Journal of Musculoskeletal and Neuronal Interactions JMNI

Case Report

Possible implication of undescribed SMN1-SMN2 genotype in chronic EMG-pattern of SMA with transitory acute denervation

Girolamo A. Vitello[1]*, Francesco Calì[1]*, Mirella Vinci[1], Carmela Scuderi[1], Francesca L'Episcopo[1], Antonino Musumeci[1], Sebastiano A. Musumeci[1], Antonio G. Nicotera[1,2]

[1]Oasi Research Institute – IRCCS, Troina, Italy; [2]Child Neuropsychiatry Unit, Department of Human Pathology of the Adult and Developmental Age University Hospital "G. Martino" Messina, Italy
* equal contribution

Abstract

Spinal muscular atrophy (SMA) refers to a group of genetic neuromuscular disorders affecting lower motor neurons causative of numerous phenotypes. To date, according to the age of onset, maximum muscular activity achieved, and life expectation four types of SMA are recognized, all caused by mutations in the SMN1 gene with SMN2 copy number influencing disease severity. Herein, we describe the case of a 31-year-old young male with normal psychomotor development who has experienced fatigue, cramps, and muscle fasciculations in the lower limbs for a period of 2 months. Based on electrophysiological and clinical findings we performed SMA genetic, clinical exome and RNA expression of candidate genes which led us to suggest SMN1-SMN2 genes [(2+0) and (0+0)] combination as possibly being implicated in the phenotype.

Keywords: Electromyography, Hereditary Motor Neuropathies, SMA, SMN1, SMN2

Introduction

Spinal muscular atrophy (SMA) is an inherited autosomal recessive neuromuscular disorder characterized by progressive degeneration of the anterior horn cells of the spinal cord, leading to both symmetrical weakness and atrophy of the voluntary muscles. SMA is one of the most important genetic cause of infant mortality, with an incidence of 1 per 6000–10000 live births and with a carrier frequency of 1/40 individual[1-3]. SMA is caused by deletion (~95% of SMA patients) or compound heterozygotes intragenic mutation (~5% of SMA patients) of survival motor neuron 1 (SMN1) gene[4]. According to the age of onset, maximum muscular activity achieved, and life expectation the following four types of SMA are recognized: a) type I, severe infantile acute form (Werdnig-Hoffman disease); b) type II, intermediate infantile chronic; c) type III, mild juvenile form (Wohlfart-Kugelberg-Welander); d) and type IV, adult-onset[5]. All four types are caused by mutations in the SMN1 gene[6]. The SMN gene exists as two highly homologous copies, SMN1 and SMN2. Differently to SMN1, SMN2 has one nucleotide difference at the position +6 in exon 7 (c.840C in SMN1 gene, and c.840T in SMN2 gene), modifying the splicing pattern and producing only 10% of full-length active transcripts[7]. While high SMN2 copy number influences disease severity in SMA, SMN1 copy number varies among healthy individuals [1+1 or 2+1][8,9]. SMA carriers generally have one copy of SMN1 [1+0]. However, a small subset of carriers may have two copies of SMN1 on the same chromosome referred to as "2+0" genotype. SMN1-two-copy alleles are relatively common, with variability among ethnic groups ranging from 3.6% in Caucasians up to 27.5% in African-Americans[10]. Based on exon 7 experimental quantification testing, these individuals are indistinguishable from wild-type (1+1). However, identification of two copies of SMN1 (2+0) significantly improved the accuracy of estimated

The authors have no conflict of interest.

Corresponding author: Dr. Francesco Calì, Oasi Research Institute – IRCCS, Via Conte Ruggero 73, 94018Troina, Italy
E-mail: cali@oasi.en.it

Edited by: G. Lyritis
Accepted 23 April 2020

residual risk, with implications of SMA carrier screening[11]. Lastly, *de novo* rearrangements were also found in 2% of index patients with SMA[12]. Herein, we are reporting a SMA genotype [SMN1 (2+0) and SMN2 (0+0)] in a patient with chronic EMG-pattern of SMA with transitory acute denervation.

Case report

A 31-year-old male with a positive clinical history of Cluster of Differentiation 4 (CD4) immunodeficiency, episodic joint pain, migraine and night sweats was admitted to our hospital for a suspected muscular disease. He reported to be an athlete participating in events like marathons. During marathon training, the patient experienced fatigue, cramps, and muscle fasciculations in the lower limbs (>gastrocnemius). Blood screening, including creatine phosphokinase (CPK), resulted to be within the normal range. At the presentation, neurological examination revealed mild hypotrophy of periscapular muscles (symmetric), right quadriceps femoris and left tibialis anterior. Pes cavus was absent. The strength was conserved. The muscle tone was normal. Brisk and irradiating tendon biceps and brachio-radial reflexes bilaterally, hyporeflexia of triceps, pronator teres and Achilles reflexes bilaterally, and brisk in patellar reflex bilaterally were also noted. No signs of upper motor neuron were present. No pain or alteration of sensitivity were reported, nor any history of facial nerve injury. Electromyography (EMG) revealed the presence of chronic neurogenic denervation with increased duration and amplitude 6/7 millivolt (mV) (collateral re-innervation) of motor unit potentials (MUP), some polyphasic potentials, reduced motor-unit recruitment during maximal voluntary contraction (reduced number of motor units) as reduced by axonal loss in the four limbs (proximal e distal muscles) and craniofacial district. In addition, EMG showed fasciculation potentials and acute denervation (slightly fibrillations and positive sharp waves) in the gastrocnemius muscles, just slightly fibrillations and positive sharp waves in extensor digitorum communis. No apparent gradient of greater gravity proximal/distal was revealed. Upper limbs appeared less severely affected than lower limbs. Brain and spinal cord Magnetic Resonance Imaging (MRI) resulted normal. The clinical symptoms resolved in about 2 months after the interruption of the competitive physical activity (see Table).

The multiplex ligation-dependent probe amplification (MLPA) was performed using the SALSA P021-A2, P060-A1 kits and P460-A1 from MRC-Holland. Analysis of MLPA data was performed using Software Coffalyser NET by MRC-Holland (Amsterdam, The Netherlands; www.mrc-holland.com). P021-A2 contained a probe mixture for SMN1 exons 7 and 8, SMN2 exons 7 and 8 and several control fragments located on different chromosomes. P460-A1 probemix included probes for the rare allele of two polymorphisms of this SMN1 haplotype: g.27134T>G and g.27706-27707delAT. These probes do not give a signal on the great majority of DNA samples tested. A signal is usually present in samples that contain three or four SMN1 copies. Whether two probes give a signal on a sample that has only two copies of SMN1, there is an increased risk, in accordance to the ethnic background, that the person tested is a silent SMA carrier who carries two SMN1 copies on one allele and none on the other. The MLPA analysis performed on the patient with the P460-A1 kit (confirmed with MLPA P021-A2) showed two copies of exon 7-8 of the SMN1 gene with the absence of both copies of SMN2 gene. The analysis of marker loci close to the gene (g.27134T>G and g.27706-27707delAT) suggested the possibility of being a "silent carrier" (2+0). The two copies of SMN1 in *cis* were inherited from the mother. Therefore, we considered the patient and his mother as healthy carriers of SMA with only one functional copy (SMN1) in one of the two alleles. However, the patient produced only one full-length active transcripts, without the contribution, even if minimum (~20%), of the SMN2 full-length active transcripts (high copy number of SMN2 modulated, as it is known, clinical severity

Table. Clinical presentation of our patient.

Clinical signs	
Onset	31 years
Family history	negative
Cognitive impairment	-
Proximal muscular atrophy	+ (periscapular bilaterally, right quadriceps femoris)
Distal muscular atrophy	+ (left tibialis anterior, right asymmetric)
Cramps	++
Pes cavus	-
Fasciculations	+++
Sensivity disturbances	-
Facial nerve injury	-
Upper motor neuron signs	-
Tendon reflexes	Asimmetrical (see clinical description)
Weakness	+ (mild, proximal, periscapular and simmetric)
Instrumental, laboratory and Electrophysiological findings	
Spinal cord MRI	Normal
Brain MRI	Normal
CPK	Normal
EMG chronic neurogenic signs	+++
NCV	Normal
Axonal neuronopathy	+
Fasciculation potentials	+++
Fibrillation/sharp waves	+
MUP	Large (6/7 mV)
Polyphasic potentials	Few (< 15%)
Legend: +++ severe; ++ mild; + present; - absent.	

among SMA patients with homozygous deletion of SMN1). Moreover, the healthy mother showed two SMN2 copies [SMN1 (2+0) and SMN2 (two copies)] acting as a carrier. The healthy father reported two SMN1-SMN2 copies. We supposed that a *de novo* rearrangement was occurring in paternal meiosis, as described in other patients (2%)[12]. In addition, research of deletion/duplication in 17p11.2 region performed with MLPA (P033-B4), resulted negative, ruling out the possibility of Charcot Marie Tooth disease type 1 (CMT1). The Clinical Exome Solution by SOPHiA GENETICS was performed following the protocol in the Thermo Fisher Scientific NGS machine, covering 4493 genes related to the most common inherited diseases, including amyotrophic lateral sclerosis and neuropathies. NGS detected heterozygous missense mutations of LRSAM1 (c.1298C>T, p.Ser433Leu) and RTN2 (c.764G>T, p.Cys255Phe) genes inherited from the mother. Finally, the Ion AmpliSeq Human Gene Expression Kit panel was used to analyze gene-level expression. Barcoded cDNA library was generated with SuperScript VILO™ cDNA Synthesis kit from 10 ng of total RNA (from peripheral blood). Then cDNA was amplified using Ion AmpliSeq™ technology of following targeted possible candidate genes implicates in phenotype: AIFM1, ATP7A, BSCL2, CCNA2, CMT1, FBXO38, GARS, HARS, HMN6, HSPB1, HSPB3, HSPB8, LAS1L, LRSAM1, PLS, RBM7, RTN2, SMN1, SMN2, TRPV4, UBE1, VRK1, WARS. Amplified cDNA of patient (SMN1=2+0; SMN2=0 copies) was compared to a healthy control (SMN1=1+1; SMN2=2 copies). Four independent experiments for patient and control were pooled equally (diluted to 100pM) and amplified using emulsion PCR on Ion Torrent CHEF instrument and enriched following manufactures instructions. Templated libraries were then sequenced on ION S5plus (Thermo Fischer) sequencing system, using 520 chip, obtaining >800K sequences for each.

Differential expression analysis was performed based on the difference in gene counts between the patient and control using Transcriptome Analysis Console (TAC). A gene was considered differentially expressed if it had a p-value less than 0.05 and an absolute log2 fold change greater than or equal to two. Only SMN2, as expected (patient=0 copies and control=2 copies), was found differentially expressed. Finally, the SMN1 fold change between the patient (SMN1=2+0) and control (SMN1=1+1), showed fold change -1.75 (p-value=0.0197) suggesting a decrease in expression probably compatible with the genotype (two copies in *cis*). This data should be verified in other individuals with SMN1 (1+0 or 2+0) and SMN2=0 copies; however this genotype is rare in our population (more than two hundred subjects analyzed).

Discussion

SMA refers to a group of genetic neuromuscular disorders affecting lower motor neurons. The motor neuron degeneration is causative of numerous phenotypes. The age of onset of these disorders is variable, ranging from the neonatal period to adulthood, and leading to significant mortality and morbidity[6]. Herein, we describe the case of a 31-year-old young male with normal psychomotor development and no-familiar history of neurological or muscular diseases. Based on electrophysiological and clinical findings we performed SMA genetic test by MLPA method. We hypothesized that this condition might be caused by the identified SMA genotype: SMN1 (2+0) and SMN2 (0+0). To reinforce our hypothesis, we further investigated the possible involvement of other genes through the evaluation of clinical exome (SOPHiA) and 22 AmpliSeq targeted RNA candidate genes expression analysis in peripheral blood. Moreover, no different expression (p>0.05, at least two-fold change in expression) was found when compared 22 candidate target genes between patient and control. However, the clinical picture of our patient was not correlated with the detected variants in LRSAM1 and RTN2 genes (4493 genes analyzed), inherited from the healthy mother. Indeed, doubts are on the pathogenicity of the detected LRSAM1 mutation, usually correlating with a sensorimotor neuropathy[13]. Furthermore, RTN2 mutation, a gene correlated to SPG12, has been already detected in healthy patients (ExAc: 0.001669%; gnomAD: 0.00123613%). The patient's mother appeared clinically asymptomatic and her EMG resulted normal. Therefore, we considered these missense mutations not relevant in our patient. Our patient reported a clinical picture of late-onset and, probably, the iterate excessive muscular effort (the patient was an athlete) favored the onset of disease (environmental factors?). Moreover, the interruption of physical activity for two months revealed the disappearance of fasciculations and fatigue. In addition, needle EMG detected fasciculation potentials on the gastrocnemius muscles, increased duration and amplitude of MUPs in the four limbs and craniofacial district and reduced motor-unit recruitment during maximal voluntary contraction as axonal loss suspecting a SMA diagnosis. Generally, evidence suggests that physical exercise training can improve muscle and cardiorespiratory function in SMA severe forms by optimizing aerobic capacity and counteracting the muscle deterioration that occurs secondary to motor neuron loss and inactivity in SMA. However, in their recent study, by adopting Cochrane methodological procedures, authors revealed that physical exercise training for SMA 3, one of the mildest form of SMA, had no statistically significant effects on fatigue and quality of life, as the quality of evidence is very low[14]. Moreover, Noto et al.[15] showed that prominent fatigue can contribute to the motor neuron damage in patients with SMA and spinal and bulbar muscular atrophy. Similarly, our patient experienced a mild form of SMA with transitory acute denervation probably unmasked by iterate physical exercise that subjecting spinal motor neurons to intolerable stress.

This study provides evidence for clinical and mutational heterogeneity of SMN1-SMN2 genes, adding a possible implication of these genes to transitory acute EMG denervation on EMG-pattern of SMA. However, further investigations needed to clarify and confirm this novel phenotype-genotypic.

Acknowledgements

Special acknowledgements for this paper are due to Mrs. Eleonora Di Fatta for her valuable assistance in the translation, preparation and formatting of the text. We would like to thank Valeria Chiavetta, Alda Ragalmuto, Angelo Gloria, and Rosanna Galati for their technical contribution.

Financial support

This work was partially supported by the Italian Ministry of Health – Ricerca Corrente 2017 – and '5 per mille' funding.

References

1. Mostacciuolo ML, Danieli GA, Trevisan C, Muller E, Angelini C. Epidemiology of spinal muscular atrophies in a sample of the Italian population. Neuroepidemiology1992;11:34-38.
2. Burd L, Short SK, Martsolf JT, Nelson RA. Prevalence of type I spinal muscular atrophy in North Dakota. Am J Med Genet 1991;41:212-215.
3. Calì F, Ruggeri G, Chiavetta V, Scuderi C, Bianca S, Barone C, Ragalmuto A, Schinocca P, Vitello GA, Romano V and Musumeci S. Carrier screening for spinal muscular atrophy in Italian population. J Genet 2014;93:179-181.
4. Yamamoto T, Sato H, Lai PS, Nurputra DK, Harahap NI, Morikawa S, Nishimura N, Kurashige T, Ohshita T, Nakajima H, Yamada H, Nishida Y, Toda S, Takanashi J, Takeuchi A, Tohyama Y, Kubo Y, Saito K, Takeshima Y, Matsuo M, Nishio H. Intragenic mutations in SMN1 may contribute more significantly to clinical severity than SMN2 copy numbers in some spinal muscular atrophy (SMA) patients. Brain Dev 2014;36:914-20.
5. Kolb SJ, Kissel JT. Spinal muscular atrophy: a timely review. Arch Neurol 2011;68:979-84.
6. Lunn MR, Wang CH. Spinal muscular atrophy. Lancet 2008;371:2120-2133.
7. Monani UR, Lorson CL, Parsons DW et al. A single nucleotide difference that alters splicing patterns distinguishes the SMA gene SMN1 from the copy gene SMN2. Hum Mol Genet 1999;8:1177-1183.
8. Lefebvre S, Burlet P, Liu Q, Bertrandy S, Clermont O, Munnich A, Dreyfuss G, Melki J. Correlation between severity and SMN protein level in spinal muscular atrophy. Nat Genet 1997;16:265-269.
9. Saito M, Chen Y, Mizuguchi M, Igarashi T. Quantitative analysis of SMN2 based on real-time PCR: Correlation of clinical severity and SMN2 gene dosage. No To Hattatsu 2005;37:407-412.
10. Sugarman EA, Nagan N, Zhu H, Akmaev VR, Zhou Z, Rohlfs EM, Flynn K, Hendrickson BC, Scholl T, Sirko-Osadsa DA, Allitto BA. Pan-ethnic carrier screening and prenatal diagnosis for spinal muscular atrophy: clinical laboratory analysis of >72,400 specimens. Eur J Hum Genet 2012;20:27-32.
11. Luo M, Liu L, Peter I, Zhu J, Scott SA, Zhao G, Eversley C, Kornreich R, Desnick RJ, Edelmann L. An Ashkenazi Jewish SMN1 haplotype specific to duplication alleles improves pan-ethnic carrier screening for spinal muscular atrophy. Genet Med 2014;16:149-56.
12. Wirth B, Schmidt T, Hahnen E, Rudnik-Schöneborn S, Krawczak M, Müller-Myhsok B, Schönling J, Zerres K. De novo rearrangements found in 2% of index patients with spinal muscular atrophy: mutational mechanisms, parental origin, mutation rate, and implications for genetic counseling. Am J Hum Genet 1997;61:1102-11.
13. Laššuthová P, ŠafkaBrožková D, Krůtová M, Neupauerová J, Haberlová J, Mazanec R, Dřímal P, Seeman P. Improving diagnosis of inherited peripheral neuropathies through gene panel analysis. Orphanet J Rare Dis 2016;11:118.
14. Bartels B, Montes J, van der Pol WL, de Groot JF. Physical exercise training for type 3 spinal muscular atrophy. Cochrane Database Syst Rev 2019;3:CD012120
15. Noto Y, Misawa S, Mori M, Kawaguchi N, Kanai K, Shibuya K, Isose S, Nasu S, Sekiguchi Y, Beppu M, Ohmori S, Nakagawa M, Kuwabara S. Prominent fatigue in spinal muscular atrophy and spinal and bulbar muscular atrophy: evidence of activity-dependent conduction block. Clin Neurophysiol 2013;124:1893-8.