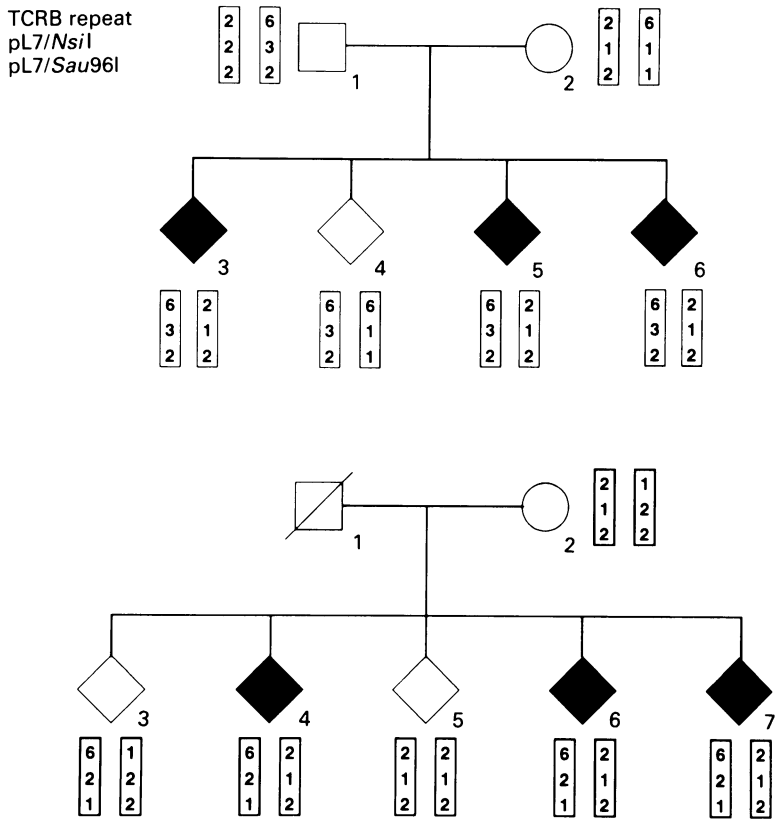
The allele A3 (pL7/*Nsi*I), representing a T

to G point mutation, was found in five families. All affected family members were compound heterozygotes, having in addition an as yet unidentified second mutation.

Of the 12 well defined isolated GM patients two exhibited this missense mutation on one chromosome. Together with the two previously reported families[20] this mutation was found in 15% of the 66 GM chromosomes counted.

HETEROGENEITY TESTS

The lod scores for each family, obtained from the LINKAGE results, were used in the HOMOG programs. Analysis of the data under the assumption of heterogeneity (H_2 *v* H_1) gave negative results (table 3A): χ^2 values for heterogeneity are not significant, the maximum ln likelihood values are not different for the two hypotheses, and therefore the α values are 1·0. The computer program HOMOG2 was used to perform a statistical A test for the presence of two sets of families, one set linked to TCRB and one set linked to CLCN1. The likelihood ratios for heterogeneity were calculated under the hypothesis of heterogeneity versus homogeneity. The hypothesis of linkage with only one family type (H_1) is supported (table 3B), while H_2 (=heterogeneity) is rejected ($\chi^2 = 0{\cdot}00$). The approximate 95% confidence interval presented by the HOMOG program is $\theta = 0{\cdot}00$–$0{\cdot}08$ ($\alpha = 0{\cdot}55$–$1{\cdot}00$) for CLCN1 and $\theta = 0{\cdot}00$–$0{\cdot}04$ ($\alpha = 0{\cdot}80$–$1{\cdot}00$) for TCRB. The 95% confidence interval for the A test performed using HOMOG2 includes the region from $\theta = 0{\cdot}00$–$0{\cdot}02$ ($\alpha = 0{\cdot}05$–$1{\cdot}00$).



Genotypes produced by the dinucleotide repeat polymorphism in the TCRB gene and the probe pL7 (partial CLC-1 cDNA) in two families. Alleles are as described in table 1.

Table 3A Tests of linkage homogeneity (HOMOG, version 3·0) in 21 GM families for loci TCRB and CLCN1.

		CLCN1			TCRB		
Tests	df	χ^2	Max ln(L)	α	χ^2	Max ln(L)	α
H2 *v* H1 (heterog)	1	0·0	24·62	1·0	0·0	24·89	1·0
H1 *v* H0 linkage	1	49·2	24·62	(1)	49·8	24·89	(1)
H2 *v* H0 total	2	49·2	(0)	(0)	49·8	(0)	(0)

Table 3B Tests for linkage homogeneity (HOMOG2, version 2·75) in 21 GM families for linkage to two different loci, TCRB and CLCN1.

Tests	df	χ^2	Max ln(L)	α	θ_1	θ_2
H2 *v* H1 (heterog)	2	0·0	49·5	1·0	0·0	0·0
H1 *v* H0 linkage	1	99·0	49·5	(1)	0·0	0·0
H2 *v* H0 total	3	99·0	(0)	(0)	(0·5)	(0·5)

Discussion

The results of the present study show linkage between the disorder GM and the CLCN1 gene, coding for the skeletal muscle chloride channel CLC-1, and the TCRB gene on the long arm of human chromosome 7.[19 20] The data thus confirm our recent results for linkage in this chromosomal region in seven GM families and extend the study to 21 GM families in total.

Our pooled data provide no evidence for a model with two different loci independently causing the disease, either on different chromosomes or in the same chromosomal region. The latter situation was shown for retinitis pigmentosa on the short arm of the X chromosome.[27] The homogeneity data for GM are in agreement with the clinical picture for the disorder, which is quite uniform and shows no extensive intra- or interfamilial variability. This does not, of course, exclude mutational heterogeneity at the actual GM disease locus. This does exist since the identified missense mutation is present in a homozygous state in only one family, the heterozygous parents being third cousins.[20] The remaining eight affected subjects exhibit the mutation on one chromosome only, which suggests compound heterozygosity.

Autosomal dominant myotonia congenita (MC, Thomsen disease) has also been shown to be linked to the CLCN1 and TCRB genes and a mutation has been identified.[20 23 28] These data suggest that the two phenotypes which appeared at first to represent separate entities are, in fact, allelic disorders. Therefore the two diseases are now classified as muscle chloride channel disorders, in a similar manner to the designation of the different phenotypes in muscle sodium channelopathies.[29]

Data from the clinical and genetic studies of Thomasen[2] and Becker[4] suggested that about 80% of the reported MC families do have the autosomal recessive form of GM. The diagnosis of GM as opposed to MC should not be difficult in typically affected subjects. The classical GM patient will exhibit a transient muscle weakness and a family history of autosomal recessive inheritance.[30] Our study showed that transient muscle weakness is a good diagnostic criterion which differentiates GM from MC. About 75% of the affected subjects studied have this symptom. However,

in isolated cases without transient muscle weakness it may not be possible to make a distinction between GM and Thomsen disease.

The muscle chloride channel disorders, GM and MC, have to be added to the increasing number of diseases that are sometimes dominantly and sometimes recessively inherited, even though they are linked to the same gene locus and show mutations within the same gene, as shown recently for hypoparathyroidism and retinitis pigmentosa.[31 32] Future studies into these chloride channel disorders may show a possible basis for the differences in inheritance. The reported translation mutation (phe to cys) in the CLCN1 protein product probably results in a null allele with respect to the ability to participate in channel function. Since it is predicted that the gene product is a homomeric multisubunit protein in a single dose, a partial loss of functional gene product might be relatively harmless and may not change channel function; the heterozygote consequently would not show an abnormal phenotype. However, a heterozygous condition which produces an abnormal gene product of a dominant negative type which interferes with the expression of the normal allele will have a deleterious effect, such as alterations in the physical properties of channel function, and thus the phenotype will be changed.

The identification of additional mutations in the CLCN1 gene might therefore allow correlations between specific mutations, genotypes, and phenotypes. However, many mutations may not be observed in sufficient numbers to allow definition of their precise phenotypes, nor to draw conclusions for altered channel function.

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1 Thomsen J. Tonische Krämpfe in willkürlich beweglichen Muskeln in Folge von ererbter psychischer Disposition (Ataxia muscularis?). *Arch Psychiatr* 1876;**6**:702–18.
2 Thomasen E. *Myotonia. A clinical and heredobiologic investigation.* Danmark: Universitetsforlaget i Aarhus, 1948.
3 Becker PE. Die Heterogenie der Myotonien. *Excerpta Medica International Congress Series* 1961;**32**:D93–4.
4 Becker PE. Myotonia congenita and syndromes associated with myotonia. In: *Topics in Human Genetics.* Stuttgart: Georg Thieme Verlag, 1977.
5 Harper PS, Johnston DM. Recessively inherited myotonia congenita. *J Med Genet* 1972;9:213–15.
6 Prabhakar S, Chopra JS. Autosomal recessive generalised myotonia (a case report). *J Assoc Physicians India* 1981;**29**:791–3.
7 Sun SF, Streib EW. Autosomal recessive generalised myotonia. *Muscle Nerve* 1983;**6**:143–8.
8 Yonekura J, Yazaki S, Takahashi Y, *et al.* A case of Becker type congenital myotonia with myalgia. *Rinsho Shinkeigaku* 1989;**29**:1140–3.
9 Zellweger H, Pavone L, Biondi A, *et al.* Autosomal recessive generalized myotonia. *Muscle Nerve* 1980;**3**:176–80.
10 Ricker K, Haass A, Hertel G, Mertens HG. Transient muscular weakness in severe recessive myotonia congenita. *J Neurol* 1978;**218**:253–62.
11 Lipicky BJ, Bryant SH, Salmon JH. Cable parameters, sodium, potassium, chloride, and water content, and potassium efflux in isolated external intercostal muscle of normal volunteers and patients with myotonia congenita. *J Clin Invest* 1971;**50**:2091–103.
12 Rüdel R, Ricker K, Lehmann-Horn F. Transient weakness and altered membrane characteristic in recessive generalized myotonia (Becker). *Muscle Nerve* 1988;**11**:202–11.
13 Jockusch H. Molecular aspects of myotonia: the ADR mouse as a model. *J Neurol Sci* 1990;**98**(suppl):9.
14 Rüdel R. The myotonic mouse – a realistic model for the study of human recessive generalized myotonia. *Trends Neurosci* 1990;**13**:1–3.
15 Füchtbauer EM, Reininghaus J, Jockusch H. Developmental control of the excitability of muscle: transplantation experiments on a myotonic mouse mutant. *Proc Natl Acad Sci USA* 1988;**85**:3880–4.
16 Mehrke G, Brinkmeier H, Jockusch H. The myotonic mouse mutant adr: electrophysiology of the muscle fiber. *Muscle Nerve* 1988;**11**:440–6.
17 Reininghaus J, Füchtbauer EM, Bertram K, Jockusch H. The myotonic mouse mutant adr: physiological and histochemical properties of muscle. *Muscle Nerve* 1988;**11**:433–9.
18 Steinmeyer K, Ortland C, Jentsch TJ. Primary structure and functional expression of a developmentally regulated skeletal muscle chloride channel. *Nature* 1991;**354**:301–4.
19 Steinmeyer K, Klocke R, Ortland C, *et al.* Inactivation of muscle chloride channel by transposon insertion in myotonic mice. *Nature* 1991;**354**:304–8.
20 Koch MC, Steinmeyer K, Lorenz C, *et al.* The skeletal muscle chloride channel in dominant and recessive human myotonia. *Science* 1992;**257**:797–800.
21 Sambrook J, Fritsch EF, Maniatis T. *Molecular cloning. A laboratory manual.* 2nd ed. New York: Cold Spring Harbor Laboratory Press, 1989.
22 Litt M. PCR of TG microsatellites. In: McPherson MJ, Quirke P, Taylor GR, eds. *PCR, a practical approach.* Oxford: Oxford University Press, 1991.
23 Abdalla JA, Casley WL, Cousin HK, *et al.* Linkage of Thomsen disease to the T-cell-receptor beta (TCRB) locus on chromosome 7q35. *Am J Hum Genet* 1992;**51**:579–84.
24 Ott J. *Analysis of human genetic linkage.* Baltimore: Johns Hopkins University Press, 1991.
25 Ott J. Linkage analysis and family classification under heterogeneity. *Ann Hum Genet* 1983;**47**:311–20.
26 Wolf F, Koch MC, Steinmeyer K, Jentsch TJ. A Sau96I RFLP at the human skeletal muscle chloride channel locus (CLCN1). *Hum Mol Genet* (submitted).
27 Ott J, Bhattacharya S, Chen JD, *et al.* Localizing multiple X chromosome-linked retinitis pigmentosa loci using multilocus homogeneity tests. *Proc Natl Acad Sci USA* 1990;**87**:701–4.
28 George AL, Crackower MA, Abdalla JA, *et al.* Molecular basis of Thomsen's disease (autosomal dominant myotonia congenita). *Nature Genet* 1993;**3**:305–10.
29 Ptacek LJ, Johnston KJ, Griggs RC. Genetics and physiology of the myotonic muscle disorders. *N Engl J Med* 1993;**328**:482–9.
30 Becker PE. Heterozygote manifestation in recessive generalized myotonia. *Hum Genet* 1979;**6**:325–9.
31 Parkinson DB, Thakker RV. A donor splice site mutation in the parathyroid hormone gene is associated with autosomal recessive hypoparathyroidism. *Nature Genet* 1992;**1**:149–52.
32 Rosenfeld PJ, Cowley GS, McGee TL, *et al.* A null mutation in the rhodopsin gene causes rod photoreceptor dysfunction and autosomal recessive retinitis pigmentosa. *Nature Genet* 1992;**1**:209–13.