Ophthalmic features of *PLA2G6*-related paediatric neurodegeneration with brain iron accumulation

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ABSTRACT

Background Neurodegeneration with brain iron accumulation (NBIA) refers to genetically heterogenous paediatric neurodegenerative disorders characterised by basal ganglia iron deposition. One major cause is recessive mutations in the *PLA2G6* gene. While strabismus and optic nerve pallor have been reported for *PLA2G6*-related disease, the ophthalmic phenotype is not carefully defined. In this study we characterise the ophthalmic phenotype of *PLA2G6*-related NBIA.

Methods Prospective cohort study.

Results The eight patients were 4–26 years old when examined. All had progressive cognitive and motor regression first noted between 9 months and 6 years of age that typically first manifested as difficulty walking (ataxia). Ophthalmic examination was sometimes limited by cognitive ability. Four of eight had exotropia, 7/7 bilateral supraduction defect, 5/7 poor convergence, 6/8 saccadic pursuit, 4/8 saccadic intrusions that resembled square-wave jerks, and 8/8 bilateral optic nerve head pallor. All patients lacked Bell phenomenon.

Conclusions Upgaze palsy, although not a previously reported finding, was confirmed in all patients (except in one for whom assessment could not be performed) and thus can be considered part of the phenotype in children and young adults. Other frequent findings not previously highlighted were abnormal convergence, saccadic pursuit, and saccadic intrusions. Optic nerve head pallor and strabismus, previously reported findings in the disease, were found in 100% and 50% of our cohort, respectively, and the strabismus in our series was always exotropia. Taken together, these clinical findings may be helpful in distinguishing *PLA2G6*-related neurodegeneration from the other major cause of NBIA, recessive *PANK2* mutations.

INTRODUCTION

Paediatric neurodegeneration with brain iron accumulation (NBIA) is a genetically heterogeneous disorder that is usually first suspected when a child with progressive neurological regression has brain MRI abnormalities suggestive of abnormal brain iron accumulation, particularly in the basal ganglia.[1] The neurological phenotype shows variable expressivity that includes progressive ataxia, dystonia, spasticity, parkinsonism, and cognitive dysfunction. Mutations in at least seven different genes to date have been associated with the disease[2]; however, the major causes are recessive mutations in *PANK2* (Mendelian Inheritance in Man (MIM) *606157; pantothenate kinase 2) or in *PLA2G6* (MIM *603604; phospholipase A2 calcium-independent group VIA).[1 2] PANK2 is an essential regulatory enzyme in coenzyme A biosynthesis; recessive gene mutation affects coenzyme A dependent processes including fatty acid metabolism which likely leads to NBIA. PLA2G6 catalyses hydrolysis of the sn-2 acyl-ester bonds in phospholipids, leading to the release of arachidonic acid and other fatty acids; recessive gene mutation disrupts cell membrane structure and promotes apoptosis which likely leads to NBIA.

The ophthalmic phenotype for *PANK2*-related disease (formerly Hallervorden-Spatz syndrome) has been characterised and includes Adie-like pupils, abnormal vertical saccades, saccadic pursuit, and retinopathy.[3] However, for *PLA2G6*-related neurodegeneration (also known as PLAN),[4] although optic neuropathy and strabismus have been reported (11/14 optic neuropathy and 13/14 unspecified strabismus in one paediatric cohort),[4] ocular features are not well defined. We characterise the *PLA2G6*-related ophthalmic phenotype in a cohort of patients with genetically confirmed disease.[5]

METHODS

Institutional board approval was granted for this study. A cohort of known Saudi Arabian patients from consanguineous families and affected by *PLA2G6*-related NBIA[5] underwent prospective ophthalmic examination. Their genetic diagnosis was established by homozygosity analysis guided candidate gene testing, which revealed homozygous *PLA2G6* mutations to segregate with their phenotypes.[5] At the time of ophthalmic examination, their mutation status was unknown. Slit-lamp or penlight anterior segment assessment, pupillary assessment (for reactivity and afferent defect), indirect ophthalmoscopy (using a 20 dioptre lens), and cycloplegic refraction (following tropicamide 1% and phenylephrine 2.5%) were performed. In addition, before pupillary dilation an ocular motility evaluation was performed. This included evaluation for fixation preference, strabismus, ductions, versions, convergence (ability to bilaterally adduct to a target brought slowly toward the patient's nose), pursuit (ability to follow a slow moving target in the horizontal and vertical planes), saccades (ability to generate a fast eye movement from straight ahead to an object of regard in the eccentric horizontal or vertical plane), and the ability to maintain fixation on an object of regard straight ahead while his/her head was forcefully turned horizontally or moved vertically (vestibulo-ocular reflex ± smooth pursuit). Bell phenomenon was assessed for all patients one eye at a time by observing whether the eye would supraduct above the midline during forced eyelid opening by the

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examiner while the patient was closing his/her eyes tightly. In addition, for all patients flash visual evoked potential (MetroVision Digital Evoked Potential Machine; MetroVision, Perenchies, France) was performed under chloral hydrate sedation.

RESULTS

Eight patients (four families) harbouring four different homozygous *PLA2G6* mutations were assessed by a single ophthalmologist (AOK). Details regarding their neurological findings are described in a companion publication.[5] Age at examination ranged from 4–26 years. All had progressive cognitive and motor regression first noted from 9 months to 6 years of age that typically first manifested as difficulty walking (ataxia); neuroimaging revealed evidence for brain iron accumulation in the basal ganglia and cerebellar cortical atrophy (figure 1). Neuroimaging did not reveal any orbital or cranial nerve abnormalities. Visual acuity could not be assessed reliably because of lack of ability or unwillingness to cooperate; however, no patient had evidence for ocular preference. All patients were found to have poor elevation during duction testing or limited cooperation in regard to vertical eye movement testing (ie, lack of interest in following a visual target vertically or in maintaining fixation on a target while the head was moved vertically); because of this limited cooperation in regard to vertical eye movement testing, vertical pursuit and vertical saccades could not be reliably assessed. Four out of eight had exotropia, 7/7 bilateral symmetric supraduction defects, 5/7 poor convergence, 6/8 saccadic pursuit, 4/8 saccadic intrusions (resembling square wave jerks), and 8/8 bilateral optic nerve head pallor. All patients lacked Bell phenomenon. No globe retraction was observed when Bell phenomenon was assessed. All eight patients were able to maintain fixation on an object of regard straight ahead while his/her head was forcefully turned to the right or left; while this suggested an intact horizontal vestibulo-ocular reflex, there may have been a contribution from smooth pursuit during this manoeuvre and thus we cannot state this conclusively. Flash visual evoked potentials were abnormal in three patients and within normal limits in five patients. Clinical features and gene mutations are summarised in table 1 and clinical examples are shown in figure 2.

DISCUSSION

Although it has not been previously documented for *PLA2G6*-related disease, bilateral supraduction defect without Bell phenomenon was uniformly present in our case series (with one patient who could not cooperative for such testing) and thus can be considered part of the *PLA2G6*-related phenotype

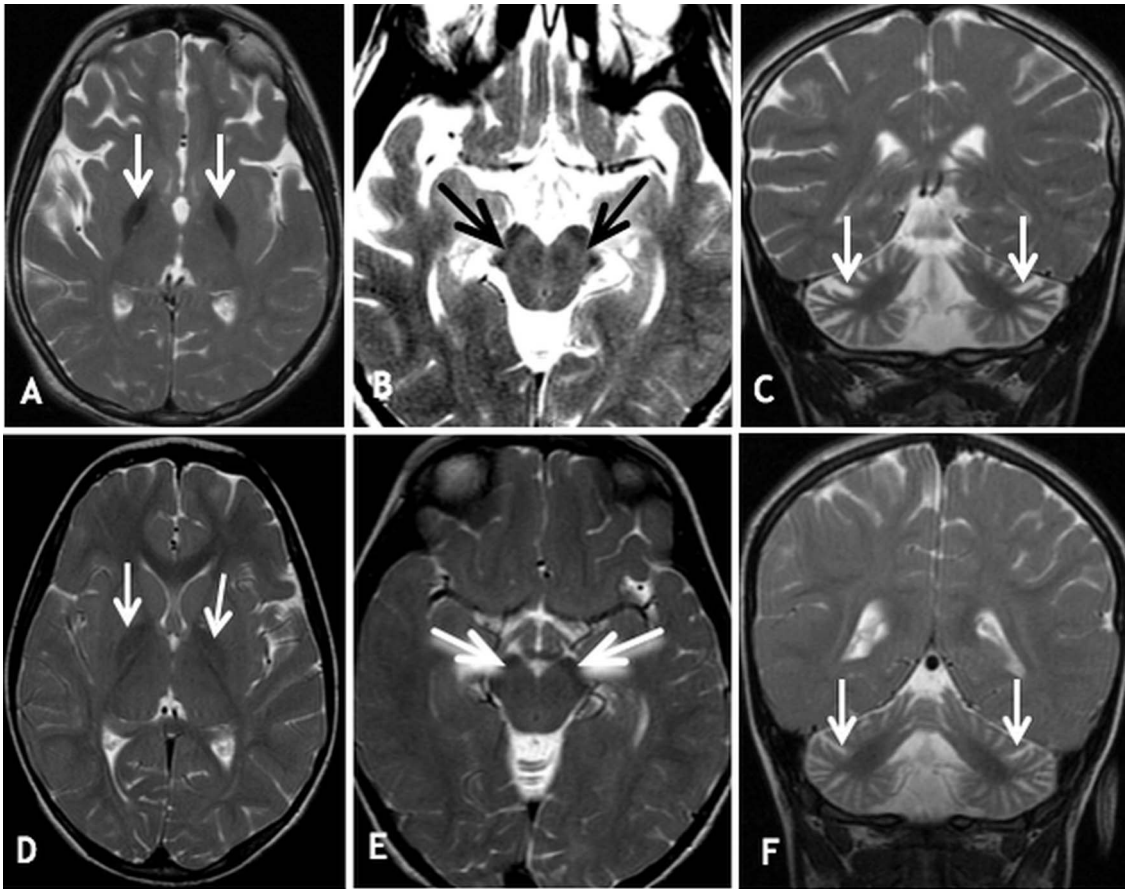

Figure 1 Neuroimaging examples. (A,B,C) Patient 7 at 10 years old. MRI brain axial T2WI (A) shows significant dark signal intensity at globus pallidus bilaterally consistent with excessive iron deposition (arrows) and (B) dark signal intensity in the substantia nigra at the level of the midbrain, also consistent with excessive iron deposition (arrows). Coronal T2WI (C) shows dilated cerebellar folia (arrows) due to cerebellar atrophy. (D, E, F) Patient 8 at 4 years old. MRI brain axial T2WI (C) shows mild dark signal intensity at globus pallidus bilaterally consistent with mild iron deposition (arrows) and also (D) at the substantia nigra (arrows) at the level of the midbrain, also consistent with iron deposition. Coronal T2WI (F) shows dilated cerebellar folia (arrows) due to cerebellar atrophy.

Table 1 Summary of clinical findings

ID	Mutation	Age	Sex	Primary	Ductions	Pursuit	Saccades	Conv	Nyst	Discs	Retinoscopy	fVEP	Comments
1 (F6, P1)	c.673C>T (p.H225Y)	26	F	XT	?	?	?	No	?	Temporal pallor	?	Non-recordable OD; delayed OS	Wheelchair bound and poor cognition/ cooperation; sister of patient 2 and cousin of patients 3 and 4
2 (F6, P2)	c.673C>T (p.H225Y)	21	F	Ortho	−4 upgaze	Saccadic	WNL	WNL	?	Pallor	Plano-1.00×180	WNL	Wheelchair bound and poor cognition; sister of patient 1 and cousin of patients 3 and 4
3 (F6, P3)	c.673C>T (p.H225Y)	16	M	XT	−3 upgaze	Saccadic	Intrusions	No	Pendular	Temporal pallor	0.75	WNL	Brother of patient 4 and cousin of patients 1 and 2
4 (F6, P4)	c.673C>T (p.H225Y)	16	M	Ortho	−3 upgaze	Saccadic	WNL	WNL	Pendular	Temporal pallor	−0.25	WNL	Brother of patient 3 and cousin of patients 1 and 2
5 (F5, P1)	c.2218G>A (p.G740R)	10	M	X(T)	−4 upgaze	Saccadic	WNL	?	No	Temporal pallor	?	WNL	Wheelchair bound and poor cognition; pupils constrict to near
6 (F1, P1)	c.1125delA (p.E376WfsX14)	9	F	XT	−4 upgaze	?	Intrusions	?	No	Pallor and 0.9 cupping	Plano-1.00×180	WNL	Wheelchair bound and poor cognition/ cooperation; pupils sluggish
7 (F3, P1)	c.1772G>A (p.R591Q)	9	F	Ortho	−4 upgaze and downgaze	Saccadic	Intrusions	No	No	Temporal pallor	1.00–0.50×180	WNL OD; delayed OS	Wheelchair bound and poor cognition; sister of patient 8
8 (F3, P2)	c.1772G>A (p.R591Q)	4	M	Ortho	−4 upgaze; −2 downgaze	Saccadic	Intrusions	No	No	Pallor	3.75–0.75×180	Delayed OU	Brother of patient 7

Age, when examined, in years; Conv, convergence; Ductions, on a scale of 0 to −4 with the latter being no movement in the attempted direction of gaze; F, female; fVEP, flash visual evoked potential; ID, patient identification number (details in parentheses refer to reference 5); M, male; Nyst, nystagmus; OD, right eye; OS, left eye; OU, both eyes; Ortho, orthotropia; Retinoscopy, cycloplegic refraction in each eye; WNL, within normal limits; XT, exotropia; X(T), intermittent XT; ?, not able to be assessed because of patient cognition/cooperation.

in children and young adults. Other recurrent findings not previously highlighted for the condition were abnormal convergence, saccadic pursuit, and saccadic intrusions that resembled square wave jerks. Strabismus and optic nerve head pallor, previously reported findings in the disease, were found in 50% and 100% of our cohort, respectively, and all strabismus in our series was exotropia.

PANK2-related disease is the only genetically confirmed form of NBIA for which the ophthalmic phenotype has been previously characterised (although 3/16 patients in that report[3] had only one confirmed *PANK2* mutation). In that study,[3] 4/10 had poor convergence, 10/10 patients had saccadic pursuit, and 2/10 had square wave jerks. Strabismus was only described in one patient, an adult with periodic alternating skew deviation. Eight of 10 had sector iris paralysis consistent with bilateral Adie's pupil, 4/10 had evidence for pigmentary retinopathy, and 10/16 had electroretinographic evidence of retinal dystrophy. Of note, optic nerve head pallor was not seen in any patient. Because of this the authors suggested that *PANK2* should not be considered a candidate gene in children affected by NBIA who have optic nerve head pallor.[3]

In the current series of patients with *PLA2G6*-related disease, although some eye movement findings overlapped with those of *PANK2*-related disease, the ophthalmic phenotype was distinct. Abnormal convergence, saccadic pursuit, and saccadic intrusions that resemble square-wave jerks are seen in both conditions. Upgaze palsy, however, is a previously unrecognised feature of *PLA2G6*-related disease that is not part of *PANK2*-related phenotype. Interestingly, there is a report of two brothers affected by NBIA who both had documented upgaze palsy; they tested negative for *PANK2* mutation and were labelled as Karak syndrome (after their town in Jordan) but not tested for *PLA2G6* mutation.[6] We are unable to confirm whether the upgaze palsy we observed in our case series was congenital or acquired, but because it was present in all patients including the youngest child (4 years old) we expect that it was early onset or congenital. Neurodegeneration affecting one or more centres for upgaze seems likely to have been responsible. The fact that Bell phenomenon was absent suggests the palsy was not limited to a supranuclear mechanism—that is, that the midbrain was affected as well. Although the substantia nigra was involved bilaterally in the midbrain, this would not account for an isolated deficit of upgaze. Among the known neurological centres associated with upgaze palsy are the dorsal midbrain and the pretectal area.[7] Another possibility is that the inability to look upward could have been related to bilateral primary inferior rectus muscle fibrosis; however, this explanation seems less likely, particularly in the context of confirmed neurodegeneration in these patients. In addition, MRI did not demonstrate any abnormalities of the extraocular muscles. Moreover, had bilateral inferior rectus muscle fibrosis been present, attempted elicitation of Bell phenomenon would be associated with some degree of globe retraction, and this was not observed. Forced duction testing could have further assessed this unlikely possibility of bilateral inferior rectus muscle fibrosis, but practically was not able to be performed. Paediatric upgaze palsy as observed in our cohort is in general a rare clinical sign. Other paediatric neurological contexts in which it has been reported include hydrocephalus,[7] Parinaud syndrome,[7] the Miller-Fisher variant of Guillain-Barre syndrome,[8] vitamin B12 deficiency,[9] and Niemann-Pick disease type C.[10]

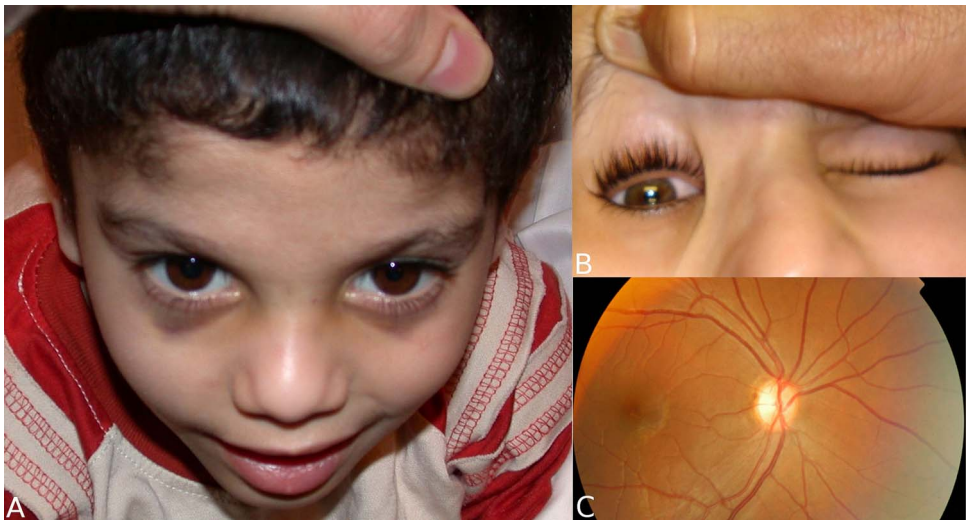

Figure 2 Clinical examples. (A) Patient 8 at 4 years old. With his head held in a chin down position, the child cannot move his eyes upward to view an object of regard. (B) Patient 5 at 10 years old. There is no Bell phenomenon (no supraduction reflex during forced eyelid opening while the patient is closing his eyes tightly). (C) Patient 2 at 16 years old. Optic nerve head pallor can be appreciated.

Strabismus was frequent, present in 50% of our cases, and always exotropia. Strabismus has been previously reported for *PLA2G6*-related disease but has not been previously specified as exotropia (or as anything more specific than 'strabismus').[4] Our patients/families could not be precise as to exactly when the exotropia was first evident, but all agreed it was within the first few years of life, consistent with what has previously been reported.[4] This exotropia could have been related to poor vision (as could occur in any individual with poor vision) or to the progressive neurodegeneration affecting one or more brain control mechanisms for binocular vision.

Optic nerve head pallor, another previously described feature of *PLA2G6*-related disease, was present bilaterally in 100% of our cases and thus like upgaze palsy is characteristic for the phenotype. We are unable to confirm whether this optic nerve head pallor was congenital or acquired, but because it was present in all patients including the youngest child (4 years old) we expect that it was early onset or congenital. As mentioned above, the frequency of exotropia in *PLA2G6*-related disease may be related to the frequency of poor vision; this in turn may be related to optic nerve atrophy. Accurate visual acuity and/or visual field testing could not be performed because of patient comprehension/cooperation issues. The visible optic nerve head pallor may have been from optic neuropathy or in the context of concurrent retinal dystrophy without visible retinal dystrophic changes, which can result in optic nerve head pallor. Proper investigation of optic nerve and retinal function would require electrophysiological studies of the retina (standard electroretinography), macula (pattern or multifocal electroretinography), and optic nerve (visual evoked potential in the context of macula evaluation results); however, comprehension/cooperation issues precluded our ability to perform complete electrophysiological testing. Gross flash visual evoked potential confirmed central visual pathway abnormality in three of the eight patients.

In summary, for the genetically heterogeneous condition known as NBIA we define the ophthalmic phenotype of *PLA2G6*-related disease in children and young adults. Upgaze palsy and bilateral optic disc pallor are characteristic and distinguish *PLA2G6*-related disease from the other major form, *PANK2*-related disease. Exotropia is frequent. Eye movement findings that are less specific for this genotype but are recurrent include poor convergence, saccadic pursuit, and saccadic intrusions that resemble square wave jerks.

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Patient consent Obtained.

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