



Case report

Novel EGR2 mutation R359Q is associated with CMT type 1 and progressive scoliosis

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Abstract

Mutations in the early growth response 2 gene (EGR2) cause demyelinating neuropathies differing in severity and age of onset. We tested 46 unrelated Czech patients with dominant or sporadic demyelinating CMT neuropathy for mutations in the EGR2 gene. One novel de-novo mutation (Arg359Gln, R359Q) was identified in heterozygous state in a patient with a typical CMT1 phenotype, progressive moderate thoracolumbar scoliosis and without clinical signs of cranial nerve dysfunction. This patient is presently less affected compared to previously described Dejerine–Sottas neuropathy (DSN) patients carrying another substitution at codon 359 (Arg359Trp, R359W). This report shows that EGR2 mutations are rare in Czech patients with demyelinating type of CMT and suggests that different substitutions at codon 359 of EGR2 can cause significantly different phenotypes.

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1. Introduction

Hereditary motor and sensory neuropathies (HMSN) or Charcot–Marie–Tooth (CMT) diseases have been traditionally classified into more frequent demyelinating (CMT1 or HMSN I) and less frequent axonal (CMT2 or HMSN II) subtypes [1].

Mutations in the early growth response 2 gene (EGR2) were reported to be rare causes of demyelinating HMSN including congenital hypomyelinating neuropathy (CHN), Dejerine–Sottas neuropathy (DSN) and demyelinating type of Charcot–Marie–Tooth disease—type 1 (CMT1) [1,2]. CMT1 caused by EGR2 mutations has been classified as CMT1D [1]. Only nine different pathogenic mutations and one silent mutation (polymorphism) have been reported in the EGR2 gene (<http://www.molgen.ua.ac.be/CMTMutations/>).

Almost all the reported mutations are autosomal dominant or de novo heterozygous mutations, while only one was described to cause recessive CHN in homozygosis in a consanguineous family [2].

EGR2 encodes a zinc finger transcription factor [3] controlling early myelination of peripheral nerves. EGR2 is activated in the Schwann cells before the onset of myelination and its disruption blocks Schwann cells at an early stage of their differentiation [4].

The aim of this study was to establish the frequency of EGR2 mutations among Czech patients with demyelinating type of CMT and to extend our knowledge of CMT phenotype caused by EGR2 mutations.

2. Patients and methods

2.1. Patients

The study included 46 unrelated patients with demyelinating type of CMT. All were initially tested negative for the CMT1A duplication/HNPP deletion using a set of

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13 microsatellite markers [5]. Mutations in the Cx32, MPZ, PMP22, SIMPLE and GDAP1 genes were excluded in some of the tested patients. Our selection criteria were: median motor NCV ≤ 40 m/s, dominant or sporadic occurrence of CMT in the family and age of onset before the age of 50.

2.2. Sequencing analysis

In all 46 patients, bidirectional direct sequencing of both coding exons of the EGR2 and flanking intron sequences was performed. Nine overlapping fragments were amplified by PCR using published primers [6]. PCR products were directly sequenced using the Big Dye Terminator V3.1 Kit and analyzed on the ABI 3730 sequencer (Applied Biosystems, USA). The sequences were evaluated using SeqMan software (DNASTAR Inc., USA).

2.3. Restriction mutation analysis

Restriction enzyme BceAI (BioLabs Inc., USA) was used to test DNA samples of healthy controls for the occurrence of the Arg359Gln mutation.

2.4. Neurophysiology

All used neurophysiologic measurements performed according to standard procedures.

3. Results

3.1. DNA testing

We found one novel missense heterozygous mutation, c.1076G > A, predicting an aminoacid exchange Arg359Gln (R359Q). EGR2 mutation was detected in one of the 46 patients, thus representing approximately 2% of patients with non-duplicated demyelinating CMT.

The Arg359Gln mutation was not found in the parents and in the older sister of the patient who did not show any signs of peripheral neuropathy and have normal results in nerve conduction studies. Correct parentage of both parents was confirmed using a set of 10 microsatellite markers. This indicates that the mutation Arg359Gln originated de-novo.

Furthermore, the mutation was absent in 138 control chromosomes tested by restriction analysis with BceAI indicating that this variant is not a common polymorphism.

The previously described polymorphism c.1086A > C (Arg362Arg, R362R) [6–8] was additionally detected in heterozygous state in two of the 46 tested patients.

3.2. Clinical and electrophysiological features of the patient carrying the Arg359Gln mutation

The patient is now 17-years-old. She is the second child of healthy unrelated parents. Birth weight, postpartum

adaptation and early motor and intellectual development were all normal. No physical handicap was noticed until the age of 12.

At age 12.5, the patient showed recurrent ankle sprains, at age 13 she noticed an episode of weakness in her legs during sprinting and in the same period the family reported the first signs of gait disorder (shuffling type of gait). Bilateral pes cavus with hammer toe deformities and thoracolumbar scoliosis were detected on orthopedic examination. The progression of the gait disorder, the foot deformities and the scoliosis was fast in the first year after the onset of the symptoms, but then slowed down. Diagnosis of HMSN I was established at age 13 after detection of severe nerve conduction slowing in nerve conduction studies.

Neurological examination at age 17 showed no clinical signs of cranial nerve involvement. Muscle atrophies are mild and apparent only in the feet. We observed bilateral distal hypesthesia and mild distal muscle weakness of wrist flexors (4/5) and of extensor hallucis longus (4/5). The patient has bilateral pes cavus with restriction of maximum active (105°) and passive (90°) foot dorsiflexion (Fig. 1). She is unable to stand and walk on heels and has no evident signs of ataxia. Deep tendon reflexes were absent in all four extremities. She has moderate progressive thoracolumbar scoliosis with a right thoracic curve of 36° and a left lumbar curve of 24°. The lumbar curve progressed at 8° per past 18 months (Fig. 2). The patient has no respiratory problems at this moment.

Nerve conduction studies at age 17 showed overall slowing of motor and sensory nerve conduction velocities (MNCV, SNCV) (Table 1). The amplitudes of compound



Fig. 1. Lower limbs of the 17-years-old patient carrying the Arg359Gln mutation in EGR2. The picture shows bilateral pes cavus with hammer toes and minimal muscle atrophies. At age 15, varus of the left calcaneus was corrected surgically (op. sec. Steindler and Dwyer). The surgery improved the gait pattern and, therefore, a correction of the right foot deformity is planned.

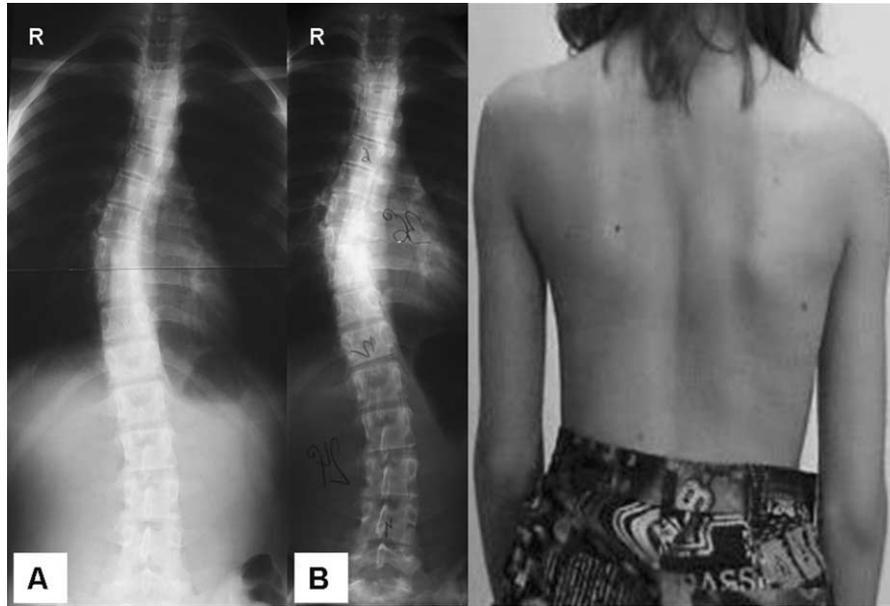


Fig. 2. AP radiographs and photograph of thoracolumbar scoliosis in the patient carrying the Arg359Gln mutation in EGR2. At age 15.5, she had a left lumbar curve of 16° (image A) and at the time of presentation (17 years), the lumbar deformity had progressed to 24° (image B). The thoracic curve of 36° did not show any progression in this period.

muscle action potentials and sensory nerve action potentials were at the lower limit of normal range in the upper limbs and significantly decreased or completely absent in the lower limbs (Table 1). Examination of the blink reflex showed demyelinating lesion of facial and trigeminal nerves. The wave latencies of the brain stem auditory evoked potentials (BAEP) and the visual evoked potentials (VEP) were normal. The CSF protein concentration was normal (310 mg/l).

4. Discussion

We show that mutations in EGR2 are very rare among Czech patients with demyelinating type of CMT. We examined 46 patients and found one novel missense mutation Arg359Gln. The number of EGR2 mutations found in our screening is similar to previous findings [2,6–9].

The age of onset and clinical phenotype in our patient carrying the Arg359Gln mutation is most compatible with

Table 1

Nerve conduction study in the patient carrying the R359Q mutation in EGR2 showing motor and sensitive primary demyelinating lesion

			Normal	Patient
Motor nerves	Median nerve	DML (ms)	≤4.0	8.9
		MNCV (m/s)	≥51	13
		Amplitude (mV)	≥4.2	5.2
		F-wave (ms)	≤30	78.4
	Ulnar nerve	DML (ms)	≤2.8	6.5
		MNCV (m/s)	≥50	11
		Amplitude (mV)	≥5.5	5.5
		F-wave (ms)	≤30	50.9
	Peroneal nerve (m. tibialis ant.)	DML (ms)	≤5.0	13.3
		MNCV (m/s)	≥39	16
		Amplitude (mV)	NA	2.5
	Tibial nerve	DML (ms)	≤5.5	14.3
MNCV (m/s)		≥40	9	
Amplitude (mV)		≥2.5	0.1	
Sensory nerves	Median nerve	SNCV (m/s)	≥48	16
		Amplitude (uV)	≥10	5.0
	Ulnar nerve	SNCV (m/s)	≥48	19
		Amplitude (uV)	≥10	12
	Sural nerve	SNCV (m/s)	≥38	Not recordable
		Amplitude (uV)	≥4.4	Not recordable

DML, distal motor latency; MNCV, motor nerve conduction velocity; SNCV, sensory nerve conduction velocity; NA, not available. Pathologic values are bold.

the classical CMT1 phenotype. The patient presented first signs of CMT1 and thoracolumbar scoliosis in her early teens. She shows marked slowing of nerve conduction velocities (NCVs), bilateral pes cavus with hammer toes deformities and very mild muscle atrophies. She shows no clinical signs of cranial nerve involvement.

In comparison with phenotypes caused by other EGR2 mutations, the age of onset and clinical manifestation in our patient resembles most closely to CMT1 phenotypes associated with the Asp355Val (sporadic case) and Arg409Trp (dominant CMT1 family) heterozygous substitutions in EGR2 [10,2]. Carriers of these mutations had very similar age of onset of CMT1 as our patient, but NCVs in our patient seem to be even more markedly reduced. Other two mutations in EGR2, both at codon 381, have been associated with CMT1 phenotype, but their phenotypes differ notably from the phenotype in our patient [11,12]. The carrier of the Arg381Cys heterozygous mutation had NCVs comparable with our patient but his age of onset of CMT was very late (59 years) [11]. The second mutation, Arg381His, was reported in heterozygous state in a family affected by severe CMT1 with a unique combination of cranial nerve deficits in one member [12]. It must be emphasized that another mutation at codon 359 of EGR2 (Arg359Trp, R359W) has been previously described in four patients with Dejerine–Sottas neuropathy [6,13,14]. They developed the first symptoms very early, their MNCV were below 8 m/s or undetectable and two of them died at a young age of respiratory failure. In three of them, clinical cranial nerve involvement was reported [6,13] and one of them developed severe thoracolumbar scoliosis [13]. Phenotype in our patient is undoubtedly milder. The Arg359Trp mutation was thought to consistently cause DSN, however, a recent study reports on two Arg359Trp carriers from a dominant CMT1 family [8,15]. Clinical findings in the patient described here further extend the knowledge of the phenotypic spectrum caused by EGR2 mutations.

The arginine residue 359 in EGR2 plays an essential role for DNA binding recognition and specificity [16] and the Arg359Trp mutation decreases DNA binding activity of EGR2 [17]. Different substitutions at codon 359 of EGR2 were found in patients with significantly different severity and age of onset indicating that the change to glutamine at codon 359 in our patient has probably less deteriorating effect on the EGR2 protein DNA binding than the change to tryptophan at this codon detected in DSN patients. Similarly at the codon 381 of EGR2, the phenotype of the Arg381Cys carrier was milder than that of the Arg381His carriers [7,11,12]. Multiple reports on changes at adjacent bases of codon 359 of EGR2 may reflect the presence of a mutation hot spot within EGR2 at this site of a CpG dinucleotide [13].

Mutations in EGR2 seem to occur frequently de novo and, therefore, patients with demyelinating type of CMT with sporadic occurrence should be included for EGR2 testing, although the probability of finding a causal mutation even in clinically carefully characterized cohort of patients seems to be generally very low.

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