Journal of the Neurological Sciences 349 (2015) 105–109

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Analysis of the *C19orf12* and *WDR45* genes in patients with neurodegeneration with brain iron accumulation

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ARTICLE INFO

Article history:
Received 1 July 2014
Received in revised form 24 November 2014
Accepted 24 December 2014
Available online 3 January 2015

Keywords:
NBIA
C19orf12
WDR45
MPAN
BPAN
Mutation analysis

ABSTRACT

Background: Neurodegeneration with brain iron accumulation (NBIA) comprises a clinically and genetically heterogeneous group of diseases presenting with movement disorders and brain iron deposits. In addition to NBIA subtypes caused by mutations in *PANK2* and *PLA2G6*, mutations in the *C19orf12* gene were recently described as the third frequent cause of NBIA (called mitochondrial membrane protein-associated neurodegeneration, MPAN). Additionally, the X-linked gene *WDR45* was found causative for a special subtype named static encephalopathy in childhood with neurodegeneration in adulthood (also called BPAN); however, analysis of this gene in a broader spectrum of NBIA has not been reported yet.
Methods: In a heterogeneous cohort of 69 patients with suspected NBIA that did not carry mutations in *PANK2* and *PLA2G6*, the coding region of *C19orf12* was evaluated by Sanger sequencing. The *WDR45* gene was analyzed *via* high resolution melting and subsequent sequence analysis.
Results: Previously described homozygous *C19orf12* mutations were found in 3/69 NBIA patients (4.3%). Analysis of the *WDR45* gene revealed a novel heterozygous missense mutation in one female NBIA patient showing psychomotor retardation with secondary decline.
Conclusions: C19orf12 mutations were confirmed in our heterogeneous NBIA cohort, while *WDR45* mutations appear to be restricted to the subtype showing encephalopathy in childhood with neurodegeneration in adulthood.

1. Introduction

Neurodegeneration with brain iron accumulation (NBIA) is a clinically and genetically heterogeneous group of neurodegenerative diseases, typically comprising movement disorders and brain iron deposits [1]. The wide phenotypic spectrum of NBIA, as well as parallels to other disorders such as Parkinsonism or spastic paraplegia [1,2], can make diagnosis difficult and, in some cases, the underlying genetic mechanisms still remain unknown.

The first subtype of NBIA, pantothenate kinase-associated neurodegeneration (PKAN) caused by mutations in the *PANK2* gene, was described in 2001 [3]. Since then, mutations in various genes, such as *PLA2G6*, *FA2H*, *FTL*, *AP* and *ATP13A2*, have been added to the growing list of clinically and genetically distinct entities associated with brain iron accumulation [4]. Most recently, mutations in the *C19orf12* and *WDR45* genes were identified as additional causes for NBIA.

Mutations in *C19orf12* were first described in a homogeneous cohort from Poland where most cases carried an 11bp deletion founder mutation [5]. In a subsequent larger multinational study, *C19orf12* mutations were found associated with a wide spectrum of phenotypes, typically including motor neuronopathy, cognitive decline and neuropsychiatric impairment [6]. MRI scans showed T2-hypointensities in globus pallidus and substantia nigra without an eye of the tiger sign [5]. Due to localization of the gene product in mitochondria, the name mitochondrial membrane protein-associated neurodegeneration (MPAN) was proposed for this clinical entity [5]. MPAN was suggested to be the third frequent NBIA subtype after PKAN (*PANK2* gene) and PLAN

Abbreviations: NBIA, neurodegeneration with brain iron accumulation; MPAN, mitochondrial membrane protein-associated neurodegeneration; BPAN, beta-propeller protein-associated neurodegeneration; PKAN, panthothenate kinase-associated neurodegeneration; PLAN, Phospholipase A2-associated neurodegeneration; HRM, high resolution melting; SENDA, static encephalopathy in childhood with neurodegeneration in adulthood.

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http://dx.doi.org/10.1016/j.jns.2014.12.036
0022-510X/

(*PLA2G6*); however the mutational frequencies varied substantially between studies.

Mutations in the *WDR45* gene, located on the X chromosome, were recently identified in patients with a very specific phenotype that is referred to as static encephalopathy in childhood with neurodegeneration in adulthood (SENDA). This new X-linked NBIA subtype, affecting a gene product involved in autophagy [7], has been named beta-propeller protein-associated neurodegeneration (BPAN) [8]. Most affected individuals are females and all reported mutations so far occurred *de novo*. However, a few males with *WDR45* mutations and a SENDA phenotype, most of them probably showing somatic mosaicism, have also been described [8]. To our knowledge, the frequency of *WDR45* mutations in patients with a broader clinical NBIA phenotype has not been investigated yet.

In order to further evaluate the mutational and clinical spectra of these two newly described NBIA subtypes, we screened a cohort of *PANK2* and *PLA2G6* mutation-negative patients clinically presenting with NBIA (n = 69) for mutations in *C19orf12* and *WDR45*.

2. Materials and methods

2.1. Patient cohort

Patients were recruited from an internal registry of the Department of Human Genetics of the Ruhr-University Bochum, including DNA samples sent by clinicians for the diagnosis of NBIA during the years 2006–2013 which had been tested negative for mutations in the *PANK2* or *PLA2G6* genes. All samples were obtained with informed consent of probands or their legal representatives. The study was approved by the Ethics Committee of the Ruhr-University Bochum and carried out in accordance with the Declaration of Helsinki protocols.

The NBIA cohort comprised 69 individuals of different ethnicities and ages, born between 1932 and 2010 (summarized in Table 1). The clinical course varied substantially between patients, with age of onset in childhood for 45% of individuals and onset in adulthood for 42%.

Table 1
Clinical data for the NBIA cohort.

	NBIA (n = 69)
Sex	34 males/35 females
Age of manifestation	
Childhood	31 (45%)
Adolescence	8 (11.6%)
Adulthood	29 (42%)
n/a	1 (1.4%)
Major symptoms	
Ocular manifestations	4 (5.8%)
Dystonia	19 (27.5%)
Dysarthria	12 (17.4%)
Spasticity	14 (20.3%)
Gait disturbance	11 (15.9%)
Parkinsonism	4 (5.8%)
Ataxia	5 (7.2%)
Choreatic movements	6 (8.7%)
Developmental delay	10 (14.5%)
Cognitive decline	6 (8.7%)
Dementia	5 (7.2%)
Psychiatric disorders	6 (8.7%)
Seizures	7 (10.1%)
n/a	12 (17.4%)
MRI imaging	
Eye of the tiger sign	7 (10.1%)
T2 signal hypointensities of basal ganglia	11 (15.9%)
Other MRI abnormalities	13 (18.8%)
Without findings	1 (1.4%)
n/a	37 (53.6%)
Ethnicity	
European	German 53.6% French 5.8% other European 7.2%
Turkish	14.5%
Argentinian	1.4%
n/a	17.4%
Family history	
Affected relative(s)	9 (13%)
Inconspicuous	27 (39.1%)
Consanguineous	6 (8.7%)
n/a	27 (39.1%)

2.2. Mutational analysis

2.2.1. C19orf12

DNA was extracted from peripheral lymphocytes according to standard methods [9]. Mutation analysis of the *C19orf12* gene in the NBIA cohort was performed by PCR amplification of all three exons followed by direct sequencing according to the method of Sanger. Primers were used as previously described by Hogarth et al. [6]; primer sequences are listed in Supplementary Table 1. The reference sequence corresponds to the longest transcript variant (NM_001031726.3) as shown in Dogu et al. [10].

2.2.2. WDR45

Mutational analysis of the *WDR45* gene in the NBIA cohort was conducted *via* high resolution melting (HRM) analysis followed by direct sequencing of atypical curves as described before [11]. Primers were designed with the LightScanner® Primer design software, and the primer sequences are listed in Supplementary Table 1. The reference sequence used was NM_007075.3.

3. Results

3.1. C19orf12

In three patients with suspected NBIA we identified homozygous mutations in *C19orf12* that had been previously described as pathogenic (Table 2). Two patients (MPAN-1 and MPAN-2) showed the homozygous mutation c.C32T (p.Thr11Met) which only affects the longer transcript variant of C19orf12 and has been reported repeatedly [5,10,12,13]. Both affected subjects were from consanguineous Turkish families, but, to our knowledge, were not related to each other. Both presented in early adulthood with similar symptoms including dystonia, spasticity, progressive dementia and psychiatric disorders. Bilateral signal hypointensities in basal ganglia were also marked in both patients. DNA was only available from the parents of MPAN-1 who were heterozygous carriers of the Thr11Met mutation. One sister of MPAN-1 was similarly affected and equally found homozygous for Thr11Met. Since the age of 30, she had shown a rapidly progressing Parkinson-like phenotype with gait instability, frequent falls, speech difficulties, tremor and rigor, dystonia as well as dementia. Even though she was initially successfully treated with Levodopa and Amantadine, within two years she was requiring a high level of care and died from pneumonia at the age of 32 years. At least one sibling of MPAN-2 had also been diagnosed with a neurodegenerative disorder, but more detailed clinical information was not available for this family.

MPAN-3 showed the homozygous recurrent mutation p.Gly69Arg [2,5,6,14]. She presented at age 10 with gait impairment and repeated falls due to spasticity of the lower limbs. Later on she developed optic atrophy and psychiatric symptoms such as impulse control difficulties and depression. MRI scans showed bilateral signal alteration in globus pallidus and crus cerebri. A variant frequently found in combination with the Gly69Arg missense mutation, c.424A>G, p.Lys142Glu (rs146170087) [6], was also found in our patient, albeit in heterozygous state. The non-consanguineous parents of MPAN-3 were confirmed to be heterozygous carriers of Gly69Arg, and the father additionally carried the Lys142Glu variant.