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Monogenic models of migraine : from clinical phenotypes to pathophysiological mechanisms

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Chapter 5.

Two novel *SCN1A* mutations identified in families with familial hemiplegic migraine

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Abstract

Background: Familial hemiplegic migraine (FHM) is a rare monogenic subtype of migraine with aura, characterised by motor auras. The majority of FHM families have mutations in the *CACNA1A* and *ATP1A2* genes; less than 5% of FHM families are explained by mutations in the *SCN1A* gene. Here we screened two Spanish FHM families for mutations in the FHM genes.

Methods: We assessed the clinical features of both FHM families and performed direct sequencing of all coding exons (and adjacent sequences) of the *CACNA1A*, *ATP1A2*, *PRRT2* and *SCN1A* genes.

Results: FHM patients in both families had pure hemiplegic migraine with highly variable severity and frequency of attacks. We identified a novel *SCN1A* missense mutation p.Ile1498Met in all three tested hemiplegic migraine patients of one family. In the other family, novel *SCN1A* missense mutation p.Phe1661Leu was identified in six out of eight tested hemiplegic migraine patients. Both mutations affect amino acid residues that either reside in an important functional domain (in the case of Ile¹⁴⁹⁸) or are known to be important for kinetic properties of the Na_v1.1 channel (in the case of Phe¹⁶⁶¹).

Conclusions: We identified two mutations in families with FHM. *SCN1A* mutations are an infrequent but important cause of FHM. Genetic testing is indicated in families when no mutations are found in other FHM genes.

Introduction

Familial hemiplegic migraine (FHM) is a rare monogenic subtype of migraine with aura (MA) that is characterised by transient hemiparesis during the aura phase. Headache and aura characteristics are identical in FHM and MA patients, apart from the motor auras and possible longer duration of auras in FHM.¹ Mutations in three neuronal or glial ion transporter genes (*CACNA1A*, *ATP1A2*, *SCN1A*) are well-known to cause FHM.² Recently, *PRRT2* was presented as the fourth FHM gene. Although the great majority of patients with a *PRRT2* mutation, which virtually always affect the same stretch of cytosine residues, suffer from “typical *PRRT2*-related phenotypes” (i.e. paroxysmal kinesigenic dyskinesia, benign familial infantile seizures, and infantile convulsion choreoathetosis syndrome), a small number of them also have symptoms of hemiplegic migraine, MA or migraine without aura.^{3–9} Consequently, unlike for the three well-known FHM genes, more convincing evidence for the importance of *PRRT2* as a hemiplegic migraine gene is still needed. At present, more than 70 mutations in the *CACNA1A* (FHM1)¹⁰ and *ATP1A2* (FHM2)¹¹ genes have been identified, and mutations in these genes seem to account for the majority of cases with FHM.¹² Only five mutations were reported for the third FHM (FHM3) gene *SCN1A*^{12,13} that encodes the $\alpha 1$ subunit of neuronal voltage-gated $\text{Na}_v1.1$ sodium channels that are primarily expressed on inhibitory neurons.^{14,15} Of these, *SCN1A* mutations p.Leu1649Gln and p.Gln1489Lys cause pure FHM, that is without additional symptoms such as epilepsy or cerebellar ataxia.^{13,16} The other FHM3 mutations either cause FHM and seizures occurring separately from the hemiplegic migraine attacks (p.Gln1489His¹⁷ and p.Leu263Val¹⁸) or FHM and “elicited repetitive daily blindness” (p.Gln1489His and p.Phe1499Leu mutations.¹⁷ Functional analyses of pure FHM3 mutations predict a loss-of-function effect on inhibitory neurons, thereby leading to overall neuronal hyperexcitability,¹⁹ although this is debated by others.²⁰ Notably, *SCN1A* is a well-known epilepsy gene with several hundred mutations causing various forms of childhood epilepsy: Dravet syndrome (also known as severe myoclonic epilepsy of infancy (SMEI)) and the milder generalised epilepsy with febrile seizures (GEFS+).²¹ Here we report the identification of two novel FHM3 mutations in two families with pure hemiplegic migraine.

Subjects and methods

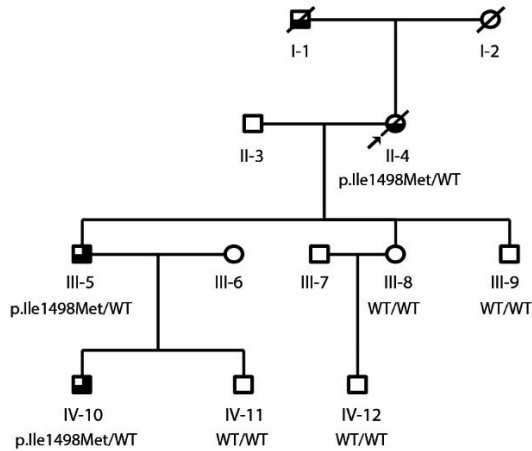
Patients

Family 1. This four-generation Spanish family includes four hemiplegic migraine patients (see Figure 1, Family 1 and Table 1). The family members were interviewed and neurologically examined (ODF). Diagnoses were established according to the International Classification of Headache Disorders, second edition (ICHD-2).¹ The diagnosis of the proband’s deceased father was based on information provided by the proband.

The proband (II-4) had her first migraine attack at age 11 and continued to have on average two attacks per month until the age of 73. These hemiplegic attacks were usually preceded by flashing lights or scotoma, speech problems and/or unilateral sensory symptoms. Auras developed gradually during five to 15 minutes, but on several occasions hemiplegia occurred instantaneously, causing her to fall. In general, hemiplegia lasted 30 to 60 minutes and then diminished gradually. After these auras, she suffered from a typical migraine headache, i.e. unilateral pulsatile headache that increased with movement and was best relieved by sleep, accompanied by photophobia and phonophobia. At the age of 69 years, she experienced an episode of benign paroxysmal positional vertigo that lasted for 15 days and then resolved gradually over the course of two weeks. At age 70, she presented with prolonged hemiplegia for 10 days, which completely recovered during the subsequent three weeks. Cerebral magnetic resonance imaging (MRI) (see Figure 2) and single photon-emission computed tomography (SPECT) (data not shown) during the hemiplegic phase showed vasogenic edema and cortical hypoperfusion in the right hemisphere. Further extensive diagnostic workup for cerebrovascular disease and epilepsy, including electroencephalography, cerebrospinal fluid analysis, echography of the heart and carotid arteries, laboratory analysis of coagulation factors and autoimmune titers, and skin biopsy for excluding cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), showed no abnormalities. From age 72, she showed progressive cognitive deterioration and was diagnosed with Alzheimer's disease. She died at the age of 79 years of urinary sepsis. Post-mortem examination of the brain showed multiple senile plaques and confirmed the diagnosis of Alzheimer's disease. No ischemic lesions were found.

The proband's father (I-1) had similar FHM attacks. He suffered from dementia from age 70 and died at age 80. Patient III-5, now 58 years old, suffered from MA and FHM with an attack frequency of two per year until he was 44. No hemiplegic attacks have occurred over the last 13 years. Patient IV-10, currently 33 years old, has experienced four hemiplegic migraine attacks from age 15 to 31. His attacks started with hypesthesia and hemiparesis during 20 minutes, followed by migraine headache. An MRI scan performed during one of these attacks showed no abnormalities. He also suffers from migraine with visual aura, with an attack frequency of one per three months.

Family 1



Family 2

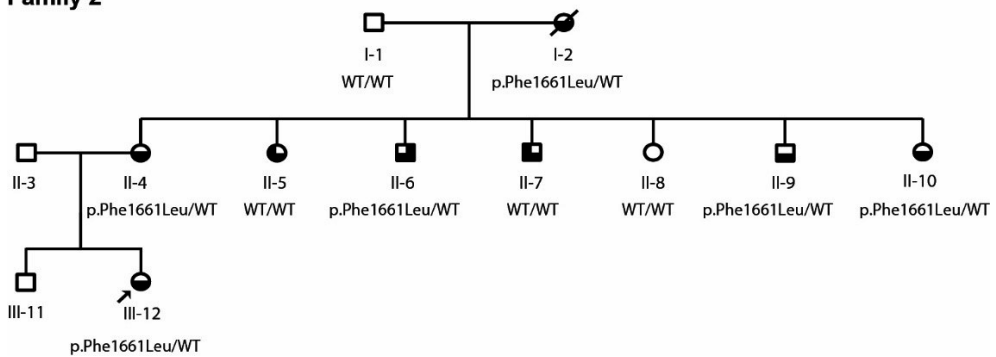


Figure 1. Pedigrees of FHM families. The arrow indicates the proband. Symbols: *black lower-half*: hemiplegic migraine; *right upper square*: migraine with aura (MA); *left upper square*: migraine without aura (MO). WT: homozygous carrier of the wild-type allele. p.Ile1498Met and p.Phe1661Leu: heterozygous carrier of the respective *SCN1A* mutations.

Family 2. This three-generation family is also of Spanish origin. Eight family members were diagnosed with FHM (see Figure 1, Family 2 and Table 1). Their diagnoses were established according to ICHD-2 criteria¹ and confirmed by a neurologist specializing in headache (JP). After the passing of the proband's grandmother, additional information was provided by her children.

The proband (III-12) had been suffering from approximately three migraine attacks per month since she was nine years old, with visual, sensory and speech auras. At the age of 16 she presented at the emergency department with right-sided hemiparesis, accompanied by scotoma, photopsia, right-

Table 2: Characteristics of Familial Hemiplegic Migraine patients in family 1 and family 2.

Gender	Age (years)	Age at onset of FHM (years)	Average attack frequency (at different ages)	Aura symptoms during FHM attacks				Other medical history	Genotype	
				Visual	Sensory	Motor	Speech			Other migraine subtypes
Family 1										
I-1	male	deceased (at age 80)	?	?	?	yes	?	?	dementia	?
II-4 (proband)	female	deceased (at age 79)	11	2x/ month	yes	Yes	yes	yes	benign paroxysmal positional vertigo, dementia (Alzheimer's)	p.Ile1498Met / WT
III-5	male	58	?	2x/ year	?	?	yes	?	MA	p.Ile1498Met / WT
IV-10	male	33	15	1x/ 4 years	no	Yes	yes	no	MA	p.Ile1498Met / WT
Family 2										
I-2	female	deceased (at age 86)	13	youth: 1-2x/ month; lower at older age	yes	Yes	yes	yes	none	p.Phe1661Leu / WT
II-4	female	53	14	14-30: 3-4x/ year >30: 1x/ several years	yes	Yes	yes	yes	none	p.Phe1661Leu / WT
II-5	female	52	20	1x/ month	yes	Yes	yes	no	MO	WT / WT
II-6	male	50	14	1x/ week	yes	Yes	yes	yes	MA	p.Phe1661Leu / WT
II-7	male	49	14	1x/ year	yes	Yes	yes	yes	MO	WT / WT
II-9	male	46	14	14-40: 1x/ month >40: 1-2x/ year	yes	Yes	yes	yes	none	p.Phe1661Leu / WT
II-10	female	43	11	2x/ year	yes	Yes	yes	yes	none	p.Phe1661Leu / WT
III-12 (proband)	female	20	9	2x/ month	yes	Yes	yes	yes	none	p.Phe1661Leu / WT
									premature ovarian failure	

MA= migraine with aura; MO= migraine without aura; WT: wild-type allele; p.Ile1498Met and p.Phe1661Leu: heterozygous carrier of the respective *SCN1A* mutations. The symbol '?' indicates this information was unavailable.

sided hypesthesia, contralateral headache and vomiting lasting for three hours. She was diagnosed with hemiplegic migraine.

The proband's mother (II-4) had three to four FHM attacks per year from age 14 to 30, later decreasing to one attack per several years. All attacks include visual, sensory, motor and speech symptoms lasting 30–60 minutes. The five affected siblings of the proband's mother experience multiple aura types, in varying combinations during separate attacks, followed by typical migraine headaches (for summary, see Table 1).

The proband's grandmother (I-2) had suffered migraine attacks since the age of 13. She always experienced visual, sensory and dysphasic auras. Hemiparesis occurred on most occasions. At age 85 she was diagnosed with dementia. She died one year later after a stroke.

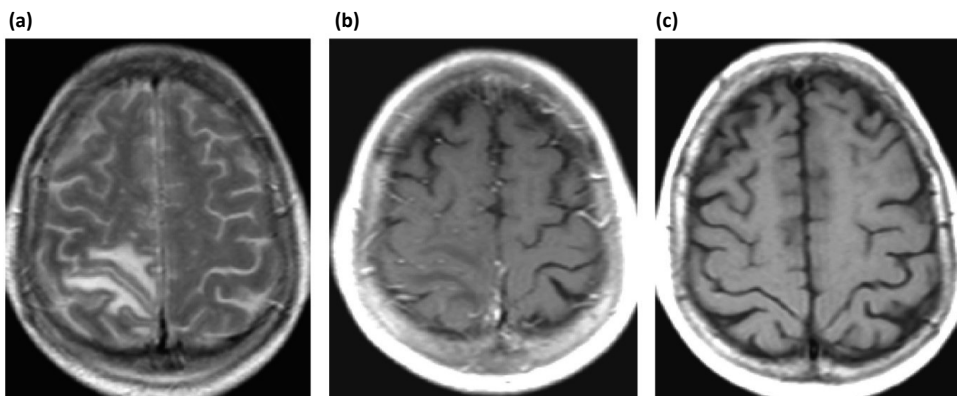


Figure 2. Cerebral MRI of the proband of Family 1 during prolonged aura and after full recovery. (a) and (b) MR FLAIR T2-weighted image in axial plane and gadolinium-enhanced T1-weighted image showing a subcortical area of white matter in the perirolandic region on the right is hyperintense on image (a) and relatively hypointense on image (b) with blurring of the cortical sulci adjacent to the convexity. (c) T1-weighted MR image showing the resolution of the radiological abnormalities, in agreement with the patient's clinical recovery.

Genetic analysis

Genomic DNA was isolated from peripheral leukocytes according to standard procedures.²² Causal mutations in the FHM genes *CACNA1A* and *ATP1A2* were excluded by direct sequencing of all coding exons (and direct flanking sequences) in the probands of both families. We also excluded a causal mutation in *PRRT2* by sequencing all four coding exons (and all direct flanking sequences) in the

probands of the families. Subsequently, we sequenced all 26 exons and flanking intronic sequences of the *SCN1A* gene. Detailed information is available from the authors upon request.

Results

The well-known FHM genes *CACNA1A* and *ATP1A2*, as well as the more recently implicated disease gene *PRRT2*, did not yield a possible causal mutation in either of the two Spanish FHM families. Therefore, we sequenced all 26 exons (and direct flanking intronic sequences) of the *SCN1A* gene in the probands of both families.

In the proband of Family 1, we identified a novel heterozygous point mutation in exon 24 (c.4460G>C; p.Ile1498Met) of the *SCN1A* gene. The mutation co-segregated with FHM in this family (Figure 1, Family 1) and was absent in our panel of 161 healthy control subjects. The p.Ile1498Met mutation is located in the intracellular loop between transmembrane domains III and IV (Figure 3a) and affects the “IFMT motif.” This motif encodes the hydrophobic latch that is responsible for adequate occlusion of the ion pore, which leads to inactivation of the Na_v1.1 channel, thereby regulating the generation and propagation of action potentials.^{14,15} The IFMT motif is named after the four amino acids encoding it, i.e. isoleucine (I), phenylalanine (F), methionine (M) and threonine (T). Mutation p.Ile1498Met leads to the substitution of isoleucine for a methionine residue at the first position of the IFMT motif. Both are hydrophobic nonpolar amino acids with similar molecular weight. Isoleucine is aliphatic while methionine is a sulfur-containing amino acid and is important for the function of the *SCN1A* encoding protein. Analysis of this amino acid in homologous and orthologous sodium channel α 1 subunits shows high conservation of isoleucine1498 (Ile¹⁴⁹⁸) (Figure 3b).¹⁷ Additional support for pathogenicity of the p.Ile1498Met mutation came from three in silico bioinformatics prediction programs, SIFT (deleterious (0)), PolyPhen2 (probably damaging (0.998)), and Mutation Taster (disease causing (0.99)), which all indicated that the mutation is likely pathogenic.

Mutation scanning of the *SCN1A* gene in Family 2 revealed a novel heterozygous point mutation in exon 26 (c.4981C>T; p.Phe1661Leu). This mutation partially co-segregated with FHM in this family as two of the eight tested clinically affected individuals (II-5 and II-7) did not have the mutation and are thus diagnosed as phenocopies (Figure 1, Family 2). The p.Phe1661Leu mutation was not found in the proband’s unaffected aunt nor in our panel of 161 healthy control subjects. Phenylalanine¹⁶⁶¹ (Phe¹⁶⁶¹) is located in domain IV, in the intracellular loop between the voltage-sensing S4 segment and part of the transmembrane pore (S5) (Figure 3a).²¹ It is highly conserved within all orthologues

and homologues except for the *SCN7A* gene, which, notably, has a leucine at the equivalent position (Figure 3b). Moreover, the importance of Phe¹⁶⁶¹ is evidenced by the observation that a change of this residue to a serine results in Dravet syndrome²³ and the fact that the mutation affects Na_v1.1 channel functioning.²⁴ Additional support for pathogenicity of the p.Phe1661Leu mutation came from the three in silico bioinformatics prediction programs, SIFT (deleterious (0)), PolyPhen2 (probably damaging (1.0)), and Mutation Taster (disease causing (0.99)), which all indicated that the mutation is likely pathogenic.

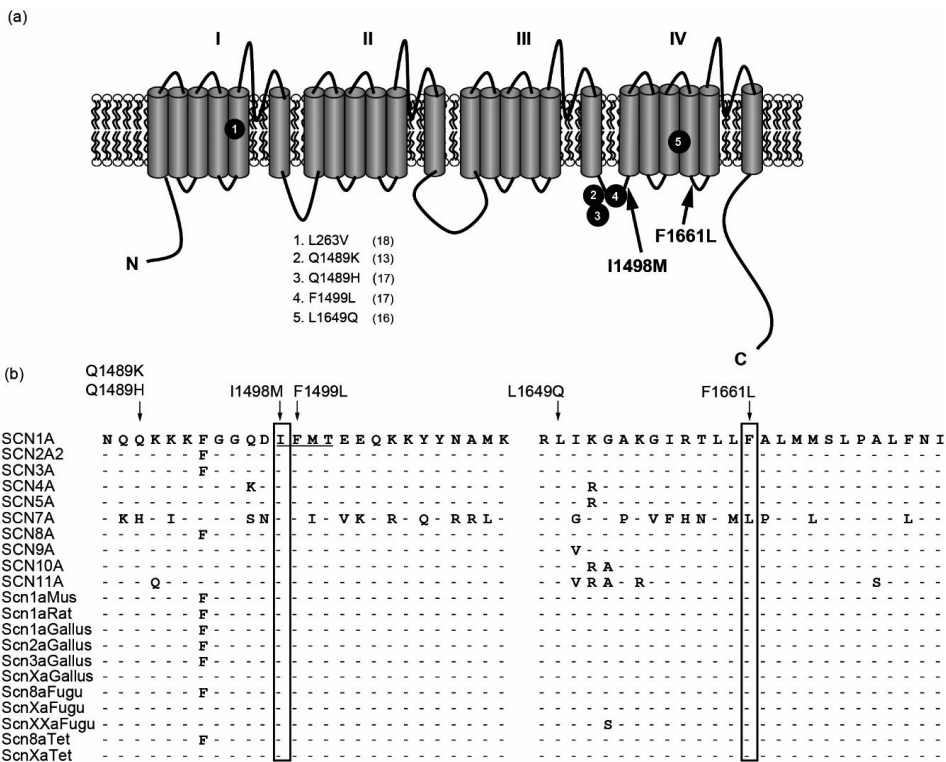


Figure 3. Predicted topology of the *SCN1A* protein and amino acid sequence alignments. (a) Arrows indicate the location of the novel p.Ile1498Met (I1498M) and p.Phe1661Leu (F1661L) FHM3 *SCN1A* mutations. Previously identified FHM3 mutations are indicated by numbers 1–5. **(b)** Alignment of the amino acid sequence of alpha subunits of sodium channels from various species.

Discussion

Here we report on two Spanish families with pure hemiplegic migraine (i.e. without other paroxysmal or permanent neurological symptoms such as epilepsy or ataxia) in which we identified two novel *SCN1A* mutations: p.Ile1498Met and p.Phe1661Leu. Attacks in both families started at a

young age, ranging from age 9 to 20 years. Attack frequency was highly variable, ranging from one attack per week to one attack per several years. Prolonged duration of auras, varying from three hours to three weeks, and co-occurrence of FHM attacks with attacks of MA and migraine without aura were observed in both families, as has been reported for other FHM families.²⁵

Ictal MRI abnormalities (Figure 2), as observed in the proband of Family 1, have not been reported in FHM3 patients, but are similar to observations made in FHM1 and FHM2.²⁵ Post-mortem examination revealed that the dementia in patient II-4 of Family 1 was due to Alzheimer's disease. Dementia in patient I-2 of Family 2 is also unlikely related to the *SCN1A* mutation, given the age at onset after 80.

Although functional testing of the mutations has not been performed, it is probable that mutations p.Ile1498Met and p.Phe1661Leu are pathogenic. First, p.Ile1498Met is present in the three family members with hemiplegic migraine and absent from a panel of 161 control subjects. Second, Ile¹⁴⁹⁸ is a highly conserved amino acid in $\alpha 1$ subunits of vertebrate sodium channels. Third, the mutation is located in the same intracellular loop as three of the five previously described FHM3 mutations (p.Gln1489Lys, p.Gln1489His, p.Phe1499Leu) (Figure 3a).^{13,17} Of these, the p.Phe1499Leu mutation is also located in the IFMT motif, which encodes the hydrophobic latch, and is essential for the function of the neuronal voltage-gated $\text{Na}_v1.1$ sodium channel.^{14,15} Finally, additional support for the pathogenicity of the p.Ile1498Met mutation came from in silico bioinformatics prediction tools that consistently predicted that the mutation is likely pathogenic. Interestingly, none of the more than 600 mutations causing Dravet syndrome or GEFS+ affect the IFMT motif.^{21,26} It is hypothesised that a defective hydrophobic latch will lead to delayed sodium channel inactivation with concomitant decreased neuronal inhibition.

Several lines of evidence indicate that the p.Phe1661Leu mutation in Family 2 is also pathogenic. The mutated Phe¹⁶⁶¹ is a highly conserved amino acid across homologues and orthologues, except for *SCN7A* a gene that shares only 52% overall sequence similarity with the *SCN1A* gene. All three tested in silico bioinformatics prediction programs indicate that the p.Phe1661Leu mutation likely has a deleterious effect on the *SCN1A* protein. In addition, the p.Phe1661Leu mutation is absent from our panel of healthy control subjects and co-segregates in six of eight hemiplegic migraine patients. The remaining two hemiplegic migraine patients should be considered phenocopies. Phenocopies have been described before in FHM families.²⁷⁻²⁹

The p.Phe1661Leu mutation is the first FHM mutation that affects the same amino acid as a known Dravet mutation, i.e. p.Phe1661Ser. In Dravet, Phe¹⁶⁶¹ is mutated into a much smaller, nucleophilic serine residue.²³ Substitution of the phenylalanine for a leucine apparently has a less detrimental effect on protein function, which seems in line with the much milder phenotype in our family. The importance of Phe¹⁶⁶¹ for sodium channel function is further illustrated by the fact that p.Phe1661Ser affects Na_v1.1 channel functioning²⁴ and that mutating the corresponding residue in *SCN4A*, which causes paramyotonia congenita, also showed alterations of the kinetic properties of the sodium channel.³⁰

In conclusion, we identified two novel *SCN1A* mutations causing pure FHM with large intra-familial variation in severity and frequency of the attacks. Our findings increase the number of FHM-causing *SCN1A* mutations to seven and illustrate the importance of this gene for genetic counseling and testing of FHM patients, especially when they test negative for *CACNA1A* and *ATP1A2* mutations.

5

Clinical implications

- Screening of *SCN1A* should be considered in hemiplegic migraine patients if no mutations are found in the *CACNA1A* and *ATP1A2* genes.
- Familial hemiplegic migraine 3 (FHM3), similar to FHM1 and FHM2, is characterised by a large intrafamilial variability in severity and frequency of attacks and accompanying symptoms.
- Decreased function of neuronal voltage-gated Na_v1.1 sodium channels may be the underlying cause of FHM3.

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Conflict of interest: C.M. Weller reports no disclosures. N. Pelzer reports support for conference visits from Menarini and Allergan UK. B. de Vries reports no disclosures. J. Pascual reports consultancy from Allergan. O. De Fàbregues, M.A. Ramos Arroyo, M. Artigas López report no disclosures. A.H. Stam reports independent research support from the NWO. S.C. Koelewijn and J. Haan report no disclosures. M.D. Ferrari reports grants and consultancy or industry support from Medtronic, Menarini, and Merck and independent support from NWO, ZonMW, the United States National Institutes of Health (NIH), the European Community, and the Dutch Heart Foundation. G.M. Terwindt reports grants and consultancy/industry support from Merck and Menarini, and independent support from the NWO. A.M.J.M. van den Maagdenberg reports no disclosures.

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