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Impact of Genomic Characterization in Patients with Non-5q Spinal Muscular Atrophy

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Abstract: Spinal Muscular Atrophy (SMA) is defined as a set of hereditary neurodegenerative disorders that cause phenotypic and genotypic variability, impacting the quality of life, psychosocial, emotional, and functional development of those affected. In Colombia, it is considered a rare disease due to its low prevalence, chronicity, and high complexity. The objective of this case report is to describe, characterize, and correlate phenotypically and genotypically a patient with clinical suspicion of neurodegenerative disease. The patient is a 32-year-old female with a clinical picture of equinus, varus, supination of the hindfoot, adduction of the right forefoot, and limitation in wrists with subsequent weakness and predominantly lower limb muscle atrophy, generalized areflexia, and positive Gowers sign. Given the suspicion of progressive degenerative neuromuscular disease, endocrine, neuromuscular, cardiovascular studies, sural nerve biopsy, and genetic testing are requested. The results show that sural nerve biopsy revealed axonal loss with little demyelination, and a genomic study using trio clinical exome sequencing performed with Illumina technology identified pathogenic variants in the Nod2 gene with heterozygous status and DYNC2H1 gene with homozygous status. Finally, a gene interaction network is created using the GeneMania program, determining gene associations. The conclusion of this study is that the diagnosis of SMA is a challenge due to its wide phenotypic-genotypic variability. Although most patients are due to variants in the SmN1 gene, there are other non-5q genes associated with this pathology. A specific diagnosis impacts treatment, prognosis, and attributed morbidity and mortality, establishing heritability risk and genetic counseling for the sake of preventive, predictive, personalized, and participatory medicine.

Key words: spinal muscular atrophy; rare disease; neuromuscular; genetic characterization; preventive medicine

1. Introduction

Spinal muscular atrophy (SMA) is defined as a set of inherited neurodegenerative disorders, which affect the cells of the anterior horn of the spinal cord and the motor nuclei in the lower part of the brain stem. It is characterized by progressive symmetrical muscle weakness and atrophy, the severity of which depends on the age of onset and long-term debilitating complications that threaten the lives of patients [1-4].

In Resolution 023 of 2023 (Ministry of Health and Social Protection, 2023) "by which the list of orphan and rare diseases in Colombia is updated", SMA is listed as an orphan disease, in relation to its low prevalence, chronicity and high complexity. It is classified into SMA associated with a genetic variant on the long arm of chromosome 5 (SMA 5q) and non-5q SMA [2].

SMA 5q accounts for 80 to 90% of inherited motor neuron disorders, with an incidence of 1 in 6,000-10,000 live births. It is caused by the deletion of the survival motor neuron gene (SMN1), which is located on chromosome 5q11.2-13.3. This gene is considered the determinant of the disease because its absence or pathogenic genetic variants support the genotype-phenotype relationship. The survival of motor neuron gene 2 (SMN2), homologous to SMN1, is a phenotypic modifier [5-6].

Non-5q SMA, on the other hand, is difficult to determine in terms of its epidemiological frequency due to the great variability of the genes involved. In Colombia, according to the National Institute of Health, in the epidemiological period 2016-2020, 2,159 cases of nervous system diseases were reported, among which three cases of non-5q SMA were identified, that is, 0.04% of the 8,555 registered as orphan and rare [7]. During 2021, the nervous system diseases reported by the Public Health Surveillance System (Sivigila) were 1,187 cases, of which 4,949 were orphan diseases, without specifying which ones correspond to each case [8]. In the epidemiological bulletin of 2022, 1,368 cases of nervous system diseases were reported to Sivigila, which included nine cases of other unspecified SMA, representing 0.14% of the 6,657 cases of orphan diseases [30].

The genes associated with non-5q SMA have expanded rapidly due to the advent of next-generation sequencing technologies. They are usually genetically classified according to the pattern of inheritance as autosomal dominant, autosomal recessive or X-linked and the distribution of muscle weakness as proximal, distal or bulbar. [9-11] (Tables 1 and 2).

Table 1. Genotypic and phenotypic characteristics associated with the presentation of early-onset non-5q SMA

	Gen	OMIM	Name	Chromosomal location	Function	Clinical presentation and onset of symptoms	Inheritance	Condition
Early start								
SMAX2	UBA1	314370	Ubiquitin-like modifier that activates enzyme 1	Xp11.3	Catalyze the first step in ubiquitin conjugation	Antenatal: Hypotonia, areflexia, thoracic deformities, facial dysmorphic features, joint contractures, bone fractures, genital abnormalities	X-linked recessive	Lethal infantile spinal muscular atrophy with arthrogryposis, congenital fractures
BVVLS1	LC52A3	613350	Solute carrier family 52 member 3	20p1.3	Encode riboflavin transporter protein	Early childhood-adulthood. Weakness of arms, hands, and face; ataxia; dysphagia; tongue atrophy, pontobulbar palsy, sensorineural deafness	Autosomal recessive	Brown Vialetto-Van Laere syndrome 1
BVVLS2	SCL52A2	613350	Solute carrier family 52 member 2	8q24.3	Encode riboflavin transporter protein	Early childhood-adulthood. Weakness of arms, hands, and face; ataxia; dysphagia; tongue atrophy, pontobulbar palsy, sensorineural deafness	Autosomal recessive	Vialetto-Van Laere Brown Syndrome 2
PCH1A	VRK1	602168	Vaccinia-related serine/threonine kinase 1	14q32.2	Encoding serine/threonine protein kinases with vaccinia	Early childhood. Microcephaly, severe hypotonia, areflexia, central visual impairment, dysphagia, respiratory failure	Autosomal recessive	Pontocerebellar hypoplasia with infantile muscular atrophy
PCH1B	EXOSC3	606489	Exosome component 3	9p13.2	Encode non-catalytic component 3 of the human exosome	Early childhood. Microcephaly, mental retardation, early death	Autosomal recessive	Pontocerebellar hypoplasia with infantile muscular atrophy

	Gen	OMIM	Name	Chromosomal location	Function	Clinical presentation and onset of symptoms	Inheritance	Condition
PCH1C	EXOSC8	607596	Exosome component 8	13q13.3	Encode non-catalytic component 8 of the human exosome	Early childhood. Microcephaly, mental retardation, early death	Autosomal recessive	Pontocerebellar hypoplasia with infantile muscular atrophy
SMAPME	ASAH1	613468	N-acylsphingosine amidohydrolase 1	8p22	Encode acid ceramidase protein family	Early childhood. Proximal muscle weakness, hypotonia, areflexia, muscle atrophy, fasciculations, sensorineural hearing loss, respiratory muscle weakness, microcephaly	Autosomal recessive	Spinal muscular atrophy with myoclonic epilepsy
LAAHD	GLE1	603371	RNA export mediator GLE1	9q34.11	Encodes a Gle1p evasion homolog polypeptide	Antenatal. Fetal immobility, hydrops, micrognathia, pulmonary hypoplasia, joint contractures	Autosomal recessive	Lethal arthrogryposis with anterior horn cell disease
SMARD1	IGHMBP2	604320	Immunoglobulin helicase μ-binding protein 2	11q13	Encodes a member of the helicase superfamily	Precocious infancy. Distal and lower extremity muscle weakness, early diaphragmatic weakness	Autosomal recessive	Spinal muscular atrophy with diaphragmatic paralysis
SMARD2	LAS1L	309585	Lanosterol synthase 1	Xq12	Activates RNA-binding activity	Precocious infancy. Distal and lower extremity muscle weakness, early diaphragmatic weakness	X-linked recessive	Spinal muscular atrophy with diaphragmatic paralysis
DSMA	TRPV4	600175	Transient receptor potential cation receptor subfamily V member 4	12q24.11	Encodes a Ca2-permeable nonselective cation channel family	Congenital. Muscle weakness, distal and proximal leg contractures, sensorineural hearing loss	Autosomal dominant	Congenital spinal muscular atrophy of the lower extremities
SMALED1	DYNC1H1	600112	Cytoplasmic dynein heavy chain 1	14q32.31	Encodes a cytoplasmic dynein heavy chain family	Congenital to adulthood. Proximal muscle deformity in legs, sparing adductors and semitendinosus, nonprogressive	Autosomal dominant	Spinal muscular atrophy in the lower extremities-1
SMALED2	BICD2	609797	BICD cargo adapter 2	9q22.31	Negative-end motility by dynein on microtubules	Congenital to adulthood. Slow progression, proximal muscle weakness in legs with some contractures	Autosomal dominant	Spinal muscular atrophy predominantly in the lower extremities-2

Source: Peeters K. Clinical and genetic diversity of SMN1-negative proximal spinal muscular atrophies. Brain. Atrófias musculares espinales no asociados a SMN1. Revista Médica Clínica Las Condes.

Table 2. Genotypic and phenotypic characteristics associated with the presentation of late-onset non-5q SMA

Type	Gen	0MIM	Name	Chromosomal location	Function	Clinic	Inheritance	Condition
Late start								
HMSNP	TFG	602498	Trafficking from the endoplasmic reticulum to the Golgi regulator	3q12.2	Encode fusion oncoprotein	Young adults. Painful muscle paresthesias, myotonia in the hands, dysphagia, muscle weakness, proximal or distal muscle atrophy, fasciculations, and slowly progressive distal sensory impairment.	Autosomal dominant	Proximal hereditary sensory-motor neuropathy, Okinawa type
LGMD1B	LMNA	150330	Lamin A/C	1q22	Encode nuclear lamina protein	Young adults. Progressive proximal muscle weakness and cardiomyopathy	Autosomal dominant	Adult-onset proximal spinal muscular atrophy followed by cardiac involvement
SMAX1	AR	Sin OMIM	Androgen receptor	Xq12	Encode androgen receptor	Adulthood. Proximal, bulbar weakness, endocrine disorder	X-linked recessive	Kennedy disease, X-linked bulbospinal atrophy
SMAJ	CHCHD10	615903	10-coiled coil-helix-coiled coil-helix domain	22q11.23	Encode mitochondrial proteins	Adulthood. Painful paresthesias, fasciculations, muscle weakness, and areflexia	Autosomal dominant	Jokela-type spinal muscular atrophy
ALS4	SETX	608465	Senataxin	9q34.13	Encodes Sen1p protein	Juvenile or adult onset: Proximal and distal weakness, hand tremor, brisk reflexes	Autosomal dominant	Juvenile- or adult-onset spinal muscular atrophy with pyramidal features
SPSM	TRPV4	605427	Transient receptor potential cation channel subfamily V member 4	12q24.11	Encodes a member of the transient receptor potential channel subfamily	Early adulthood: Progressive weakness of facial and pectoral muscles with laryngeal paralysis, sensorineural deafness, skeletal abnormalities sparing the medial gastrocnemius and biceps femoris	Autosomal dominant	Scapuloperoneal spinal muscular atrophy
SMAFK	VAPB	605704	Synaptobrevin-like protein (VAMP)-associated proteins B and C	20q13.32	Encodes type IV membrane protein	Adulthood: Paresthesias and muscle fasciculations	Autosomal dominant	Late-onset Finkel-type spinal muscular atrophy
SMAFK	HEXB	606873	Hexosaminidase beta subunit	5q13.3	Encodes hexosaminidase beta subunit	Late adult onset: Proximal muscle weakness of the lower extremities	Autosomal recessive	Late-onset pure adult-onset spinal muscular atrophy

Source: Peeters K, 2014.

Pathophysiological mechanisms secondary to motor neuron damage include: abnormalities in DNA (deoxyribonucleic acid) repair (UBA1), alteration in RNA (ribonucleic acid) processing and degradation (EXOSC3, EXOSC8), vitamin uptake (SLC52A3 and SL-C52A2), cell cycle regulation (VRK1, TFG), lipid metabolism (ASAH1), nuclear transport (GLE1), regulation of autophagy control, cytoskeletal dynamics and RNA metabolism (LAS1L, IGHMBP2, AR, SETX), molecular transport by cation channeling (TRPV4), nuclear transport (LMNA), nuclear transcription (AR), axonal transport (BICD2 and DYNC1H1), immune and inflammatory regulation (TFG), ganglioside accumulation in the lysosome (HEXB), structural and functional abnormalities of mitochondria (CHCHD10) [9, 15].

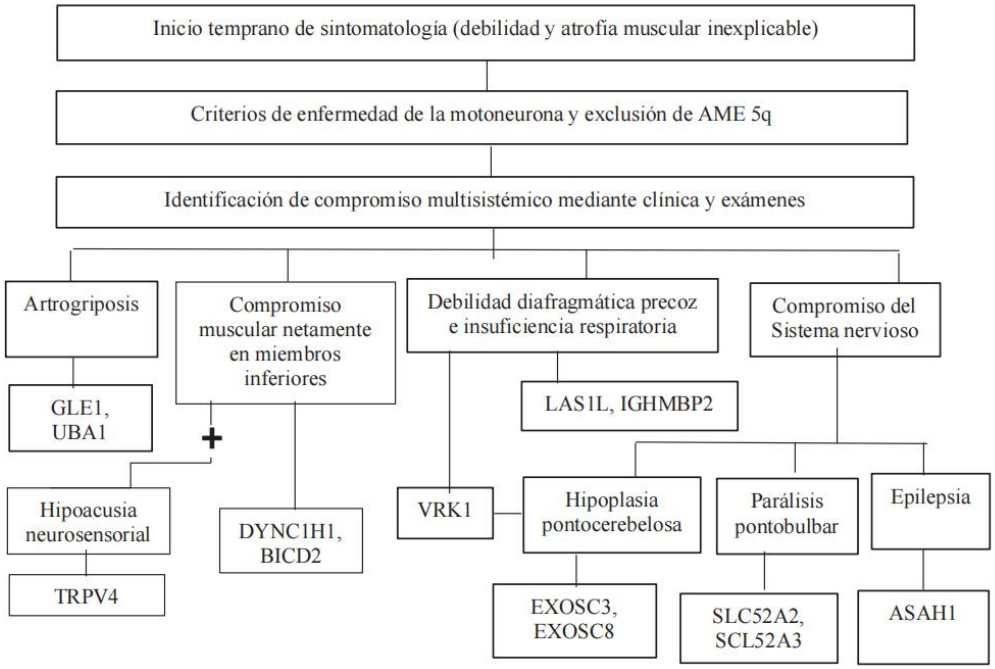

Figure 1. Algorithm for diagnostic suspicion in early-onset non-5q SMA.
Source: Peeters K. Clinical and genetic diversity of SMN1-negative proximal spinal muscular atrophies. Brain. Castiglionia C. Spinal muscular atrophies not associated with SMN1. Revista Médica Clínica Las Condes.

The clinical spectrum of SMA is broad and heterogeneous, depending on each genetic variant and the areas involved. The main clinical manifestation of lower motor neuron involvement is muscle weakness (proximal or distal, symmetric or asymmetric, in the upper or lower limb, and with or without involvement of the respiratory muscles). If bulbar motor neurons are involved, swallowing and voice disorders will be present. Finally, hyporeflexia or areflexia, muscle or tongue fasciculations, and fine hand tremors indicate second motor neuron involvement.

The clinical presentation of some types of non-5q SMA may be similar to 5q SMA, which may include fetal hypomotility, muscle weakness, hypotonia, areflexia, facial diplegia, arthrogryposis, or respiratory failure. However, the complementary testing, treatment, and genetic counseling are clearly different [2, 4, 14, 16].

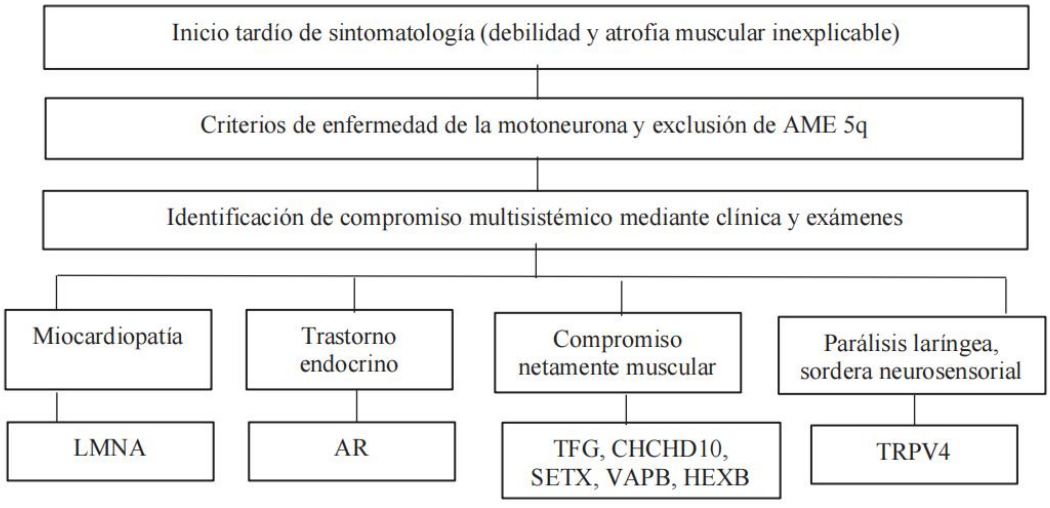

Figure 2. Algorithm for suspected diagnosis in late-onset non-5q SMA.
Source: Peeters K. Clinical and genetic diversity of SMN1-negative proximal spinal muscular atrophies. Brain. Castiglionia C. Spinal muscular atrophies not associated with SMN1. Revista Médica Clínica Las Condes.

SMA is considered a medical challenge. The diagnostic suspicion begins with a patient with symptoms consisting of: muscle weakness or hypotonia, not attributed to secondary causes; clinical signs that suggest lower motor neuron involvement (muscle atrophy, fasciculations, hypotonia, equinus foot, deforming contractures, winged scapula, hypoarreflexia or areflexia). The age of onset of clinical manifestations should also be taken into account: early (congenital, early infancy, early childhood) or late (young adult, older adult); the progression of symptoms: rapid or slow; the involvement of other systems or organs and knowledge of the different entities described in tables 1 and 2, all of which will allow correct decision making [2, 3].

Diagnosis is confirmed by molecular genetic testing that analyzes specific genetic variants such as homozygous deletion of exons 7 of SMN1, which is the most common genetic variant in SMA. However, due to the wide genetic variability of non-5q SMA, the absence of pathogenic variants in SMN1 does not rule out the presence of SMA. Electromyography and muscle biopsy were previously standard parts of the diagnostic evaluation of SMA; however, molecular genetic testing is now widely available [2, 3].

Today there is no established treatment for each of the non-5q SMA. According to the Clinical Trials, 215 studies related to SMA have been documented from 2000 to the present year, among which are two studies associated with non-5q SMA: a clinical trial with intrathecal administration of genes in phase I/IIa for diseases related to the IGHMBP2 gene with an estimated completion date of the study in November 2028; the other trial for the evaluation of the safety and efficacy of leuprorelin acetate with 11.25 mg in patients with spinal and bulbar muscular atrophy with an estimated completion date of August 31, 2025.

In the literature, Lee, Termglinchan, Diecke et al. [20] describe a study in which a Lamin A/C (LMNA) dilated cardiomyopathy model was created in vitro using patient-specific induced pluripotent stem cell-derived cardiomyocytes (iPSC-CM). They demonstrated that pharmacological and molecular inhibition of the platelet-derived growth factor (PDGF) signaling pathway improved the arrhythmia phenotypes of mutant iPSC-CM in vitro, suggesting the PDGF receptor beta as a potential therapeutic target. However, adequate doses or alternatives to these inhibitors have not been established through clinical trials. Therefore, therapeutic management of patients with non-5q SMA is limited to supportive orthopedic, nutritional, ventilatory, and rehabilitation therapies, depending on the patient's clinical presentation. [1, 17-20]

Follow-up of patients diagnosed with SMA in the presymptomatic stage requires monitoring for the development of symptoms to determine the appropriate initiation of targeted or supportive therapies. A multidisciplinary evaluation should be performed every six months to assess respiratory, motor, orthopedic, and nutritional status; this support is essential to reduce the severity of symptoms [23].

As this is a progressive neuromuscular disease, the functional prognosis, respiratory capacity, and life expectancy will depend on the duration of the disease, timely multidisciplinary management, and the development or absence of complications, including swallowing problems with subsequent nutritional impairment; gastrointestinal dysfunction presenting as constipation, gastroesophageal reflux and delayed gastric emptying; and respiratory problems consisting of airway obstruction and aspiration infections. The prognosis for a patient with complications and without multidisciplinary interventions rarely has a life expectancy greater than 2 or 3 years [4, 24].

2. Materials and Methods

A 32-year-old female patient presented with clinical symptoms of equinus, varismus, hindfoot supination, right forefoot adduction and bilateral wrist extension limitation at one year and three months of age, with subsequent onset of weakness and muscular atrophy predominantly in the lower limbs. She denied the presence of proprioceptive, auditory, visual and skin disorders. Family history included non-consanguineous parents and no relatives with degenerative

neuromuscular diseases.

Physical examination revealed symmetrical extremities, symmetrical pulses, no distal coldness, capillary refill less than two seconds, fasciculations and pain on palpation in the rhomboid muscle, bilateral hand drop posture, no hyperextension, no ligamentous hyperlaxity, with presence of splints in lower limbs, muscle atrophy, generalized areflexia, walks with support, strength according to Daniels scale with absence of contraction in flexoextensors of hands and feet, positive Gowers sign. Cannot sit up, neurologically alert, oriented in time, place and person.

With paraclinical tests showing: creatine kinase 239 IU/L, calcium 9.4 mg/dl, prolactin 19.14 ng/ml, thyroid stimulating hormone (TSH) 5.04 mIU/L, parathyroid hormone (PTH) 51.91 pg/mL, vitamin D 25 ng/ml, 17-OH-progesterone 19.7 IU/l, somatomedin C 181 U/ml, electrocardiogram, transthoracic echocardiogram and holter without cardiovascular alterations, orthoradiography of the spine with rotascoliosis of the right vertex at the level of the lumbar spine with a Cobb angle of 44 degrees, sural nerve biopsy with loss of axons with little demyelination, Schwann cell hypertrophy, without performing electromyography.

According to the degenerative neuromuscular clinical manifestations and the results of the sural nerve biopsy, the first diagnostic impression was Charcot-Marie-Tooth disease, which is why a molecular study of deletions/duplications in the PMP22 gene was performed, which resulted in the absence of alterations.

Given the suspicion of a progressive neurodegenerative polyneuropathy to be classified, and taking into account the wide phenotypic and genotypic variability associated with this medical condition, a clinical trio exome sequencing study was performed using Illumina technology from a peripheral venous blood sample. DNA was extracted using Qiagen's DNeasy package; to determine its concentration and purity, the samples were evaluated using a spectrophotometer (NanoDrop), which yielded approximate values of 500 ng/uL and a mean optical density (OD) A260/A280 of 1.80. Subsequently, massive sequencing of Nextera TM libraries was performed using the Illumina platform with 100X coverage. Alignment with the GRCh38 reference genome. All selected regions presented a depth greater than or equal to 32.2 x and a minimum mapping confidence threshold of Q30 with a total reading of 27,320,632 Nexteratm Illumina libraries. The results were compiled into a Variant Call Format (VCF) output file, where the variants found were recorded. The Exac, 1000 genomes, OMIM and gnomAD population databases were consulted to determine the existence of the reported variants; subsequently, the bioinformatics analysis and prediction of the functional effect of the variants found were carried out.

For the analysis of the reported variants, the bioinformatics software Mutation Taster (http://www.mutationtaster.org/), Protein Variation Effect Analyzer (PROVEAN) (http://provean.jcvi.org/index.php), the UMD predictor (http://umd-predictor.eu/), Polyphen (http://genetics.bwh.harvard.edu/pph2/), SIFT (https://sift.bii.a-star.edu.sg/), Human Splicing Finder (http://umd.be/Redirect.html), and Clinvar (https://www.ncbi.nlm.nih.gov/clinvar/) were used as in silico clinical prediction tools. The nomenclature used to name the variants was based on the recommendations of the American College of Medical Genetics and Genomics (ACMG). Finally, a gene interaction network was created using the GeneMania program to determine close associations with other genes that would allow determining physical interactions or co-expression levels.

3. Results

In the clinical trio exome sequencing, a pathogenic variant was found in the DYNC2H1 gene with nucleotide change: c.8365T>C, protein: p. Phe2789Leu, zygosity: homozygous, father and mother heterozygous; gene encoding cytoplasmic dyneins of the heavy chain 1, subunit of the primary motor protein responsible for retrograde axonal transport in neurons that when affected is associated with presentation of muscular involvement predominantly in the proximal lower limb [25].

On the other hand, the second variant with pathogenic clinical significance was found in the clinical trio exome sequencing in the NOD2 gene; This variant was not identified in the DNA extracted from either parent, with nucleotide change: c.1001G>A, protein: p.Arg334Gln, zygosity: heterozygous, gene that plays an important role in the function of the immune system; however, the genetic variant has not been associated with any type of clinical manifestation according to the ClinVar database and the European Bioinformatics Institute NCBI [27].

Additionally, to determine interactions between the affected genes and establish a phenotype-genotype relationship, the gene interaction network was built (Figure 3), considering the two genes in which pathogenic variants were found in the patient were observed: DYNC2H1 and NOD2. According to the analysis with GeneMania, they presented close interactions with the genes RIPK2, NLRP1, NOD1, IKBKG, DYNC2LI1, NLRC4, DCTN1 and CASP1, all with functions related to processes in the nucleotide-binding domain, leucine-rich repeat-containing receptor signaling pathway, MyD88-dependent Toll-like receptor signaling pathway, zymogen activation, Toll-like receptor 4 signaling pathway, regulation of interleukin-1 beta secretion, antigen processing and presentation of peptide or polysaccharide antigens by means of major histocompatibility complex (MCH) class II. The genes highlighted for their coexpression with the genes DYNC2H1 and NOD2 were RIPK2, NOD1 and TNFAIP3.

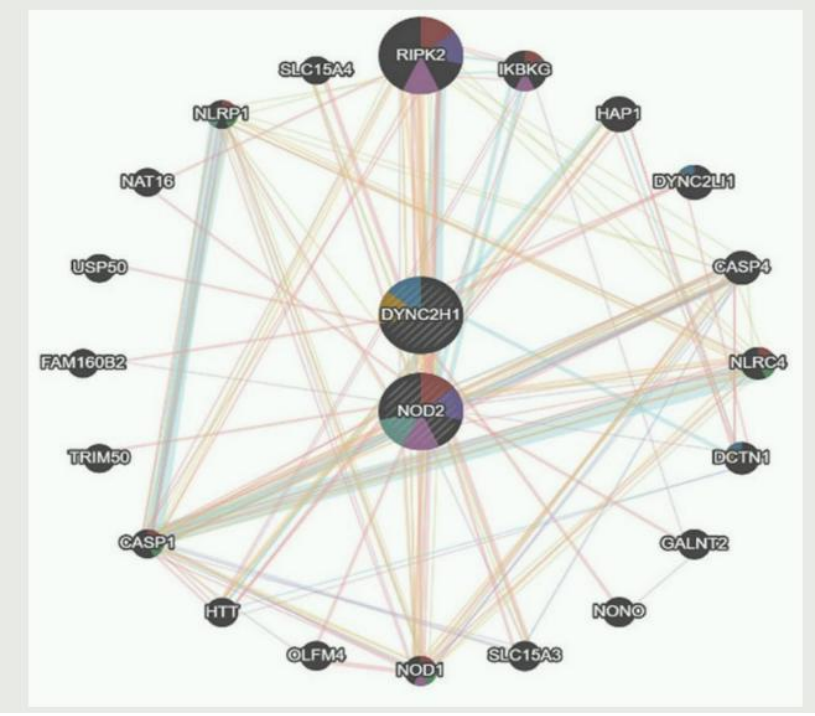

Figure 3. Interaction network between DYNC2H1, NOD2, and associated genes
Source: Prepared by the authors, GeneMania Program.

The DYNC2H1 gene showed physical interaction with six different genes, having a high interaction with one of them (DYNC2LI1). The NOD2 gene, on the other hand, was the one that reported the most physical interactions [16]. The association with the RIPK2 gene was the strongest of them.

Knowing the metabolic pathways and interactions allows us to identify the pathophysiological mechanisms involved and establish a specific diagnosis in a degenerative and progressive neuromuscular disease with phenotypic and genotypic variability such as non-5q SMA in order to offer a targeted treatment that can impact the prognosis and morbidity and mortality attributed to this pathology, establishing follow-up guidelines, education on the risk of heritability and adequate genetic counseling, bringing us closer to 4P medicine that would impact the natural history of the disease [2, 4, 28].

4. Bioethical Aspects

This case report is considered an observational descriptive study based on the review of a patient's medical history and a review of the literature. No intentional modification of the biological, physiological or psychological characteristics was made, which is why the level of this research has been categorized as minimal risk, according to Resolution 8430 of 1993. Prior to the production of the case report, the respective informed consent was obtained for the use of data in a confidential manner. The authors declare that in this article the data taken from the medical history were analyzed to protect the confidentiality and privacy of the patient.

5. Discussion

In Colombia, SMAs are considered orphan diseases that have coexisted in the population since their origin; however, they have gone unnoticed due to their low prevalence and lack of awareness among healthcare personnel, which makes diagnosis more difficult. As described by Peeters et al., [9, 32] 5q SMAs represent 80% of motor neuron disorders, but the number of causal genes associated with non-5q SMAs has expanded rapidly due to the advent of next-generation sequencing technologies. For Castiglioni and Lozano, [15] as of 2018, there were 20 associated genes. Therefore, obtaining a molecular study that reports non-pathogenic clinical significance in the SMN1 gene does not rule out the disease, and more genomic data should be included to characterize patients with suspected SMA.

Exome sequencing seeks to obtain the maximum possible genetic information from the exons present in the nearly 26,000 human genes, covering approximately 85% of the variants associated with a heterogeneous group of complex inherited genetic diseases, elucidating the diagnosis of the patient's characteristic phenotype and discovering new genetic variants associated with pathologies [28].

Currently, as part of exome analysis targeting genes of interest, so-called clinical exome sequencing is in use, using industry-developed kits such as Agilent SureSelect Focused Exome, Illumina TruSight One, Roche NimbleGen SeqCap, and EZ MedExome. These kits include approximately 5,000 genes in the Online Mendelian Inheritance in Man (ONIM) catalog and are associated with clinically relevant disease-related phenotypes. That is, they only cover 20% of the entire exome and, like gene panels, require additional analysis if the causative alteration of the symptoms is not found. Clinical use has been proposed as the first diagnostic test for neurodevelopmental disorders [28].

Clinical bioinformatics is defined as an emerging scientific discipline in biomedical sciences that uses information technology to organize, analyze, and distribute biological information, using DNA, RNA, amino acid sequences, proteins, three-dimensional molecular structures, gene interactions, metabolic pathways, among others; It emerged in the early 1990s from the databases obtained from the Human Genome Project and through in silico experimentation, which has made it possible to explain the function of various proteins, develop structural models, interaction networks between proteins, elucidate the different molecular mechanisms related to the presentation of different diseases, improve the understanding of the relationship between inheritance and the risk of suffering from a condition, reveal the genetic influence on its appearance and identify biomarkers and specific therapeutic strategies for these conditions [29].

In order to define a specific diagnosis based on the patient's heterogeneous clinical manifestations, a clinical trio exome sequencing study was performed, looking for genomic variants associated with the patient's phenotype and to correctly correlate protein function using bioinformatics techniques. In this context, in the present study, two genetic variants with pathogenic clinical significance were identified in the DYNC1H1 and NOD2 genes [28].

The DYNC1H1 gene encodes the dynein-2 protein, with subsequent development of the dynein-2 complex found in cilia, microscopic projections that protrude from the surface of cells. Dynein-2 is involved in intraflagellar transport (IFT) by which materials are transported within the cilia; Specifically, dynein-2 is a motor that uses energy from the ATP

molecule to drive the transport of materials from the tip of the cilia to the base [25].

The DYNC1H1 and DYNC2H2 gene family encodes cytoplasmic dyneins of the heavy chain 1, a subunit of the primary motor protein responsible for retrograde axonal transport in neurons. When a genetic variant with pathogenic clinical significance is present in this gene, these functions are altered, confirming the cause of non-5q SMA in patient [25].

According to studies carried out by Weedon et al. [26] who identified a nonsense mutation [p.H306R (c.917A>G)] in the DYNC1H1 gene in a four-generation family with 23 members, the most striking feature among the patients was a unique distribution of muscle involvement. The quadriceps femoris muscle was affected from the early course of the disease and the proximal lower limb was predominantly involved throughout the course of the disease.

Harms et al. [25] reported three missense variants in the tail domain of DYNC1H1 in families with dominant spinal muscular atrophy with lower limb predominance (SMA-LED, OMIM 158600), expanding the role of DYNC1H1 to motor neuron maintenance. In their work, they proposed that the non-progressive clinical course of the disease, despite early childhood onset should be another hallmark of SMA-LED; they further postulated that the gene family associated with DYNC1H1 plays an essential role in the maintenance of spinal motor neurons and their axons.

Tsurusaki et al. [10] studied two brothers: the first had a mild motor delay in infancy, an unsteady gait that persisted until age three, examinations showing proximal lower limb muscle atrophy and decreased deep tendon reflex; Gowers sign was positive. No neurological deficit was demonstrated. Motor nerve conduction velocity was within normal limits. Muscle CT demonstrated severe atrophy and lipid degeneration, predominantly in the bilateral quadriceps femoris muscle. A muscle biopsy of the quadriceps femoris muscle demonstrated severe type 2 fiber bundling atrophy.

The second patient presented delayed motor development; physical examination revealed moderate muscle weakness in the proximal lower limb, but Gowers sign was negative, muscle CT revealed severe atrophy and lipid degeneration, mainly in the bilateral quadriceps femoris. Proximal lower limb weakness and wasting is evident, but the patient did not show upper limb weakness. The researchers found missense variants in the DYNC1H1 and DYNC2H2 genes in the two patients [10].

Scoto et al. [21] describe the phenotype of patients with pathogenic clinical significance in the DYNC1H1 gene, which is usually characterized by muscle weakness with predominance in the lower limbs in proximal segments, which relatively spares the adductor and semitendinosus muscles, muscle atrophy with absence of sensory alteration, slow gait, delayed walking, and loss of distal osteotendinous reflexes.

On the other hand, the pathogenic variant in the NOD2 gene not identified in the DNA extracted from leukocytes of either parent, previously known as CARD15 according to Guzmán et al. [22], a protein that plays an important role in the function of the immune system, is active in some types of immune system cells (including monocytes, macrophages, and dendritic cells), which help protect the body against bacteria and viruses.

The NOD2 protein has several critical functions in the body's defense against foreign invaders. It is involved in recognizing certain bacteria and stimulating the immune system to respond appropriately. When activated by specific substances produced by bacteria, the NOD2 protein activates a protein complex called nuclear factor-kappa-B, which regulates the activity of multiple genes, including those that control immune and inflammatory responses. However, despite presenting genetic variants, it is not associated with any clinical manifestations [27].

6. Conclusion

Non-5q SMA is a group of inherited neurodegenerative disorders capable of causing alterations in the anterior horn cells of the spinal cord and the motor nuclei in the lower brainstem, and subsequently high phenotypic and genotypic variability, which impact the quality of life and psychosocial, emotional, and functional development of individuals.

Although most patients are due to variants in the SMN1 gene, other non-5q genes are associated with this pathology. This is why it is important to perform genomic studies, clinical bioinformatics, interaction networks, and reverse phenotyping based on the selection of a group of people with a genetic variant and the evaluation of their phenotype. These have become fundamental tools for the characterization of these complex diseases and the potential to transform reactive medicine into preventive medicine.

This allows for early diagnosis, with the possibility of initiating early and targeted treatment that impacts the prognosis and morbidity and mortality attributed to this pathology. Follow-up guidelines are established, along with appropriate genetic counseling that allows for education about the risk of heritability or disease presentation, bringing us closer to precision medicine for the sake of the 4Ps medicine that would impact the natural history of the disease [1, 15, 31].

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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