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C19orf12 mutation leads to Karak pallido-pyramidal syndrome

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Abstract

Pallido-pyramidal syndromes combine dystonia with or without parkinsonism and spasticity as part of a mixed neurodegenerative disorder. Several causative genes have been shown to lead to pallido-pyramidal syndromes, including *FBXO7*, *ATP13A2*, *PLA2G6*, *PRKN* and *SPG11*. In particular, mutations in *PLA2G6* have been identified in patients with Karak syndrome, a neurodegenerative disorder that features ataxia, dystonia-parkinsonism, dementia and spasticity with neuroradiologic evidence of cerebellar atrophy and/or brain iron deposition. Some patients with phenotypic Karak syndrome do not have demonstrable mutations in *PLA2G6*. Using homozygosity mapping and direct sequencing in a multiplex consanguineous Saudi Arabian family with Karak syndrome, we identified a homozygous p.G53R mutation in C19orf12. Our findings expand the phenotypic spectrum associated with *C19orf12* mutations.

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Introduction

In 2003, Mubaidin and colleagues reported a pallido-pyramidal syndrome that consisted of mixed ataxia, spasticity, and extrapyramidal features, which they designated “Karak syndrome” after the village the index patients hailed from[1]. Karak syndrome (MIM 610217) typically begins in school-age, and initially presents with ataxia. A mixed neurodegenerative course then results, with progressive dementia, dystonia and/or parkinsonism, and spasticity ensuing. Neuroimaging demonstrates cerebellar atrophy and hypointensity of the substantia nigra and globus pallidus on T2-weighted MRI sequences. The index family was ultimately found to harbor mutations in *PLA2G6*[2] but genetic heterogeneity has been suspected. We report the identification of mutations in *C19orf12* in a multiplex consanguineous Saudi kindred clinically characterized as *PLA2G6*-negative Karak syndrome.

Materials & Methods

Patients

The probands derived from a multiplex consanguineous Saudi pedigree (Figure 1).

Patient 1 (VII:5)—The patient was a male born at term after an unremarkable pregnancy. Early milestones were attained at appropriate ages, and the patient walked at age one. At four years of age, the patient developed an abnormal gait. A progressive motor decline ensued, leaving the patient reliant on a wheelchair for locomotion at age 17. Dementia became evident in late adolescence, with anxiety and phobias emerging at that time. Examination at age 20 disclosed persistent nystagmus on lateral gaze, distal muscle wasting of the upper and lower limbs (Figure 1), upper limb dystonia and pyramidal tract signs of the upper and lower limbs, with kyphoscoliosis of the cervical and thoracic spine, and flexion contractures of the knee joints, and pes equinovarus foot deformities bilaterally. Evoked motor potentials demonstrated slowed velocity and reduced amplitude. MRI demonstrated bilateral T2 hypointensity of the globus pallidus and substantia nigra.

Patient 2 (VII:7)—This patient is the brother of Patient 1, was also born at term after an unremarkable pregnancy. He walked at one year, and developed gait impairment at age 6 years. Gait progressively declined, and cognitive impairment was noted in adolescence. Examination at age 15 years revealed anxiety with self-injurious behaviors, insomnia, and slowed vertical saccades. Bradykinesia and pyramidal tract signs were evident, and bilateral equinovarus deformities were noted. Echocardiogram, lipid panel, and blood smear for acanthocytes were negative. Nerve conduction studies (done at the age of 15 years) showed normal motor conduction velocity (MCV) and distal motor latency (DML) of median, ulnar and tibial nerves. Compound motor action potential amplitudes (CMAPs) of median and ulnar nerves were normal, while that of tibial nerve was slightly reduced (2.3 mV). Sensory nerve action potential amplitudes (SNAPs) of median, ulnar and sural nerves were normal. Electromyography (EMG) revealed normal findings. Visual evoked potentials (VEP), electroretinography (ERG) and brain auditory evoked responses (BAER) revealed normal results. MRI exhibited T2 hypointensity of the globus pallidus and substantia nigra.

Patient 3 (VII:2)—This patient was also born at term, and walked at age 15 months. At age 9, he developed ataxia and cognitive impairment. At age 10, he developed spasticity of the lower limbs. At the time of most recent examination at age 16, the patient exhibited gait impairment, bradykinesia, and pyramidal tract signs. MRI disclosed evidence of cerebellar atrophy in addition to T2 hypointensity of the globus pallidus and substantia nigra (Figure 1).

Genotyping

Genomic DNA was extracted from blood using established methods. Sanger sequencing excluded pathogenic variants in *PANK2* and *PLA2G6* prior to genotyping. Primers were designed to span coding exons of each gene along with 10–20 bp of adjacent intronic sequences (sequences available upon request). Genotyping was performed on the Axiom platform following the manufacturer's instructions (Affymetrix, Santa Clara, CA). Homozygosity mapping was performed using autoSNPa as previously described[3]. While several runs of homozygosity were identified per patient, we focused on a large run of homozygosity on chromosome 19 found to be shared by the affected members of the family and absent in unaffected members (hg19 chr19: 28281401-39670046).

Results

Sequencing

Mutations in *C19orf12* have previously been shown to lead to a phenotype similar to that seen with *PLA2G6* mutation[4]. As *C19orf12* fell within the identified linkage interval, Sanger sequencing of the *C19orf12* gene was performed. This analysis identified a homozygous c. 157G>A, p.G53R (NM_001031726.2) mutation in all affected family members. The G53R mutation falls within the protein's putative transmembrane region as do several other reported pathogenic mutations (Figure 2).

In silico analysis

Although this sequence variant is listed as rs200133991 in dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/), the variant is predicted to be "deleterious" by SIFT (http://sift.jcvi.org) and "probably damaging" by PolyPhen2 (http://genetics.bwh.harvard.edu/pph2). The 1000Genomes database (http://www.1000genomes.org/) annotates the allele frequencies of the C (G) and T (A) nucleotides (YRI) as: C: 0.994, T: 0.006, indicating that this sequence variant represents a rare allele. In addition, c.157G>A has been previously reported as pathogenic in heterozygous form[5].

Short linear protein binding motifs (SLiMs) were predicted using SLiMPred[6], protein intrinsic disorder was predicted with IUPred[7] and three class protein secondary structure (Helix, Strand and Coil) was predicted by Distill[8]. Transmembrane regions were predicted using published algorithms[9–14]. *In silico* mutation modeling indicated that the sequence change would have little effect on secondary structure or short linear protein interacting motifs (Figure 3) suggesting that abnormal protein-lipid interactions may account for this mutation's pathogenicity, perhaps by impairing insertion within the mitochondrial

membrane. Consistent with such a paradigm, the G53R mutation is predicted to disrupt a glycine zipper motif crucial for membrane interaction (Figure 3)[15].

Discussion

We thus report a homozygous p.G53R mutation in *C19orf12*, a newly identified cause of neurodegeneration with brain iron accumulation (NBIA)[5] in a large multiplex Saudi family with pallido-pyramidal syndrome. Clinically, affected members of this family were considered to be affected with *PLA2G6*-negative Karak syndrome. A novel imaging finding not previously reported in *C19orf12*-associated NBIA seen in this family was cerebellar atrophy. In addition, all affected patients presented with ataxia that was later overshadowed by spasticity and dystonia-parkinsonism. There was no evidence of optic atrophy or peripheral neuropathy in the case (Patient 2) who had detailed neurophysiologic testing at the age of 15 years. Nevertheless, evoked motor potentials demonstrated slowed velocity and reduced amplitude in Patient 1 at the age of 20 years, suggesting that the distal muscle wasting found on examination (Figure 1) is due to a central axonopathy rather than peripheral nerve involvement.

Our findings indicate that mutations in *C19orf12* should be considered in the differential diagnosis of patients presenting with pallido-pyramidal syndromes. Unlike patients with mutations in *FBXO7, SPG11* and *PRKN*, who can also present with a pallido-pyramidal syndrome[16], patients with *C19orf12* mutations typically exhibit brain iron deposition in the globus pallidus and substantia nigra similar to many patients with *PLA2G6* mutations. Patients with *ATP13A2* typically also present with pallido-pyramidal syndrome, but only rarely demonstrate accumulation of brain iron[17]. *C19orf12* can thus be considered in cases of *PLA2G6*-negative Karak syndrome. Although Karak syndrome is classified as a form of NBIA, it is important to recognize that many patients with mutations in *PLA2G6* do not feature brain iron deposition, and it is not known whether iron deposition in the brain is an invariant feature of *C19orf12*-associated disease. Finally, it remains to be seen whether gene mutations that lead to similar clinical presentations will be found to intersect in common pathways at the molecular level.

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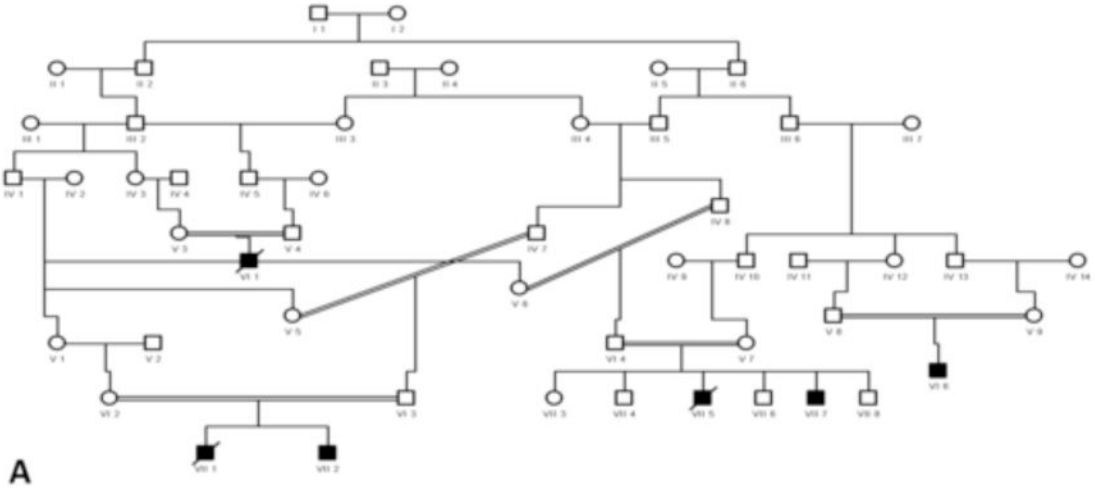
Research Highlights

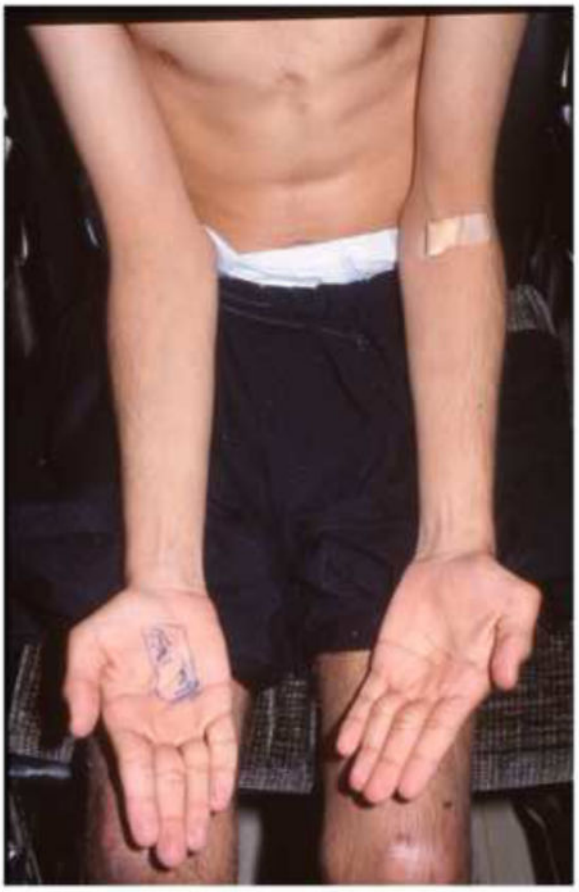
- The pallido-pyramidal syndrome is genetically heterogeneous
- We report a new family with pallido-pyramidal syndrome and mutations in *C19orf12*
- This mutation disrupts a glycine zipper motif crucial for protein-lipid interaction
- Mutations in C*19orf12* lead to a mixed movement disorder phenotype

Highlights

- Although mutations in *PLA2G6* have been shown to lead to Karak syndrome, an autosomal recessive pallido-pyramidal syndrome, the syndrome is genetically heterogeneous
- We report homozygosity mapping and candidate gene sequencing in a consanguineous family with Karak syndrome, leading to the identification of a homozygous p.G53R mutation in *C19orf12*
- This mutation is predicted to disrupt a glycine zipper motif crucial for protein-lipid interactions important for the normal function of this transmembrane protein
- Mutations in C*19orf12* can thus lead to a mixed movement disorder phenotype, combining spasticity, ataxia, dystonia, and parkinsonism with cerebellar atrophy on MRI



A



B

C

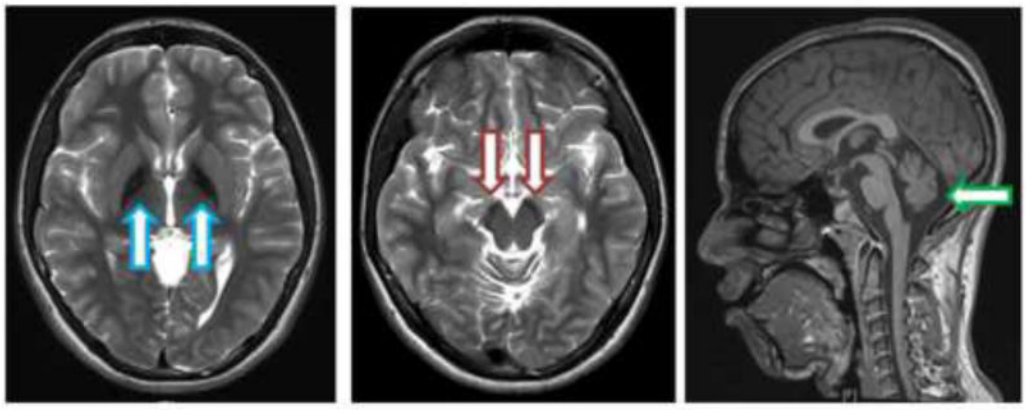


Figure 1. Index family

(A) Family pedigree. (B) Distal wasting of the upper limbs. Atrophy of the thenar and hypothenar muscles. (C) MRI features of affected individuals. MRI from patient 3 demonstrates T2 hypointensity of the globus pallidus, substantia nigra, and cerebellar atrophy consistent with Karak syndrome.

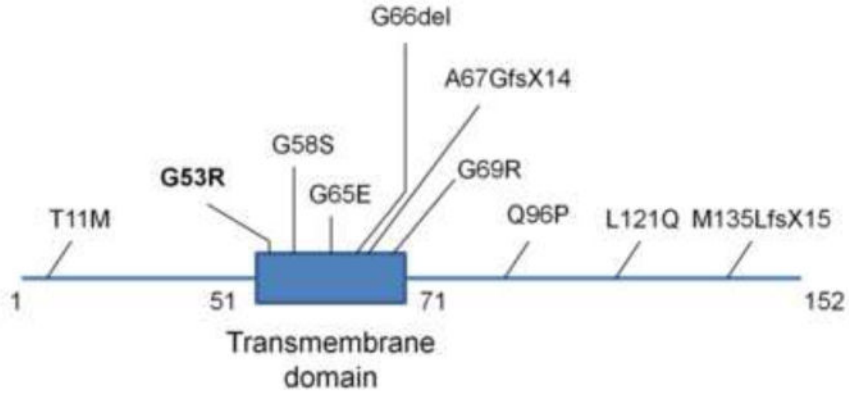


Figure 2. Catalog of *C19orf12* mutations

Shown are reported mutations[5,18–21], including the present one based on UniProtKB Q9NSK7. Mutations cluster around the putative transmembrane region.

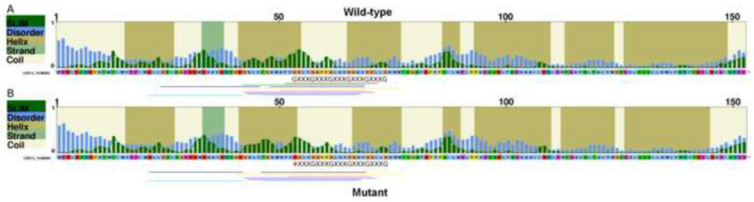


Figure 3. *In silico* analysis of the effect of p.G53R on protein binding regions, secondary structure, intrinsic disorder and transmembrane domain prediction

There is little change in the predicted secondary structure, protein disorder or short linear protein-binding motifs (SLiMs). Although some algorithms predicted no or small changes in the putative transmembrane (TM) region [MEMSAT-SVM/MEMSAT3 (magenta), PRO (cyan), PRODIV (blue), PolyPhobius (yellow), PHDhtm (orange), SMART/TMHMM (green), UniProt (red)], C19orf12 is annotated by Pfam as a glycine zipper-containing OmpA-like membrane domain. Glycine-zipper motifs (typically GxxxGxxxG repeats) strongly drive right-handed helix packing and mutations in the motif can block channel formation[15]. The p.G53R mutation disrupts this important structural motif, possibly disrupting TM region architecture (as shown by changes in TM region prediction).