



Homozygous variant, p.(Arg643Trp) in *VAC14* causes striatonigral degeneration: report of a novel variant and review of *VAC14*-related disorders

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Abstract

VAC14-related disorders include two distinct phenotypes, striatonigral degeneration [MIM# 617054] and Yunis–Varon syndrome. Striatonigral degeneration is a recently described childhood onset dystonia caused by pathogenic variants in *VAC14*. It is characterized by a period of apparent normalcy followed by abrupt onset neuroregression, dystonia, involuntary movements and degenerative brain lesions involving caudate nucleus, putamen and substantia nigra. Yunis–Varon syndrome is a well described severe condition characterised by skeletal findings and dysmorphism along with neuronal degeneration. Pathogenic variants in *FIG4* have been previously reported to cause Yunis–Varon syndrome. Recently, loss of function variants in *VAC14* were also reported in an individual affected with Yunis–Varon syndrome. Total seven individuals from four families are reported to have *VAC14*-related disorders till date. Here, we report another individual with clinical and radiological features suggestive of striatonigral degeneration with homozygous missense variant in *VAC14*. The patient fibroblasts showed extensive vacuolization, characteristic of *VAC14*-related disorders. We also review the phenotype and genotype associated with these disorders.

Introduction

Striatonigral degeneration (MIM# 617054, SND) and Yunis–Varon syndrome (YVS) are two distinct disorders caused by pathogenic variants in *VAC14*. Individuals affected with *VAC14*-related disorders present early in life. YVS is a severe condition involving skeletal abnormalities, dysmorphism, neurological deficit and brain imaging findings of pachygyria, agenesis of corpus callosum and cerebellar atrophy. While SND is characterised by abrupt onset of neuroregression, dystonia, involuntary movements and findings of degenerative basal ganglia lesions uncovered upon neuroimaging [1]. Histopathological evaluation of brain and skin tissue from individuals with both the

disorders have been described with extensive endolysosomal vacuolization [1–3]. Till date six individuals from three families with SND and a single case with *VAC14*-related YVS have been described in the literature [1–4]. We hereby report another individual with SND and review clinical and genetic findings associated with *VAC14*-related disorders.

Clinical report

We ascertained a sixteen-month-old female, first born to a consanguineously married couple (Fig. 1a). She was born at term by normal vaginal delivery with birth weight of 2.5 kg (–2 SD). She attained age appropriate milestones till 8 months of age, however the former were subsequently lost. On examination, her occipito-frontal circumference was 46 cm (normal), weight 8.2 kg (–2.5 SD) and length 72 cm (–2.1 SD). She had spasticity, dystonia, clonus and exaggerated deep tendon reflexes. Rest of the systemic examination was unremarkable. Auditory brainstem response test revealed bilateral profound sensorineural hearing loss. Metabolic testing (gas chromatography-mass spectrometry and tandem mass spectrometry) returned normal results. On magnetic resonance imaging (MRI) of

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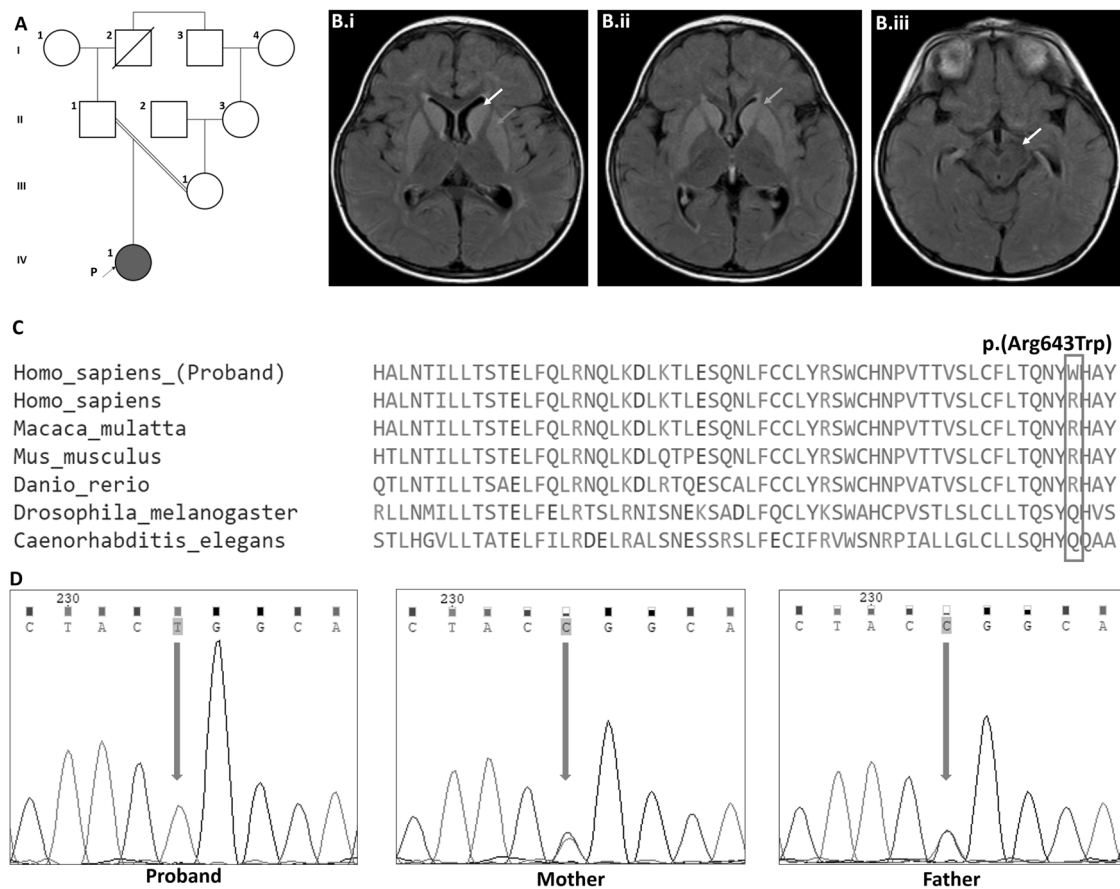


Fig. 1 **a** Pedigree of the family. **b** Magnetic resonance imaging of brain in the proband revealed T2 hyperintensities in caudate nucleus (yellow arrow), putamen (red arrow) (b.i) and periventricular white matter (b.ii). T2 hypointense foci were noted in substantia nigra (white

arrow, b.iii). **c** Multiple sequence alignment representing the conservation of amino acid residue Arg643 across vertebrates. **d** Sanger chromatograms demonstrate bi-allelic segregation of the variant c.1927C > T p.(Arg643Trp) in exon 16 of *VAC14* (NM_018052.5)

brain, T2-weighted imaging showed bilateral hyperintensities in caudate nucleus, putamen and periventricular white matter without diffusion restriction and hypointense foci in substantia nigra (Fig. 1b).

Methods

Informed consent approved by the institutional ethical committee was taken following detailed clinical and radiological evaluation. Exome sequencing (Illumina, Inc. San Diego, California, USA) was performed for the index patient as described previously [5]. Variant prioritization strategy used to analyse the data is outlined in Tables S1 and S2. Validation of the candidate variant identified by exome sequencing and segregation analysis was done by Sanger sequencing.

To assess the structural impact of the pathogenic variant we performed in silico 3D modelling of both wildtype and mutant *VAC14* (HGNC ID: 25507) using I-TASSER (Iterative Threading ASSEmbly Refinement), as its protein

structure is absent in Protein Data Bank (PDB). I-TASSER generated five models with each input and those with C score of -0.50 (wildtype) and -0.57 (mutant) were retrieved in PDB format and loaded on PyMOL (The PyMOL Molecular Graphics System, Version 2.0.6, Schrödinger, LLC) to compare the polar interactions of both wildtype (Arg643) and mutant (Trp643) residue.

To investigate the previously reported vacuolization phenotype associated with SND, the patient and control fibroblasts were cultured in RPMI-1640 medium, trypsinized and fixed using 4% paraformaldehyde. Subsequently the fixed cells were evaluated using Axio Vert.A1 FL microscope (Carl Zeiss, Germany).

Results

The proband presented with neuroregression, spasticity, dystonia, hearing loss and characteristic neuroimaging findings of SND. Detailed clinical and radiological phenotype is described in Table 1. Exome sequencing in her

Table 1 Phenotypic and genotypic comparison of individuals with *VAC14*-related disorders

Family	Present study	Lenk et al. [1]		Stuttered et al. [3]		Lyon et al. [4]		Lines et al. [2]
		Proband 1		Proband 2		Family 1		
		Individual 1	Individual 2	Proband	Affected sibling			
Variant in <i>VAC14</i> (NM_018052.5)	c.1927C>T; p.(Arg643Trp)	c.1271G>T; p.Trp424Leu c.1528 + 1G>A	c.1744G>T; p.Ala582Ser c.1748C>T; p.Ser583Leu	c.1271G>T; p.Trp424Leu	c.1271G>T; p.Trp424Leu	c.2005G>T p.Val669Leu	c.1895C>T; p.Thr632Met, c.923T>A; p.Leu308Ter	
Disease entity	SND	SND	SND	SND	SND	SND	YVS	
Age at examination	16 months	5 years	2 years	NA	NA	4 years	Neonate	
Gender	Female	Male	Male	Male	Male	Female	Female	
Birth history	Not significant	Not significant	Not significant	Not significant	Not significant	Not significant	Small for gestation	
Birth weight (SD)	2.5 kg (-1)	3.9 kg (normal)	3.6 kg (normal)	NA	NA	2.8 (-2 SD)	2.3 kg (-1.6 SD)	
Onset of neuroregression	8 months	3 years	18 months	3 years 6 months	2 years	NA	Neonate	
OFC (SD)	46 cm (normal)	Normal	Normal	Normal	Normal	NA	Macrocephaly	
Weight (SD)	8.2 kg (-2)	NA	NA	NA	NA	NA	NA	
Length (SD)	72 cm (-2)	NA	NA	NA	NA	NA	NA	
Dysmorphism	-	-	-	-	-	+	+	
Bilateral cortical cataract	-	-	-	-	-	+	+	
Retinitis pigmentosa	-	-	-	-	-	+	-	
Dystonia	+	+	+	+	+	-	-	
Ataxia	+	+	+	+	+	+	+	
Dysarthria	-	+	+	+	+	Delayed speech	-	
Muscle spasms	-	+	-	+	+	-	-	
Joint contractures	-	-	-	+	+	-	-	
Hearing evaluation	Profound hearing loss	NA	NA	NA	NA	NA	NA	
Skeletal findings	Not done	NA	NA	NA	NA	Short fingers	Osteopenia, hypoplastic bones	
Neuroimaging findings								
T2W hyperintensities in basal ganglia	+	+	+	-	-	+	Progressive cystic degeneration of the basal ganglia	
T2W hyperintensities in periventricular white matter	+	+	+	-	-	-	-	
T2W hypointense foci in substantia nigra	+	+	+	-	-	+	-	

Table 1 (continued)

Family	Present study		Lenk et al. [1]		Stuttered et al. [3]		Lyon et al. [4]		Lines et al. [2]	
	Proband 1	Proband 2	Family 1		Family 1		Family 1			
			Individual 1	Individual 2	Individual 1	Individual 2	Proband	Affected sibling		
Diffuse leukoencephalopathy	–	–	–	–	–	–	–	–	NA	+
MRS	Not done	Normal	Normal	NA	NA	NA	Normal	Normal	NA	Small N-acetylaspartate (NAA) peak

SND striatonigral degeneration, YUS Yunis–Varon syndrome, + present, – absent, NA not available, MRS magnetic resonance spectroscopy

revealed a previously unreported missense variant c.1927C >T p.(Arg643Trp) in homozygous state in exon 16 of *VAC14* (NM_018052.5). Her parents were found to be heterozygous carriers for the same (Fig. 1d). This variant is not observed in the population database gnomAD and our in-house data of 583 exomes. Multiple in silico analysis tools (MutationTaster, Sorting Intolerant from Tolerant and Combined Annotation Dependent Depletion) are consistent in predicting the variant to alter protein function. Genomic evolutionary rate profiling and Clustal Omega (Fig. 1c) analyses demonstrated that this variant occurs at an amino acid that is highly conserved across species. In silico protein modelling of wild-type and mutant *VAC14* revealed loss of polar contacts due to the mutant residue with Arg603, Asn604 and Asp647 and gain of interaction with Gln640 (Fig. 2a). On microscopy, patient fibroblast cells demonstrated extensive vacuolization as compared with the control cells (Fig. 2b).

Discussion

VAC14-related disorders are a recently described group of developmental conditions. Till date seven individuals of four families, including the present case, have been identified with SND [1, 3, 4] and a single case is reported to have YVS [2]. All individuals with SND presented in early childhood with abrupt onset of neuroregression. Lenk et al. reported two probands from two families. Proband 1 was apparently normal till 3 years 1 month of age followed by abrupt onset regression. He developed lower limb dystonia, which became generalised by the age of 5 years. Proband 2 also had abrupt onset of regression at 18 months with weakness and dystonia. In addition, he had slowing of speech although his receptive language was normal [1]. In the family reported by Stutterd et al., individual 1 had normal development for first 3 years of life followed by neuroregression. Thereafter, left leg dystonia was noted, which subsequently extended to involve all four limbs and truncal ataxia. He had progressive dystonia, painful muscle spasms, joint contractures and died at 5 years of age. Individual 2 had regression at 2 years of age and followed a similar course of sudden deterioration at 4 years and death [3]. In the family reported by Lyon et al., the proband had regression by the age of 2 years with unsteady gait and unclear speech. He had walking difficulty, which progressed to spastic paraparesis and spastic ataxic gait. However, frank dystonia was not noted. At 5 years of age, he developed visual difficulty and was diagnosed with retinitis pigmentosa. His 4-year-old sibling was also noted to have developmental difficulties and retinitis pigmentosa [4]. The present proband had an earlier age of presentation as compared with the other individuals reported in the

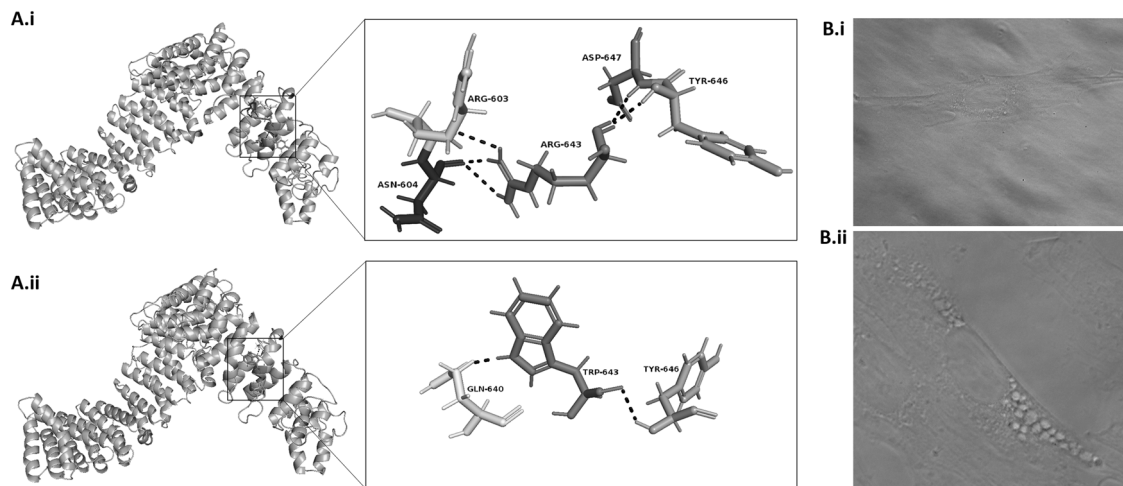


Fig. 2 **a** In silico predicted model of wildtype Vac14 and its corresponding magnified view (a.i) demonstrate Arg643 (wildtype) interacts with four neighbouring residues (Arg603, Asn604, Asp647 and Tyr646). In silico predicted model of mutant Vac14 and its corresponding magnified view (a.ii) demonstrate Trp643 (mutant) lost polar

interactions with Arg603, Asn604, Asp647 and gained an interaction with Gln640. **b** Images of control (b.i) and patient (b.ii) Fibroblasts demonstrate significantly increased vacuolization in patient cells as compared with control cells

literature. Speech could not be evaluated in her since she was 16-months old at the time. Hearing evaluation revealed profound bilateral hearing loss, which was not screened in any of the earlier reported individuals. The progression of the disease appears to be variable given that out of two individuals in a family, one succumbed to the illness in childhood [3], while the other was alive at 21 years of age [4].

Characteristic MRI findings of degenerative changes in basal ganglia and periventricular region were noted in five subjects with SND, including the present proband [1, 4]. Of note, one family had normal neuroimaging findings, however, their data were not available for review [3]. Basal ganglia histopathology studies in them revealed neuronal degeneration.

The reported subject with YVS, presented shortly after birth with hypotonia, developmental delay, facial dysmorphism, bilateral cortical cataract and skeletal abnormalities [2]. She had extensive cavitating changes in basal ganglia and diffuse leukoencephalopathy with decreased NAA peak on neuroimaging.

Exome sequencing in the proband from the current study revealed a homozygous missense variant, c.1927C>T p.(Arg643Trp) in *VAC14*. *VAC14* encodes for a scaffold protein that interacts with *FIG4* encoded phosphatidylinositol 3,5-bisphosphate phosphatase to activate *PIKFYVE* encoded phosphatidylinositol 3,5-bisphosphate synthesizing enzyme [6–8]. All three proteins are essential for phosphatidylinositol 3,5-bisphosphate synthesis and its dynamic regulation. Phosphatidylinositol 3,5-bisphosphate is an essential component of the eukaryotic endolysosomal membranes and is involved in intracellular fission and

fusion events, thus playing a crucial role in intracellular vesicle transport.

Knockdown of *VAC14* expression in HEK293 cells has been previously shown to result in decreased levels of phosphatidylinositol 3,5-bisphosphate and large endolysosomal vacuoles [6]. Similar metabolic and histopathological findings were noted in patients with bi-allelic disease-causing variants in *VAC14* [1–3]. Previously, *Vac14*-null mouse model showed neonatal lethality, neurodegeneration of the central nervous system and peripheral nervous system [8, 9]. It recapitulated the phenotype described in individuals with *VAC14*-related disorders. In silico comparative study of polar contacts of both the wildtype (Arg643) and the mutant residue (Trp643) predicted altered molecular interactions. This variant is likely to result in loss of interactions with three of the four neighbouring residues and gain of an interaction with Gln640 residue. This is likely to result in altered protein structure, thus affecting its activity.

Bi-allelic variants in *FIG4* have been reported to cause complete loss of protein function and resulted in the severe phenotype of YVS [10]. While variants, which impaired the protein function but do not cause complete loss of activity, resulted in moderately severe Charcot–Marie–Tooth disease, type 4J [11] and bilateral temporo-occipital polymicrogyria [12]. Similar genotype–phenotype correlation has not been observed for *VAC14*-related disorders. Bi-allelic missense variants and truncating variants in *trans* with the missense variants (Table 1) in *VAC14* are known to cause SND and YVS [1–4].

Individuals with pathogenic variants in *VAC14* present with recognisable and characteristic but variable clinical phenotypes. However, further report of individuals with

these rare disorders would aid in understanding the cause of variability and absence of genotype–phenotype correlation.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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