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Case report

Charcot-Marie-Tooth disease type 2CC due to a frameshift mutation of the neurofilament heavy polypeptide gene in an Austrian family

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Abstract

Neurofilaments are structural components of motor axons. Recently different variants resulting in translation of a cryptic amyloidogenic element of the neurofilament-heavy polypeptide (*NEFH*) gene have been described to cause Charcot-Marie-Tooth disease type 2CC (CMT2CC) by forming amyloidogenic toxic protein aggregation. Until now only few CMT2CC patients have been described. Clinical features include progressive muscle weakness and atrophy mainly affecting the lower limbs, hyporeflexia and distal sensory impairment. In addition to classic CMT features, some patients were reported to have increased serum creatine kinase levels, an electrophysiologic pattern suggestive for myopathies, and pyramidal signs. Ambulation is progressively impaired, most patients are non-ambulant in the 5th decade. Nerve conduction testing shows a symmetrical, distal and proximal sensorimotor axonal neuropathy. Here we describe the first Austrian pedigree suffering from CMT2CC and give an overview on the phenotype of CMT2CC described so far.

Keywords: Charcot-Marie-Tooth Type 2CC; Neurofilament heavy chain; Protein aggregation.

1. Introduction

Neurofilaments are components of the neuronal cytoskeleton and are composed of three subunits: the neurofilament heavy chain *NFEH*, the medium chain (NEFM), and the light chain (NEFL). They are crucial for the growth of axons, the maintenance of axon caliber and the transmission of electrical impulses along axons [1]. Abnormal accumulation of neurofilament occurs in pathological conditions such as neurofilament inclusion disease (NFID), giant axonal neuropathy (GAN), diabetic neuropathy, spinal muscular atrophy (SMA), spastic paraplegia, Alzheimer's disease (AD) and Parkinson's disease (PD) [1]. In amyotrophic lateral sclerosis (ALS) phosphorylated neurofilament heavy chain (pNEFH) has been proposed as promising biomarker at the diagnostic phase [2–5].

Charcot-Marie-Tooth disease (CMT) is the most frequent hereditary form of neuropathy with an esteemed prevalence of 10–28/100 000, a great variety of phenotypes, inheritance patterns, and over 80 associated genes [6,7]. Two main forms of CMT can be distinguished according to median (or ulnar) motor nerve-conduction velocity; the demyelinating form with MNCV < 38 m/s (CMT1) and the axonal form with MNCV > 38 m/s (CMT2) [6]. Gene mutations in NEFL have been found to underlie demyelinating CMT1F [8] or axonal CMT2E form. [9,10] Rebelo et al. first reported two frameshift variants c.3010–3011delGA (p.Asp1004Glnfs*58) and c.3017–3020dup (p.Pro1008Alafs*56) in the 3′ untranslated region (UTR) of the neurofilament-heavy polypeptide (*NEFH*) gene to cause axonal Charcot-Marie-Tooth neuropathy type 2CC (CMT2CC) by forming amyloidogenic toxic protein aggregation. [11]

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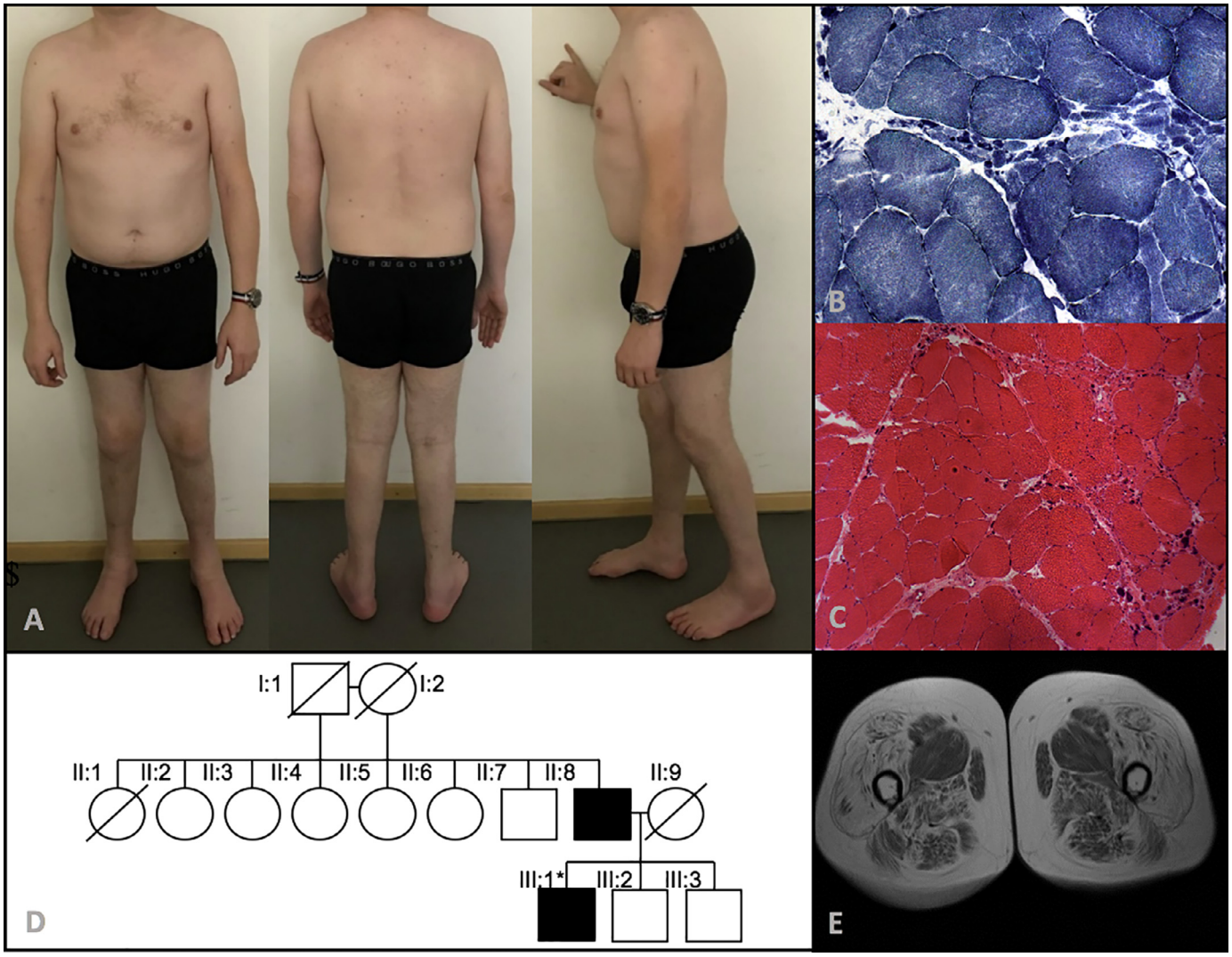

Fig. 1. A: Clinical phenotype of the patient. Symmetric atrophy of the ventral thighs and extensor and flexor muscles of the feet, mild distal atrophy of the upper limbs. Clubfeet. B&C: HE and NADH: Scattered neurogenic small group atrophy (x20). D: Pedigree of the family. Affected members are shown in black. I:1 died at age 74 due to a fire, I:2 died at the age of 55 due to a traffic accident. II:1 died at age 60 due to a melanoma, none of them is reported to have had gait disturbance. E: MRI of the lower limbs. Fatty atrophy of all thigh muscles except M. gracilis and caput brevis of biceps femoris muscle.

Clinical features include progressive muscle weakness and muscle wasting, predominantly affecting the lower limbs. Mild sensory deficits and absent deep tendon reflexes are also common. Electrophysiological studies show a symmetrical, distal and proximal sensorimotor axonal neuropathy.

The present report summarizes the literature on CMT2CC phenotype features and describes the first Austrian autosomal dominant CMT2CC family with two affected members (Fig. 1) due to a frameshift mutation in the *NEFH* gene.

2. Methods

2.1. Patients

The patient with a family history suggesting an autosomal dominant trait was first seen in our neuromuscular outpatient clinic at Friedrich-Baur-Institute, Dep. of Neurology, Ludwig-Maximilians-University of Munich, in May 2014. Written informed consent was obtained for participation in this investigation. Our index patient (III.1) displayed a clinical and electrophysiological phenotype of axonal motor and sensory neuropathy; at that time, no mutations in commercially available CMT2 genes had been detected.

2.2. Clinical assessment

Patient III.1 was seen in our neuromuscular center and assessed by a senior neurologist (MW) specialized in neuromuscular disorders. Patient II.8 was seen by an external neurologist. Clinical assessment included medical history and neurological examination

2.3. Electrophysiological study

Neurophysiological studies included nerve conduction studies in upper and lower limbs and electromyography by concentric needle electrodes.

2.4. Histological study

A muscle biopsy was performed in patient III.1 and II.8. A sural nerve biopsy was performed in Patient II.8. Muscle and nerve biopsies were processed according to standard techniques. [12]

2.5. Molecular analysis

Next generation sequencing revealed a novel mutation in index patient III.1, the mutation was confirmed by Sanger sequencing. The father (II.8.) mother (II.9) and siblings (III.2 and III.3) were tested by Sanger sequencing.

3. Results

3.1. Clinical features

3.1.1. Patient III.1, Fig 1

We report on a 34-year-old male patient with an uneventful birth and normal early development; the parents marriage was not consanguineous. Around the age of 8 years, he experienced small difficulties in running and arising from squatting position. Over the following years, he developed progressive weakness of the distal lower limbs and clubfeet. Neurological examination at age 34 years revealed a marked symmetric atrophy of the ventral thighs, along with atrophy of feet extensors and flexors and a mild distal atrophy of the upper limbs. Muscle strength testing showed a severe weakness of both proximal and distal leg muscles. Medical Research Council Scale (MRC) was as follows in the lower limb muscles: iliopsoas 3–4/5, hamstring muscles 4/5, quadriceps 2/5, gluteus 4/5, tibialis anterior and gastrocnemius 0/5. Heel or tiptoe walking was not possible. No facial involvement, no considerable weakness of the upper extremities was noted. Deep tendon reflexes were absent. A distal symmetric sensory deficit (vibration and touch) was noted on upper and more prominent lower limbs. Creatine kinase levels were mildly elevated between 400 and 700 U/l (normal <180 U/L).

3.1.2. Patient II.8, Fig 1

The father of the patient was reported to be similarly affected; he had normal developmental milestones, and first noted weakness and atrophy of the distal lower limbs resulting in an unsteady gait around age 16. At the age of 34 years, he developed a mild weakness of the distal upper limbs. Examination at age 35 revealed mild weakness and atrophy of the distal upper limbs as well as marked distal atrophy of the lower limbs, along with severe distal muscle weakness of 0/5 in tibial and gastrocnemius muscles. Deep tendon reflexes of the upper limbs were diminished, and absent on the lower limbs. There was a mild symmetric sensory deficit (vibration and touch) on upper and lower limbs. He became wheelchair bound by age 64. Additionally, he suffered from a mitral valve replacement and atrial fibrillation, potentially associated with the family disease. CK levels were mildly elevated (228 U/l).

3.2. Electrophysiological findings

3.2.1. Patient III.1

Nerve-conduction velocity studies (NCV) were consistent with a motor and sensory axonal neuropathy predominantly affecting the lower limbs. (Table 1)

Electromyography (EMG) revealed in proximal and distal muscles, chronic neurogenic changes in combination with features reminiscent of myopathy such as polyphasic short small unit motor potentials, fibrillations and positive sharp waves with full to slightly reduced recruitment pattern and complex repetitive discharges. (Table 2)

3.2.2. Patient II.8

Nerve-conduction-velocity studies showed decreased NCV on motor median nerves at age 34, unfortunately, no follow-up was available. Electromyography showed a chronic neuropathic pattern, along with giant potentials. (Table 2)

3.3. Histological findings

3.3.1. Patient III.1

At the age of 16 years, a first muscle biopsy of the tibialis anterior muscle was obtained showing mild to moderate fibrosis, an increase of centrally placed myonucleii with myofiber diameter variation and fiber splitting was found. No large or small grouped atrophy beyond a type-1 fiber predominance was seen, however some angulated atrophic fibers were evident. Based on the upregulation of neural cell adhesion molecules, a neuropathic process with secondary myopathic changes was alternatively discussed. Immunoblotting for dystrophin, alpha- and gamma-sarcoglycan, merosin, and emerin revealed no abnormities. Interestingly, a second muscle biopsy from the left biceps brachialis muscle at the age of 32 years revealed a chronic neuropathic muscular atrophy with large and small fiber group atrophy. (Fig. 1B&C) No inflammatory infiltrates or mitochondrial abnormalities were observed. Cox staining was normal. Immunhistochemical analysis for dystrophin, alpha-sarcoglyan, merosin, emerin, caveolin-3, alpha-B-crystallin/desmin, dysferlin, and alpha-dystroglycan revealed no abnormities.

3.3.2. Patient II.8

A muscle biopsy taken from the right gastrocnemius muscle at age 34 years showed grouped fiber atrophy suggestive of peripheral denervation with secondary myopathic changes. However, interpretation of his biopsy is limited since the patient had been diagnosed with poliomyelitis at the age of 16 years.

Table 1
Nerve-conduction velocity studies (NCV) of Patient III.1 were consistent with a motor and sensory axonal neuropathy predominantly affecting the lower limbs. The right side of the nerves is represented in this table. CMAP = compound motor action potential (in mV). CV = conduction velocity (in m/s). NO = Not obtained. NA = Not available.

Motor nerve conduction		*Sensory nerve conduction*									
Median nerve		*Ulnar nerve*		*Tibial nerve*		*Median nerve*		*Radial nerve*		*Sural nerve*	
CMAP	CV	CMAP	CV	CMAP	CV	CMAP	CV	CMAP	CV	CMAP	CV
8	52	7	40	0,1	40	NO	NO	NO	NO	NO	NO

Table 2
Electromyography revealed mixed chronic neurogenic and myopathic changes. CN = chronic neurogenic changes. SP MUP = short polyphasic motor unit potentials. CRD = complex repetitive discharges. FDIO = first dorsal interosseous. The right side of the muscles is represented in this table.

	Patient III.1	*Patient II.8*
Age at examination	32 years	53/35* years
Biceps	CRD/SP MUP	NA
FDIO	CRD/CN	CN/SP MUP
Extensor digitorum	SP MUP	CN
Vastus lateralis	CN/SP MUP	CN/SP MUP*
Tibialis anterior	CN	CN

A biopsy of the sural nerve was without pathological findings.

3.4. Molecular analysis

Next generation sequencing identified an autosomal dominant heterozygous frameshift mutation c.3057dupG (p.Lys1020Glufs*43) in the *NEFH* gene in Patient III.1. The variant was confirmed in other affected family members by Sanger sequencing. His symptomatic father (Patient II.8.) carried the same variant in *NEFH*, while his two asymptomatic siblings and the asymptomatic mother of the patient did not carry the mutation, in line with dominant family co-segregation. The mutation constitutes a duplication of one nucleotide within the last exon of the *NEFH* gene, causing a frameshift and elongation of the transcript, coding a C-terminal altered protein containing a cryptic amyloidogenic element (Gln-Phe-Ser-Leu-Phe-Leu-Ser-Leu) leading to aggregation and accumulation of neurofilament protein.

4. Discussion

We identified a two generation family carrying a c.3057dupG (p.Lys1020Glufs*43) mutation in *NEFH* with mid-adult onset and an autosomal dominant trait.

Until now, only seven pedigrees have been reported worldwide. Rebelo et al. first reported two frameshift variants c.3010-3011delGA (p.Asp1004Glnfs*58) and c.3017-3020dup (p.Pro1008Alafs*56) causative for CMT2CC [11]. Recently, four new mutations in the *NEFH* gene causing CMT2CC were identified. Two mutations were found in two different French families, c.3008_3009del (p.Lys1003Argfs*59) and c.3043_3044del (p.Lys1015Glyfs*47) (13), another mutation was found in a Chinese family c.3015_3027dup (p.Lys1010Glnfs*57) [14]. While we were in preparation of this paper, Bian et al. reported an additional Chinese family carrying a novel mutation c.3057insG (p.Lys1020Glufs*43) that is identical to the mutation identified in our family (Table 3) [15]. So far, published mutations in *NEFH* result in a frameshift leading to a stop and translation of a cryptic amyloidogenic element encoded by the 3′ UTR [11]. Jacquier et al. reported mutant *NEFH* protein to cause insoluble perinuclear protein aggregation, so-called aggresomes, in transfected human neuroblastoma cells and primary motoneurons, addressing the autophagic pathway and triggering caspase-3 activation and apoptosis *in vitro*. Using electroporation of chick embryo spinal cord, they also found that mutant *NEFH* proteins form aggresomes and trigger apoptosis of spinal cord neurons *in vivo* [13]. Interestingly, protein aggregates and dysregulated autophagy contribute to several neuromuscular diseases such as motoneuron and muscle disorders [16–18]. Aggregation of *NEFH* seems to be a distinctly cytotoxic event with harmful effects on motor neurons [11]. Finding a way to minimize or dissolve these aggregates could be a rational approach for the treatment of affected patients.

The frequent duplication/deletion events within the neurofilament heavy gene in close proximity to each other and in patients without ethnic predisposition might be caused by a hairpin structure of the partial palindromic DNA sequence [12].

Clinical features of CMT2CC include primarily distal, later also proximal muscle weakness and muscle wasting, predominantly affecting the lower limbs; Table 3 gives an overview about the reported patients in the literature. Disease onset varies between 3 and 43 years, with a median of 24 years. In the majority of patients, the weakness progresses to the proximal muscles, predominantly the iliopsoas within the 3rd decade causing ambulation difficulties with loss of ambulation around the 7th decade. The upper limbs are usually less affected with distal predominance. Mild sensory deficits and absent deep tendon reflexes are also common, but not clinically meaningful for the patients. Clubfeet and cramps are consistent features.

In addition to classic CMT features, some patients, including ours, were also reported to have increased serum creatine kinase, changes reminiscent of myopathy in electromyographic studies as well as pyramidal signs. [11] The predominance of muscle weakness and muscular

Table 3
Summary of characteristics of CMT2CC Patients. M = male. F = female. Y = years. UL = upper limb. LL = lower limb. (a) Muscle wasting: - none; + below wrist or ankle; ++ below elbow or knee; +++ above elbow or knee. (b) Muscle weakness: - none; + >4 distal muscle groups (first dorsal interosseous, abductor pollicis brevis, ankle dorsiflexion, plantar flexion, or below); ++ <4 distal muscles; and +++ proximal weakness (knee flexion and extension, elbow flexion and extension, or above). (c) Reflexes: - absent; +normal; ↑ increased/brisk; ↓ decreased. (d) Sensory examination: - normal; + mild deficit; ++ moderate deficit; +++ severe deficit.

Family/ Patient (Sex, Age)	*Age at onset (y)*	*Initial symptoms*	*Ambulation (age)*	*Muscle wasting*[a]	*Muscle weakness*[b]	*Reflexes*[c]	*Sensory involvement*[d]	*Additional signs*	*NFH mutation*
1/I.1 (M,80)	NA	NA	Walking support (50 s) Wheelchair bound (70 s)	NA	NA	NA	NA	Hearing loss (50 s)	c.3010_3011delGA p.Asp1004Glnfs*58[11]
1/II.1 (F,79)	20	Unable to stand on tiptoes	Walking support (50 s) Wheelchair bound (70 s)	UL+ LL+++	UL++ LL+++	UL− LL−	UL+LL +++	Hearing loss (70 s)	
1/III.2 (M,57)	15	Shoes with ankle support	Tripping (40) Walking support (53)	UL+LL++	UL+ LL+	UL+LL −	UL+LL ++	Cramps	
1/IV.1 (M,26)	≈3	Frequent falling	Walking support (20 s)	UL+ LL++	UL+ LL+++	UL+LL−	UL+LL ++	High arches, tone ↑ LL, cramps, stiff gait, Gower's maneuver	
2/III.1 (M,56)	38	Walking difficulties	Without help	UL − LL +	UL− LL+	UL↓ LL↓	UL− LL+	Clubfeet	c.3017-3020dup p.Pro1008Alafs*56[11]
3/II.1 (F, 70)	40	Walking difficulties	Bedridden	NA	UL++ LL+++	LL↑	UL+ LL+		c.3008_3009del p.Lys1003Argfs*59[12]
3/II.5 (M,68)	38	Walking difficulties	Without help	NA	UL+LL ++	UL− LL −	UL− LL+		
3/III.2 (F,55)	30	Walking difficulties	Without help	NA	UL− LL+++	UL+ LL↓	UL− LL+		
3/III.3 (F,49)	27	Difficulties climbing stairs	Wheelchair for longer distances	UL+LL++	UL+ LL+++	UL+ LL+	UL − LL +	Gower's maneuver clubfeet, hypophonia	
3/III.4 (M,50)	30	Walking difficulties	Without help	NA	UL+ LL++	UL+ LL+	UL− LL+		
3/III.5 (M,49)	43	Walking difficulties	Without help	NA	UL+ LL ++	UL+ LL−	UL− LL+		
3/III.6 (F,46)	40	Walking difficulties	Without help	NA	UL − LL++	UL+ LL−	UL− LL−		
3/III.7 (F,44)	34	Walking difficulties	With one cane	NA	UL − LL+++	UL+ LL↑	UL− LL +		
3/IV.1 (M,23)	15	Running difficulties	Without help	NA	UL − LL+	UL↑ LL↑	UL− LL−		
3/IV.5 (F/23)		None	Without help	–	UL− LL−	UL+ LL−	UL− LL−		
3/IV.6 (M,17)		None	Without help	–	UL− LL−	UL+ LL−	UL− LL−		
4/II.1 (M,23)	5	Walking difficulties	Without help	NA	Ul+ LL+++	UL− LL−	UL− LL+	Gastroparesis, clubfeet, pectum excavatum, scapular winging Cramps	c.3043_3044del p.Lys1015Glyfs*47[12]
5/II.2 (F/32)	12	Problems standing on toes	With one cane (20 s)	UL+ LL +++	UL+ LL+++	UL− LL−	++	Cramps, Gower's maneuver	c.3015_3027 dup p.Lys1010GInfs*57[13]
5/III.1 (F/6)		None	Without help	UL − LL−	UL− LL−	UL+ LL↓	+	Cramps	
6/I.1 (M,72)	27	NA	Wheelchair bound	UL+LL+++	UL+LL++	UL − LL−	UL − LL−	Clubfeet	c.3057insG p.Lys1020Glufs*43[14]
6/II.1 (F,49)	28	NA	Without help	UL − LL+	UL − LL+	Ul+ LL↓	UL − LL−	Cramps/ fatigability Clubfeet	
6/II.3 (F,46)	27	NA	Without help	UL − LL+	UL − LL+	UL+ LL↓	UL − LL−	Cramps/fatigability Clubfeet	
6/II.5 (F,45)	25	NA	Without help	UL+LL++	UL+LL+	UL− LL−	UL − LL−	Cramps/fatigability Clubfeet	
6/II.8 (F,40)	25	NA	Without help	UL+LL++	UL − LL+	UL − LL−	UL − LL−	Cramps/fatigability Clubfeet	
6/III.3 (M,20)	20	None	Without help	UL − LL+	UL − LL+	UL+ LL↓	UL − LL−	Clubfeet	
6/III.4 (M,14)	10	NA	Without help	UL − LL+	UL − LL+	UL ↓ LL−	UL − LL−	Cramps/fatigability Clubfeet	
7/II.8 (M/74)	*16*	*Weakness of lower limbs*	*Wheelchair bound (60 s)*	*UL++ LL+++*	*UL+ LL+++*	*UL↓ LL↓*	*UL+ LL++*		*[c.3057dupG] p.Lys1020Glufs*43*
7/III.1 (M,35)	*8*	*Running difficulties*	*Without help*	*UL − LL+++*	*UL− LL+++*	*UL− LL−*	*UL+ LL+*	*Clubfeet*	

atrophy in patient II.8 led to the misdiagnosis of spinal muscular atrophy, while the clinical phenotype of patient III.1, the electrophysiological changes also frequently seen in myopathy like short and polyphasic motor unit potentials as well as complex repetitive discharges in the electromyography and elevated CK-levels prompted us to initially study different genes associated with muscular dystrophy. Rebelo et al. also found changes in the electromyography that prompted towards

a myopathic disorder. [11] CK levels also were reported to be elevated ranging from 424 to 1288 IU/L by other groups [11,14]. The Chinese family carrying the same mutation as identified in our pedigree seems to be generally less severely affected in comparison to our observation and the other reported cases. However concordant to our results the clinical features presenting with muscle weakness and atrophy also prompted towards a muscular disease since no sensory deficits were reported. This highlights the importance of an elaborate examination including a precise electrophysiological study, that in CMT2CC reveals a symmetrical, sensorimotor axonal neuropathy, frequently before clinical symptoms are even noted.

Other authors suggested a pattern of anticipation, given that symptoms and onset appear to be more severe and earlier from one generation to another [11,13]. Our pedigree supports the hypothesis of anticipation, as the weakness was more pronounced in the son (III:1) compared to the father (II:8) at the same age. However, the descendants might be more alert to the development of symptoms, so this may have contributed to the earlier diagnosis.

5. Conclusion

Here we report of the first Austrian pedigree with autosomal dominant trait diagnosed with CMT2CC due to a mutation in *NEFH* c.3057dupG (p.Lys1020Glufs*43). The father of our patient had been misdiagnosed with spinal muscular atrophy, the electrophysiological changes in our patient that can also frequently be seen in myopathy like short and polyphasic motor unit potentials as well as complex repetitive discharges and the elevated CK-levels prompted us to initially study different genes associated with muscular dystrophy; therefore, the clinical spectrum can range from mimicking motoneuron or myopathy phenotypes. Our observation further expands the phenotypic spectrum of *NEFH* mutations and indicates that *NEFH* mutations should be considered in the differential diagnosis of patients presenting with axonal neuropathy, gait disturbances, muscular weakness and muscle wasting predominantly affecting the lower limbs along with proximal muscle involvement.

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