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Tremor and Other Hyperkinetic Movements

Brief Reports

PLA2G6 Mutations Related to Distinct Phenotypes: A New Case with Early-onset Parkinsonism

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Abstract

Background: *PLA2G6*-associated neurodegeneration (PLAN) is a recessive neurodegenerative disorder characterized by three distinct phenotypes: infantile neuroaxonal dystrophy (INAD), atypical neuroaxonal dystrophy (atypical NAD), and *PLA2G6*-related dystonia–parkinsonism.

Methods: A consanguineous index case from Turkey was diagnosed with early-onset Parkinsonism at the Istanbul Faculty of Medicine. She and her unaffected brother were subjected to whole-genome sequencing.

Results: In this report, we describe a 33-year-old index case with parental consanguinity and early-onset Parkinsonism. Whole-genome sequencing of this individual revealed that a homozygous p.R747W mutation in *PLA2G6* segregates with the disease in this family

Discussion: This result supports the importance of prioritizing this gene in mutational analysis of autosomal recessive Parkinsonism, and confirms the clinical heterogeneity of PLAN.

Keywords: *PLA2G6*, parkinsonism, *LRRK2*, *DCNT1*

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Ethics Statement: This study was performed in accordance with the ethical standards detailed in the Declaration of Helsinki. The authors institutional ethics committee has approved this study and all patients have provided written informed consent. All patients that appear on video have provided written informed consent; authorization for the videotaping and for publication of the videotape was provided.

Introduction

Mutations in phospholipase A2, group VI (*PLA2G6*) were initially discovered in a set of families with infantile neuroaxonal dystrophy (INAD),[1] and also in a large consanguineous Pakistani family with neurodegeneration with brain iron accumulation (NBIA) who did not have mutations in *PANK2*.[2] In 2009, Paisán-Ruiz et al.[3] using magnetic resonance imaging (MRI) identified mutations in *PLA2G6* in two unrelated families with adult-onset levodopa-responsive dystonia–parkinsonism with generalized cerebral atrophy but no brain iron accumulation. After this discovery, mutations in this gene have been associated with a broad spectrum of phenotypes, ranging from generalized dystonia to pure recessive Parkinsonism.[4–6] Because of this heterogeneity, the term *PLA2G6*-associated neurodegeneration (PLAN) has been proposed for all phenotypes associated with mutations in this gene. The nature of this mutational pleiotropy is largely unknown.

In this article we describe a 33-year-old Turkish patient with early-onset Parkinsonism in whom a homozygous p.R747W *PLA2G6* mutation was identified.

Methods

Subjects

This study was approved by the Istanbul Faculty of Medicine Ethics Committee. The family consisted of four members, including two offspring of whom one is affected. Blood was taken from all available family members after obtaining informed consent. All subjects underwent detailed clinical examination including the Unified Parkinson's Disease Rating Scale, Geriatric Depression Scale, and Mini Mental State Examination.

Exclusion of PARK2 mutations

To rule out the possibility that mutations in *PARK2* are the cause of disease in this family, the complete gene coding sequence and exon–intron boundaries in the index case were examined by Sanger sequencing (primer sequences available on request). In addition, multiplex ligation-dependent probe amplification (MLPA) with the SALSA MLPA P051 Parkinson mix 1 probemix (MRC-Holland, www.mlpa.nl) was used to exclude exonic rearrangements in this gene. This experiment was performed according to the manufacturer's protocol.

Whole-genome sequencing

The index case and her unaffected brother were subjected to whole-genome sequencing (WGS) at Macrogen (http://www.macrogen.com/). In brief, samples were prepared according to the Illumina TruSeq Nano DNA library preparation guide and libraries were sequenced using Illumina HiSeqX sequencer (www.illumina.com).

Bioinformatic analysis

Sequence reads were aligned to the reference genome (hg19) using Isaac Genome Alignment software (https://github.com/sequencing/isaac_aligner). Sorting, indexing, and polymerase chain reaction duplicate marking was also performed with this software. Variant calling and indel recalibration were performed with Isaac Variant Caller v.1.0.7 (https://github.com/sequencing/isaac_variant_caller). A single Variant Call format (VCF) file with variants identified by the Whole Genome Study was then generated for a total of two individuals from this family. KGGSeq (http://grass.cgs.hku.hk/limx/kggseq/) was used to apply quality control (QC) filters on individual genotypes and genomic variants. In brief, genotypes with a Phred quality score below 10, a read depth under 5, the second smallest normalized Phred-scaled genotype likelihood below 20, those with the fraction of reads carrying $\geqslant$5% of the alternative allele at a reference allele homozygous genotype, the fraction of reads carrying $\leq$25% of alternative allele at a heterozygous genotype, or the fraction of the reads carrying $\leq$50% of the alternative allele at an alternative-allele homozygous genotype were set to missing (./.). Variants with the "FILTER" field not matching the label "PASS," a minimum overall sequencing Phred quality score below 50, an overall mapping quality Phred score below 20, an overall strand bias Phred-scaled p-value (using Fisher's exact test) above 60, and those with a disease allele frequency above 1% in the 1000 Genomes database (www.1000genomes.org), dbSNP138, dbSNP141, the Exome Variant Server (http://evs.gs.washington.edu/EVS/), or ExAC http://exac.broadinstitute.org/), were removed from further analysis with KGGSeq. After this extensive QC, we were left with a total of 4,228,886 variants. Gene-based and impact score annotations were assigned to these variants with KGGSeq, using RefSeq (hg19) and dbNSFP (https://sites.google.com/site/jpopgen/dbNSFP) respectively. Intronic and synonymous variants were consequently removed from further analysis, leaving us with a total of 16,645 variants in the index case and her unaffected brother.

Runs of homozygosity (ROH) were identified using PLINK 1.9 (https://www.cog-genomics.org/plink2). A window of 50 single nucleotide polymorphisms (SNPs) was defined as homozygous if it contained, at most, one heterozygous genotype and five missing genotypes. Such windows were moved across the genome, and an SNP was eligible for inclusion in an ROH if the hit rate of all scanning windows containing the SNP was at least 0.05. These are the values suggested in PLINK 1.9, designed to minimize the probability of a window being called homozygous by chance, while ensuring that SNPs on the edge of a true ROH will be assigned to that ROH. Each ROH had to contain on average at least one SNP per 50 kb. The minimum length of the ROH was set at 1 Mb.

In silico analysis

Multiple sequence alignment of *PLA2G6*, *LRRK2*, and *DCTN1* was performed using Clustal omega (http://www.ebi.ac.uk/Tools/msa/clustalo/). RaptorX (http://raptorx.uchicago.edu/) was used to predict the three-dimensional conformation of wild type and mutated *LRRK2* and *DCTN1* proteins.

Results

The index case is a 33-year-old female patient with parental consanguinity (Figure 1A) who presented with left-sided foot shuffling and reduced arm swinging at the age of 27 years. When diagnosed with Parkinson disease (PD), the patient was started on levodopa treatment (levodopa/carbidopa/entacapone 100 mg/25 mg/200 mg, five times a day), but this was changed to dopamine agonists (ropinirole 8 mg/day and cabergoline 4 mg/day) after the appearance of irritability and restlessness. After 2 years the patient developed severe generalized bradykinesia, experienced difficulty walking with frequent falls, and had a bilateral hand tremor. After increasing the doses of the anti-parkinsonian therapy (levodopa/benserazide 750 mg/187.5 mg and amantadine 400 mg per day), the patient developed impulse control disorder including ablutomania, pathological spending, testiness, and hypersexuality. These symptoms improved after medication and the introduction of neuroleptics (quetiapine 25 mg/day). However, the motor symptoms worsened again, and oromandibular dyskinesia appeared. At the time of admission in our clinic, the patient presented with generalized severe bradykinesia, hypomimia, hypophonia, and mild dysarthria, and was not able to stand up or walk on her own. Intermittent micturition disturbances, postural

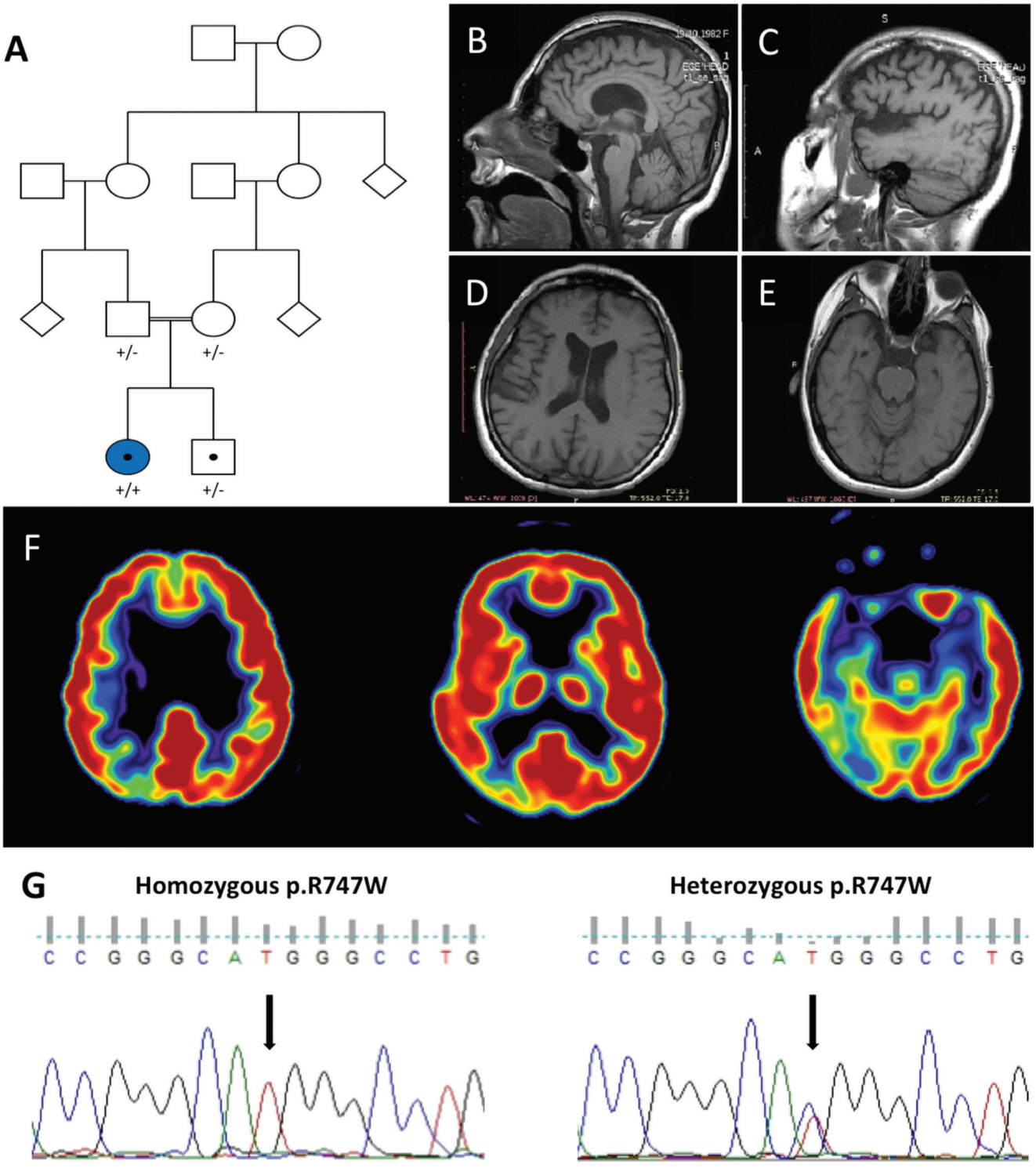

Figure 1. **Pedigree structure and brain imaging of the index case; and electropherogram of *PLA2G6* p.R747W mutation in the index case and her unaffected brother.** (A) Pedigree of the index case (individual with blue fill) with *PLA2G6* p.R747W mutation. Samples with a dot were subjected to whole-genome sequencing. Mutant alleles are shown with a "+" symbol, and wild-type alleles are indicated with "−." (B–E). T1 images of brain magnetic resonance imaging of the index case, performed at the age of 30, showing no atrophy of the brainstem and cerebellum (B), marked atrophy in the right side of the frontal and temporal cortical areas (C), asymmetrical atrophy of the right perisylvian region (D), and no atrophy of the medial, lateral, and anterior temporal areas, occipital lobes or cerebellum (E). (F) Positron emission tomography showing significant hypometabolism in the right parietal and right temporal areas. (G) Electropherogram of *PLA2G6* p.R747W mutation in the index case (homozygous; left panel) and her unaffected brother (heterozygous; right panel). The position of this mutation is marked with a black arrow.

Video 1. **Index case video clip.** The first part of the video shows the patient before starting any treatment. She had generalized severe bradykinesia, hypomimia, and intermittent arm tremor, and was not able to stand up or walk on her own. The second part of the video shows the patient after the establishment of a subcutaneous apomorphine pump (5mg/hour). Although she was able to get up and walk again, mild bradykinesia and hypomimia, and mild postural instability are still noted.

instability, and tremor were noted. Deep tendon reflexes and eye movements were normal. At the age of 30, brain MRI without contrast enhancement, including T1, T2, fluid-attenuated inverse recovery (FLAIR), and T2* images, revealed atrophy of the frontal and temporal cortices, predominantly in the right side (Figure 1B–E). There was no evidence of pathological iron accumulation in T2* images. A positron emission tomography (PET) scan showed significant hypometabolism in the right parietal and temporal areas (Figure 1F). The research for metabolic diseases and neuroacanthocytosis was normal, as were electroencephalogram (EEG) and electromyography (EMG) examinations. Transthoracic echocardiography revealed mild mitral valve degeneration. Neuropsychological evaluations showed impairments in language, memory, and visuospatial domains. After the establishment of a subcutaneous apomorphine pump (5 mg/hour), the patient was able to get up and walk on her own again. At the age of 31 the patient suffered from an epileptic seizure after bupropion therapy (150 mg/day) for depression was introduced. This was followed by intermittent visual hallucinations. A new cranial MRI did not show any changes (data not shown). A video related to this patient is provided (Video 1). The parents of the patient are first cousins (Figure 1A) and originate from Eskisehir, a city in northwestern Turkey. They, and the 31-year-old brother of the index case, are healthy without any signs of neurological disease.

After excluding point mutations and exonic rearrangements in *PARK2* as the cause of disease in this family, the index case and her unaffected brother were subjected to WGS followed by a screen of ROH and mutation identification. A homozygous c.2239C→T transition in a 1.04-MB ROH exclusive to the index case (Supplementary Table S2) was identified as a result of this analysis. This variant is located in exon 16 of *PLA2G6*. After Sanger sequencing, all available family members with disease could be identified (Figure 1G). This mutation results in a p.R747W substitution in a highly conserved region of the encoded protein (Supplementary Figure S1). It is not present in the 1000 Genomes database (http://www.1000genomes.org/), the Exome Variant Server (http://evs.gs.washington.edu/EVS/), the Turkish Genome Project (http://turkiyegenomprojesi.boun.edu.tr/starteng.html),[7] our in-house whole-genome/exome database from more than 3,000 neurologically normal individuals (including 10 of Turkish descent), and has only one allele in the ExAC database (South Asian: 1/7,878, allele frequency = 0.0001269; http://exac.broadinstitute.org/). It had been predicted to be pathogenic using the SIFT,[8] CADD,[9] and PolyPhen2[10] algorithms and a rare disease causal by a logistic regression model described elsewhere.[11] Because of this, and because of recent functional data showing that cultured fibroblasts of a patient with this mutation have lipid peroxidation, mitochondrial dysfunction, and subsequent mitochondrial membrane abnormalities,[12] we believe that this mutation is the cause of disease in this family.

This is the same mutation that Paisan-Ruiz et al.[3] described in a consanguineous index case from Pakistan, which presented with different clinical features. In order to explain this phenotype variability, we hypothesized that variation in genes associated with different PLAN phenotypes, as well as PD and other neurodegenerative disorders (Supplementary Box S1), may be acting as genetic modifiers. To test this possibility, variation in these genes was further investigated in the WGS data generated. Results derived from this analysis are summarized in Supplementary Table S1. A novel heterozygous c.3883T→C transition in exon 28 of *LRRK2* that is present in the index case and her unaffected brother deserves special mention. This variant is not present in public databases or our in-house database. It results in a p.W1295R substitution in a highly conserved region (Supplementary Figure S2) within the Leucine-Rich Repeat domain of the *LRRK2* protein, which is known to participate in

interactions with other proteins through binding to its extended solvent-accessible surface.[13] The identified substitution alters the three-dimensional conformation of this domain as predicted by *in silico* analysis (Supplementary Figure S2) and affects its charge and hydrophobicity, which may affect interaction with other proteins. Another variant of interest was identified in *DCTN1*, which is associated with Perry syndrome, an autosomal dominant neurodegenerative disorder characterized by late-onset parkinsonism.[14] The identified variant is a heterozygous c.35G→A transition in exon 2 of the gene, resulting in a p.T12M substitution that is present in ExAC with a frequency of 0.00003398 (European: 2/64,482, allele frequency = 3.102E-5; Latino: 2/11,478, allele frequency = 0.0001742). It is in a well-conserved region among human, macaque, mouse, and rat, and slightly alters its three-dimensional conformation (Supplementary Figure S3).

Discussion

In this report, we describe a 33-year-old index case with parental consanguinity (Figure 1A) who presented with early-onset Parkinsonism. WGS of this individual and an unaffected brother revealed that a homozygous p.R747W mutation in *PLA2G6* segregates with the disease in this family. A recent publication by Kinghorn et al.[12] shows that knockout of the *PLA2G6* homologue in Drosophila results in reduced survival, locomotor deficits, and organismal hypersensitivity to oxidative stress, as well as mitochondrial respiratory chain dysfunction, reduced ATP synthesis, abnormal mitochondrial morphology, and increased lipid peroxidation levels. Replication of these findings in cultured fibroblasts of a patient with p.R747W mutation strongly supports that the mutation described herein is the cause of disease in this family.

Paisán-Ruiz et al.[3] identified the same mutation in a consanguineous index case from Pakistan. This individual, however, had an earlier age at onset (18 vs. 27 years) and different clinical symptoms, including rapid cognitive decline with personality changes, foot dystonia, blepharoclonus, and asymmetric pyramidal features with spasticity.[3,15] The clinical differences between these two patients suggest an absence of a genotype–phenotype correlation for this mutation, unlike the one proposed for p.D331Y mutation carriers.[5] In order to explain this phenotype variability, we contemplated the possibility that variation in genes associated with different forms of neurodegeneration (including PLAN and PD) may be acting as genetic modifiers. Further examination of the WGS data generated revealed the presence of two heterozygous missense variants in *LRRK2* and *DCTN1* in both the index case and her unaffected brother. The role of these variants as genetic modifiers of p.R747W deserves further investigation in a larger dataset.

The phenotypes associated with mutations in this gene are heterogeneous, ranging from INAD to autosomal recessive Parkinsonism. The index case presented herein was diagnosed with levodopa-responsive PD and did not present any signs of brain iron accumulation, which is in line with a recent literature review by Karkheiran et al.[15] in which only eight patients (33%) presented iron depositions. A summary of the clinical features and cerebral imaging results in all individuals with levodopa-responsive PD and no signs of brain iron accumulation reported to date is shown in Supplementary Figure S3. This shows that both the associated clinical symptoms and the imaging findings (other than brain accumulation) vary considerably among these individuals, suggesting that no specific clinical or cerebral imaging pattern can be associated with *PLA2G6* mutations.

In summary, we present a consanguineous PD case from Turkey with genetically confirmed PLAN. This result supports the importance of prioritizing this gene in mutational analysis of autosomal recessive PD cases, and confirms the clinical heterogeneity of PLAN. We also found two heterozygous variants in *LRRK2* and *DCTN1* in both the index case and her unaffected brother. The role of these variants in families with this mutation deserves further investigation.

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Supplementary Material

All supplementary data referenced in this article is available here: http://dx.doi.org/10.7916/D86973FQ.

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