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WARS2 mutations cause dopa-responsive early-onset parkinsonism and progressive myoclonus ataxia

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ABSTRACT

Introduction: Sixteen subjects with biallelic *WARS2* variants encoding the tryptophanyl mitochondrial aminoacyl-tRNA synthetase, presenting with a neonatal- or infantile-onset mitochondrial disease, have been reported to date. Here we present six novel cases with *WARS2*-related diseases and expand the spectrum to later onset phenotypes including dopa-responsive early-onset parkinsonism and progressive myoclonus-ataxia.

Methods: Six individuals from four families underwent whole-exome sequencing within research and diagnostic settings. Following the identification of a genetic defect, in-depth phenotyping and protein expression studies were performed.

Results: A relatively common (gnomAD MAF = 0.0033) pathogenic p.(Trp13Gly) missense variant in *WARS2* was detected in trans in all six affected individuals in combination with different pathogenic alleles (exon 2 deletion in family 1; p.(Leu100del) in family 2; p.(Gly50Asp) in family 3; and p.(Glu208*) in family 4). Two subjects presented with action tremor around age 10–12 years and developed tremor-dominant parkinsonism with prominent neuropsychiatric features later in their 20s. Two subjects presented with a progressive myoclonus-ataxia dominant phenotype. One subject presented with spasticity, choreo-dystonia, myoclonus, and speech problems. One subject presented with speech problems, ataxia, and tremor. Western blotting analyses in patient-derived fibroblasts showed a markedly decreased expression of the full-length WARS2 protein in both subjects carrying p.(Trp13Gly) and an exon-2 deletion in compound heterozygosity.

Conclusions: This study expands the spectrum of the disease to later onset phenotypes of early-onset tremor-dominant parkinsonism and progressive myoclonus-ataxia phenotypes.

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1. Introduction

Mitochondrial aminoacyl-tRNA synthetases (mt-ARSs) are essential components of the translation process in mitochondria, charging tRNAs with their cognate amino acids during translation of mitochondrial genes [1]. A total of 19 mt-ARSs have been described; they all have been linked to human mitochondrial diseases with a broad range of clinical manifestations [2]. Recently, biallelic pathogenic variants in *WARS2* (OMIM *604733) encoding the tryptophanyl mt-ARSs (mtTrpRS) have been reported in 16 individuals with neonatal or infantile-onset disease presenting with phenotypes ranging from intellectual disability [3], to infantile parkinsonism [4–6], leukoencephalopathy [7,8], complex hyperkinetic disorder [9], and neurodevelopmental disorder, mitochondrial, with abnormal movements and lactic acidosis, with or without seizures (NEMMLAS) [10].

Here we present six more cases, expanding the spectrum of *WARS2*-related disease to later onset phenotypes including early-onset levodopa-responsive parkinsonism with abnormal nigrostriatal presynaptic dopaminergic imaging, clinically diagnosed as early-onset Parkinson's disease (PD) and a progressive myoclonus-ataxia phenotype.

2. Methods

2.1. Patients

Subjects with whole-exome sequencing (WES) data were recruited within research (families 1 and 2) or clinical diagnostic (families 3 and 4) settings. Clinical information and WES data were collected after obtaining signed informed consent forms from all participating individuals and families. The study was performed according to Declaration of Helsinki principles and under protocols approved by local ethic committees.

2.2. Genetic studies

Genetic studies in families 1 and 2 have been described [11,12]. WES on genomic DNA isolated from the blood lymphocytes of the subjects in families 3 and 4 was carried out using previously reported methods [13]. In short, exome capture was performed using Agilent SureSelect v6 kits, and sequencing on an Illumina HiSeq4000 system was done with 100-bp paired-end reads at an average depth of coverage of >100x. Sequence-data analysis and interpretation were performed at the Helmholtz Center Munich and Technical University of Munich (Munich, Germany) using a validated in-house-developed bioinformatics pipeline, as described [14]. In family 1, the exomes of two affected siblings and the unaffected father had been sequenced [11,12]; in family 2, quartet WES (two affected siblings plus unaffected parents) had been performed [11]; in family 3, we performed trio WES (index patient plus unaffected parents); and in family 4, only the index patient underwent WES. The affected individuals' entire WES data sets were searched for pathogenic or likely pathogenic variants in reported disorder-associated genes, as detailed earlier [13,15]. Sanger sequencing was carried out to test for the biallelic status of the identified *WARS2* variants in the index patient from family 4.

2.3. Functional studies

Patient-derived skin fibroblasts from case P1, case P2, and unrelated controls were expanded in culture, and the expression of the WARS2 protein in cell lysates was investigated using the Western blotting method. The detailed methodology is summarized in the supplementary materials. Statistical analyses were carried out using GraphPad Prism 9 (San Diego, USA). One-way ANOVA with post-hoc Tukey test was applied.

3. Results

A total of six individuals from four families with biallelic variants in *WARS2* were identified (Fig. 1). For detailed clinical characteristics and a review of previously reported cases, see Table 1 and Supplementary Table 3. Genotype information from families 1 and 2 has been published previously [11,12].

Family 1, Case P1 is a 36-year-old male of Slovakian descent born after an uneventful pregnancy and delivery. His first symptoms started at the age of 12 years with bilateral action hand tremor, which progressed to bilateral resting tremor (age 18 years) and bradykinesia with rigidity (age 20 years). His symptoms gradually progressed until, at the age of 27 years, he was referred to a movement disorders specialist and was diagnosed with early-onset tremor-dominant PD with a correlate of abnormal DaT scan (Fig. 2A, Supplementary Table 2). His MRI was repeatedly normal. He was initially treated with rotigotine 8mg/daily with good effect on bradykinesia and rigidity, while tremor was improved only partially; levodopa (up to 1000mg/daily) was added with additional partial improvement of his tremor. The tremor finally required adding propranolol to achieve acceptable compensation. Over the follow-up, the patient presented with prominent psychiatric features including major depression (BDI-II 28 pts), anxiety, apathy, and occasional irritability with associated outbursts of rage. He was not compliant to medication, and psychotic symptoms required repeated psychiatric hospitalizations. Rotigotine was discontinued and the patient is now well-compensated in terms of psychotic symptoms and irritability on a combination of clozapine and quetiapine. Currently he presents with prominent symmetric resting and postural jerky hand tremor, with mild dystonic posturing of arms and legs, hypokinesia and akinesia on repetitive upper and lower limb movements, no axial symptoms or freezing, occasional mild dyskinesia, mild cognitive impairment (MoCA 21 pts), depression, anxiety, apathy, mild urine retention and paroxysmal sinus tachycardia (supplementary video segment 1). A next-generation sequencing-based dystonia panel [16] performed at the age of 32 years did not identify a known genetic cause at that time.

Supplementary data related to this article can be found online at https://doi.org/10.1016/j.parkreldis.2021.11.030

Family 1, Case P2, the younger sister of subject P1, is a 34-year-old female of Slovakian descent. Her first symptoms started at the age of 10 years with asymmetric jerky action hand tremor. At the age of 17 years, she developed cervical dystonia that required botulinum toxin A injections with good clinical effect. At the time of her first evaluation in our center (at the age of 26 years), she presented with a jerky dystonic action hand tremor with a mild resting component and mild cervical dystonia, without rigidity and bradykinesia. On sonography, she had hyperechogenicity of substantia nigra. At the age of 32 years, she developed rigidity and bradykinesia, satisfying the MDS clinical criteria for PD; her DaT scan was abnormal in this period (Fig. 2B, Supplementary Table 2), while her brain MRI has been repeatedly normal. From the age of 27 years, she also presented with gradually worsening prominent neuropsychiatric features, specifically severe anxiety disorder, including a persistent and episodic component, and major depression (BDI-II 24 pts). She recently presented with visual hallucinations requiring neuroleptic treatment. Currently her motor features except for tremor are well compensated without any motor fluctuations on a combination of levodopa/carbidopa/entacapone (900 mg levodopa/daily) and rasagiline; she presents with prominent neuropsychiatric features, with psychotic symptoms well compensated on quetiapine, but anxiety and depression are persistent. Her cognition is normal (MoCA 28 pts). She also complains of insomnia and constipation. Features indicative of atypical complex parkinsonism [17] were not present in any of the siblings from family 1.

Family 2, Case P4 is a 24-year-old male of Czech origin. From the age of 3 years, he has presented with a slowly progressive mainly distal severe myoclonus of the arms and lower extremities that increases with

action. There is also an increase with intention; clinically this may fit into the spectrum of both mild ataxia and myoclonus. The pattern of the myoclonus suggests a cortical origin of the myoclonus, although no face jerks are present. He also manifests with intermittent mild cervical and axial dystonia and dystonic posturing of his upper extremities with a right-sided preponderance, more pronounced since the age of 11 years. However, posturing of the arms in particular could represent a mechanism to suppress the myoclonic jerks in severe cortical myoclonus. Neuropsychological testing reveals mild to moderate mental retardation. On neurological examination he additionally shows slow horizontal saccades, and spasticity of lower extremities. He has social phobia (he is relatively well compensated on SSRI and cognitive behavioral therapy). Paraclinical examinations screening for metabolic disorders were unrevealing; he had normal alpha-fetoprotein, normal brain MRI, and normal liver and abdominal sonography. His myoclonus transiently improved on levodopa (300 mg per day) but did not respond to clonazepam or gabapentin.

Family 2, Case P3 is a 28-year-old female of Czech origin with a milder manifestation and progression of the same disease as in her younger brother since the age of 6 years – abnormal movements of hands more pronounced on the left side that she calls "tremor" but clinically present as distal myoclonus. When stretching out the arms, there is some abnormal posturing; again, it is difficult to say whether this is dystonia or attempts to suppress myoclonic jerks in cortical myoclonus. The pattern with distal myoclonus with a clear increase with intention fits best with progressive cortical myoclonus (supplementary video segment 3). Her brain MRI was normal and her intellectual capacity is borderline to slightly below average with decreased performance in attention, executive functions, and verbal memory domains. She managed to finish primary school with assistance and later was trained to become a gardener.

Family 3, Case P5 is a 14-year-old male of Swiss origin, who was born after an uneventful pregnancy and delivery and had a normal early development in the first year of his life. At 18 months of age, he developed myoclonus, reported by his parents as "uncontrollable shaking" during excitement, fever, etc., which progressed to continuous myoclonus by the age of 2 years. Metabolic testing and brain MRI were unrevealing at this age. At 3 years of age, he also developed action tremor, limb hypertonia, and Babinski sign, and subsequently dystonic and choreic involuntary movements. His cognition was reportedly normal, although there was a clear speech developmental delay, especially in the expressive language skills domain. By 6–8 years of age, the disease had significantly progressed in terms of dysarthria and dysphagia requiring PEG placement; the patient was wheelchair-bound. By 11 years of age, his weight, height, and head circumference were below the 3rd percentile and his clinical findings were dominated by spasticity more pronounced on the upper limbs, generalized dystonia, chorea, and myoclonus. His EEG was normal. He experienced transient improvement of his symptoms on pramipexole; he did not experience therapeutic benefits from a combination of tetrabenazine, levodopa, gabapentin, and baclofen.

Family 4, Case P6 is an 8-year-old male of German descent, who was born by acute c-section in the 35th week of pregnancy. Problems with weight gain and unstable body temperature were noted in the postnatal phase. His developmental milestones, including speech as well as gross and fine motor skills, were slightly delayed. He currently presents with a combination of mild speech problems, mild ataxia, and dominant action hand tremor that is intensified by emotional excitement and physical exertion. After prolonged exercise such as swimming, he seems to have less strength in his right leg based on history from his parents, although no weakness or pyramidal signs are present on objective examination. His recent EEG was pathologic due to bifrontal polyspikes and irregular generalized spike-wave paroxysms up to 2–3 s without clinical correlation. There were no reported clinical seizures in his history. Otherwise,

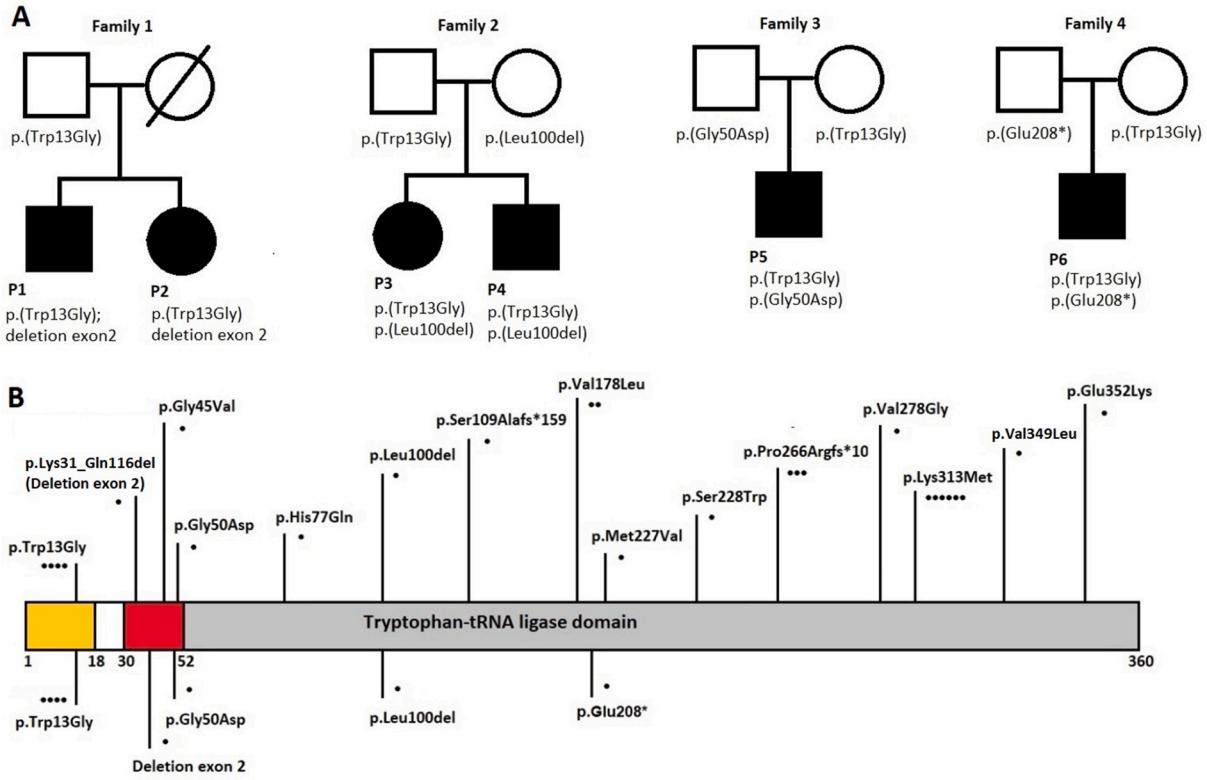

Fig. 1. Genetic and clinical characteristics A) pedigrees of included families with biallelic variants in WARS2; B) location of disease-causing variants on the WARS2 protein scheme. Previously reported variants are reported above the image; currently identified variants are reported below the image; dots indicate number of reported families with a given variant. The mitochondrial targeting sequence is shown in yellow and the aminoacyl-tRNA synthetase conserved site is shown in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Clinical characteristics of the study subjects and review of previously reported cases with biallelic *WARS2* variants.

Patient ID	P number	Gender	Origin	Age	Onset	Initial presentation	Parkinsonism	Ataxia	Dystonia	Myoclonus	Chorea	Action tremor	Muscle tone	Seizures	Mental retardation	Growth retardation	Other symptoms
P1 (this study)	p.Trp13Gly Del exon 2	M	Slovak	36 y	12 y	Action hand tremor	X		X		X*	X	↑				Sinus tachycardia, aggressive behavior, anxiety, depression, psychosis
P2 (this study)	p.Trp13Gly Del exon 2	F	Slovak	33 y	10 y	Action hand tremor	X		X	X		X	↑				Psychosis, anxiety, depression
P3 (this study)	p.Trp13Gly p.Leu100del	M	Czech	28 y	3 y	Myoclonus		X	X	X		X			X		
P4 (this study)	p.Trp13Gly p.Leu100del	F	Czech	24 y	6 y	Myoclonus		X	X	X		X	↑		X		Social phobia, slowed saccades, spasticity
P5 (this study)	p.Trp13Gly p.Gly50Asp	M	Swiss	14 y	18 m	Myoclonus			X	X	X	X	↑			X	Speech delay, spasticity, dysphagia
P6 (this study)	p.Trp13Gly p.Glu208*	M	German	8 y	Neonatal	Developmental delay		X				X			X	X	Unstable body temperature, paroxysmal exercise-induced leg weakness
Theisen 2017	p.Lys313Met p.Leu100del	M	European	24y +	Neonatal	limited head control		X	X			X	↓	X	X	X	exotropia, amblyopia, horizontal nystagmus, diffuse weakness, spastic quadriplegia
Musante 2017 Fam 2V7	p.Ser109Alafs*159 p.Trp13Gly	F	Iranian	16 y	NA	NA		X			X				X		Aggressive behavior, weakness, long philtrum
Musante 2017 Fam 2V8	p.Ser109Alafs*159 p.Trp13Gly	F	Iranian	16 y	NA	NA		X			X				X		Aggressive behavior, weakness, long philtrum
Wortmann 2017 I1	Del exon 2, 36 kb p. Val349Leu	F	Slovak	23d +	Neonatal	NA							↓		NA	X	intestinal pseudo-obstruction
Wortmann 2017 I2	p.Pro266Argfs*10 p.Lys313Met	M	Dutch	3.5y +	Neonatal	Developmental delay			X				↑	X	X	X	
Wortmann 2017 I3	p.Pro266Argfs*10 p.Lys313Met	M	Dutch	1.5y +	Neonatal	Developmental delay							↑	X	X	X	
Wortmann 2017 I4	p.His77Gln p.Glu352Lys	F	French	3y +	4 m	Hypotonia, strabismus							↓	X	X		cardiomyopathy, retinitis pigmentosa
Wortmann 2017 I5	p.Val178Leu p.Val178Leu	M	Iraqi	3 y	13 m	periods of altered consciousness, hypotonia			X				↓↑		X		hepatosplenomegaly with CMV infection
Wortmann 2017 I6	p.Gly45Val p.Lys313Met	F	Canadian	10 y	18 m	delayed speech and fine motor skills		X					↑		X		mild nystagmus, optic atrophy
Vantroys 2018	p. Pro266ArgfsTer10 p. Lys313Met	F	Belgian	6.5 y +	<1 y	axial hypotonia, hypertonia, dystonia			X		X		↓	X	X	X	optic atrophy, weakness, acute hepatopathy, thin upper lip, low set ears, broad nasal bridge, hypertelorism, ogival palate
Burke 2018	p.Trp13Gly p.Ser228Trp	M	European	17 y	1 y	Left leg tremor	X		X		X*	X					chronic headache
Virdee 2019	p.Pro266Argfs*10 p.Lys313Met	F	Dutch	8 y	Infantile	NA	X	X				X			X	X	
Hubers 2019	p.Trp13Gly p.Gly50Asp	M	German	31 y	15 m	ballistic movements and			X		X				X		

(*continued on next page*)

Table 1 (*continued*)

Patient ID	P number	Gender	Origin	Age	Onset	Initial presentation	Parkinsonism	Ataxia	Dystonia	Myoclonus	Chorea	Action tremor	Muscle tone	Seizures	Mental retardation	Growth retardation	Other symptoms
						developmental regression											
Maffezzini 2019 II:1	p.Val278Gly p.Lys313Met	F	Swedish	15 y	<1 y	general muscle weakness, hypotonia, ataxia		X					↓↑	X	X	X	congenital atrial septum defect, strabismus, visual impairment, weakness, severe obstipation, sleep problems
Maffezzini 2019 II:2	p.Val278Gly p.Lys313Met	F	Swedish	10.5 y	<1 y	Developmental delay							↓↑	X	X	X	congenital septal defect, strabismus, obstipation, sleeping problems
Martinelli 2020	p.Trp13Gly p.Met227Val	M	Italian	11 y	6–9 m	trunk instability, occasional jerks	X		X		X*	X		X			oculogyric crisis, ptosis, supranuclear gaze palsy, exotropia

+ – died, NA – not assessed, ↑ – spasticity, ↓ – hypotonia, * – drug-induced dyskinesia.

brain MRI, ophthalmological and endocrinological examinations, and metabolic screening were unremarkable.

3.1. Genetic studies

WES revealed compound heterozygous *WARS2* variants in all affected subjects (variant nomenclature refers to RefSeq accessions NM_015836.3 and NP_056651.1). Each of the identified *WARS2* sequence changes qualified as a "pathogenic" variant according to the American College of Medical Genetics & Genomics guidelines [15]. A recurrent (gnomAD MAF = 0.0033) missense variant, c.37T > G, p.(Trp13Gly), for which a disease-causing effect has been demonstrated in several previous reports [3,4,6,9] was shared by all six affected subjects. As reported previously [11,12], this variant occurred in compound heterozygosity with an exon 2-deletion in both affected siblings from family 1 [10]. and a known c.298_300delCTT, p.(Leu100del) variant predicted to result in the deletion of a highly conserved amino acid in both affected siblings from family 2 [8]. In addition, we identified this variant *in trans* with a known c.149G > A, p.(Gly50Asp) missense variant predicted to result in the substitution of a highly conserved amino acid in the index patient from family 3 [9] and a novel c.622G > T, p.(Glu208*) nonsense variant predicted to result in premature termination of protein translation in the index patient from family 4.

3.2. Expression studies

To determine the effects of the variants observed in patients P1 and P2 (c.37T > G, p.(Trp13Gly); deletion exon 2) on WARS2 protein expression, Western blotting analysis was performed. These data demonstrate significantly lower levels of full-length WARS2 protein in fibroblasts from patients P1 and P2 when compared to three unrelated controls (Fig. 3).

4. Discussion

Here we present clinical, genetic, and expression studies of six subjects from four families having *WARS2*-related diseases and expand the phenotypic spectrum of this disorder to later-onset phenotypes presenting with early-onset dopa-responsive parkinsonism with abnormal dopaminergic imaging, clinically diagnosed as early-onset PD and to a progressive myoclonus ataxia phenotype.

Using an unbiased WES approach, we identified compound heterozygous variants in *WARS2* in all the six subjects. The p.(Trp13Gly) variant found in all six affected subjects is a relatively common variant compared to variants causing rare Mendelian disorders. It is present in the gnomAD database in 922 heterozygous carriers and in six subjects in a homozygous state. This variant has been defined as pathogenic in several previous reports [3,4,6,9] and was shown to impact the correct localization of WARS2 protein in cells [5] and to variably affect the OXPHOS system [7]. Cumulative evidence therefore strongly suggests that p.(Trp13Gly) is a hypomorphic allele, which is disease-causing only when present *in trans* with loss-of-function alleles; due to this nature, the p.(Trp13Gly) variant often presents with milder disease phenotypes and later disease onset, as shown in several of our subjects. The occurrence of the variant in multiple families with WARS2-related disease combined with the demonstration of a deleterious effect in cellular assays provides genuine evidence for a causative role of this allele in human disease conditions. We note, however, that the variant may not be disease-causing when present in a homozygous state alone, given that six apparently healthy carriers are listed in gnomAD.

A larger deletion involving exon 2 was previously described in a single subject presenting with a severe fatal neonatal form of *WARS2*-related disease [10], in contrast to a rather late (10–12 years old) disease onset in our cases. This may be explained by presence of another variant, the p.(Val349Leu), with a more drastic deleterious effect and possibly other modifiers in their cases, in contrast to the presence of the p.

(Trp13Gly) variant with a rather mild pathogenicity in our subjects. Deletion of the exon 2 is predicted to result in an in-frame deletion of 86 amino acids (amino acid 31 to amino acid 116, (p.Lys31_Q116del), consistent with a loss-of-function effect.

The in-frame deletion of c.298_300delCTT, p.(Leu100del), identified in subjects P3 and P4, results in the deletion of a highly conserved amino acid [8] that is located in the tryptophanyl-tRNA synthetase catalytic core domain (TrpRS core), described previously in a case with infantile onset leukoencephalopathy. In contrast to the previously described case, MRI in all our subjects was unremarkable, without signs of white matter abnormalities.

The c.149G > A, p.(Gly50Asp) missense variant, which was not found in any of the control databases, predictably results in the substitution of a highly conserved amino acid, and was also previously described in a subject with a similar phenotype dominated by hyperkinetic movement disorders [9]. Additionally, our case initially presented with myoclonus, which was observed throughout his disease course and later developed a severe spastic phenotype eventually leading to a wheelchair-bound state and dysphagia requiring PEG placement.

The c.622G > T, p.(Glu208*), nonsense variant has not been described previously and it was absent from all examined control databases. This sequence alteration is predicted to result in a stop-codon located in exon 5 leading to a truncated and non-functional WARS2 protein, or, via nonsense-mediated mRNA decay (NMD), to severely decreased protein level. Similar to several of the previously described cases, this subject had a neonatal onset of symptoms and delay in developmental milestones, dominated by speech problems, ataxia, tremor, and paroxysmal exercise-induced leg weakness. While seizures were reported in previous cases of *WARS2*-related diseases [4,7,10], clinical seizures were not reported in relation to the pathological EEG findings in subject P6.

4.1. Functional studies

Western blotting analyses in patient-derived fibroblasts showed a significantly decreased expression of the full-length WARS2 protein in the mitochondria of both subjects (P1 and P2), who were compound heterozygous carriers of the p.(Trp13Gly) and p.(Lys31_Q116del) variants. The in-frame deletion of 86 amino-acids, p.(Lys31_Q116del), removes part of the stable core of the enzyme, but leaves the mitochondrial targeting sequence (MTS) intact. This variant (if expressed) will result in a non-functional protein due to the lack of part of the domain where the tRNA and Trp bind, which is consistent with a loss-of-function effect. If expressed, the *WARS2* p.(Lys31_Q116del) variant would result in a protein of reduced predicted molecular weight (30 kDa), as opposed to the 40 kDa full length wildtype protein. We did not detect a protein of such a reduced molecular weight in our blots (data not shown). This is in line with the findings of the study by Wortmann et al. showing that in the mitochondria of the fibroblasts derived from patient F1, I1 carrying the same p.(Lys31_Q116del) variant in combination with another *WARS2* missense variant p.(Val349Leu), the WARS2 protein expression cannot be detected despite the presence of a functional MTS [10]. Taken together, these data show that the *WARS2* p.(Lys31_Q116del) variant most likely leads to a loss of protein expression. It has been shown that the p.(Trp13Gly) variant located within the mitochondrial targeting sequence (MTS) leads to impaired mitochondrial localization [3]. That study showed that WARS2 protein levels in cells over-expressing the p.(Trp13Gly) variant are reduced in purified mitochondria, but are unchanged in total cell protein extracts when compared to cells over-expressing wildtype WARS2 protein. Other studies reported reduced endogenous WARS2 protein expression in purified mitochondria from patient fibroblasts carrying the p.(Trp13Gly) in combination with the p.(Met227Val) variant [4], and also in total cell extracts from subjects carrying the p.(Trp13Gly) variant in combination with the p.(Ser228Trp) variant [6]. However, these previous studies and our findings on endogenously expressed *WARS2* p.(Trp13Gly) protein cannot distinguish between an effect of the p.(Trp13Gly) variant on mitochondrial localization or protein stability, because these studies were performed in cells from subjects carrying the p.(Trp13Gly) variant in a compound heterozygous state carrying an additional missense or deletion variant on the other allele. To determine the effect of the *WARS2* p.(Trp13Gly) variant on protein stability, WARS2 expression in total cell extracts from heterozygous p(Trp13Gly) variant carriers (e.g. a parent or sibling carrying only this variant together with a wildtype allele). should be analyzed. Unfortunately, the parent carrying the heterozygous p.(Trp13Gly) in our study was not available for skin biopsy. Although the pathogenicity of the *WARS2* p.(Trp13Gly) variant requires further investigation, previous studies and our work together support the contention that biallelic loss-of-function *WARS2* variants lead to mitochondrial dysfunction and disease.

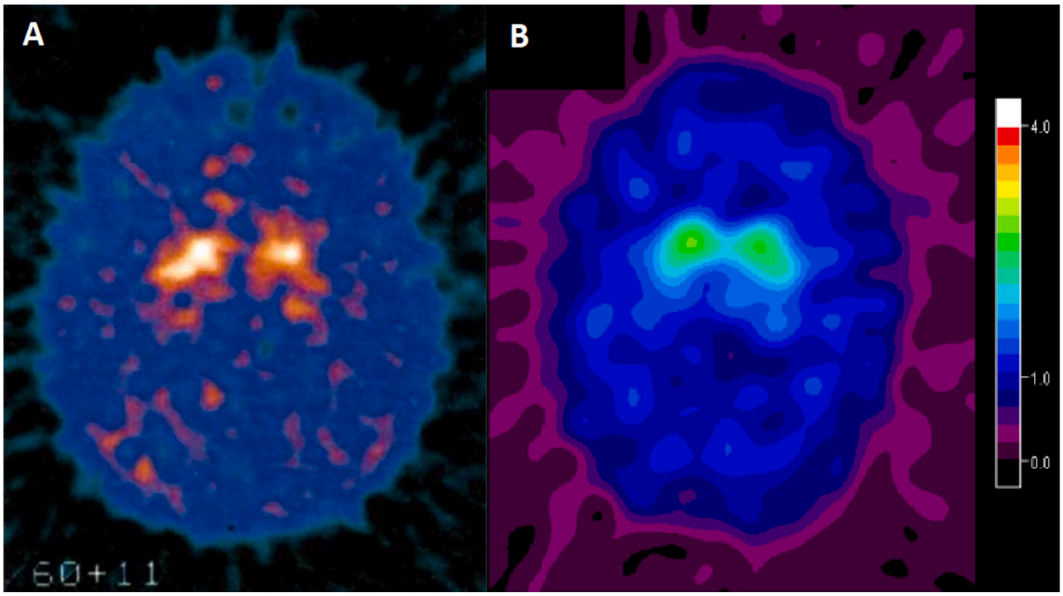

Fig. 2. DaT scans in subjects P1 and P2 presenting with early-onset parkinsonism: A) reduced radiotracer uptake on DaT scan of subject P1 at the age of 27 years; B) reduced radiotracer uptake on DaT scan of subject P2 at the age of 32 years.

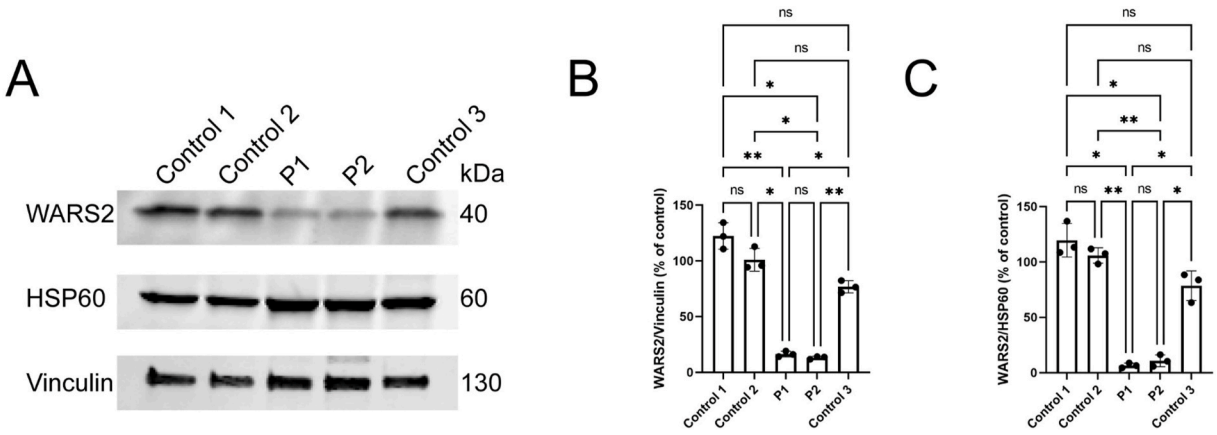

Fig. 3. A. Representative Western blot analyses of fibroblast-derived mitochondrial protein extracts from individuals with WARS2 mutations (compound heterozygous p.(Trp13Gly) variant; deletion of exon2)(P1, P2) and unrelated controls (Control 1–3). Vinculin and HSP60 are shown as loading controls. Molecular weights (kDa) are indicated. **B, C.** Quantitative analysis of WARS2 protein levels in subject P1 and subject P2 fibroblasts, and three unrelated controls after normalization to Vinculin (B) or HSP60 (C). Data are expressed as mean ± SD (*: $p \leq 0.05$, **: $p \leq 0.01$, $n = 3$, One way ANOVA, Tukey's multiple comparisons test).

Three cases with infantile onset parkinsonism caused by biallelic *WARS2* variants have been described previously, all presenting also with action limb tremor, treatment-induced dyskinesia in 2 subjects [4,6] and seizures along with oculogyric crisis, ptosis, supranuclear gaze palsy, exotropia, and severe derangement of DaT scan in one subject [4]. In contrast to the previous reports, subjects P1 and P2 presented with a significantly later onset of parkinsonism, starting uniformly as action hand tremor around 10–12 years of age, later accompanied by cervical dystonia in subject P2. Clinical diagnosis of PD was made at 27 years of age in P1 and 32 years of age in P2. The differential diagnosis of early onset parkinsonism is broad. Our patients presented with a slowly progressive parkinsonism and their presentation largely overlaps with phenotypes of the typical early-onset PD forms with autosomal recessive inheritance, including those caused by biallelic PRKN, PINK1, and DJ1 [17,18]. Although the parkinsonism in our patients is well responsive to dopaminergic medication, a jerky dystonic hand tremor is persistent and dominates the motor features of their disease. Dopamine agonists had to be discontinued in both cases due to psychotic complications, and their non-motor phenotype is dominated by prominent neuropsychiatric symptoms, including anxiety and depression. Abnormal DaT scans in both siblings indicate a neurodegenerative background with nigrostriatal denervation.

Myoclonus, not previously described in relation to *WARS2* gene mutations, was a presenting feature in cases P3, P4, and P5, further expanding the spectrum of *WARS2*-related disease. Transient cervical, trunk and upper limb dystonia would suggest myoclonus-dystonia (M-D)-like syndrome in P3 and P4, although body part "posturing" could also represent compensatory mechanisms in severe myoclonic jerks. The character of myoclonus with distal limb engagement with provocation by action would fit cortical myoclonus in a progressive myoclonus-ataxia syndrome. However, ataxia was not an initial symptom – it was rather mild in both siblings. The most common and best characterized diseases with progressive myoclonus ataxia syndrome are Unverricht-Lundborg disease (MYC/ATX-CSTB), Lafora disease (MYC/ATX-EMP2A), neuronal ceroid lipofuscinosis (multiple genes), sialidosis (MYC/ATX-NEU′1), and dentatorubral-pallidoluysian atrophy (ATX-ATN1). Typical additional symptoms such as cherry-red spots, epileptic seizures, psychosis, and drop attacks were not present in our patients [19]. Several other disorders involving mitochondrial dysfunction, e.g. myoclonic epilepsy with ragged red fibers (PMA-MERRF) [20], as well as other pathophysiological mechanisms may present both with parkinsonism and myoclonus ataxia phenotypes, including Lafora disease (Ragona 2020), neuronal ceroid lipofuscinosis type 2, ATP13A2, PLA2G6, TMEM240, NIID, and SLC18A2 [17,21]. Although initially diagnosed as M-D syndrome, the clinical phenotype of subjects P3 and P4 is also not fully compatible with the classic myoclonus dystonia caused by SGCE mutations due to the presence of several additional features such as intellectual disability, ataxia, and spasticity. Also, SGCE carriers present with a subcortical rather than cortical type of myoclonus, which is typically located in the proximal part of upper limbs (and trunk) [22]. While a transient improvement after levodopa in subject P4 and after pramipexole but not levodopa in subject P5 were observed in our cohort, the involvement of the dopaminergic system may play a wider role in WARS2-related disease. Since these families did not agree to further diagnostic testing, we are not able to determine the degree of dopaminergic cell loss in these non-parkinsonian cases.

In conclusion, the prevalence of *WARS2* mutations in the above-mentioned phenotypes warrants further study; nevertheless, in case of suspected *WARS2*-related disease, relaxing of exome filters might be crucial in order to prioritize the recurrent causative p.(Trp13Gly) variant found in all of our cases. To that end, in the context of suspected *WARS*-related diseases, variant prioritization should not exclude homozygous variants found in control subjects (e.g., gnomAD) and needs to consider variants with a MAF above 0.1%.

Author roles

MS – 1ABC, 2C, 3A, IR – 1C, 2C, 3A, WM – 1C, 2AB, 3A, MW – 1C, 2C, 3B, RS – 1C, 2C, 3B, LO – 1C, 2C, 3B, VH – 1C, 2C, 3B, PP – 1C, 2C, 3B, AM – 1C, 2C, 3B, KK – 1C, 2C, 3B, MO – 1C, 2C, 3B, ZG – 1C, 2C, 3B, BP – 1C, 2C, 3B, TB – 1C, 2C, 3B, RB – 1C, 2C, 3B, VB – 1C, 2C, 3B, PH – 1C, 2C, 3B, MAJT – 1C, 2C, 3B, RK – 1C, 2C, 3B, MR – 1C, 2C, 3B, HH – 1C, 2C, 3B, JW – 1C, 2C, 3B, VB – 1ABC, 2C, 3B, MZ – 1ABC, 2C, 3A, RJ – 1ABC, 2C, 3B

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2021.11.030.

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