Ataxia with Oculomotor Apraxia Type 4 with *PNKP* Common "Portuguese" and Novel Mutations in Two Belarusian Families

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Abstract

Ataxia with oculomotor apraxia type 4 (AOA4) is a rare autosomal recessive, *PNKP*-related disorder delineated in 2015 in Portugal. We diagnosed AOA4 by next generation sequencing (NGS) followed by Sanger's sequencing in three boys from two unrelated Belarusian families. In both families, one of the heterozygous *PNKP* mutations was c.1123G>T, common in Portuguese patients; biallelic mutations, c.1270_1283dup14 and c.1029+2T>C, respectively, were novel. These are the first reported AOA4 Slavic cases and the first with a "Portuguese" *PNKP* mutation outside Portugal. Distinction in two brothers was microcephaly but their disease was not severe in contrast to *PNKP*-related "microcephaly, seizures, and developmental delay" and reported cases with features of both phenotypes.

Keywords
- ataxia with oculomotor apraxia type 4
- microcephaly
- mutation frequency

Introduction

The *PNKP* gene (MIM*605610, locus 19q13.33) encodes polynucleotide kinase 3′-phosphatase, a protein involved in DNA damage repair, and it is associated with two autosomal recessive disorders: ataxia with oculomotor apraxia type 4 (AOA4; MIM#616267) recognized in Portuguese families[1] and "microcephaly, seizures, and developmental delay" (MCSZ; MIM#613402) delineated in families of various ethnicity.[2] Though different, the diseases have cross-points, and mixed phenotypes have been reported.[3,4] *PNKP*-related cases with predominant congenital or adult-onset axonal polyneuropathy and later-appearing mild AOA4 features adjoin AOA4.[5,6] Some *PNKP* mutations are shared by different phenotypes. The *PNKP* missense mutation c.1123G>T (p.Gly375Trp) was common in a Portuguese AOA4 group[1] but not found elsewhere. We diagnosed AOA4 in two Belarusian families with compound heterozygosity for c.1123G>T and novel mutations. These cases and our data on frequency of the detected mutations in a population-matched control are presented.

Families and Methods

The two families are of Belarusian ethnicity, nonconsanguineous, unrelated, and come from different Belarus regions.

DNA testing in patients 1 and 2–1 was performed by targeted next generation sequencing (NGS) panel Illumina TruSight One (clinical exome sequencing, CES) performed on an Illumina NextSeq 500 instrument in 2×151 bp paired-end mode in the Laboratory of Molecular Pathology (Genomed Ltd., Moscow). The bioinformatics pipeline of NGS data analysis was described previously.[7] Further filtering was performed by functional consequences, population frequencies, and clinical relevance. All variants were named according to the NM_007254.3(*PNKP*_v001) reference transcript variant. NGS findings of definite/probable diagnostic value were verified by Sanger's sequencing in the patients and their parents.

For the population study, peripheral blood samples were collected from 116 unrelated healthy individuals of Belarusian origin. The population frequency of detected *PNKP* mutations was studied in the control sample by PCR-RFLF

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for c.1123G>T (by restriction with *Bss*T1I endonuclease) and c.1029+2T>C (by restriction with *Hph*I) and by AFLP for c.1270_1283dup14. Primer sequences are listed in ►**Supplementary Table S1** (available in online version only).

Informed consent of the families for obtaining blood samples and for publication was obtained. The study was approved by the Ethics Committee of the Research Centre for Medical Genetics.

Results

Clinical Findings

The case of Patient 1, a 9-year-old boy, was partially reported (for pedigree see ►**Fig. 1A**).[8] Early development was normal except for non-progressing dysarthria. He walked at 10 months, at 2 years moderate unsteadiness appeared and was stable up to 8 years, initial diagnosis was mild cerebral palsy. At 8 years, ataxia progressed and hyperkinesia became evident. Brain magnetic resonance imaging (MRI), which was previously normal, revealed cerebellar atrophy (►**Fig. 1C**). Electroneuromyography (ENMG) revealed axonal polyneuropathy. Examination showed normal height and occipitofrontal circumference (OFC), vertical ophthalmoparesis, oculomotor apraxia (OA), peroneal paresis (inability to stand on heels), low arm and patellar reflexes, Achilles areflexia, normal sensation, unsteadiness in Romberg's position, slow unsupported gait with features of paretic and ataxic, moderate generalized dystonic hyperkinesia (particularly in hands), and dystarthria; there was impression of mild/moderate cognitive deficit. Additional information was received from parents 1.5 years later: neurological disorders progressed, the boy moved with a support indoors and in a

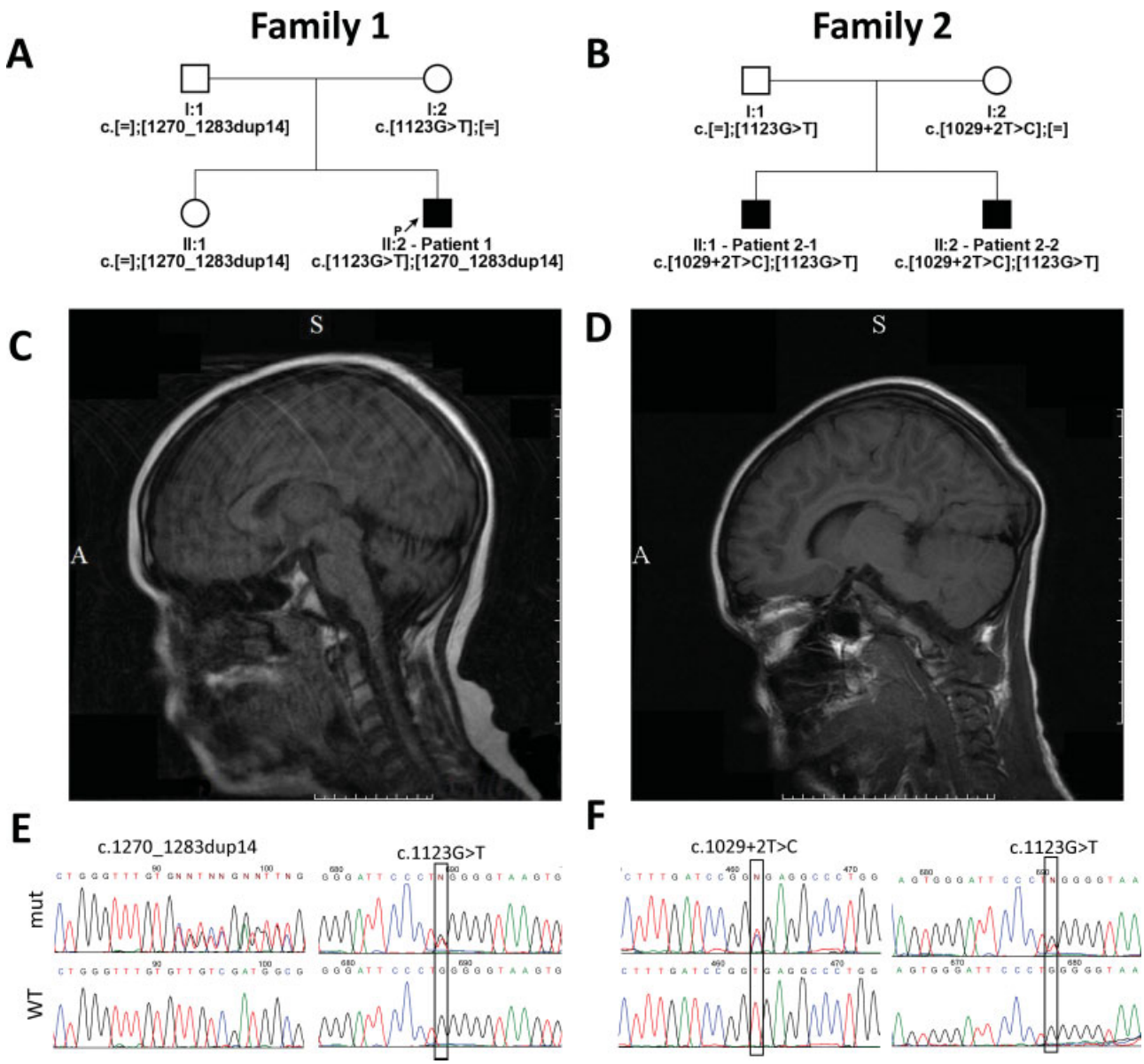

Fig. 1 Pedigrees of families 1 and 2 are shown in (**A**) and (**B**), respectively. T1-weighted FLAIR mode sagittal slice images of brain MRI of patients 1 and 2–1 are shown in (**C**) and (**D**), respectively. Sequenograms of the identified mutations in patients 1 and 2–1 are shown in (**E**) and (**F**), respectively. FLAIR, fluid attenuated inversion recovery; MRI, magnetic resonance imaging.

wheel-chair outdoors. He had learning difficulties at school for children with special needs but managed reading, writing, and simple arithmetic. Psychological and psychiatric examination showed moderate total mental underdevelopment, particularly in verbal tests, and stiffness of thinking with no behavioral disturbances. AOA type 1 (MIM#20820, gene *APTX*) characterized by average onset at 4 to 5 years, ataxia, dysarthria, OA, axonal polyneuropathy, cerebellar atrophy on MRI and some facultative signs (hyperkinesia, cognitive deficiency, hypoalbuminemia, and hypercholesterolemia) seemed most probable, and NGS was performed.

Family 2 had two affected children, 2–1 and 2–2 (for pedigree see ►**Fig. 1B**). We examined them twice: at 5 (2–1) and 3 (2–2) years, and at 8 (2–1) and 6 (2–2) years; additional information from parents was received 1 year later. Though patient 2–1 had pre-/perinatal complications, the boys developed similarly and achieved early milestones timely but ataxia was evident from the age of independent walking. At 5 to 6 years of age, moderate hyperkinesia appeared. Other signs were microcephaly, speech delay, and dysarthria. Examination revealed normal height, microcephaly (on second visit OFC 48 cm, under 3rd percentile, < -2 standard deviation (SD) in both), OA, retained muscle power, reflexes, and sensation, moderate ataxia (unsteadiness in Romberg's position, intention tremor in limbs, unsupported ataxic gait), dysarthria, and moderate dystonic hyperkinesia. In addition, since 5.5 years, patient 2–1 had attacks of severe headache, abdominal pain, and retching, sometimes with a short phase of tonic tension, blurred (not lost) conscience, and incoherent stereotypical murmuring. They occurred after falling asleep and could be suppressed by instant analgesics injection. Over 2.5 years, there were only five attacks. Regular electroencephalography (EEG) registered no epileptic activity, epilepsy was rejected and complicated migraine was diagnosed. On recent information, with schooling onset at 8.5 years, the attacks provoked by fatigue or emotional stress occurred monthly, twice accompanied by urination, and once by defecation. Though there was no loss of conscience or epileptic EEG activity, epilepsy was suggested again. Over 4 months on levetiracetam the boy is free of attacks, yet their epileptic nature remains questionable.

Since 8.5 years (patient 2–1) and 7 years (patient 2–2) the boys began studying at school with sparing regimen. Results of neuropsychological examination performed at that time were similar. Both boys contacted adequately. Insufficiency of neurodynamic activities (split attention and failing concentration) was detected. Signs in emotional sphere were infantilism and emotional lability. Impairment of visuoconstructive and graphic praxis was detected by tests for recoding and head tests and simultaneous agnosia was a sign of fragmentary perception. There were difficulties in performing tests related to logical and grammatical constructions comprehension. Detected disturbances are of functional nature due to deprivation of mediobasal temporal regions, temporo-parieto-occipital (TPO) zone and limbic subcortex. Mental development is within normal limits, yet educational capacities need medical, pedagogical, and psychological corrections.

Repeated ENMG and brain MRI in both boys were normal. MRI picture of patient 2–1 is shown in ►**Fig. 1D**. Tests for several neurometabolic disorders were negative. NGS was recommended with no concrete preliminary diagnosis.

Molecular Findings

NGS in patient 1 revealed two heterozygous *PNKP* mutations: c.1123G>T (p.Gly375Trp) in exon 16, common in Portuguese patients, and a novel small deletion c.1270_1283dup14 in exon 14 leading to frame shift, p.Ala429Gln*fs**43 (►**Fig. 1E**). According to Bras et al,[1] c.1123G>T is located in a potential ATP nucleotide-binding domain within the kinase region of *PNKP*. The latter variant is predicted to disrupt the second half of the kinase domain of PNKP protein and is thus regarded as probably pathogenic. Neither mutation was registered in the Genome Aggregation (gnomAD) database. Sanger's sequencing in the family detected biallelic mutations in the patient, heterozygous c.1123G>T in the mother, and heterozygous c.1270_1283dup14 in the father.

NGS in patient 2–1 also detected two heterozygous *PNKP* mutations: c.1123G>T and an intronic mutation, c.1029+2T>C (rs199919568), affecting an invariant donor splicing site dinucleotide of intron 11 (►**Fig. 1F**), which was not previously described in association with human diseases and thus considered as probably pathogenic. Disrupting the donor splicing site should lead to entire exon 11 skipping as predicted by Human Splicing Finder v. 3.1 (http://www.umd.be/HSF3/) and MutationTaster, which would result in in-frame deletion p.(Phe313_Pro343del) affecting the phosphatase domain of PNKP protein. The latter variant is present in the total gnomAD database with an allele frequency of 0.001067, but has never been found in the homozygous state. Sanger's sequencing in the family revealed biallelic mutations in patient 2–1, an identical *PNKP* genotype in patient 2–2, and heterozygosity in the parents: paternal c.1123G>T, maternal c.1029+2T>C. Thus, AOA4 was diagnosed in both families.

Population screening for the three detected mutations was performed: c.1123G>T and c.1270_1283dup14 were not detected in 232 population-matched control chromosomes tested, while c.1029+2T>C was found in one case.

Discussion

Families 1 and 2 are the first reported AOA4 Slavic cases. Features of the disease are summarized in ►**Table 1**. Patient 1 presents typical AOA4 and vertical ophthalmoparesis is his only neurodegenerative sign, unreported in AOA4 previously. Family 2 has some distinctions from AOA4, namely microcephaly in patients 2–1 and 2–2 and probable nonsevere epilepsy in patient 2–1. Both features are signs of MCSZ but, in contrast to the disease in the brothers, MCSZ is characterized by progressing microcephaly, severe development delay, and infantile-onset refractory epilepsy, but no ataxia or OA.[2,9,10] In few reported cases of mixed MCSZ-AOA4 phenotypes, MCSZ features were predominated.[3,4] Though AOA4 with microcephaly and/or epilepsy was not reported previously, we consider the phenotype in the brothers as AOA4 variant. Absence of cerebellar atrophy on MRI and of polyneuropathy may be other distinctions of AOA4 in family 2, or these signs may appear later, like polyneuropathy in Swedish

Table 1 AOA4 features in families 1 and 2 and in other reported cases

	Family 1	Family 2		Reported cases[a] (17 patients/13 families)
	Patient 1	Patient 2–1	Patient 2–2	
Gender	Male	Male	Male	6 males, 11 females
Family ethnicity	Belarusian	Belarusian		Portuguese (8), Swedish, Norwegian, German, Brazilian, Arabic (one each)
Age at diagnosis (y)	9	8	6	Variable, up to 50
Age at onset (y)	2	1 (since age of walking)		Average, 4–5 (range, 1–14)
First sign	Ataxia	Ataxia		Variable: hyperkinesia, ataxia, OA, polyneuropathy
Ataxia	+[b]	+[c]		+
Dysarthria	+	+		+
Oculomotor apraxia	+	+		± (absent in 3 patients)
Axonal polyneuropathy	+	−		++
Hyperkinesia	+	+		+
Independent walking	+	+		Loss in 2nd–3rd decades
Cognitive impairment	+ (mild)	+ (mild)		±
Microcephaly	−	+		−
Other signs	Vertical ophthalmoparesis	Epilepsy (?,[e])	−	Edema (1); pilocytic astrocytoma (1)
MRI: cerebellar atrophy	+[d]	−		+
MRI: brainstem atrophy	−	−		±
Obesity	−	−		±
Hypercholesterolemia	+ (6.6 mg/dL; $n < 5.2$)	−		±
Hypoalbuminemia	–	Not tested		±
Serum α-fetoprotein	↑(17.7 ME/mL; $n < 7.3$)	Normal		Normal/raised (rarely)
PNKP mutations	c.[1123G>T]; [1270_1283dup14]	c.[1123G>T]; [1029+2T>C]		Ten reported mutations; c.1123G>T common in Portugal

Abbreviations: AOA4, Ataxia with oculomotor apraxia type 4; MCSZ, microcephaly, seizures, and developmental delay; MRI, magnetic resonance imaging.
[a]MCSZ cases with some AOA4 signs (Poulton et al[3], Taniguchi-Ikeda et al[4]) and Costa Rican cases regarded as hereditary neuropathy (Leal et al[6]) are not included.
[b]Deterioration at 8 years.
[c]No deterioration.
[d]Appeared at 8 years.

and Norwegian patients,[11,12] like cerebellar signs in Latin American cases with predominant polyneuropathy,[5,6] or like cerebellar atrophy in patient 1. In spite of some differences, the disease in the brothers is similar, but intrafamilial AOA4 variability may be more pronounced as in the Arab family.[13]

To our knowledge, families 1 and 2 are the first reported cases with *PNKP* mutation c.1123G>T outside Portugal, where it was found homozygous in four and heterozygous in two of eight AOA4 families.[1] Belarusian origin of both families is of interest (the more as families from Belarus are infrequent among our patients) but may be coincidental.

Another mutation in the Portuguese AOA4 group, c.1253_1269dup17 (p.Thr424fs), was reported also in MCSZ,[2] in mixed MCSZ-AOA4 phenotype,[3] and in a German patient with AOA4 and pilocytic astrocytoma.[14] The mutation together with mutation c.1270_1283dup14 (p. Ala429fs), identified also in Family 1, was detected by CES in our third *PNKP*-related case. This 1-year-old Russian boy had typical MCSZ: congenital microcephaly (OFC; 29 cm at birth, 39 cm in 1 year), profound mental and motor delay, severe epilepsy since 4 months, and no malformations on brain computed tomography. As the parents' DNA was unavailable for genotype verification, the family was not reported yet. Thus, both mutations can be found in AOA4 as well as in MCSZ. Another example of identical *PNKP* mutation in differing phenotypes is c.1221_1223delCAC (p. Thr408del) found in typical AOA4[1] and in cases with predominant polyneuropathy.[5,6]

The phenotype of the brothers in family 2 is in line with the opinion that MCSZ and AOA4 present a clinical continuum rather than independent phenotypes.[4] *PNKP* mutations shared by both disorders support this opinion. Genetic and/or epigenetic modifiers are most likely reasons for inter- and intrafamilial variability of *PNKP*-related disorders.[15]

Conclusion

The two reported Belarusian families have several points of interest: they are the first Slavic AOA4 cases; the heterozygous *PNKP* mutation c.1123G>T, found in both families, proves that the mutation is not only "Portuguese"; two novel *PNKP* mutations were detected; microcephaly in affected brothers and probable nonsevere epilepsy in one of them are novel AOA4 features expanding its phenotype and adding to the opinion of absence of strict borders between *PNKP*-related disorders.

Authors' Contribution

Authors G.E.R. and A.V.M. have designed the study; authors G.E.R., O.A.S., E.L.D., I.A.A., N.V.P. have collected data for the study; authors G.E.R., A.V.M., O.A.S., E.R.L., F.A. K. have done data analysis; and authors G.E.R., A.V.M. have prepared the manuscript.

Conflict of Interest

None declared.

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