

Short communication

A hereditary spastic paraplegia predominant phenotype caused by variants in the *NEFL* gene

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ABSTRACT

Introduction: This study reports a large series of patients with a clinical picture dominated by spastic paraplegia in whom variants in the *NEFL* gene, a known cause for Charcot-Marie-Tooth disease, were identified.

Methods: Index patients referred for a suspicion of hereditary spastic paraplegia (HSP) were clinically assessed and genetic analysis by next-generation sequencing was undertaken. Additional family members were clinically examined and subjected to targeted testing.

Results: We identified two different heterozygous dominant variants in the *NEFL* gene in 25 patients from 14 families. Most of them (21/25) had a clinical diagnosis of HSP, often with a concomitant clinical diagnosis of polyneuropathy (16/21). Two patients were identified with a polyneuropathy with a pyramidal reflex pattern, but without spasticity. Two patients had isolated polyneuropathy. Out of the 21 patients with a diagnosis of HSP, two had co-occurring cerebellar signs. The c.262A > C p.(Thr88Pro) variant was detected in 13 families. Genealogical analysis showed shared ancestors or a similar geographical origin in 12, suggesting a founder effect. The other variant, c.296A > C p.(Asp99Ala), was found in only one family, in which limited segregation analysis could be performed.

Discussion: Variants in the *NEFL* gene can cause HSP, with or without co-existing polyneuropathy, and should be included in diagnostic testing strategies for HSP patients.

1. Introduction

Pathogenic variants in the neurofilament light chain gene *NEFL* are a cause of autosomal dominant Charcot-Marie-Tooth disease (CMT), first identified in 2000 [1,2]. The phenotypic spectrum of *NEFL*-related CMT is wide, with reported additional features such as hearing loss, ataxia and pyramidal signs [3,4]. Here, we report a large series of patients with a clinical picture dominated by adult-onset, hereditary spastic paraplegia (HSP) caused by a variant in the *NEFL* gene.

2. Methods

2.1. Patients

Patients were referred to the Expert center for Rare and Genetic movement disorders of the Radboud university medical center, or to the Genetics or Neurology departments of the University Medical Center Utrecht. All index patients were referred for the analysis of progressive pyramidal features, often with a specific suspicion of HSP, or because of a combination of spastic paraparesis and polyneuropathy within the family. Diagnostic procedures were undertaken as considered

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appropriate, which included clinical assessment and genetic analysis by either clinical exome sequencing or targeted next-generation sequencing (panels). Clinical exome sequencing was approved by the Medical Review Ethics Committee, Region Arnhem-Nijmegen, Number 2011/188. Where indicated and available, family members were clinically and genetically investigated. Patient files were systematically reviewed to obtain clinical and imaging data. In-depth genealogical analysis was performed to identify common ancestors of patients with identical *NEFL* variants. Pedigrees were generated using Dutch civil registers and church books.

2.2. Genetic testing

The indexes from families 1 and 2 underwent targeted analysis for HSP/CMT genes, including the *NEFL* gene, at the University Medical Center Utrecht, the Netherlands. In brief, next generation sequencing was performed with a SOLiD 5500XL sequencing system (Applied Biosystems) after enrichment with the Sure Select XT enrichment kit (Agilent). Subsequent DNA testing in affected relatives was performed by Sanger sequencing. In the other families, clinical exome sequencing was essentially performed as previously described [5]. Briefly, capture of exons was done using an Agilent SureSelect Human All Exon 50 Mb Kit (Santa Clara, CA, USA). Sequencing was performed using an Illumina HiSeq 2000 or 4000 (San Diego, CA, USA). Read mapping and variant calling were done using BWA and GATK, respectively. Initially, a filter for a ‘movement disorders’ gene panel was applied. This panel consists of ~200 genes implicated in various forms of cerebellar ataxia, HSP, genetic dystonia, and other hyperkinetic movement disorders. Only genes with substantial evidence (multiple families, functional evidence, and/or literature reports) were included in this panel. Genes with repeat expansions as the only mutational mechanism were not included. The genes in this panel and coverage statistics can also be found at www.genomediagnosicsnijmegen.nl/exome. Variants were prioritized based on the following criteria: frequencies (<1% dbSNP, <0.1% in-house database of >30,000 exomes), nucleotide and amino acid conservation (based on alignments), relation of the gene to disease (per family), and inheritance patterns. Genome build HG19, reference sequence NM_006158.4 and HGVS were used for the nomenclature of the *NEFL* variants.

3. Results

3.1. Genetic findings

We identified 25 patients from 14 families with one of two different variants in the *NEFL* gene (NM_006158.4). Twenty-two patients carried

the same heterozygous missense variant (c.262A > C p.(Thr88Pro)), located on the HEAD domain of the protein [6]. Three patients from one family carried another missense variant (c.296A > C p.(Asp99Ala)) in the coil 1 domain. Combined Annotation Dependent Depletion (CADD) PHRED scores for c.262A > C p.(Thr88Pro) and c.296A > C p.(Asp99Ala) are 23.3 and 22.9, respectively [7]. CADD.PHRED scores larger than 20 indicate that the variants belong to the <1% most deleterious variants in the human genome. Both variants are absent in control populations (gnomAD, <https://www.biorxiv.org/content/10.1101/531210v3>) and have not been described before in the literature. Both Thr88 and Asp 99 are conserved in all vertebrate orthologues, though a Ser was found at position 88 in chicken. More importantly, segregation analysis performed in four families with multiple affected family members showed that the variant segregated with the clinical features (Fig. 1). One family member in family 2 without any neurological symptoms underwent presymptomatic genetic testing at age 68 and tested negative for the *NEFL* variant. Using ACMG guidelines for the classification of the variants [8] in combination with the information above, the p.(Thr88Pro) variant is considered likely pathogenic (PS4, PM2 and PP1), while the p.(Asp99Ala) variants remains a VOU until additional lines of evidence become available. Genealogical analysis in families with the p.(Thr88Pro) variant, showed 10 of 13 families to be related, with shared ancestors after 3 till 6 generations. Furthermore, all indexes of these 13 families except one, originated from the same geographical region in the eastern part of the Netherlands. However, no common ancestor for all families could be detected by genealogy.

3.2. Clinical characteristics

We identified 25 patients from 14 families who carried one of the variants in the *NEFL* gene. Twenty-one of these patients (15 males, age range 37–77 years) presented with an adult-onset slowly progressive bipyramidal syndrome (dysfunction of the corticospinal tracts resulting in upper motor neuron signs, i.e. spasticity and/or a pyramidal pattern of tendon and plantar reflexes, in the lower extremities), consistent with a clinical diagnosis of HSP. A summary of clinical characteristics per patient is presented in Table 1. These patients all had lower limb spasticity, balance difficulties, and pyramidal reflex patterns. Age of symptom onset varied from 20 to 70 years old (median 50 years). Most of them (16/21) had a concomitant clinical diagnosis of polyneuropathy, which was confirmed by EMG in 9 patients (axonal, sensorimotor polyneuropathy). The indication for genetic testing of the index patient in 13 of 14 families, was the diagnosis of a bipyramidal syndrome, with or without polyneuropathy. In family 1, the indication was a polyneuropathy in the index patient (patient 7), with a family history of both polyneuropathy and bipyramidal syndrome. This index patient aged 34

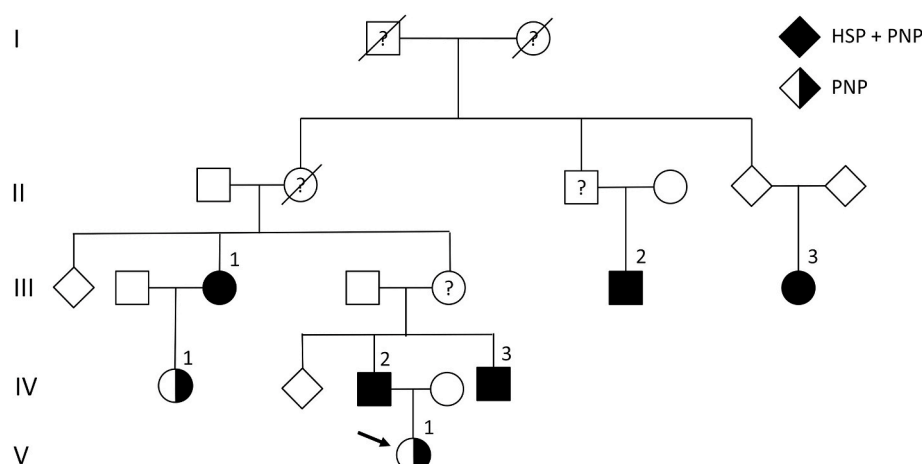


Fig. 1. Pedigree of family 1. HSP: hereditary spastic paraplegia; PNP: polyneuropathy; arrow: index patient.

Table 1
Clinical characteristics of patients with a NEFL-variant.

Patient	1	2	3	4	5	6	7	8	9	10	11 [†]	12 [†]	13 [†]	14	15	16	17	18	19	20	21	22	23	24	25
Family	III-1	III-2	III-3 [#]	IV-1	IV-2	IV-3	V-1	II-1	II-2	II-3	II-1	III-1	III-2	II-1	I-1	M	M	M	M	M	M	M	M	F	M
Pedigree ID	73	66	58	50	57	48	34	77	75	73	69	51	49	37	66	62	58	53	74	49	65	65	65	77	63
Sex	F	F	F	F	M	M	F	M	F	M	M	F	M	M	F	M	M	M	M	M	M	M	M	F	M
Age at study (y)	68	51	51	48	50	27	31	n/a	n/a	n/a	70	49	35	46	30	54	50	50	69	20	50	57	20	70	48
Age at onset (y)	+	+	+	+	+	+	+	?	?	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HSP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LL spasticity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Proximal LL weakness	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Balance difficulties	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PTD	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Extensor plantar response	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary sphincter problems	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gait ataxia	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Limb ataxia	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MRI brain	n	n/a	n/a	n/a	n	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
MRI spine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyneuropathy	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Distal vibration sensation	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Distal touch sensation	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Distal LL weakness	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pes cavus, claw toes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ATR	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EMG abnormalities	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

n = normal; y = years; abs = absent; n/a = not available; HSP = hereditary spastic paraplegia; LL = lower limbs; PTR = patella tendon reflex; ATR = ankle tendon reflex; EMG = electromyography; CA = cerebellar atrophy; SVD = small vessel disease; [†]patient with p.Asp99Ala variant; [#]no genetic testing performed; *patient underwent bladder surgery.

years, and one other family member from family 1 aged 50 years, had an isolated polyneuropathy without any signs of upper motor neuron involvement. Two patients from one family (family 2) were identified with a polyneuropathy and a pyramidal reflex pattern, but without spasticity. Two of the patients with a bipyraximal syndrome were initially diagnosed with, and genetically tested for, genetic ataxia. Both had hypermetric saccadic eye movements, gait ataxia, sway during stance, dysmetria on finger chase and heel-shin slide tests, and intention tremor on nose-finger testing. One of them additionally had a dysarthric speech and mild cerebellar atrophy on brain MRI. In one other patient with an otherwise pure HSP phenotype, dysmetria was noted on the heel-shin slide. Two patients had sensory ataxia; one of them was diagnosed with both HSP and polyneuropathy, the other with isolated polyneuropathy. Upper limb muscle weakness was rare. Two of the patients with a combined phenotype of spastic paraplegia and polyneuropathy had mild weakness of some distal forearm muscles. No cognitive deficits were noted.

4. Discussion

HSP is clinically and genetically heterogeneous. An overlap between HSP and other neurogenetic disorders like CMT and genetic forms of ataxia or parkinsonism is increasingly recognized and various of these HSP overlap syndromes are caused by specific genes. This study reports a series of 25 patients with an HSP-predominant phenotype in whom variants in the *NEFL* gene were identified. Pathogenic *NEFL* variants are a recognized cause for CMT, which is in line with the finding in our study that many, though not all, of the HSP patients had a concomitant diagnosis of polyneuropathy [3,6]. Although central nervous system signs, like pyramidal reflex patterns or ataxia, have been previously reported in CMT patients with *NEFL* variants, previous studies showed that these patients usually do not present with a predominantly central nervous system phenotype [3]. In the literature, 13 patients from 7 kindreds with *NEFL* variants and evidence of pyramidal tract involvement in addition to polyneuropathy have been described [3,9–13]. In only 4 of these patients, all from one family, spasticity was a more prominent clinical feature than polyneuropathy [14]. Seven patients described in the literature had a phenotype consistent with and were initially tested for spinocerebellar ataxia or Friedreich's ataxia [3]. In our series, the majority of patients had predominant spastic paraplegia. Intrafamilial variability was remarkable in this study, with different phenotypes (isolated HSP, isolated polyneuropathy, or an overlapping phenotype) occurring within the same family. Based on its function, it is not surprising that variants in the *NEFL* gene can cause both central and peripheral nervous system pathology. The *NEFL* gene encodes the light chain neurofilament protein (NFL), one of the subunits that compose neurofilaments. Neurofilaments are essential for the radial growth of axons during development, the preservation of axon diameter, and the transmission of electrical impulses [15]. NFL are subunits of neurofilaments in both the central and the peripheral nervous system and as such, dysfunction of the NFL protein could give rise to pathology in either of the two parts of the nervous system [15]. Although the p.(Thr88Pro) variant in this study has not been previously reported, it is likely pathogenic because of its abundance in patients and absence in control databases and the co-segregation of symptoms with the variants within multiple families. While other *NEFL* variants that cause polyneuropathy with pyramidal tract signs were mostly located in the end portion of the rod domain and the tail subdomain of the *NEFL* gene, the p.(Thr88Pro) variant in this study is located in the head domain. The number of patients (three in a single family) with the p.(Asp99Ala) does not allow a higher formal classification than a variant of uncertain significance. Future detection of families with this variant is needed to determine its pathogenicity according to current guidelines. The fact that most of the identified patients carried an identical variant, and that all of these patients originated from the same small geographical area in the Netherlands, suggests that the p.(Thr88Pro) variant is a founder variant.

Indeed, this is supported by detection of genealogical links between the majority of families. To conclude, specific pathogenic variants in the NEFL gene cause a spectrum of neurological diseases ranging from CMT to HSP. The NEFL gene should therefore not only be included in the diagnostic genetic testing strategies for CMT patients, but also for HSP patients.

Author disclosures

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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.2020.09.016>.

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