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Clinical short communication

Elevated hydroxycholesterols in Norwegian patients with hereditary spastic paraplegia SPG5

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ABSTRACT

Spastic paraplegia type 5 (SPG5/HSP-*CYP7B1*) is an autosomal recessive hereditary spastic paraplegia (HSP) caused by biallelic variants in the *CYP7B1* gene, resulting in dysfunction of the enzyme oxysterol-7-α-hydroxylase. The consequent accumulation of hydroxycholesterols in plasma seems to be pathognomonic for SPG5, and represent a possible target for treatment. We aimed to characterize Norwegian patients with SPG5, including clinical examinations, genetic analyses, measurements of hydroxycholesterols, electrophysiological investigations and brain imaging. Five unrelated patients carried presumed disease-causing variants in *CYP7B1*, three of the variants were novel. Four patients presented with pure HSP, one with complex HSP. The three tested patients all had markedly increased levels of 25- and 27-hydroxycholesterol in plasma. Our results suggest that the clinical examination is still the best approach to classify disease severity in patients with SPG5. Plasma hydroxycholesterols were elevated, thus presenting as potentially valuable diagnostic biomarkers, in particular in patients where genetic analyses are inconclusive.

1. Introduction

Spastic paraplegia type 5 (SPG5/HSP-*CYP7B1*) is an autosomal recessive form of hereditary spastic paraplegia (HSP). HSP is characterized by progressive gait problems due to spasticity and weakness in the lower limbs, with or without additional neurological features, and so far ~80 genetic forms have been described. There are no curative or disease-modifying treatment available for HSP and reliable biochemical biomarkers are largely lacking [1]. However, for SPG5 there is increasing evidence of hydroxycholesterols as reliable biomarkers [2–4], there has also been performed one preclinical trial investigating treatment targeting the molecular mechanism [5].

SPG5 most often present as a pure form of HSP comprising progressive spasticity and muscle weakness in the lower extremities, which can be accompanied by bladder dysfunction and posterior column sensory impairment. The clinical features can be heterogeneous, and additional symptoms such as ataxia may occur [2].

The cause of SPG5 is biallelic variants in the *CYP7B1* gene. This gene encodes the enzyme oxysterol-7-α-hydroxylase, which is involved in the degradation of cholesterol into primary bile acids [6,7]. The disease-causing *CYP7B1* variants lead to loss of function of the enzyme, and plasma accumulation of its substrates, 25-hydroxycholesterol (25-OHC)

Abbreviations: CYP7B1, Cytochrome P450, family 7, subfamily B, polypeptide 1; HSP, hereditary spastic paraplegia; OHC, hydroxycholesterol; SPG5, Spastic paraplegia type 5; SPRS, Spastic Paraplegia Rating Scale.

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Table 1
Characteristics of Norwegian patients with SPG5.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Phenotype	Pure HSP	Pure HSP	Pure HSP	Complex HSP	Pure HSP
Variant 1 in *CYP7B1*	c.1250G > A (p.Arg417His)	c.1322C > T (p.Pro441Leu)	c.1456C > T (p.Arg486Cys)	c.793C > T (p.Gln265*)	c.1456C > T (p.Arg486Cys)
Variant 2 in *CYP7B1*	c.321-324del (p.Lys107Asnfs)	c.1456C > T (p.Arg486Cys)	c.1456C > T (p.Arg486Cys)	c.1456 > T (p.Arg486Cys)	c.1456C > T (p.Arg486Cys)
Age at onset[a]	2	1	2	10	35
First symptom	Stiff legs	Gait disturbance and stiff legs	Unsteadiness	Stiff legs	Stiff legs
Age at last examination[a]	22	57	69	60	40
DD (years)	20	56	67	50	5
SPRS score [b]	14	31	37	NA	10
Gait spasticity	Severe	Severe	Severe	Severe	Moderate
Resting spasticity LL	Moderate	Severe	Severe	Severe	Moderate
Proximal weakness LL	Moderate	Severe	Severe	Severe	Mild
Distal weakness LL	Moderate	Severe	Mild	Severe	None
Patellar reflexes	Increased	Increased	Increased	Increased	Increased
Achilles reflexes	Increased	Diminished	Diminished	Increased	Increased
Plantar responses	Extensor	Extensor	Extensor	Extensor	Extensor
Vibration sense	Slightly reduced	Moderately reduced	Strongly reduced	Strongly reduced	Slightly reduced
Superficial sensation	Slightly reduced	Slightly reduced	Slightly reduced	Slightly reduced	Normal
Eye findings	None	Optic atrophy	None	Saccadic pursuit	None
Other findings	None	None	Dysarthria	Cognitive impairment, truncal ataxia	None
Cerebral MRI [a]	Normal (22)	WML (60)	WML (68)	WML (67)	Normal (40)
EMG/NCV [a]	Normal (22)	Bilat. CTS (51)	NA	Bilat. CTS (61)	Normal (40)
SEP [a]	Absent cortical response right, very delayed left (22)	Inconclusive (57)	NA	NA	Normal (40)
BAEP, VEP[a]	Normal (22)	Normal (57)	NA	NA	Normal VEP, unilat. lack of response in BAEP (40)
BoNT LL	None	Yes, with effect	Yes, no effect	None	None

BAEP, Brainstem auditory evoked potential; bilat., bilateral; BoNT, botulinum neurotoxin treatment; CTS, Carpal tunnel syndrome; DD, Disease duration; EMG, electromyography; LL, lower limbs; NA, Not available; NCV, Nerve conduction study; SEP, Sensory Evoked Potential; Unilat., unilateral; VEP, Visual Evoked Potential; WML; white matter lesions of unspecific origin.

[a] At age, in years.

[b] Spastic Paraplegia Rating Scale (0–52), scores obtained at last examination.

and 27-hydroxycholesterol (27-OHC). Of these, only 27-OHC accumulates in CSF [3].

It has been demonstrated that 27-OHC impairs metabolic activity and viability of human cortical neurons at concentrations found in patients with SPG5, indicating that the elevated levels of hydroxycholesterols are the key pathogenic factors in SPG5 [2]. As there is a correlation between cerebrospinal fluid (CSF) levels of 27-OHC and plasma levels, the plasma levels represent potential biomarkers of the disease [2].

To date, there are no drugs that target hydroxycholesterols directly. However, existing cholesterol-lowering statins target precursors of 27-OHC in plasma, and may present possible treatment options for SPG5. Indeed, correlation between plasma and CSF-levels has been demonstrated, also as response to statin therapy [2]. Hence, plasma hydroxycholesterols may be of use as a diagnostic tool, and possibly as a biomarker in clinical trials. In this study we provide the first thorough characterization of Norwegian patients with SPG5.

2. Material and methods

All patients diagnosed with SPG5 in an ongoing project on spinocerebellar degenerative disorders in Norway [8] were included in this study, signed informed consents and were thoroughly examined by a project neurologist. The study is approved by the Regional ethical committee of South-Eastern Norway, REK no 2010/1579.

Variants in *CYP7B1* were identified by routine genetic investigations, applying high-throughput sequencing based gene panels for movement disorders at the Department of Medical genetics, Oslo University Hospital or Telemark Hospital Trust, using standard diagnostic procedures [9]. To investigate a possible founder effect of the c.1456C > T variant, a simple haplotype analysis was performed with the exome data from patients 2–4. Outer limits for shared haplotypes were established by discordantly homozygous variants on each side of the variant (Supplementary table 1A).

Full blood was obtainable from three fasting patients (patients 1–3) for biochemical analyses of plasma hydroxycholesterols. Ultra-performance liquid chromatography-tandem mass spectrometer with isotopic dilution was used to measure plasma 25-OHC and 27-OHC, in addition to cholesterol and triglycerides, in three patients with methods previously described [4].

Nerve conduction studies (NCS) were performed in four patients. Brainstem auditory evoked potentials (BAEPs), somatosensory evoked potentials of the median nerve (SEPs) (N20 wave) and visual pattern evoked potentials (VEPs) (reversed checkerboard) were performed in three patients. Magnetic Resonance Imaging (MRI) of the brain was performed in all patients.

3. Results

Five presumed unrelated patients with SPG5 were identified, representing 2.1% of 238 probands (families and isolated cases) with HSP included in the Norwegian research cohort. A summary of the clinical characteristics of the patients is presented in Table 1 and more details are reported in the supplementary material. The presenting symptoms were stiff legs, unsteadiness and gait disturbances. Age at onset was in the first decade in four patients, while one was asymptomatic until the

Table 2
Variants in *CYP7B1* identified in Norwegian patients with SPG5.

Variant in *CYP7B1* (NM_004820.4)	c.1250G > A	c.321_324delACAA	c.1456C > T	c.1322C > T	c.793C > T
Protein change	p.Arg417His	p.Lys107Asnfs	p.Arg486Cys	p.Pro441Leu	p.Gln265*
Present in patients	1	1	2–5	2	4
Type of variant	Missense	Frameshift	Missense	Missense	Nonsense
Reference	[2, 6]	This paper	[2,4,6,11]	This paper	This paper
Allele frequency, Europe [a]	0.00003112	0.00003153	0.0009783	0.00006663	0.00001766
Allele frequency Norway [b]	Absent	Absent	0.002518892	Absent	Absent
Polyphen/ SIFT	Probably damaging/ deleterious	NA	Probably damaging/ deleterious	Probably damaging/ deleterious	Possibly damaging/ deleterious
Classification by Franklin [c]	Pathogenic	Pathogenic	Likely pathogenic/ pathogenic	VUS	VUS

VUS, variant of uncertain significance.
[a] gnomAD [18].
[b] https://invivo.hpc.uio.no/vcf-miner/. Norwegian Cancer Genomics Consortiums database of normal variation in the Norwegian population, containing 1590 normal chromosomes of cancer patients.
[c] https://franklin.genoox.com. The suggested classification of c.1322C > T and c.794C > T is between VUS and likely pathogenic, the strict classification is due to lack of functional and clinical data on the variants.

Table 3
Cholesterol and hydroxycholesterol levels in Norwegian patients with SPG5.

	Patient 1	Patient 2	Patient 3	Reference range
Cholesterol (mmol/l)	3.30	4.97	3.25	4.14–6.22
Triglycerides (mmol/l)	0.87	1.19	1.42	0.4–1.65
25-OHC (nmol/ml)	876	304	462	2.5–50
27-OHC (nmol/ml)	2403	1769	2150	310–610
27-OHC/cholesterol	782.2	355.9	661.5	

OHC, hydroxycholesterol.

age of 35. Neurological examination revealed spasticity and muscle weakness in the lower extremities, consistent with a pure, slowly progressive spastic paraplegia in the majority of the patients (4/5). One patient also presented with cognitive impairment and cerebellar signs, compatible with complex HSP. The majority of patients (3/5) were walking-aid-depended. In addition, all patients had posterior column sensory impairment and reduced surface sensations, four of five patients had bladder dysfunction, and one patient had signs of optic atrophy.

In total, five different variants in *CYP7B1* were observed (NM_004820.4), of which two were previously described as disease-causing and three were novel (Table 2) [6,10,11]. The variants c.321_324delACAA and c.793C > T introduces a premature stop codon, predicted to degrade CYP7B1 mRNA and/or produce a shortened protein product. The missense variant c.1322C > T leads to substitution of a conserved amino acid, most likely inactivating the enzyme [12,13]. Four patients shared the variant c.1456C > T, and analyses of exome data showed that the maximum length of a hypothetical shared haplotype was 2.54 Mb (Supplementary Table 1B).

Levels of 25-OHC and 27-OHC in plasma were markedly elevated in all three patients [1–3] and up to four times the upper reference value for 27-OCH, despite normal levels of cholesterol in plasma (Table 3).

Electrophysiological and MRI scan data are summarized in Table 1, and elaborated in the Supplementary material. Two patients had electrophysiolocal evidence of carpal tunnel syndrome with reduced motor and sensory nerve conduction velocities, while none had signs of neuropathy in the lower limbs. BAEPs and VEPs were without relevant findings. SEPs (Median nerves) revealed an absent cortical response (N20) on the left side and delayed signal on the right side in patient 1. Patient 5 had normal SEPs, and in patient 2 the response was inconclusive due to technical artefacts. MRI scan of the brain in patients 2, 3 and 4 showed subtle white matter lesions of unspecific origin (Supplementary Fig. S1). In patient 2, retinal imaging revealed decreased vision and thinned nerve fibres compatible with optic atrophy.

4. Discussion

SPG5 is a relatively rare form of HSP, in our cohort SPG5 was the third most common recessive HSP subgroup, after SPG7 (11 families) and SPG11 (7 families). Four patients shared the same variant, c.1456C > T, one of the more commonly observed variants in SPG5 [2]. Haplotype analyses suggested that this was not the result of a recent founder, however more data would be needed to resolve whether the variant derives from a distant founder in the Norwegian population. The majority of the Norwegian patients shared the core features typical of SPG5, comprising a pure, slowly progressive HSP, mainly with symptom onset in early childhood, combined with posterior column sensory impairment and bladder dysfunction. The phenotypes coincide with observations in other patients with SPG5 [2,4].

Unspecific white matter changes, the most common MRI abnormality in patients with SPG5., were observed in three out of the five Norwegian patients, concurring with previous studies [2]. The white matter changes are likely caused by neurodegeneration, but inflammation, vascular ischemia or a combination of these, may also contribute [6].

Current literature claims that absent cortical responses, measured by SEP, predict the likelihood of non-awakening of comatose patients with a high level of certainty [14]. One of the two patients in this study with reliable findings in SEPs, had complete loss of cortical response. Loss of central response potentials has previously been described in six patients with SPG5 [15,16]. This finding suggests that SEPs may not be a reliable tool for prediction of non-awakening in comatose patients with SPG5. Further studies are needed to clarify this issue.

Plasma levels of 25-OHC and 27-OHC were elevated up to as much as 17,5 times the upper reference value for 25-OCH, and four times for 27-OCH, in the three patients available for testing in this study. The levels were markedly elevated compared to healthy controls and heterozygotes carriers [2]. This coincides with all previously reports on hydroxycholesterols levels in patients with SPG5 [2–4,10]. No other known metabolic effect has been shown to increase plasma 25-OHC or 27-OHC [4]. Thus, elevation of these hydroxycholesterols seems to have a high sensitivity and specificity for the diagnosis SPG5. To illustrate the potential benefit of this, three patients in our study had novel variants which had not been previously described in patients with SPG5. Two of the variants were classified as borderline between variants of uncertain significance (VUS) and likely pathogenic, and functional studies of pathogenicity were not feasible. In such cases, the value of plasma hydroxycholesterols as a biomarker is of particular importance as elevated levels supports the pathogenicity of the variant. Hence, plasma OHC can be useful diagnostic biomarkers, both as a first-line investigation guiding diagnostic work-up, and also aiding the evaluation of identified variants classified as VUS.

In larger studies, Schöls et al. found an association between plasma 27-OCH and both Spastic Paraplegia Rating Scale (SPRS) [17] score and disease duration [2], while Marelli et al. did not find any correlation between plasma hydroxycholesterols to age at onset, disability stage or disease duration [4]. In our small study, the youngest patient, with the shortest disease duration and least severity measured by SPRS, showed the highest concentrations of hydroxycholesterols. However, the two elder patients were treated with cholesterol-lowering drugs, which are likely to affect the results. Hence, whether 27-OHC levels are associated with disease duration and severity has yet to be established.

Our results suggest that thorough clinical examination, including SPRS scoring, is still the best way to classify disease severity, in the lack of biomarkers reflecting the severity of symptoms. Norwegian patients with SPG5 share common biochemical and clinical features with previously described patients. This study confirm that plasma hydroxycholesterols are an important biological validation test for SPG5, and a robust diagnostic biomarker, being of particular use in patients where genetic analysis were inconclusive [4]. 27-OHC could be a future drug target and a biomarker to evaluate treatment response. SPG5 is one of few neurodegenerative diseases with promising near-future treatment opportunities, thus these patients are important to recognize and diagnose. More patients should be included in larger, multinational cohort studies in order to come closer to a treatment strategy for this patient group.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jns.2020.117211.

Declaration of Competing Interest

The authors declare that they have no competing interests relevant to the current study. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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