
Original articles

Genetics of congenital nemaline myopathy: a study of 10 families

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Abstract

In order to investigate the inheritance in congenital nemaline myopathy (CNM), we studied the family histories and pedigrees of 13 patients with CNM from 10 families, and the 20 parents, by physical examination, single fibre electromyography, ultrasonography of muscles, measurement of serum creatine kinase, muscle biopsy, and electrophoresis of muscle proteins. None of the parents was affected. In three families there were two affected children. Of the parents, 15 showed deficiency of type 2B muscle fibres, and all except one father showed some other minor neuromuscular abnormality. These may represent heterozygous manifestations of a recessive gene. Most of the ancestors came from sparsely populated rural communities in the west of Finland.

We conclude that in the Finnish CNM patients, the mode of inheritance appears to be recessive. Apart from a few instances of dominant inheritance, most cases published also seem compatible with recessive inheritance.

Congenital nemaline myopathy (CNM) was first described in 1963.^{1,2} It is characterised by generalised

muscular weakness and hypotonia from birth or infancy. Further characteristics are myopathic facies, high arched palate, and often chest deformities and scoliosis. The diagnosis is confirmed by a skeletal muscle biopsy stained by the Gomori trichrome method, which shows the presence of abundant, thread-like (Greek nema=thread) particles, the nemaline bodies, in the myofibres, and often predominance of type 1 muscle fibres.

The clinical picture of CNM is uniform, but the severity varies widely. A subclassification of nemaline myopathy into an early fatal form, a more benign congenital form,³ and an adult onset form⁴ has been suggested. No clear histological differences have been found between these subgroups.⁵ However, the adult onset form differs from the other two in several ways. Besides having a later age of onset, it is usually preceded by an inflammatory disease, and characterised by a progressive course and absence of dysmorphic features. Thus, the adult onset form probably represents a different disease entity.

CNM is generally thought to be a hereditary disease, but the mode of inheritance is not clear. In some families the trait shows an autosomal dominant pattern (McKusick *16180),⁶⁻⁹ and in others seems to be recessive (McKusick *25603).^{6,7} In a survey of 50 published cases,¹⁰ autosomal dominant inheritance with reduced penetrance was suggested.

In this study we examined the family history and pedigree data of 10 families with 13 CNM patients. In addition, we report the results of clinical examination of the 20 parents, including single fibre electromyography, ultrasonography of muscles, serum creatine kinase, muscle biopsy, and electrophoresis of muscle proteins.

Subjects and methods

PATIENTS

We examined all 13 CNM patients known in Finland at the time of this study. Their diagnoses were based on clinical features consistent with congenital myopathy¹¹ and the presence of nemaline bodies in a muscle biopsy. Twelve of the patients had been

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ascertained among the 400 patients under 16 years of age with neuromuscular disease diagnosed at the University of Helsinki Children's Hospital in 1964 to 1985.¹² The thirteenth patient was a mildly affected sister of a proband; she was diagnosed at the time of the follow up study. Nine of the patients were female and four were male (aged 5 to 40 years, mean 18 years). The results of the patients have been published previously¹²⁻¹⁵ and a summary is provided in table 1.

PARENTS

All the parents (10 mothers and 10 fathers aged 31 to 64 years, mean 45 years) of the patients with CNM were studied by physical examination, single fibre electromyography, ultrasonography of the lower limb muscles, serum creatine kinase determination, muscle biopsy, and electrophoresis of muscle proteins.

FAMILY HISTORIES

Family histories were taken and, if positive for any neuromuscular symptoms or chronic diseases, checked from hospital records. From the family histories and the population register, the grandparents and great grandparents were sought for determination of the geographical distribution of their birthplaces and any close consanguinity between ancestors.

SINGLE FIBRE ELECTROMYOGRAPHY

Measurements of fibre density were performed by a computerised method in the extensor digitorum communis muscle with a concentric needle electrode.^{13 16}

ULTRASONOGRAPHY

Ultrasonography was done with a real time apparatus with a 7.5 MHz sector scanner. Longitudinal and

*Table 1 Characteristic findings in patients with congenital nemaline myopathy.*¹²

Muscle weakness most pronounced in face, neck, trunk, and distal parts of limbs (13/13).
Inability to run (13/13).
Hypermobility of joints (7/12).
Absence of tendon (12/12) and palatal (9/12) reflexes.
Diadochokinesis slow or clumsy (6/12).
Abnormally high fibre density at single fibre electromyography (9/10).
Abnormally high (grade 2-4) echogenicity on ultrasonography of thigh (10/12) and shin (12/12) muscles.
Nemaline bodies in muscle biopsy (13/13).
Predominance (>55%) of type 1 (slow) fibres in muscle biopsy (12/13).
Absence of type 2B (fast fatiguable) fibres in muscle biopsy (10/12).
Abnormally shaped histograms of muscle fibre diameter (10/13).
Abnormally high variability coefficients of muscle fibre diameter (12/13).
Internal nuclei (7/12), splitting of fibres (2/13), or endomysial fat or fibrosis (9/12) in muscle biopsy.
Deficiency of α -actinin (9/13) and a 65 kd (6/13) protein on electrophoresis of muscle proteins.

transverse scans at mid-thigh and mid-calf were taken from the quadriceps femoris muscles, the plantar flexors of the feet, and the dorsiflexors of the feet. The findings were graded from 1 to 4, using a modification of the scale of Heckmatt and Dubowitz,¹⁷ grade 1 representing normal scans and grades 2 to 4 pathological scans of increasing abnormality.¹⁴

SERUM CREATINE KINASE

Serum creatine kinase was determined by a routine spectrophotometric method, the upper limit of normal in our laboratory being 150 IU/l.

MUSCLE BIOPSY

Biopsies were obtained from the quadriceps femoris muscle under local anaesthesia with a Bergström or Tru-Cut^R needle at a site one third of the distance from the joint space of the knee to the trochanter major. The samples were immediately orientated under a dissecting microscope and frozen in freon cooled with liquid nitrogen. Cryostat sections (10 μ m) were stained with the haematoxylin-eosin (HE), Gomori trichrome, and myosin adenosine triphosphatase (ATPase) methods, the latter after pre-incubation at pH 4.3, 4.6, and 9.4. Trichrome and HE stained sections were examined for nemaline bodies and secondary signs of myopathy, respectively.

Two hundred fibres in each sample were classified as type 1, 2A, 2B, or 2C and measured from photomicrographs of ATPase stained sections. Histograms of fibre diameters were plotted separately for each fibre type.¹⁵ The mean fibre diameters, standard deviations, variability coefficients, and atrophy and hypertrophy factors were calculated.^{18 19}

ELECTROPHORESIS OF MUSCLE PROTEINS

Muscle proteins were extracted from the biopsies according to Stuhlfauth *et al.*²⁰ One dimensional sodium dodecyl sulphate polyacrylamide gradient gel electrophoresis was performed, using a modification of the methods of Laemmli²¹ and O'Farrell.²²

CONTROL STUDIES

Ultrasonography of the lower limbs was done in 14 healthy control subjects from the medical staff (aged 18 to 65 years, mean 37 years). Results of single fibre electromyography in 64 healthy controls (38 females and 26 males aged 20 to 84 years, mean age 46 years) have been published previously.¹⁶ With the method used, the mean fibre density for the extensor digitorum communis muscle is 1.17, the upper limit of normal being 1.27 (mean + 2 SD). Five muscle biopsies from controls (aged 13 to 32 years, mean 21 years) were processed in the same way as the parents' biopsies. Control biopsies 1 to 3 were obtained during orthopaedic surgery for unrelated causes, and control

biopsies 4 and 5 were from healthy volunteers from the medical staff. Three of the biopsies were from the quadriceps femoris muscle, no 2 was from the gracilis, and no 3 from the hamstring muscles. Parts of the control biopsies were used for electrophoresis in the same way as those from the patients and parents.

Results

FAMILY HISTORIES

The 13 patients came from 10 families (fig 1). In three families there were two affected sibs, in whom the disease differed somewhat in severity, and the other seven patients were the only affected persons in the family. None of the parents reported any symptoms of muscular disease. Grandparents, aunts and uncles, and the children of these were also reportedly unaffected. One mildly affected patient had two healthy children (family 8, fig 1), and a severely affected patient (family 9, fig 1) gave birth to a clinically healthy son in June 1989. Physical examination of the boy at birth and at 2 months of age showed no signs of CNM.

No consanguineous marriages were found and the families were not found to be related. However, several of the grandparents originated from sparsely populated rural communities in the west of Finland (fig 2).

There were four male and nine female patients; thus the sex ratio was 0.44. The 13 patients had 12

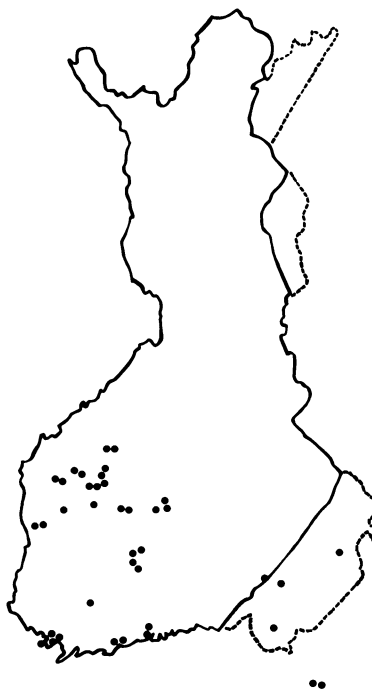


Figure 2 The birthplaces of the grandparents of the patients with CNM, showing clustering in sparsely populated rural communities in the western part of Finland. Each dot marks the birthplace of one grandparent. The areas enclosed by the broken line were parts of Finland before the second world war.

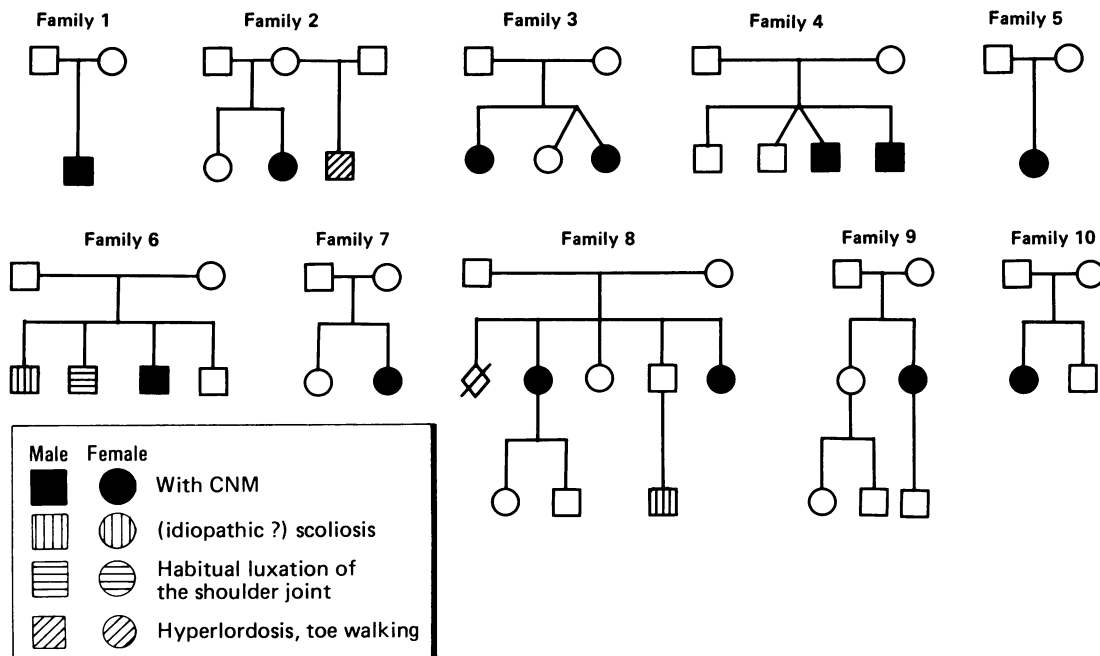


Figure 1 Pedigrees of the 10 families of patients with CNM.

unaffected sibs. In addition, in family 8 there was one stillborn sib whose cause of death was not known. A brother (family 6) and a nephew (family 8) had scoliosis. Another brother (family 6) had habitual luxation of the shoulder joint. Both the brothers and the nephew allegedly had normal muscle power. A half brother (family 2) had hyperlordosis of the spine and was a toe walker, but on examination at the age of 17 his muscle strength was normal. Two of the unaffected sibs were twins of patients. DNA fingerprint analysis confirmed that the twins were non-identical.

The proportion of affected sibs was 0.268 when corrected for truncate complete ascertainment by Apert's a priori method, and 0.20 when corrected for truncate single ascertainment by omitting one affected sib from each sibship.²³ The stillborn sib was omitted from these calculations.

CLINICAL FINDINGS IN THE PARENTS

None of the parents had CNM, but judged by a

careful physical examination seven mothers and seven fathers showed minor neuromuscular abnormalities (table 2) of which, as a rule, they were not aware.

SINGLE FIBRE ELECTROMYOGRAPHY

On single fibre electromyography of the extensor digitorum communis muscle, two fathers showed fibre densities higher than normal (1.35 and 1.40, respectively) (table 2). In all the other parents, fibre density was normal. The probands had a mean fibre density of 1.54 (range 1.29 to 2.07).¹³

ULTRASONOGRAPHY

In the thigh muscles all parents showed normal echogenicity, and in the calves or shins five mothers and two fathers showed slightly greater (grade 2) echogenicity than normal. Ultrasonography of the controls showed completely normal scans in 10 and a slightly greater echogenicity than normal (grade 2) in the thighs or calves in four.

Table 2 Minor neuromuscular abnormalities in healthy parents of patients with congenital nemaline myopathy.

Mother No	Clinical finding	Hist 2B-/ other	El ph	EMG	Father No	Clinical finding	Hist 2B-/ other	El ph	EMG
1	Deviant facial appearance* Facial weakness† High arched palate Habitual subluxation of patella	+/+	+	-	1	-	-/-	-	-
2	Subluxation of hip Pain in elbows after carrying heavy loads	+/+	+	-	2	-	+/+	+	-
3	DDK clumsier on dominant side	+/+	-	-	3	Weak tendon reflexes	+/-	-	+
4	Dorsiflexors of right foot thin	+/+	-	-	4	Facial weakness	-/+	-	-
5	-	+/+	+	-	5	DDK slow, clumsy‡	-/+	-	-
6	-	+/+	-	-	6	Weak palatal reflex	+/+	+	-
7	Facial weakness Dorsiflexors of left foot thin	+/+	-	-	7	Inability to run fast	+/-	-	-
8	Facial weakness Biceps reflexes weak	-/+	+	-	8	-	+/+	-	-
9	-	+/+	-	-	9	Slight facial and finger weakness	-/+	-	+
10	DDK slow	+/+	-	-	10	Weak fingers Clumsiness (DDK and fine motor) Right calf 1 cm thinner	+/+	+	-

Hist 2B-/ other + = muscle biopsy shows deficiency of type 2B fibres or some other abnormality, ie above normal numbers of internal nuclei, splitting of fibres, abnormally shaped histograms of fibre diameter, abnormally great variability of fibre diameter and/or abnormal atrophy factors. El ph = electrophoresis of muscle proteins, + = deficiency of α -actinin and/or a 65 kd protein. EMG = single fibre electromyography, + = abnormally high fibre density. * = Elongated face with small muscle bulk, micrognathia, and expressionless appearance at rest, but with normal facial expressions during conversation. † = Facial weakness slight in all cases, mostly inability to keep air in cheeks against pressure. ‡ = Possibly owing to toxic agent. DDK = diadochokinesis. Blank = examination not done.

CREATINE KINASE

Serum creatine kinase concentrations were slightly higher than normal in two mothers (243 IU/l in mother 6 and 183 IU/l in mother 8).

MUSCLE HISTOLOGY

No nemaline bodies were found in the parents' muscle biopsies. Five mothers and four fathers showed some of the following abnormalities: fibre type grouping (3/20), small angulated fibres (3/20), above normal numbers of internal nuclei (5/20), or splitting of fibres (4/20). None of the parents had endomysial or perimysial fat or fibrosis and none had necrotic or regenerating fibres.

Four of the 10 mothers and five of the 10 fathers had predominance (>55%¹⁹) of type 1 fibres (table 3, figs 3 and 4). Eight mothers and five fathers showed deficiency (<10%¹⁹) of type 2B fibres, and one further mother and one father showed total lack of type 2B fibres. One mother and four fathers had a normal fibre type composition.

Three controls (no 3, 4, and 5) showed slight predominance of type 1 fibres (table 3). One control (no 4, one of the healthy volunteers) had deficiency of type 2B fibres.

As in the probands,¹⁵ the histograms of muscle fibre diameter in the parents showed abnormal distributions of three types (fig 3): a flattened and bilaterally broadened curve, skewing towards the

Table 3 Fibre type composition in parents of CNM patients and in controls.

Mother No	Age	Fibre type (%)				Father No	Age	Fibre type (%)			
		1	2A	2B	2C			1	2A	2B	2C
1	31	54	39.5	5.5	1	33	32	25	43		
2	38	41.5	55	3	2	37	72	24.5	3.5		
3	41	74.5	25.5	—	3	40	73	25.5	1.5		
4	42	53.5	46	0.5	4	43	54	26	19.5	0.5	
5	41	74.5	21	3	5	45	34.5	53	12.5		
6	48	34.5	64	1.5	6	52	39.5	59	1.5		
7	47	38.5	52	9.5	7	45	63.5	36.5	—		
8	64	46.5	22	29.5	8	60	81.5	15.5	2.5	0.5	
9	50	64.5	33	1	9	52	50.5	15.5	33.5	0.5	
10	48	72	21.5	6.5	10	47	76.5	13	9.5	1	
Female control						Male control					
1	13	47	37.5	15.5	2	13	41.5	13.5	43.5	1.5	
3	13	61	23	16							
4	31	68.5	27.5	4	5	32	57	27	16		

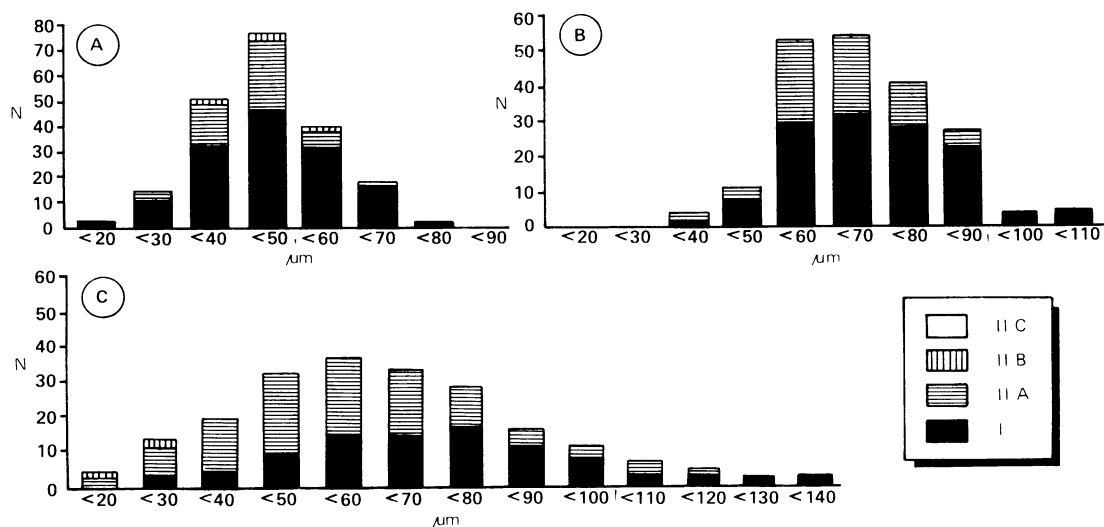


Figure 3 Histograms of muscle fibre diameters in one healthy female control (A) and in two fathers (B, C) of patients with CNM. The histogram of the control has the normal bell shaped appearance (A). Note in father 7 (B) the total lack of type 2B fibres, and in father 6 (C) the broad and flat shape of the histogram as well as the smallness and deficiency of type 2B fibres.

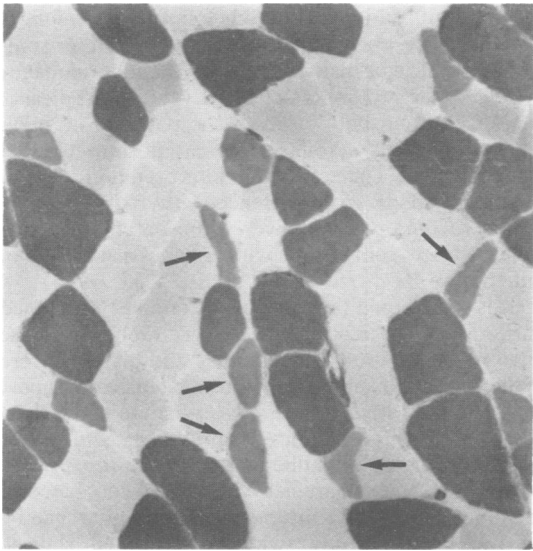


Figure 4 Section of muscle biopsy from mother 7 stained by the ATPase method after preincubation at pH 4.6, showing a few small, grey type 2B fibres (arrows) scattered among larger type 1 (black) and type 2A (white) fibres. The fibre types were determined from adjacent sections stained with ATPase after preincubation at pH 9.4 and 4.3, respectively.

atrophic end, or bimodal or trimodal histograms. Parent and proband did not always show the same abnormality. Two mothers and five fathers had the normal bell shaped histogram characteristic of the controls. In two of the parents the mean fibre diameters deviated clearly from those of the controls, the type 2B fibres being smaller than normal in these parents, both of whom had type 2B deficiency. The mean fibre diameters of our controls compared well with published normal values.^{18 24-27}

The variability coefficients ($VC = SD/\text{mean fibre diameter} \times 1000$) were greater than normal (250)¹⁹ for the type 1 fibres in five mothers and four fathers, for the type 2A fibres in five mothers and three fathers, and for the type 2B fibres in six of the seven mothers and five of the nine fathers for whom they could be calculated. Thus, all the mothers and six of the fathers had an abnormally high variability coefficient for at least one fibre type. The controls had normal or nearly normal variability coefficients.

ELECTROPHORESIS OF MUSCLE PROTEINS

Three mothers and one father showed deficiency or total lack of α -actinin, and two mothers and two fathers showed total lack of a 65 kd protein. Both these proteins were present in all controls but deficient in nine and six of the probands, respectively.

Summarising the investigations in the parents,

none of them had CNM, but in all parents except father 1 some of the neuromuscular abnormalities characteristic of the probands were found (table 2). In family 1 paternity was confirmed with DNA fingerprint analysis.

Discussion

It is generally accepted that CNM is a hereditary disorder, and an exogenic cause for the characteristic congenital symptoms and signs is seldom even discussed. In our series there were two pairs of non-identical twins discordant for CNM. Thorough histories of pregnancy failed to disclose any possible risk factor common to the 10 mothers. Thus our results are in keeping with the view that an exogenic cause for CNM is unlikely.

CNM is a rare disease and articles on CNM mostly consist of case reports. This series of extensively examined parents of patients with CNM is to our knowledge the largest one published hitherto, and therefore enables us to shed some new light on the mode of inheritance in CNM. However, it was not possible to examine the healthy sibs of the patients. Another difficulty was the lack of a large normal series of human muscle biopsies, hampering interpretation of the many different slightly pathological features in the muscle biopsies of the parents. The series published²⁴⁻²⁷ have all been relatively small and ours consisted of only five biopsies. A reference series widely in use comprises biopsies obtained on suspicion of neuromuscular disease but found to be normal.¹⁸

Among the methods tried in this study to search for minor neuromuscular abnormalities in the parents, ultrasonography does not seem to be a useful one. The small excess of serum creatine kinase in two mothers could be from causes unrelated to CNM, especially since the patients showed normal levels. Fibre type grouping or small angulated fibres were not included among the pathological muscle biopsy features in table 2, since these were not found in the probands, and fibre type grouping is a normal feature of ageing muscle.

Increased fibre density in single fibre electromyography indicates altered distribution of the muscle fibres of a motor unit. It is known to be a sensitive quantitative detector of neuromuscular abnormality.^{16 28} Specificity is not very good, however, and high fibre densities are found in both neurogenic and myopathic disorders and as late sequelae of various disorders. In this study only two fathers showed fibre densities higher than normal. The significance of the electrophoretic findings remains to be clarified.

One abnormality was present in 75% of the biopsies of the parents, that is, deficiency or total lack of type 2B fibres, mostly combined, as in the probands, with predominance of type 1 fibres (table 3, figs 3 and 4).

One of our healthy controls from the medical staff exhibited a similar deficiency. We do not know the reason for this. The control differed, as far as we are aware, from all previously published controls²⁴⁻²⁷ of compatible age. In a study of 34 healthy male subjects biopsied twice, the proportion of type 1 fibres varied by as much as 6.2 to 12.3%.²⁹ This variation is naturally to be expected in the parents as well, but hardly accounts for the presence of the deficiency in as many as 15 of the parents. Predominance of type 1 fibres has previously been noted in three mothers of CNM patients,^{7 30 31} but most reports of parental biopsies fail to describe fibre type distribution.

The two main findings in this study were that in none of the families were two generations affected, and that in all but one of the families physical examination, electromyography, histological examination of muscle biopsies, or electrophoresis of muscle proteins (table 2) showed minor neuromuscular abnormalities in both parents. Thus, these minor abnormalities probably represent heterozygous manifestations of an autosomal recessive gene. This interpretation is corroborated by the fact that all the parents had normal muscle power. The only exceptions are those listed in table 2, and the parents themselves were unaware of them. The proportion of affected sibs was 0.268 and 0.20, respectively, when corrected for truncate complete and single ascertainment. These figures are in accord with recessive inheritance,³² and suggest that the ascertainment was between complete and single. The small number of families, however, reduces the value of segregation analysis. The sex ratio is also compatible with a recessive mode of inheritance. Most of the families originated from sparsely populated rural areas in the western parts of Finland (fig 2) that were permanently settled in the 16th century by relatively few founders. This is a common finding in corresponding maps of rare recessive disorders in Finland: most of the ancestors come from sparsely populated areas consisting of local isolates.³³ Thus, this map (fig 2) can be considered to support a recessive mode of inheritance.

Family histories published in 50 cases of CNM have been analysed by Kondo and Yuasa.¹⁰ Among the 44 families reported, three showed a dominant mode of inheritance with clinically overt CNM verified by biopsy in two generations.⁷⁻⁹ However, one of these⁷ could have been pseudodominant, as the parents of the affected person in the second generation were remotely related. Altogether, parental consanguinity was reported in five families. Arts and de Groot³⁴ calculated that when families with CNM in two or more generations were excluded the proportion of consanguineous marriages was 5/43, a high proportion which suggests recessive inheritance. In the study by Kondo and Yuasa,¹⁰ there were 37 families in which both parents were healthy, and in eight of these there were two or more affected children. In 12 families

both parents underwent muscle biopsy, and in four of these families one of the parents showed either some of the physical or histological abnormalities (nemaline bodies or large numbers of internal nuclei) typical of CNM, in four both parents showed one or other abnormality, and in four neither parent showed either abnormality. The authors' interpretation was a dominant mode of inheritance with reduced penetrance. In our opinion, however, most of the pedigrees analysed are compatible with a recessive mode of inheritance.

It is to be noted that four of the families³⁵ included in the report by Kondo and Yuasa¹⁰ took part in the present study, and the patients and parents were rebiopsied and examined with more extensive methods. The parents who at the first examination showed no histological abnormalities (families 1 and 3 of Kuitunen *et al*,³⁵ families 6 and 7 in the present study), and the parents who were not biopsied (families 2 and 4 of Kuitunen *et al*,³⁵ families 5 and 9 in the present study) when re-examined showed minor physical or histological abnormalities typical of CNM. Thus it is possible that more extensive methods of investigation, if applied to other apparently healthy parents, might show manifestations of heterozygosity in these too.

We conclude that in most cases of CNM, including the Finnish patients, the most likely mode of inheritance is recessive. However, it should be borne in mind that there have been a few instances of dominant inheritance.

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