Clinical heterogeneity of PLA2G6-related Parkinsonism: analysis of two Saudi families

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Abstract

Background: Recessive mutations in PLA2G6 have been associated with different neurodegenerative disorders, including infantile neuroaxonal dystrophy, neurodegeneration with brain iron accumulation and more recently, early-onset dystonia parkinsonism.

Method: Targeted-next generation sequencing using a custom Neurology panel, containing 758 OMIM-listed genes implicated in neurological disorders, was carried out in two index cases from two different Saudi families displaying early-onset levodopa-responsive Parkinsonism with pyramidal signs and additional clinical features. The detected mutations were verified in the index cases and available family members by direct sequencing.

Results and conclusion: We identified a previously described PLA2G6 homozygous p.R741Q mutation in three affected and two asymptomatic individuals from two Saudi families. Our finding reinforces the notion of the broadness of the clinical spectrum of PLA2G6-related neurodegeneration.

Keywords: PLA2G6, Parkinsonism, Saudi patients

Background

Oxidative stress is considered a key pathophysiological mechanism underlying dopaminergic neuronal loss in Parkinson’s disease (PD). Excess iron is one main contributor to oxidative stress that promotes the formation of harmful free radicals via the Fenton reaction. High iron content, in addition to other factors, including the presence of reactive oxygen species (ROS)-generating enzymes and dopamine oxidation, renders dopaminergic neurons of the substantia nigra vulnerable to oxidative stress [1]. Another source of ROS in the brain is the metabolism of arachidonic acid, which is released from membrane phospholipids by the hydrolytic activity of calcium-independent group VI phospholipase A₂ (iPLA₂-VI) [2].

Recessive mutations in PLA2G6 [MIM 603604], the gene encoding (iPLA₂β/iPLA₂-VI), have been associated with different neurodegenerative disorders, including infantile neuroaxonal dystrophy (INAD), neurodegeneration with brain iron accumulation (NBIA) and more recently, early-onset dystonia parkinsonism [3]. The involvement of iPLA₂-VI in oxidative stress [2], the identification of PLA2G6 mutations in patients with parkinsonian features [3], and the presence of α-synuclein Lewy body pathology in five dystonia-parkinsonism cases with PLA2G6 genetic abnormalities [4] suggest a possible role for this gene in PD pathogenesis.

Utilizing targeted-next generation sequencing (NGS) of 758 OMIM-listed neurological disorders-associated genes, we identified a homozygous p.R741Q mutation in three cases with early-onset Parkinsonism [MIM 612953] displaying additional clinical features. Our finding reinforces the notion of the broadness of the clinical spectrum of PLA2G6-related neurodegeneration.

Methods

Subjects

A total of 17 individuals (3 affected and 14 unaffected) from two families originating from the Eastern province...
of Saudi Arabia were recruited after obtaining written informed consent from all study subjects for participation in the study and for the publication of their genetic and clinical data. This study was approved by the King Faisal Specialist Hospital and Research Center (KFSHRC) IRB, Research Ethics Approval Committee (RAC# 2110035).

Clinical assessment
Standardized clinical investigation was undertaken in all patients by senior neurologists. Neuroimaging examinations were performed using Axial brain fluid attenuated inversion recovery (FLAIR) MRI and F-18-fluorodeoxyglucose positron emitting tomography (F18-FDG-PET). Mini Mental State examination (MMSE) and Hoehn & Yahr (H&Y) stage were used to evaluate cognitive function and disease progression, respectively. Clinical features of the patients are summarized in Table 1. For detailed clinical history, see Additional file 1.

Targeted-NGS
Pathogenic mutations in parkinsonism-related genes, such as PARKIN [MIM 602544], PINK1 [MIM 608309], DJ-1 [MIM 602533], SNCA [MIM 163890], LRRK2 [MIM 609007], UCHL1 [MIM 191342], and FBXO7 [MIM 605648], had previously been excluded in all index cases by means of Sanger sequencing before undergoing targeted-NGS [5]. Custom Neurology panel, developed by the Saudi Mendeliome Group as part of the Saudi Human Genome Program [6], was utilized to evaluate peripheral blood DNA from the probands of both families. The panel includes 758 OMIM-listed genes implicated in neurological disorders.

| Table 1 Summary of the demographic, clinical and neuroimaging information of the study subjects |
|----------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Patient ref no.                  | Family 66                      | Family 66                      | Family 66                      | Family 97                       | Family 97                       |
|                                  | 66-E                           | 66-K                           | 66-L                           | 97-E                            | 97-E                            |
| Gender/AAE (y)                   | Female/35                      | Male/28                        | Male/31                        | Female/30                       |                                 |
| AAO (y)                          | 26                             | 22                             | 23                             | 25                              |                                 |
| Initial symptoms                 | Depression/bradykinesia        | Depression/tremor              | Bradykinesia                   | Neuropsychiatric symptoms       |                                 |
| Bradykinesia                     | +++                            | ++                             | +++                            | ++                              |                                 |
| Tremors                          | Yes                            | Yes                            | No                             | No                              |                                 |
| Postural instability             | +++                            | ++                             | +++                            | +++                             |                                 |
| Dystonia                         | No                             | No                             | No                             | No                              |                                 |
| Loss of ambulation               | 3 y after onset                | ambulatory                     | 3 y after onset                | 2 y after onset                 |                                 |
| Pyramidal signs                  | ++                             | +                              | ++                             | +                               |                                 |
| Cerebellar signs                 | No                             | No                             | No                             | No                              |                                 |
| Levodopa response                | Yes -minimal                   | Yes -minimal                   | Yes -minimal                   | Yes -minimal                    | Yes -moderate                   |
| Levodopa-induced dyskinesia      | ++                             | –                              | ++                             | –                               | –                               |
| Frontotemporal atrophy on MRI    | ++                             | +                              | ++                             | ++                              | ++                              |
| Iron accumulation                | No                             | No                             | No                             | No                              |                                 |
| PET scan                         | Frontotemporal lobe            | Frontotemporal lobe            | Not done                       | Frontotemporal lobe             |                                 |
| Hoehn & Yahr stage               | 4.5                            | 4                              | 5                              | 4                               |                                 |
| Neuropsychiatric symptoms        | ++                             | ++                             | +                              | +++                             |                                 |
| Emotional liability              | +++                            | +                              | +                              | +++                             |                                 |
| Cognitive decline                | +                              | ++                             | +                              | +                               | +                               |
| Semantic disorder                | +                              | +                              | +                              | –                               | –                               |
| Autonomic symptoms               | Yes                            | Yes                            | Yes                            | No                              |                                 |
| Bladder disturbances             | Urgency                        | Urgency                        | Urgency                        | –                               | –                               |
| Sweating                         | Yes                            | Yes                            | No                             | –                               | –                               |
| Flushing                         | No                             | Yes                            | No                             | –                               | –                               |
| Sleep disorders                  | Yes                            | Yes                            | Yes                            | Yes                             |                                 |
| REM-sleep behavior disorder      | Yes                            | No                             | No                             | No                              | –                               |
| Vivid dreaming                   | No                             | Yes                            | No                             | Yes                             |                                 |
| Sleep fragmentation              | Yes                            | Yes                            | Yes                            | –                               | –                               |
| Death                            | Alive                          | Alive                          | Died after 8 y                  | Alive                           |                                 |

AAE age at examination; y years; AAO age at onset; REM rapid eye movement; + mild; ++ moderate; +++ severe
Library building, NGS, and bioinformatics analysis was carried out as previously described [6]. Short-listed variants (those that passed the filtering criteria) were validated by Sanger sequencing and subsequently screened in available affected and unaffected family members.

**Genotyping**

Parents and the index case from each family were genotyped using the Affymetrix Axiom Arrays according to the manufacturer’s recommendations (Affymetrix, Santa Clara, CA 95051, USA), genotypes were called using Genotyping Console™ (GTC version 4.2), and generated files were further integrated for markers flanking the PLA2G6 locus; 72 markers were selected in the haplotype analysis. In addition to the Axiom analysis, conventional genotyping was performed using five previously reported microsatellite markers within chromosome 22 flanking PLA2G6 (D22S426, D22S1045, D22S445, D22S1156, D22S423) [7]. The PCR amplicons were electrophoretically separated on ABI Prism 3100 Genetic Analyzer and the data was analyzed using GeneMapper 5 (Applied Biosystems, Foster City, CA, USA).

**Results**

**Mutation detection and haplotype analysis**

Targeted-NGS analysis revealed a previously described PLA2G6 homozygous mutation [c.2222G > A (p.R741Q)] [8] (Fig. 1a) shared by both probands (66-E and 97-E) as well as an affected sibling (66-K) (Fig. 1b, c). The mutation was absent in the 1000 Genomes Project database, in addition to our in-house Saudi human genome database (~1000 controls), and was confirmed by bidirectional Sanger sequencing. We next sought to assess the segregation of this mutation, and, to that end, DNA samples from affected and unaffected family members of both families (FM 66 and FM 97) were screened for the p.R741Q mutation. The mutation appears to segregate with the disease in FM 97 as clinically unaffected members were either heterozygotes or wild-type (Fig. 1c). As for FM 66, the presence of this homozygous mutation in two asymptomatic members (66-D and 66-G) is suggestive of incomplete penetrance (Fig. 1b).

Since both families originate from the same geographical area and share a specific mutation, we suspected them to have descended from a common ancestor. This notion was examined by genotyping 72 SNP markers along with five previously described microsatellite markers [7] spanning a 3.3 Mb segment on chromosome 22 harboring the PLA2G6 locus. The analysis revealed a shared haplotype between the father of 66-E and the parents of 97-E spanning a 0.49 Mb segment on chr22q13.1 containing part of PLA2G6. The probands, on the other hand, shared the same haplotype across a larger region (1.4 Mb) (Fig. 2). Overall, the genotyping results suggest that the p.R741Q mutation is likely to be inherited from a common ancestor.

![Fig. 1](image_url) p.R741Q mutation of PLA2G6 detected in two families with early-onset parkinsonism. a Chromatogram of the c.2222G > A mutation. b, c Pedigrees of the two families showing the genotypes of the mutation. d–g Example radiological imaging of the affected individuals. d, f Axial brain fluid attenuated inversion recovery (FLAIR) MRI sequence of the frontotemporal region of 66-E (d) and 66-K (f) showing moderate (d) and mild (f) atrophy. e 18F-FDG-PET scan of 66-E showing moderate decrease in glucose uptake in the frontoparietal regions. g Coronal FLAIR MRI sequence showing moderate frontotemporal atrophy of 97-E. * denotes no DNA sample available.

AAE age at examination in years; AAO age at onset in years
Clinical features of the patients homozygous for p.R741Q mutation

At the age of 26 years, the proband of FM 66 (patient 66-E) presented with bradykinesia, tremors, neuropsychiatric symptoms, and sleep disturbances. A year later, she became almost anarthric, using sign language, and was confined to a wheelchair due to poor balance. She showed marked generalized rigidity affecting axial more than appendicular muscles; however, no dystonia was observed. During her admission, she was started on Levodopa/Carbidopa therapy. This caused a stereotyped, predictable episode of agitation, crying and moaning with semi-rhythmic dyskinetic and dystonic movements— including craniofacial dystonia— with clenching of hands, scratching, and mutilating movements. These movements would start 30–40 min after a Levodopa dose and last for 1.5–2 h. This occurred even with the smallest possible dose of Levodopa, i.e. 50 mg/day. Alternatively, the patient was switched to dopamine agonist therapy in the form of Pramipexole. The dose was gradually increased to 3 mg/day with some improvements in the bradykinesia; her walking improved moderately, but she still required assistance with standing and walking. However, her agitation and emotional lability did not match the symptomatic motor improvement. MRI brain scan showed moderate atrophy in the frontotemporal region (Fig. 1d) with absence of iron deposition in the basal ganglia which was confirmed by T2* sequence. Dopamine transporter single-photon emission computed tomography (DaT-SPECT) scan revealed symmetrical reduced uptake bilaterally in the basal ganglia (image not available) and 18-FDG-PET showed moderate decrease in glucose uptake in the frontoparietal regions (Fig. 1e).

At the age of 22 years, the affected sibling of the proband (patient 66-K) started showing signs of motor dysfunction, including bradykinesia, poor balance, and symmetrical tremors, that led him to have three road traffic accidents in one year. Signs of autonomic dysfunction, sleep disorder, and psychiatric problems were reported. Brain MRI (Fig. 1f) and PET imaging revealed similar but milder findings compared to the proband (66-E). Moderate cognitive deficit was shown on neurological assessment. The patient manages limited daily activities on a combination therapy of dopamine agonist, amantadine, MAO-Inhibitors, and atypical antipsychotic. The proband also has another affected sibling (patient 66-L—deceased) that was a former patient at KFSHRC. See Additional file 1 for detailed clinical history.

The proband from the second family (97-E) was leading a normal life as a full time nurse until the age of 25 when she developed neuropsychiatric symptoms. A few months later, motor symptoms, such as rigidity,
bradykinesia and poor balance, were evident. The initial
diagnosis was drug-induced Parkinsonism, and, subse-
sequently, she was treated with anticholinergic and electro-
convulsive therapy. She became relentlessly dependent,
needing two people to help her stand up and walk on her
tip-toes. The examination revealed severe Parkinson-
ism (H&Y stage >4.5) with mild cognitive impairment
(MMSE score of 18). Brain MRI showed moderate fron-
totemporal lobar atrophy (Fig. 1g) with no iron deposi-
tion in the basal ganglia and 18-FDG-PET scan showed
bilateral frontal and parietal reduction of glucose metab-
olism. Like in the previous cases (66-E and 66-L), Lewo-
dopa therapy triggered adverse emotional–side effects
in this patient. She had frequent episodes of loud crying,
tearing, clenching of the mouth at times or bringing her
head and neck backward, refusal to eat, self-scratching
and stereotyped repetitive movements involving the
arms and legs. She became more disturbed after Levo-
dopa therapy and developed craniofacial dystonia and,
consequently, was started on a combination of second
line medication, including Clozapine, Amantadine, Clon-
zepam and Pramipexole.

Discussion

Here we report on the clinical and neuroimaging findings
of patients from two different families presenting with
early-onset Parkinsonism in whom the reported PLA2G6
mutation (c.2222G > A; p.R741Q) [8] was detected. The
two families, with no reported relationship, originate
from the Eastern province of Saudi Arabia; however, hap-
lotype analysis suggests that the mutation may be inher-
ited from a common ancestor (Fig. 2).

The clinical features of our patients overlap with what
have been reported in the original cases harboring the
p.R741Q mutation [8] with the exception that there was
no dystonia reported. Additional features, including sleep
and autonomic problems, were also noticed (Table 1). Of
note, response to levodopa was limited by the adverse
emotional side-effects.

All recruited patients, two of which are siblings (66-E
and 66-K), were born to consanguineous parents (Fig. 1b,
c). The autosomal recessive transmission of this muta-
tion, as demonstrated by the genotypes and pedigrees,
is consistent with the first report of this mutation in a
Pakistani/Indian family and with other reports on dif-
ferent PLA2G6 mutations in PARK14-linked Parkinson-
ism patients from other populations [3, 8]. The parents
of 97-E were heterozygous carriers and the siblings were
either heterozygotes or wild-type; they all have passed
the expected age of disease-onset at the time of examina-
tion without showing any symptoms (Fig. 1c) which sug-
gests that the mutation co-segregates with the disease as
previously reported [8]. Unlike FM 97, the mutation does
not appear to segregate with the disease in FM 66 as not
only the affected individuals were homozygous carriers,
but also two asymptomatic individuals; one (66-G) above
and the other (66-D) under the age of disease-onset
reported in the proband (Fig. 1b). Defining the pene-
trance of PLA2G6 mutations in PARK14-linked Parkin-
sonism patients is complicated by the age of onset, that
can vary widely among patients (8–37 years) and within
families [3, 8, 9], which could lead to pseudo-incomplete
penetrance. Pseudo-incomplete penetrance is a term
used when an inaccurate assumption of non-penetrance
has been made due to incomplete clinical examination
or absence of symptoms at the time of examination [10].
Moreover, asymptomatic homozygous mutation carriers
may exhibit preclinical signs; for instance, Shi and col-
leagues described a slightly reduced uptake in the right
posterior putamen of a homozygous carrier of p.D331Y
mutation in PLA2G6 who was clinically unaffected at the
time of examination [9]. In this regard, including DAT
imaging to the initial clinical assessment and follow-up
asymptomatic homozygous mutation carriers should
warrant accurate penetrance determination.

PLA2G6 is ubiquitously expressed with widespread
presence in all areas of mammalian brain [11]. Its prod-
uct, iPLA2-VI, which catalyzes fatty acids hydrolysis from
phospholipids and lysophospholipids, plays a key role in
maintaining cell membrane homeostasis. The high lipid
content of the brain renders the CNS especially sensi-
tive to lipid metabolism dysregulation. For instance,
arachidonic acid, a product of PLA2 activation, contrib-
utes to the generation of ROS which indirectly induce
cellular lipid peroxidation compromising membrane
integrity and fluidity and/or disrupting permeability
and ion homeostasis [2]. These alterations may under-
lie axonal dystrophy and iron accumulation typical of
INAD and NBIA, respectively [11]. Similarly, the con-
nection between defective phospholipid metabolism and
Parkinsonism was speculated on the basis of iPLA2-VI
role in ROS generation and lipid peroxidation. This was
supported pathologically by the widespread Lewy body
presence that is most pronounced in the neocortex docu-
menced in postmortem analysis of patients with PLA2G6
mutations and clinically by the presence of Parkinsonism
features [4].

Distinct PLA2G6 mutations give rise to various phe-
notypes and have different effects on iPLA2-VI catalytic
activity. For instance, impaired enzyme catalytic activity
was reported in iPLA2-VI proteins extracted from 293FT
cells expressing different mutations associated with
either INAD or NBIA. However, results on the effect of
dystonia-parkinsonism associated mutations were con-
troversial. Engel et al. [12] reported normal catalytic
activity of recombinant iPLA2-VI proteins containing
Additional file

Additional file 1. Detailed clinical history of the patients.

Abbreviations

Ang: arginine; CNS: central nervous system; DaT‑SPECT: dopamine transporter single‑photon emission computed tomography; 18FDG‑PET: F‑18‑fluorodeoxyglucose positron emitting tomography; FLAIR: fluid attenuated inversion recovery; Gln: glutamine; H6Y: hoehn & yahr; INAD: infantile neuroaxonal dystrophy; iPLA2‑VI: calcium‑independent group VI phospholipase A2; IRB: Institutional Review Board; KFSHRC: King Faisal Specialist Hospital and Research Center; MAO: monoamine oxidase; MMSE: mini mental state examination; MRI: magnetic resonance imaging; NBA: neurodegeneration with brain iron accumulation; NGS: next generation sequencing; OMIM: online Mendelian inheritance in man; PD: Parkinson’s disease; RAC: Research Advisory Council; ROS: reactive oxygen species; Trp: tryptophan.

Authors’ contributions

NAA conceived and designed the study. SAB and HA contributed with patients recruitment, diagnosis and clinical assessment. DM, SAH, MA, ME and TF carried out the targeted‑NGS experiments and analysis. EAA preformed the mutation validation experiments. AEM and DSK preformed genotyping experiments. NAA, AIT, EAA and BRA interpreted the data. BRA and SAB drafted the manuscript. NAA critically reviewed the manuscript. All authors read and approved the final manuscript.

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Consent to publish

Written informed consent was obtained from all study subjects for the publication of their genetic and clinical data including images.

Ethics and consent to participate

Written informed consent was obtained from all study subjects for the publication of their genetic and clinical data. This study was approved by the King Faisal Specialist Hospital and Research Center (KFSHRC) IRB, Research Ethics Approval Committee (RAC# 2110035).

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Availability of data and materials

The data supporting the results of this article are included within the article and its additional files.

Competing interests

The authors declare that they have no competing interests.
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