# A 71-nucleotide deletion in the periaxin gene in a Romani patient with early-onset slowly progressive demyelinating CMT

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*Background:* Mutations in the periaxin (*PRX*) gene cause autosomal recessive demyelinating neuropathy Charcot–Marie–Tooth (CMT) type 4F. To date, 10 non-sense or frameshift *PRX* mutations have been reported in patients with early-onset neuropathy and further disease course consistent with either Dejerine–Sottas neuropathy or slow-progressive demyelinating CMT. *Methods:* We sequenced 59 patients from 55 Czech families including four unrelated patients of Romani (Gypsy) origin with early-onset CMT displaying decreased nerve conduction velocities. *Results:* We identified a novel homozygous mutation c.3286_3356del71 (K1095fsX18) in one Romani patient showing very slow disease progression. Amongst non-Romani Czech CMT patients, *PRX* mutations have been proven to be very rare.

## Introduction

Inherited neuropathy Charcot–Marie–Tooth (CMT) is characterized by slowly progressive distal muscle weakness and atrophy, sensory loss and absence of deep tendon reflexes. Based on electrophysiological examination, the demyelinating CMT1 and axonal CMT2 types are distinguished. CMT1 is characterized by the median nerve motor conduction velocity (MCV) reduced below 38 m/s. In CMT2, nerve conduction velocities (NCV) are normal or slightly reduced [1,2]. CMT1 and CMT2 are further subclassified according to inheritance patterns and to the underlying molecular genetic cause [3].

Charcot–Marie–Tooth type 4F neuropathy (CMT4F) is caused by mutations in the periaxin (*PRX*) gene mapped on 19q13 [4,5]. So far, 10 different frameshift or non-sense *PRX* mutations were reported to cause autosomal recessive (AR) early-onset demyelinating neuropathy [6]. It develops further in the course either as severe Dejerine–Sottas syndrome [7,8] or demyelinating CMT with slow or no progression [8–10].

*PRX* encodes two PDZ-domain proteins, L-periaxin and S-periaxin, that are expressed in myelinating Schwann cells [11]. In the mature myelin, L-periaxin links the basal lamina to the cytoskeleton and stabilizes the myelin sheath [12–14]. The protein has four characteristic domains: PDZ, nuclear localization signal, repeat and acidic domains [13–15]. Loss of the acidic domain is shared by all pathogenic mutations in the gene reported so far and presumably plays a role in the disease pathogenesis [7–10,16].

We give results of a screening study of *PRX* mutations in Czech CMT patients including Romani (Gypsy) families. We report a novel homozygous mutation c.3286_3356del71 (K1095fsX18) in a patient of Romani origin with early-onset slowly progressive demyelinating CMT.

## Patients and methods

Fifty-nine patients from 55 Czech families with demyelinating CMT (median nerve MCV < 38 m/s) were screened for mutations in *PRX*. The patients were either affected siblings born to healthy parents based on history (eight families) or isolated cases (47 families). There was one adopted child in the cohort without precise information on the clinical status of his biological parents and siblings. Three families and the adopted child had Romani origin. Consanguinity was reported for one of the Romani families. All patients presented the first neuropathy symptoms by the end of the second decade. Patients were previously tested negative for CMT1A duplication and most of them also for mutations in the *CX32*, *MPZ*, *PMP22*, *EGR2*, *NEFL* and *SIMPLE* genes.

All four coding exons and intron–exon boundaries of *PRX* were PCR amplified (primer sequences are available on request). PCR products were directly sequenced using the BigDye Terminator v3.1 kit and analyzed on the ABI 3100 and ABI 3730 capillary sequencers (Applied Biosystems, Foster City, CA, USA). Ninety-four

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unrelated Romani subjects without neuromuscular disease history were screened for c.3286_3356del71 mutation.

Clinical and electrophysiological evaluations of a patient with *PRX* mutation were performed.

All tested individuals signed an informed consent and the study was approved by the Central Ethical Committee of the University Hospital Motol in Prague.

## Results

One patient was homozygous for an extensive deletion including 71 base pairs between positions 3286 and 3356 in the last exon 7 of *PRX* (Fig. 1). The mutation causes a frameshift after amino acid lysine at codon 1095 and terminates the protein at codon 1113. Parental DNA was not available. The mutation was not detected in 94 healthy unrelated Romani controls.

The patient is an adopted child of Romani (Gypsy) origin (Fig. 2). We lack closer information on his biological family except that his mother is reportedly unaffected. At the age of 3 months, the boy showed muscular hypotonia and delayed motor milestones. He walked without support at 21 months. The gait was unsteady, broad-based with frequent falls. At age 2, he developed mild bilateral pes cavus. Nerve conduction studies (NCS) performed at age 3 showed unelicitable action potentials at the lower limb nerves and the upper limb sensory nerves and severely reduced NCV and compound muscle action potentials (CMAP) at the ulnar and median nerves (Table 1). On the basis of the early hypotonia, delayed walking acquisition and slow NCVs, the diagnosis of CMT was established in the patient. At age 4, following tonsillitis, he suffered from 3-day myalgia of the calves. Laboratory work-up showed elevated blood myoglobin 315.8 ng/ml. The episode was evaluated as parainfectious myositis. At age 5, the patient underwent surgical release of the plantar aponeurosis. Neurological examination revealed normal findings on cranial nerves, normal hand muscle bulk and dexterity, mild bilateral pes cavus, normal distal lower limb strength, no limb ataxia, absent deep tendon reflexes, normal vibration and touch sensation and mildly unsteady gait. The NCS showed similar findings to the previous examination (Table 1). Neither cerebrospinal fluid studies nor MRI of CNS were performed.

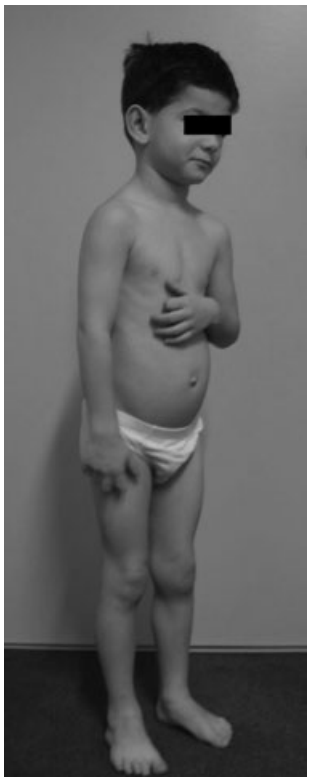

**Figure 2** Patient carrying the *PRX* mutation K1095fsX18.

## Discussion

We identified a novel frameshift *PRX* mutation in one Romani patient with demyelinating CMT. As in most

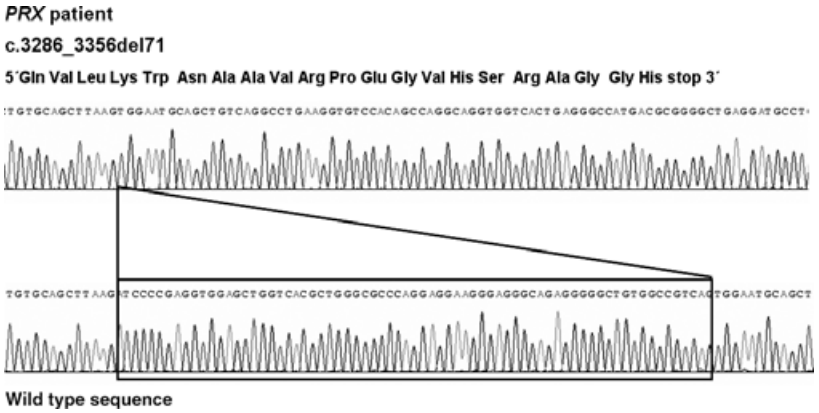


**Figure 1** Chromatogram of the homozygous mutation c.3286_3356del71 (K1095fsX18) in *PRX*.

 

**Table 1** Summary of electrophysiological findings in the patient carrying the K1095fsX18 mutation in *PRX*

| Age (years) | 3 | 5 |
|---|---|---|
| *Motor nerves* | | |
| Tibial nerve | NR | NR |
| Median nerve | | |
| DML (ms) | 24.8 | 11 |
| MNCV (m/s) | 19 | 17 |
| CMAP (mV) | | |
| Wrist | 0.05 | 0.6 |
| Elbow | 0.5 | 0.8 |
| | Temporal dispersion | |
| F-wave | ND | ND |
| Ulnar nerve | | |
| DML (ms) | 19.1 | 23 |
| MNCV (m/s) | [a] | 23 |
| CMAP (mV) | | |
| Wrist | 0.4 | 0.05 |
| Elbow | 0.2 | 0.1 |
| | Temporal dispersion | |
| F-wave | ND | ND |
| *Sensory nerves* | | |
| Sural nerve | NR | ND |
| Median nerve | NR | ND |
| Ulnar nerve | NR | ND |

DML, distal motor latency; MNCV, motor nerve conduction velocity; CMAP, compound muscle action potential; NR, not recordable; ND, not done.
[a]Not evaluated.

of the *PRX* mutations previously described, the c.3286_3356del71 (K1095fsX18) located in the 3′ terminal of the last exon most probably escapes from nonsense mediated decay [7–10]. It is the most downstream located mutation so far reported truncating L-periaxin inside the acidic domain just at its beginning. It predicts L-periaxin lacking the most part of the functional C-terminal acidic domain. Acidic domains are generally known to mediate protein–protein interactions and the L-periaxin lacking this motif may not be able to bind to the cytoskeleton of Schwann cell and to form stable myelin. The c.3286_3356del71 thus broadens the spectrum of *PRX* loss-of-function mutations.

Considering the single ethnic origin of the parents, we assume the homozygote state and AR effect of the mutation in the patient.

Consistent with previous reports, the disease in our *PRX* patient had early onset with delayed motor milestones. The last neurological examination at age 5, except for mild bilateral pes cavus, absent deep tendon reflexes and unsteady gait, was otherwise unremarkable. Further disease progression up to now is, therefore, consistent rather with the slow-progressive disease course [8–10]. Unlike in most *PRX* patients, vibration, touch and pinprick sensation was not impaired in the patient possibly due to its involvement later in the course in CMT4F patients (second to sixth decade) [8–10]. NCS performed at early age showed severely reduced NCVs. Discrepancy between very low median and ulnar nerve CMAPs and the hand muscle strength that is utterly normal is probably due to marked temporal dispersion.

The c.3286_3356del71 (K1095fsX18) is the first *PRX* mutation reported in the Romani (Gypsy) population, suggesting testing the gene in Romani patients with demyelinating type of CMT. We identified no *PRX* mutations amongst the remaining 51 isolated cases or AR families, indicating their rare occurrence in the Czech population of non-Romani origin.

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