

Research letter

Palmoplantar keratoderma and Charcot–Marie–Tooth disease: combination of two independent genetic diseases? Identification of two point mutations in the *MPZ* and *KRT1* genes by whole-exome sequencing

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DEAR EDITOR, Charcot–Marie–Tooth disease intermediate (CMT) and palmoplantar keratoderma (PPK) are two different diseases that have been described as being associated.¹ Charcot–Marie–Tooth hereditary neuropathy type 2 (CMT2) is an axonal (nonmyelinating) peripheral neuropathy characterized by distal muscle weakness and atrophy, mild sensory loss, and normal or near-normal nerve conduction velocities. PPK represents a group of disorders characterized by thickening of the skin on the palms of the hands and soles of the feet of affected individuals.^{2,3}

A clinical condition in which these two different diseases define an interesting complex phenotype (OMIM 148360) has been described. So far only two families have been identified, six members in the family reported by Rabbiosi *et al.*¹ and two patients showing a double phenotype in the family described by Powell *et al.*⁴ In fact, in 1983 and 1988, a similar complex phenotype – punctate keratoderma and spastic paralysis – was defined with autosomal dominant inheritance.^{4,5}

Herein we describe an Italian family, from the Sardinia region, showing a complex phenotype characterized by the association of palmoplantar keratoderma of Thost–Unna and late-onset motor and sensory polyneuropathy cosegregating in all but two family members.

In all affected individuals, PPK manifested in early childhood, between 3 and 5 years of age (Fig. 1). After 5 years of disease, all affected family members showed a marked involvement of the distal lower-limb muscles, with calf hypotrophy, inability of dorsiflexion of the toes and feet, and marked weakness in plantar flexion of the feet and toes. There was also associated mild proximal weakness of the iliopsoas and gluteus muscles. The upper limbs were involved later in the course of the disease, with weakness of intrinsic muscles of the hands and marked interosseous muscle hypotrophy, more evident at the first dorsal interosseous muscle. Associated sensitive disturbances were moderate hypoesthesia with stocking and glove distribution, loss of the sense of vibration and deep tendon reflexes, paraesthesias and pain. Walking was characterized by foot drop with steppage gait and sensitive ataxia. Electroneurography (ENG) showed a symmetrical

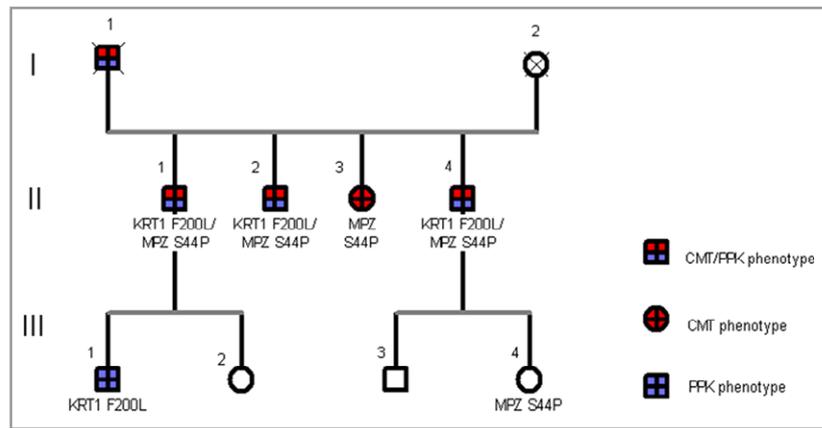
motor and sensory polyneuropathy with nerve conduction parameters within the CMT2 range, affecting primarily the motor fibres.

Patient II.3 did not have PPK (Fig. 2). She exhibited gait disturbance at the age of 41 years and was the only wheelchair-bound individual of the family. At 53 years of age she manifested epileptic seizures, treated with levetiracetam administration. Brain magnetic resonance imaging, tilt table tests and electrocardiogram were all normal. Comparative genomic hybridization array did not show chromosomal imbalances. Patient III.1 had PPK but not neuropathy. Neurological examination and ENG recordings at the age of 37 years were normal. Individuals III.1, III.2 and III.4 had neither PPK nor neuropathy (Fig. 2).



Fig 1. Clinical picture of cutaneous lesions in palmoplantar keratoderma.

Fig 2. Detection of MPZ and KRT1 mutations in the Italian family with Charcot–Marie–Tooth hereditary neuropathy type 2 (CMT) and palmoplantar keratoderma (PPK). Filled symbols represent affected individuals and open symbols nonaffected individuals. Diagonal slashes indicate that the person is deceased. The presence of the MPZ mutation is reported in red, while the KRT1 mutation is indicated in blue. The members affected by both diseases are shown in blue/red.



DNA libraries were constructed using the Illumina TruSeq DNA Sample Preparation Kit (Illumina, San Diego, CA, U.S.A.). Sequencing was performed with an Illumina HiSeq 1000 Sequencer. The genetic variants were confirmed by Sanger sequencing. Raw reads were filtered by removing low-quality reads, and filtered reads were mapped to the human reference genome (build hg19) using Burrows–Wheeler Aligner 0.6.2-r126. Multisample variant calling was performed using the Unified-Genotyper module in GATK with the -glm BOTH parameter set (Broad Institute, Cambridge, MA, U.S.A.). The annotated VCF files were then filtered using GATK VariantFiltration, and pedigree analysis were performed using SNP and Variation Suite (SVS) 7.7.5 (Golden Helix, Bozeman, MT, U.S.A.).

We sequenced the exomes of five individuals: one with both PPK and CMT2 (II-2), one with CMT2 (II-3), one with PPK (III-1) and two who were healthy (III-2 and III-3). From exome analysis we identified a nonsynonymous mutation Ser44Phe in the myelin protein zero gene (MPZ), responsible for CMT2 clinical signs, and a new mutation Phe200Leu in the keratin 1 gene (KRT1), which is causative of the PPK phenotype. Both genes, MPZ and KRT1, are autosomal dominant.

Genetic analysis by direct sequencing of all the members of the family showed the presence of the mutation Ser44Phe in MPZ in patients with CMT2 (II-1, II-2, II-3, II-4); moreover, we detected a new mutation, Phe200Leu, in keratin 1 in patients affected by PPK (II-1, II-2, II-4, III-1). The patients with the CMT–PPK phenotype (II-1, II-2, II-4) were carriers of both mutations. These mutations were absent from healthy family members (III-2, III-3). Patient III-4 showed the presence of the MPZ Ser44Phe variant, although she is currently healthy. She is 33 years old, and we cannot exclude a CMT phenotype in the future.

The MPZ mutation Ser44Phe was described for the first time in 1993, associated with CMT1B. Moreover, MPZ mutations also have been linked to CMT1 and Dejerine–Sottas syndrome.^{6–8}

In particular, the mutation Ser44Phe has been associated with the classic CMT2 phenotype (CMT2I) in an Italian family.⁹

Regarding KRT1, mutations in this gene have been associated with PPK.¹⁰ The mutation Phe200Leu is undescribed. Different predicted programs, such as SIFT Pred (<http://sift.jcvi.org/>), Polyphen2 (<http://genetics.bwh.harvard.edu/pph2/>) and Mutation Taster (<http://www.mutationtaster.org/>), defined this mutation as damaging or disease causing. The mutant residue is smaller than the wild-type residue, which might cause a loss of external interactions. The differences in amino acid properties can disturb this region and its function; in fact the mutated residue is located in a domain that is important for the activity of the protein and is in contact with residues in another domain. The novel identified mutation was not found in 100 healthy Italian individuals.

In this present study we report a large Italian family affected by PPK and hereditary motor and sensory neuropathy (OMIM 148360), a pathology first described in 1980.¹ We found two different mutated genes responsible for the two main clinical signs. Exome analysis detected two missense mutations, one in KRT1 and one in MPZ. This paper demonstrates that next-generation sequencing may be fundamental to solving complex clinical cases, as in the family described here.

We characterized a complex phenotype that, so far, has been considered a specific unique disease, and instead we have demonstrated that there are two different pathologies not genetically associated. In fact, the clinical condition is the consequence of a combination of two heterozygous mutations in different genes, and not in a common mutant one.

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