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Autosomal dominant early-onset spastic paraparesis with brain calcification due to *IFIH1* gain-of-function

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Abstract

We describe progressive spastic paraparesis in two male siblings and the daughter of one of these individuals. Onset of disease occurred within the first decade, with stiffness and gait difficulties. Brisk deep tendon reflexes and extensor plantar responses were present, in the absence of intellectual disability or dermatological manifestations. Cerebral imaging identified intracranial calcification in all symptomatic family members. A marked upregulation of interferon stimulated gene (ISG) transcripts was recorded in all three affected individuals and in two clinically unaffected relatives. A heterozygous *IFIH1* c.2544T>G missense variant (p.Asp848Glu) segregated with interferon status. Although not highly conserved (Supporting figure 1; CADD score 10.08 versus MSC-CADD score of 19.33) and predicted as benign by *in silico* algorithms, this variant is not present on publically available databases of control alleles, and expression of the D848E construct in HEK293T cells indicated that it confers a gain-of-function. This report illustrates, for the first time, the occurrence of autosomal dominant spastic paraplegia with intracranial calcifications due to an *IFIH1*-related type 1 interferonopathy.

Keywords: *IFIH1*, interferonopathy, spastic paraparesis, brain calcification

Introduction

Hereditary spastic paraparesis (HSPs) are Mendelian neurodegenerative diseases characterized by progressive spasticity and hyperreflexia of the lower limbs (Esteves et al., 2014). At least 76 loci and 58 corresponding genes linked to HSPs have been identified so far (Parodi et al., 2017). Autosomal-dominant HSPs have been described due to mutations in 19 SPG genes (Klebe et al., 2015). Brain calcification has been reported in subjects with *CYP2U1* mutations (Tesson et al., 2012), but this imaging feature is otherwise rarely described in the context of HSP. The molecular basis of a number of familial cases of HSP remains undefined.

Aicardi-Goutières syndrome (AGS) is a severe childhood onset degenerative encephalopathy associating brain calcification, white matter disease, cerebral atrophy and psychomotor retardation (Aicardi and Goutières, 1984). Seven genes have so far been described which, when mutated, can cause an AGS phenotype: *TREX1* (AGS1) (Crow et al., 2006a), *RNASEH2A* (AGS3), *RNASEH2B* (AGS2), *RNASEH2C* (AGS4) (Crow et al., 2006b), *SAMHD1* (AGS5) (Rice et al., 2009), *ADARI* (AGS6) (Rice et al., 2012) and *IFIH1* (MIM# 606951) (AGS7) (Rice et al., 2014). AGS is most frequently transmitted as an autosomal recessive trait, although cases of inherited and *de novo* autosomal dominant occurrence are also documented. An underlying activation of the interferon signalling pathway is considered as a direct pathogenic driver, although definitive proof of this in the human context is yet to be determined (Crow and Manel, 2015). Assessment of interferon status has proven to be a very reliable disease marker. This method gives an interferon score comparing the expression of six interferon-stimulated genes using quantitative PCR in peripheral blood in patients and controls (Rice et al., 2013; Rodero et al., 2017). Using this screening assay, a remarkably broad spectrum of associated neurologic phenotypes has been revealed to be associated with

mutations in *AGS1-7*, with isolated spastic paraparesis reported in a limited number of patients with mutations in *ADARI*, *IFIH1* and *RNASEH2B* (Crow et al., 2014, 2015).

Here we describe a Portuguese family segregating an autosomal dominant HSP phenotype with intracranial calcification due to a gain-of-function mutation in *IFIH1* and demonstrating clinical non-penetrance in certain individuals.

Clinical histories

This Portuguese family includes three clinically affected individuals: a 14 year old girl, her father and her paternal uncle. The proband developed walking difficulties with moderate spasticity of the lower limbs from 3 years of age. On examination aged 10 years, she was able to stand on one foot, had brisk deep tendon reflexes, extensor plantar responses and a stiff gait. Her growth was delayed, with a height at -3SD. Growth velocity deceleration was noticed from the age of 10 years, contrasting with normal weight (3rd percentile) and OFC (-1.5 SD). She was proficient at school with good social integration. At age 14 years she is unable to walk on tip-toes and unipodal balance is unstable. Muscle strength is normal. Her brain CT-scan is remarkable for extensive calcifications in the deep white matter of the frontal lobes and at the white-gray junction (Figure 1G-I). Her twin brother is asymptomatic, with unremarkable clinical examination including normal deep tendon reflexes. Her twin brother and her father have height in the normal range.

The father of the proband reported onset of walking difficulties from 2 years of age, associated with an increased frequency of falls. A slowly progressive course was noted, still allowing him to run when aged 24 years. When evaluated at 41 years of age he had severe spastic weakness, MRC grade 4, with marked hyperreflexia and bilateral Achilles clonus.

Dysarthria and occasional dysphagia were also noted. He has normal higher intellectual functions. Brain CT performed at age 24 years revealed extensive calcifications in the deep white matter (centrum semi-ovale), involving frontal and temporal regions. Punctate deposits were also present in the posterior fossa, globi pallidi and dentate nuclei (Figure 1A-C).

The maternal uncle experienced progressive lower limb motor impairment since the age of 13 years. On examination, aged 38 years, an MRC grade 4 spastic weakness with marked hyperreflexia and bilateral Achilles clonus was noted. Cognition was considered as normal. His brain CT-scan indicates subtle changes similar to those of his niece (Figure 1D-F). His height is in the normal range.

The paternal grandparents of the proband are asymptomatic. Cerebral CT of the grandfather revealed likely non-pathological falx calcifications.

Methods

Written, informed consent for DNA analysis was obtained from patients and parents for the children. The study was approved by our local ethics committee.

Interferon signature

The expression of six interferon-stimulated genes (ISGs), namely *IFI27* (Hs01086370_m1), *IFI44L* (Hs00199115_m1), *IFIT1* (Hs00356631_g1), *ISG15* (Hs00192713_m1), *RSAD2* (Hs01057264_m1), and *SIGLEC1* (Hs00988063_m1) was measured by quantitative PCR according to a previously reported method (Rice et al., 2014). The median fold change, when compared with the median of 29 healthy controls, was used to create an interferon score for

each patient. Scores higher than the mean of the controls plus two SD ($>2 \cdot 466$) were designated as positive.

Sanger sequencing of IFIH1

DNA was extracted from whole blood samples using standard methods, and sequencing performed using primers designed to amplify the coding exons of *IFIH1*. Purified PCR amplification products were sequenced using BigDye Terminator chemistry and an ABI 3130 DNA sequencer. Mutation description is based on the reference cDNA sequence NM_022168.3, with nucleotide numbering beginning from the first A in the initiating ATG codon. Variants were assessed using the *in silico* programs SIFT (<http://sift.jcvi.org>) and Polyphen2 (<http://genetics.bwh.harvard.edu/pph2/>), and population allele frequencies obtained from the gnomAD (<http://gnomad.broadinstitute.org>) database.

Interferon reporter assay

The pFLAG-CMV4 plasmid encoding IFIH1 has been described elsewhere (Rice et al., 2014). The indicated mutations were introduced using KAPA HiFi DNA polymerase. Human embryonic kidney (HEK) 293T cells (American Type Culture Collection, ATCC) were maintained in 48-well plates in DMEM (Cellgro) supplemented with 10% heat-inactivated FCS and 1% L-glutamine. Cells were tested for mycoplasma. At ~80% confluence, cells were cotransfected with pFLAG-CMV4 plasmids encoding wild-type or mutant IFIH1 (10 ng), IFN- β promoter-driven firefly luciferase reporter plasmid (100 ng) and a constitutively expressed Renilla luciferase reporter plasmid (pRL-CMV; 10 ng) using Lipofectamine 2000 (Life Technologies) according to the manufacturer's protocol. Medium was changed 6 hours after transfection, and cells were subsequently stimulated with poly I:C (0.5 $\mu\text{g/ml}$;

Invivogen) using Lipofectamine 2000. Cells were lysed 16 hours after stimulation, and IFN- β promoter activity was measured using the Dual-Luciferase Reporter assay (Promega) and a Synergy2 plate reader (BioTek). Firefly luciferase activity was normalized against Renilla luciferase activity. Each experiment (n = 4) was performed in triplicate and data are presented as mean \pm SEM. Statistical significance was determined by two tailed, unpaired Student's *t* test with * and ** indicating p values <0.05 and <0.01 respectively. Comparable expression of construct proteins was verified by western blotting (data not shown).

Results

Based on the lower limb spasticity with early onset, slow progression and the presence of brain calcifications, an initial diagnosis of Fahr syndrome was considered in the proband and her father. Standard karyotypes were 46,XX and 46,XY and array CGH (Agilent 180K) was normal. *SPG4* and *SPG3* genes, encoding spastin and atlastin were sequenced without identifying any likely pathogenic variant. Subsequently, the possibility of an interferonopathy was considered. Quantitative RT-PCR (qPCR) demonstrated persistent and robust upregulation of ISG expression in whole blood in the proband, her affected father and her affected uncle, with scores ranging from 10 to 53 (NR<2.44). Furthermore, the proband's asymptomatic twin brother and her unaffected paternal grandfather also demonstrated significant upregulation of interferon signalling (Figure 2A).

Sequencing of *IFH1* identified a heterozygous c.2544T>G sequence alteration resulting in a missense p.Asp848Glu change in every family member displaying abnormal ISG expression (Figure 2B). This variant is predicted as benign according to *in silico* analyses, and has not been reported in other patients. However, the variant is not present in 240 000 control alleles recorded in the gnomAD cohort. Furthermore, interferon β (IFN- β) reporter stimulatory

activity of wild-type and variant *IFIH1* in HEK293T cells indicated that the p.Asp848Glu confers a gain-of-function, comparable to that recorded with other known *IFIH1* mutations (Figure 2C) (Rice et al., 2014). The aspartate at position 848 is not highly conserved (Supporting figure 1), the p.Asp848Glu substitution is predicted as benign according to in silico analyses (CADD score 10.08 versus MSC-CADD score of 19.33), and this variant has not been reported in other patients.

Discussion

IFIH1 gain-of-function mutations were first reported in 2014 in patients with a variety of neuro-immunological phenotypes including AGS (Rice et al., 2014). Subsequently, *IFIH1* mutations were also identified in patients with non-neurological phenotypes, including complex dermatological and skeletal anomalies in the context of Singleton-Merten syndrome (Bursztejn et al., 2015; Rutsch et al., 2015; Buers et al., 2017; De Carvalho et al., 2017), manifesting dental dysplasia, glaucoma, psoriasis, aortic calcification and skeletal abnormalities. Patients with *IFIH1* mutations have been previously described to demonstrate lower limb spasticity (Oda et al., 2014; Rice et al., 2014; Bursztejn et al., 2015; Hacoheh et al., 2015; Marguet et al., 2016; Buers et al., 2017; Pettersson et al., 2017). Isolated spastic paraplegia was observed in the 31 year old father of a girl presenting with a complex phenotype encompassing spasticity and a lupus-like illness (Rice et al., 2014; Hacoheh et al., 2015). Of note, apparently isolated spastic paraplegia was reported in three patients belonging to two unrelated families from North Africa with mutations in *RNASEH2B*, and in a singleton of European ancestry harbouring a *de novo ADARI* mutation (Crow et al., 2014), both being AGS-associated genes.

In addition to variable clinical expression already described in the type I interferonopathy context, our family underlines the possibility of discordant penetrance between relatives, including twins inheriting the same mutation from their affected father, one of them being clinically asymptomatic despite a robust interferon signature. The follow-up of this brother will determine if he eventually develops a phenotype. Such a contrast between non-identical twins points to the effect of environmental triggers or genetic modifiers.

Although the *IFIH1* variant that we describe was predicted as benign according to SIFT and Polyphen softwares, functional studies with dosage of IFN- β receptor activity were in favour of pathogenicity, raising issues about the interpretation of *IFIH1* variants based solely on prediction algorithms.

Summarising, our observations suggest that an *IFIH1*-related interferonopathy should be taken into consideration in the differential diagnosis of autosomal dominant spastic paraparesis, either in the presence of normal neuroimaging or in association with the presence of intracranial calcification.

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Figure 1 CT scans of the father of the proband (panels A-C) show slightly prominent sulci and ventriculomegaly indicating a mild degree of cerebral atrophy, as well as prominent calcifications bilaterally in the cerebellar dentate nuclei (A) and basal ganglia (B), and in the central white matter (centrum semiovale) of the frontal and anterior parietal lobes (C). **Scans** from the paternal uncle of the proband (D-F), and the proband (G-I) herself, demonstrate no atrophy, absence of calcification in posterior fossa (G) and only small or subtle calcifications in the basal ganglia (E) and frontal white matter (F, **H**, I)

Figure 2 A: Quantitative RT-PCR (qPCR) of a panel of six ISGs in whole blood measured in IFIH1 mutation-“positive probands and mutation-negative relatives and controls. Note that not only the proband (in pink), her affected father (in green) and her affected uncle (in purple) have markedly abnormal values, but also her unaffected twin brother and unaffected paternal grandfather. B, Pedigree of family showing affected members and interferon signaling status. Normal interferon signatures are marked with a black asterisk, whilst abnormal results are annotated in red. C, Functional analysis of interferon \hat{I}^2 (IFN- \hat{I}^2) reporter stimulatory activity of wild-type and putative mutant (p.D848E) IFIH1 in human embryonic kidney (HEK) 293T cells. The p.R927H construct is unable to bind dsRNA and therefore acts as a negative control.

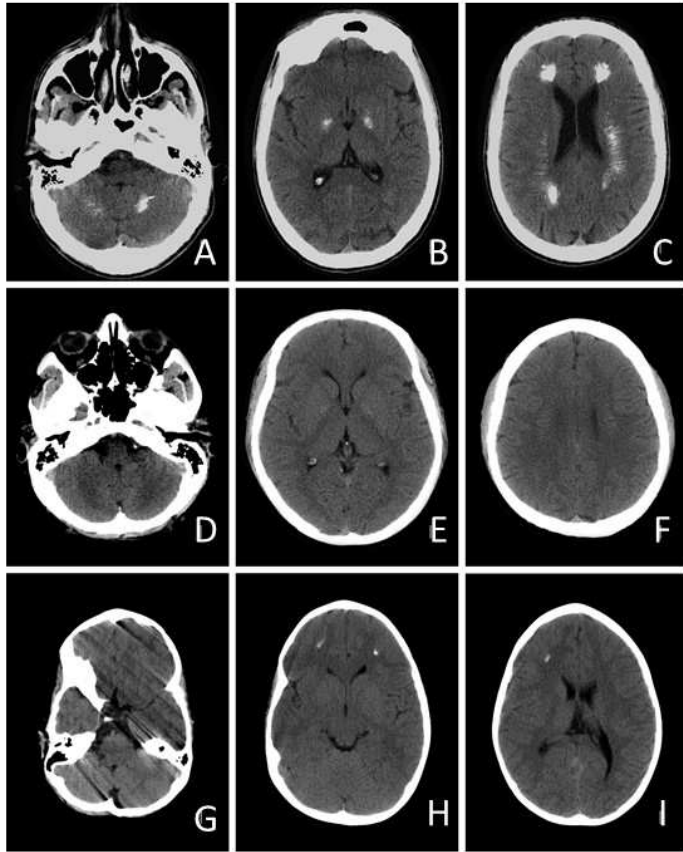


Figure 1

Figure 2

