ANDES PEDIATRICA
REVISTA CHILENA DE PEDIATRÍA
www.scielo.cl

Andes pediatr. 2023;94(4):520-528
DOI: 10.32641/andespediatr.v94i4.4039

ORIGINAL ARTICLE

Characterization of strength, endurance and lung function in subjects with neuromuscular diseases with the R577X polymorphism of the ACTN3 gene

Caracterización de fuerza,resistencia y función pulmonar en sujetos con enfermedades neuromusculares con el polimorfismo R577X del gen ACTN3

Eduardo Cifuentes-Silva[a,b,e], **Daniel Bueno-Buker**[b], **Constanza Pastene-Maureira**[c,e], **Alejandro Pacheco-Valles**[d,f]

[a]Escuela de Kinesiología, Facultad de Ciencias Médicas, Universidad de Santiago de Chile (USACH). Santiago, Chile
[b]Hospital Dr. Exequiel González Cortés. San Miguel, Chile.
[c]Hospital Dr. Eduardo Schütz Schroeder. Puerto Montt, Chile.
[d]Departamento de Kinesiología Facultad de Ciencias de la Salud, Universidad Católica del Maule. Talca, Chile.
[e]Kinesiólogo.
[f]Tecnólogo Médico.

Received: September 15, 2021; Approved: March 26, 2023

What do we know about the subject matter of this study?

The ACTN3 R577X polymorphism determines the expression of alpha-actinin 3 protein in human muscles. Subjects without the ACTN3 gene have less muscle strength. Neuromuscular diseases (NMD) generate an accelerated loss of muscle strength by their natural course. The interaction of both variables may be of great interest.

What does this study contribute to what is already known?

We present the results of ACTN3 R577X genotyping in a group of patients with NMD and its characterization based on pulmonary function, strength, and muscular endurance. The subjects presented restrictive respiratory spirometric alterations and decreased muscle strength when compared with reference values. It was not possible to establish a relationship with the ACTN3 gene polymorphism. This is the first study on the ACTN3 R577X polymorphism and NMD in Chile.

Abstract

The ACTN3 R577X polymorphism determines the expression of alpha-actinin 3 protein in human muscle. The homozygous XX genotype fails to synthesize alpha-actinin 3 and is associated with lower muscle strength than the RR genotype. Neuromuscular diseases (NMD) generate an accelerated loss of muscle strength, and their relationship with the ACTN3 gene has not been established. Objective: To describe the variables of strength, respiratory muscle endurance, and lung function in patients

Keywords:
ACTN3 Protein;
Genetic Polymorphism;
Neuromuscular Disease;
Respiratory Muscles;
Muscle Strength

Correspondence:
Alejandro Pacheco-Valles
apacheco@ucm.cl

Edited by:
Luisa Schonhaut Berman

How to cite this article: Andes pediatr. 2023;94(4):520-528. DOI: 10.32641/andespediatr.v94i4.4039

with NMD who present the ACTN3 R577X polymorphism. **Patients and Method:** Descriptive observational study. Six subjects between 10 and 14 years old, with a diagnosis of NMD, treated at the *Hospital Dr. Exequiel González Cortés* in Santiago, Chile, were evaluated. They were genotyped with the ACTN3 R577X polymorphism by polymerase chain reaction (PCR). Lung function was measured by spirometry. Muscle strength was evaluated with maximal inspiratory pressure (MIP), maximal expiratory pressure (MEP), and grip strength (GS). Respiratory muscle endurance was evaluated by time limit (TLim). **Results:** The median and 25–75th percentile [Med(p25-p75)] of the lower limit percentages (%Li) for GS, MIP, and MEP were: 36.01% (16.88-53.30), 68.88% (41.07-89.59), and 38.74% (27.74-56.90), respectively. The Med(p25-p75) of TLim was 299.0 (113.3-356.3) seconds. Regarding the genotyping of the ACTN3 R577X polymorphism, in 2 subjects it was XX, in 2 RX, and in 2 RR. **Conclusions:** The subjects presented restrictive ventilatory spirometric alterations and decreased muscle strength when compared with the reference values. No relationship could be established with the ACTN3 gene polymorphism.

Introduction

Muscle strength has an important influence on functional abilities and has been positively associated with improved sports performance, longevity, and quality of life[1].

Although environmental stimuli are an important element that can determine muscle size and muscle strength, the genome plays an important role in determining phenotypes [2]Research has demonstrated that genetic factors contribute to 44 to 58% of individual variations in muscle strength and average muscle mass[3], with the alpha-actinin 3 (ACTN3) gene being one of the key genes that can significantly impact these muscle-related traits[4,5].

Alpha-actinins constitute a family of actin-binding proteins that share a resemblance to dystrophin and are responsible for associating with the cytoskeleton in various tissues throughout the body[6]. Currently, 4 types of alpha-actinins are known: alpha-actinins 1 and 4 which are found in all cells of the body, mainly in the cytoskeleton and act as membrane binders[7], and alpha-actinins 2 and 3 which are located in the sarcomere Z-disc, even interacting with membrane proteins[6]. Alpha-actinin 2 is found mainly in cardiac muscle and oxidative fibers while, in contrast, alpha-actinin 3 is restricted to a group of type II fibers (all IIA fibers and 50% of IIB fibers)[8].

The ACTN3 R577X (or rs1815739) polymorphism generates 3 possible genotypes: homozygous RR for the synthesis of alpha-actinin 3, heterozygous RX which synthesizes the functional protein in the same way, and homozygous XX which fails to synthesize alpha-actinin 3[9]. To understand the function of alpha-actinin 3, studies have been performed in ACTN3 knockout (KO) mouse models (ACTN3-KO) homologous to the lack of alpha-actinin 3 in the human R557X polymorphism, observing that the absence of this protein generates an increase in alpha-actinin 2 as a compensatory phenomenon[10]. In addition, ACTN3-KO mice do not show physical alterations upon examination, but a decrease in total weight, lean mass, and isolated muscle mass, altering the proportion and diameter of glycolytic fibers, but not the total amount of muscle fiber[11]. When subjected to endurance exercise, they tend to run more meters on a treadmill than wild type (WT). In contrast, ACTN3-KO mice have lower grip strength, and therefore, lower muscle power[10,11].

Studies investigating the relationship of the ACTN3 R577X polymorphism in athletes and in the general population report that the allele R (expressing the functional protein) is overrepresented in explosive strength and speed athletes when compared with their control group in a Greek population[12] and Australian[4], Finnish[13], and Israelis[14] long-distance runners. Other authors have reported this overrepresentation in Spanish professional soccer players[15], Italian artistic gymnasts[16], and Russian power athletes[17]. From these same studies, it has been shown that carriers of ACTN3 expression (XX genotype carriers) have lower explosive strength and speed compared with the control group or non-carrier athletes[4, 13-17].

Neuromuscular diseases (NMD) are a group of mostly inheritable pathologies that can affect the neuromuscular junction and muscle fiber. Functionally, there is a decrease in muscle strength and endurance associated with the natural course of the disease, having consequently a restrictive functional respiratory alteration[18].

Some studies have tried to link the ACTN3 gene to NMD, however, they fail to establish the absence of it as a primary cause of muscle weakness[19-21]. Of note is the 2017 study by Hogarth et al.[22] where they retrospectively analyzed subjects with Duchenne Muscular Dystrophy (DMD), the presence or absence of ACTN3 as a factor in early loss of ambulation (LoA), showing that heterozygous subjects presented LoA 1-2 years earlier compared with RR and XX homozygous ones.

A similar condition is observed in grip strength assessed over 4 years in subjects aged 6 to 10 years when comparing the initial versus final measurement[22].

Considering that alpha-actinin 3 influences the musculature of athletes and healthy subjects and that NMD accelerates the loss of muscle strength associated with its natural course, it would be interesting to characterize patients with NMD according to ACTN3 to describe NMD to about their genetic load since, as of this writing, no studies were found that describe respiratory muscle strength and/or endurance variables in subjects with NMD carriers of the different genotypes of the R577X polymorphism.

The objective of this study was first to describe the behavior of strength, respiratory muscle endurance, and pulmonary function in patients with NMD carriers of ACTN3 R577X polymorphism, and second, to describe the allelic and genotypic frequency of the ACTN3 R577X polymorphism in the group evaluated.

Patients and Method

A descriptive observational study in a cohort of patients seen at the *Hospital Dr. Exequiel González Cortés* (HEGC) in Santiago, Chile.

Participants

A convenience sample of subjects with a medical diagnosis of NMD, referred for respiratory rehabilitation at the Physical Medicine and Rehabilitation Unit of the HEGC, by a physician specializing in neurology, physiatry, or pediatric bronchopulmonary medicine. The subjects were included after signing the informed assent and informed consent of their legal guardians.

Recruitment of the full cohort meeting the inclusion criteria (confirmed NMD and understanding of simple commands) of 15 subjects was not possible in the context of the COVID-19 pandemic. The final sample was 6 subjects [5 males and 1 female; median 12 years; 10 - 14 years (p25-p75)].

Exclusion criteria were inability to understand the test instructions, acute pulmonary exacerbation, body mass index (BMI) over 30kg/m^2, and diagnosis of cardiometabolic pathologies that generate confounding variables.

Procedures

First stage

A kinesthetic evaluation was performed. The assessment of grip strength was considered as a predictor of loss of muscle function, according to the protocol of Gómez-Campos et al, using a Baseline® hydraulic hand dynamometer[23]. The grip strength was recorded in a seated position, shoulder adducted and neutrally rotated, and elbow flexed at 90°. The test was performed 3 times with each hand, recording the best value obtained in kilograms.

To evaluate the pulmonary function, respiratory muscle strength was considered according to Black and Hyatt's protocol[24]. Ventilation at tidal volume was requested for five respiratory cycles through a T-piece connected with a 15-cm silicon unidirectional valve to a digital manometer PCE-005® where the maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP) were recorded in cmH_2O. In both, the best value was selected from a minimum of three acceptable and reproducible respiratory cycles; cough peak expiratory flow was defined according to the Lo-Mauro et al.[26] protocol using a Mini-Wright® peak flow meter; spirometry was performed according to SER protocol with a Platinum Elite RTD® plethysmograph by a pulmonary function specialist[27]. The patient was asked to take a slow and progressive maximal inspiration and then to perform a fast and forced maximal expiration until her/his lungs were completely emptied. A minimum of three technically satisfactory maneuvers (maximum 8 attempts) with variations of less than 5% between them had to be achieved. Only baseline spirometry was considered since when presenting spirometry values with a restrictive ventilatory alteration, it is understood that there are no significant changes after the administration of beta adrenergics[27].

For the calculation of the lower limit percentages (LL%) in muscle strength measurements, the average reference value according to age and sex corresponding to the variable of interest was used and two standard deviations were subtracted, recording this value as 100% of the LL.

Respiratory muscle endurance was measured with the time-limit test (TLim) according to the protocol of Zenteno et al.[28] as follows: a fatiguing load corresponding to 40% of the previously evaluated MIP was applied using a Philips® threshold Inspiratory Muscle Trainer (IMT) valve with the subject breathing through it. The time in seconds in which the patient managed to correctly execute the respiratory cycle was recorded.

Second stage

Leukocyte DNA extraction was performed using the salting-out method modified by Salazar et al.[29]. Polymerase Chain Reaction (PCR) and a restriction enzymes analysis (RFLP) were then performed which detected the presence of the ACTN3 rs1815739 polymorphism through the primers 5'-CTGGGCTGGAAGACAGGAG-3' and 5'-AGGGTGATGATGTAGGGATTGGTG-3', described by Druzhevskaya et al.[17].

PCR was performed in 25 ml of total volume containing 25 ng of DNA, 0.2 mmol/l of each primer, 200

mmol/l of each dNTP, 0.5 U/µl of EP0712 DreamTaq Green DNA polymerase (Fermentas, Lithuania), and 2.5 µl of EP0711 DreamTaq Green Buffer (Fermentas, Lithuania). PCR conditions were initial denaturation at 95°C for 5 min and 30 cycles of denaturation at 95°C for 30 sec, hybridization at 54.5°C for 30 sec, and extension at 72°C for 30 sec, followed by a final extension at 72°C for 10 min.

The amplified product generated fragments of 291 base pairs (bp), which underwent enzymatic digestion via DdeI restriction endonuclease (Promega, USA) according to the manufacturer's instructions, i.e. for 14 h at 37°C, followed by RFLP analysis on 2% agarose gel (Biotium, USA). The RR genotype was identified by the presence of a 291 bp fragment; the RX genotype by fragments of 291, 183, and 108 bp, and the XX genotype by two fragments of 183 and 108 bp. Finally, a quality control protocol was performed, where 20% of the laboratory analyses were randomly repeated to corroborate that the samples were properly analyzed.

Data analysis

Data were analyzed in GraphPad Prism 6.0 software (GraphPad Software, Inc., San Diego, CA). Results are presented as percentages, medians, and 25th-75th percentiles.

Ethical considerations

This study was approved by the Ethics Committee of the Chilean South Metropolitan Health Service and complies with the ethical standards specified in the Declaration of Helsinki.

Results

Of the 6 subjects evaluated, 2 had Duchenne muscular dystrophy (DMD), 2 had type II spinal muscular atrophy (SMA), and 2 had type III SMA. Table 1 details the demographic and anthropometric characteristics of the participants. When analyzing the characterization of pulmonary function (table 2), it should be noted that most of the sample presented restrictive alterations characterized by a Forced Vital Capacity (FVC) below predicted, while the Forced Expiratory Volume in the first second (FEV1) and FVC (FEV1/FVC ratio) was normal only in subject n°5.

Table 3 shows the muscle strength assessments and Figure 1 shows their relationship to LL%. In these evaluations, only subject n°2 could not perform the grip strength test and coincided with the lowest respiratory muscle strength assessment. It should be noted that subject n°3 was the only one with MIP over LL. Regarding respiratory muscle endurance measured by TLim, values were obtained for the total sample, median, and 25-75 percentile [Med(p25-p75)] 299.0 (113.3-356.3) seconds.

Finally, genotyping (table 4) indicates that subjects n°5 and n°6 had a total absence of ACTN3 protein. From the measurements of these two patients, subject n°5 presented the second lowest values in respiratory muscular strength and endurance, with spirometry values within the normal range, and his performance in the TLim test was the lowest. Subject n°6 was within the high values of respiratory muscular strength and endurance, with restrictive spirometry, and with a performance in the time test within the highest 50%.

Discussion

The objective of this study was to describe the behavior of respiratory muscle strength and endurance variables in patients with NMD presenting the ACTN3 R577X polymorphism. Regarding spirometry, 5 subjects presented a restrictive ventilatory alteration possibly associated with the natural course of NMD. Mayer et al. evaluated the spirometry of 60 subjects with DMD and established a 5% drop in FVC per year independent of the use or not of corticosteroids[30]. Besides, Meier et al., in an analysis of 64 subjects with DMD, determined an 8.7% drop in FVC per year after discontinuation of corticosteroids[31]; the initial spirometry values were qualified as a restrictive ventilatory alteration. In our study, corticosteroids were not discontinued in the case of DMD.

To about spirometry and ACTN3 polymorphism, Bello et al. sought to associate spirometry drop in DMD and the ACTN3 gene, failing to establish a relationship between the two variables[32]. In the case of spirometry in SMA, Khirani et al in 7 type II SMA subjects and 9 type III SMA subjects were able to determine the annual fall in FVC in 9.8% and 4.2%, respectively[33]. This deterioration and general muscular weakness of NMD causes them to present rib deformities which decrease FVC and appear as a restrictive respiratory functional pattern related to poor cough capacity, hypoventilation, hypercapnia, pulmonary hypertension, and sleep-disordered breathing[34].

Regarding grip strength in DMD, some studies propose that this would be related to functional impairment, but they are not conclusive to indicate this measurement is routine to evaluate these subjects[35]. Bulut et al., in 38 subjects with a mean age of 12.02 ± 1.99 years, correlated grip strength with functionality in non-ambulatory DMD subjects, reporting a mean strength of 1.45 ± 1.99 (kg)[36]. These values are below those obtained in our sample despite the age similarity between the two studies. In SMA, no reports of specific

Table 1. Demographic characteristics and resting physiological parameters of the studied group

General characteristics	
Men (n)	5/6
Dominance R (n)	4/6
	Median (p25-p75)
Age (years)	12 (10 - 14)
Weight (Kg)	51 (35 - 59.25)
Height (m)	1.43 (1.38 - 1.48)
BMI (Kg/m2)	24.03 (19.01 - 27)
SpO2 (%)	98 (96.5 - 98.25)
HR (beats/min)	95 (72.75 - 110.5)
RR (breaths/min)	23 (20 - 26.5)
SBP (mmHg)	119 (104.3 - 123)
DBP (mmHg)	66.5 (57.25 - 73.25)
MBP (mmHg)	81 (76.5 - 85)

Age, weight, height, BMI, SpO2, HR, FR, SBP, DBP, MBP with their respective median and 25th percentile - 75th percentile (p25-p75). R: Right, BMI: Body Mass Index, SpO2: Blood oxygen saturation and pulse, HR: Heart rate, RR: Respiratory rate, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, MBP: Mean blood pressure.

values for grip strength were found. It has been shown that both diseases share muscle weakness and may have similar functional behavior[37]. Subject n°2 scored 0 (kg) in the grip strength test and was diagnosed with type II SMA. Of the 4 subjects with Alpha-actinin 3 (+), 3 have high grip strength values, and of the 2 with Alpha-actinin 3 (-), only 1 has low values, however, due to the type of study, it is not possible to establish an association between the variables.

For the care of patients with DMD, it is recommended to assist cough and perform respiratory muscle training (RMT) when they have a MIP lower than 60 cmH_2O[34] and/or is below the lower limit of the reference values according to age and sex[28], as in the case of subjects n°2 and n°3. Of these, subject n°2 is alpha-actinin 3 (+) and was diagnosed with SMA hence RMT could be performed at a higher workload or, in practical terms, higher cmH_2O since, theoretically, it has muscle tissue with adequate levels of dystrophin, suggesting that it would respond better to muscle contraction than a subject with DMD. On the other hand, subject n°5 has DMD and alpha-actinin 3 (-), so we must be conservative in performing RMT.

When analyzing MEP, cough categorization, and alpha-actinin 3, it is observed that 2 of the 4 alpha-actinin 3 (+) subjects presented ineffective cough and low MEP. All phases of cough are altered in NMD with the most important being the inspiratory phase for mucociliary clearance, however, the expulsive

Table 2. Assessment of lung function by baseline spirometry.

Patient	FVC (L)	% T	FEV1 (L)	% T	FEV1/FVC (%)	FEF 25-75 (L/s)	% T	VENT
1	DIS	63	1.47	50	65	0.76	25	RE
2	1.41	63	1.30	66	92	1.90	77	RE
3	1.37	57	1.20	57	88	2.10	79	RE
4	2.06	75	1.72	71	83	2.29	81	RE
5	2.35	91	2.19	97	93	2.59	97	N
6	3.14	82	2.70	82	86	3.05	82	RE
Med	2.17	69.00	1.60	68.50	87.00	2.20	80.00	
p25	1.40	61.50	1.28	55.25	78.50	1.62	64.00	
p75	2.55	84.25	2.32	85.75	92.25	2.71	85.75	

FVC, FEV1, FEV1/FVC, FEF 25-75 and %T with their respective median and 25th percentile - 75th percentile (p25-p75). The VENT DIS variable is expressed as categorical. FVC: Forced Vital Capacity, FEV1: Forced Expiratory Volume in the first second, FEV1/FVC: Ratio of Forced Expiratory Volume in the first second and Forced Vital Capacity, FEF 25-75: Forced Expiratory Flow of 25% to 75%, %T: Percentage of the theoretical value (of the variable immediately to the left of it), VENT DIS: Ventilatory disorders, RE: Restrictive, N: Normal.

Table 3. Muscle strength evaluations.

Patient	HGS (Kg)	LL%	MIP (cmH2O)	LL%	MEP (cmH2O)	LL%	PCF (L/Min)	PCF CAT	TLim (s)
1	9.5	22.5	100	84.03	55	42.31	285	EF	354
2	0.0	0.0	35	35.71	22	17.19	150	IN	121
3	9.1	64.1	68	106.25	40	56.34	180	IN	296
4	9.1	49.7	70	71.43	45	35.16	280	EF	363
5	6.8	37.2	42	42.86	40	31.25	250	ME	90
6	9.1	34.9	65	66.32	75	58.59	250	ME	302
Med	9.10	36.01	66.50	68.88	42.50	38.74	250.00		299.00
p25	5.10	16.88	40.25	41.07	35.50	27.74	172.50		113.30
P75	9.20	53.30	77.50	89.59	60.00	56.90	281.30		356.30

HGS, MIP, MEP, PEFC, LL% and TLim with their respective median and 25th percentile - 75th percentile (p25-p75). The PFC CAT variable is expressed as categorical. HGS: Hand grip strength, MIP: Maximum inspiratory pressure, MEP: Maximum expiratory pressure, PFC: Peak cough flow and LL%: Percentage of the lower limit of the theoretical value (of the variable immediately to the left of it), TLim: Limit time, PFC CAT: Peak cough flow categorization, EF: Efficient, ME: Moderately efficient, IN: Inefficient

Table 4. Molecular analysis of ACTN3 rs1815739 polymorphism

Patient	Dx	Molecular analysis R577X	
		PCR-RFLP	ACTN3
1	DMD	CC	(+)
2	SMA II	CC	(+)
3	SMA II	CT	(+)
4	SMA III	CT	(+)
5	DMD	TT	(-)
6	SMA III	TT	(-)

Dx, PCR-RFLP and ACTN3 are expressed as categorical. Dx: Diagnosis, RFLP: Restriction Fragment Length Polymorphisms, PCR: Polymerase Chain Reaction, ACTN3: Alpha-Actinic 3 Gene, DMD: Duchenne Muscular Dystrophy, SMA II: Spinal Muscular Atrophy Type 2, SMA III: Spinal muscular atrophy type 3, CC: Homozygous dominant, CT: Heterozygous, TT: Homozygous recessive, (+): Present, (-): Absent.

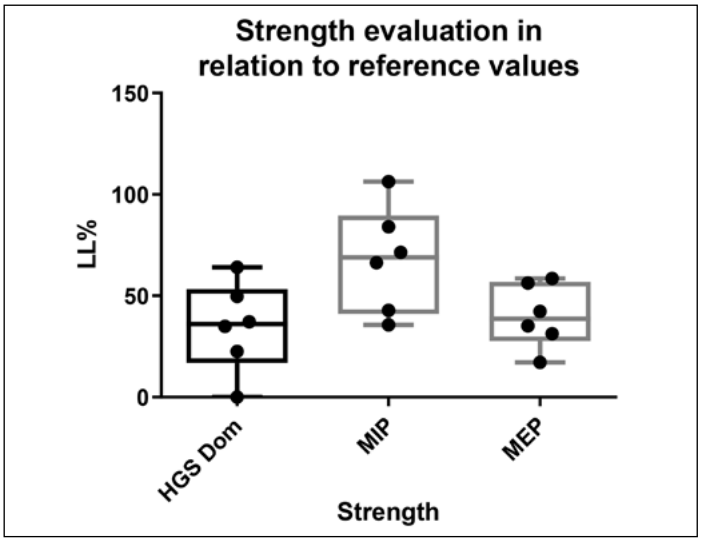

Figure 1. Strength Assessments in Relation to Baseline Values. Each one of the points represents the value obtained by each subject and the whiskers represent the minimum and maximum values. LL%: Percentage of the lower limit. HGS: Hand grip strength, MIP: Maximum inspiratory pressure, MEP: Maximum expiratory pressure.

phase also influences, and it has been shown that if a subject has an ineffective cough, the expulsive phase is also affected[38] and it has been shown that if a subject performs inspiratory RMT, it also improves the MEP[39].

Regarding the LL% of the 3 strength measurements, only subject n°3 has MIP values above the LL%. The rest of the sample in all evaluations was below the lower limit, confirming the muscle weakness of the cohort. Due to the nature of the study and the lack of a control group, it cannot be affirmed that these values below LL% are due to the presence of ACTN3 polymorphism or the natural evolution of NMD, or its different underlying diagnoses.

The TLim test was performed to evaluate respiratory muscle endurance at 40% of the MIP according to national recommendations[28], however, there are no reference values for this variable, which limits further analysis. In the clinical context, the TLim test is useful when executing an RMT protocol to evaluate its effec-

tiveness in the same subject over a given period.

Genotyping and PCR analysis showed 2 alpha-actinin 3 (-) and 4 alpha-actinin 3 (+) subjects, obtaining proportions similar to those reported in the Chilean non-athlete population[40]. The genotyping data should be analyzed with caution since 2 of the subjects were homozygous dominant (RR), 2 heterozygous (RX), and 2 homozygous recessive (XX). Hogarth et al. demonstrated the protective effect against muscle injury in subjects with DMD homozygous versus ACTN3 heterozygous. The latter lost the gait milestone 1 to 2 years earlier than those homozygous[22]. The same authors demonstrated that KO mice for DMD and ACTN3 genes show overexpression of calcineurin, AMPK (AMP-activated protein kinase), and Pgc1-α (peroxisome proliferator-activated receptor gamma coactivator 1-alpha protein), concluding that a phenotypic conversion from fast to slow muscle fibers is generated, which provides a "protective effect"[22].

Dial et al. reported the role of AMPK and Pgc1-α as key players through exercise in the maintenance of neuromuscular junction and mitochondrial biogenesis[37]; both signaling mechanisms are also activated in subjects with DMD when performing physical activity.

It is worth mentioning that what has been mentioned for both SMA and DMD patients could be relevant when prescribing physical therapy in patients with ACTN3 polymorphism although, due to the nature of the study, it is not possible to generate any type of conclusion or recommendation.

It should be noted that this is the first study in Chile on ACTN3 rs1815739 polymorphism and NMD. The main projection of the study would be to provide a tool with a molecular basis to complement the already existing clinical tools, oriented to determine the appropriate training loads, both in muscle strength and endurance.

The limitations of our study include the small sample size and the lack of follow-up to evaluate the effect of the interventions on this group of patients and the lack of a control group. The study of patients from a single health center also represents a selection bias, determining particular characteristics in the sample that are not necessarily representative of the population. Other factors to consider in obtaining the results are the baseline conditions of the subjects, clinical evolution of the NMD, or previous interventions, whether pharmacological or rehabilitative, which could influence the results obtained more than the polymorphism itself. These aspects are relevant to consider the extrapolation of our results to the rest of the pediatric population with NMD.

Conclusions

The spirometry values of the study subjects showed a restrictive ventilatory alteration. Respiratory muscle strength and grip strength were found to be decreased when contrasted with the reference values.

One-third of the sample analyzed did not express alpha-actinin 3 protein according to the genetic analysis. It would be important to confirm this frequency in a larger sample of subjects with neuromuscular diseases to relate the behavior of the pulmonary strength and function of these subjects.

Ethical Responsibilities

Human Beings and animals protection: Disclosure the authors state that the procedures were followed according to the Declaration of Helsinki and the World Medical Association regarding human experimentation developed for the medical community.

Data confidentiality: The authors state that they have followed the protocols of their Center and Local regulations on the publication of patient data.

Rights to privacy and informed consent: The authors have obtained the informed consent of the patients and/or subjects referred to in the article. This document is in the possession of the correspondence author.

Conflicts of Interest

Authors declare no conflict of interest regarding the present study.

Financial Disclosure

This project has received resources from the Kinesiology Department of the *Universidad Católica del Maule*, which provided laboratories and equipment for this study, and from the internal project UCM-434211, which provided the necessary supplies for laboratory analysis.

References

1. Newman AB, Simonsick EM, Naydeck BL, Boudreau RM, Kritchevsky SB, Nevitt MC, et al. Association of long-distance corridor walk performance with mortality, cardiovascular disease, mobility limitation, and disability. Jama. 2006;295(17):2018-26. doi:10.1001/jama.295.17.2018
2. Stewart CE, Rittweger J. Adaptive processes in skeletal muscle: molecular regulators and genetic influences. Journal of musculoskeletal & neuronal interactions. 2006;6(1):73-86.
3. Arden NK, Spector TD. Genetic influences on muscle strength, lean body mass, and bone mineral density: a twin study. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 1997;12(12):2076-81. doi:10.1359/jbmr.1997.12.12.2076
4. Yang N, MacArthur DG, Gulbin JP, Hahn AG, Beggs AH, Easteal S, et al. ACTN3 genotype is associated with human elite athletic performance. American journal of human genetics. 2003;73(3):627-31. doi:10.1086/377590
5. Clarkson PM, Devaney JM, Gordish-Dressman H, Thompson PD, Hubal MJ, Urso M, et al. ACTN3 genotype is associated with increases in muscle strength in response to resistance training in women. Journal of applied physiology (Bethesda, Md : 1985). 2005;99(1):154-63. doi:10.1152/japplphysiol.01139.2004
6. Mills M, Yang N, Weinberger R, Vander Woude DL, Beggs AH, Easteal S, et al. Differential expression of the actin-binding proteins, alpha-actinin-2 and -3, in different species: implications for the evolution of functional redundancy. Human molecular genetics. 2001;10(13):1335-46. doi:10.1093/hmg/10.13.1335
7. Honda K, Yamada T, Endo R, Ino Y, Gotoh M, Tsuda H, et al. Actinin-4, a novel actin-bundling protein associated with cell motility and cancer invasion. The Journal of cell biology. 1998;140(6):1383-93. doi:10.1083/jcb.140.6.1383
8. Kunishima S, Okuno Y, Yoshida K, Shiraishi Y, Sanada M, Muramatsu H, et al. ACTN1 mutations cause congenital macrothrombocytopenia. American journal of human genetics. 2013;92(3):431-8. doi:10.1016/j.ajhg.2013.01.015
9. Beggs AH, Byers TJ, Knoll JH, Boyce FM, Bruns GA, Kunkel LM. Cloning and characterization of two human skeletal muscle alpha-actinin genes located on chromosomes 1 and 11. The Journal of biological chemistry. 1992;267(13):9281-8.
10. MacArthur DG, Seto JT, Chan S, Quinlan KG, Raftery JM, Turner N, et al. An Actn3 knockout mouse provides mechanistic insights into the association between alpha-actinin-3 deficiency and human athletic performance. Human molecular genetics. 2008;17(8):1076-86. doi:10.1093/hmg/ddm380
11. Seto JT, Quinlan KG, Lek M, Zheng XF, Garton F, MacArthur DG, et al. ACTN3 genotype influences muscle performance through the regulation of calcineurin signaling. The Journal of clinical investigation. 2013;123(10):4255-63. doi:10.1172/jci67691
12. Papadimitriou ID, Papadopoulos C, Kouvatsi A, Triantaphyllidis C. The ACTN3 gene in elite Greek track and field athletes. International journal of sports medicine. 2008;29(4):352-5. doi:10.1055/s-2007-965339
13. Niemi AK, Majamaa K. Mitochondrial DNA and ACTN3 genotypes in Finnish elite endurance and sprint athletes. European journal of human genetics : EJHG. 2005;13(8):965-9. doi:10.1038/sj.ejhg.5201438
14. Eynon N, Duarte JA, Oliveira J, Sagiv M, Yamin C, Meckel Y, et al. ACTN3 R577X polymorphism and Israeli top-level athletes. International journal of sports medicine. 2009;30(9):695-8. doi:10.1055/s-0029-1220731
15. Santiago C, González-Freire M, Serratosa L, Morate FJ, Meyer T, Gómez-Gallego F, et al. ACTN3 genotype in professional soccer players. British journal of sports medicine. 2008;42(1):71-3. doi:10.1136/bjsm.2007.039172
16. Massidda M, Vona G, Calò CM. Association between the ACTN3 R577X polymorphism and artistic gymnastic performance in Italy. Genetic testing and molecular biomarkers. 2009;13(3):377-80. doi:10.1089/gtmb.2008.0157
17. Druzhevskaya AM, Ahmetov, II, Astratenkova IV, Rogozkin VA. Association of the ACTN3 R577X polymorphism with power athlete status in Russians. European journal of applied physiology. 2008;103(6):631-4. doi:10.1007/s00421-008-0763-1
18. Henderson RD, McCombe PA. Assessment of Motor Units in Neuromuscular Disease. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics. 2017;14(1):69-77. doi:10.1007/s13311-016-0473-z
19. North KN, Beggs AH. Deficiency of a skeletal muscle isoform of alpha-actinin (alpha-actinin-3) in merosin-positive congenital muscular dystrophy. Neuromuscular disorders : NMD. 1996;6(4):229-35. doi:10.1016/0960-8966(96)00361-6
20. Vainzof M, Costa CS, Marie SK, Moreira ES, Reed U, Passos-Bueno MR, et al. Deficiency of alpha-actinin-3 (ACTN3) occurs in different forms of muscular dystrophy. Neuropediatrics. 1997;28(4):223-8. doi:10.1055/s-2007-973704
21. North KN, Yang N, Wattanasirichaigoon D, Mills M, Easteal S, Beggs AH. A common nonsense mutation results in alpha-actinin-3 deficiency in the general population. Nature genetics. 1999;21(4):353-4. doi:10.1038/7675
22. Hogarth MW, Houweling PJ, Thomas KC, Gordish-Dressman H, Bello L, Pegoraro E, et al. Evidence for ACTN3 as a genetic modifier of Duchenne muscular dystrophy. Nature communications. 2017;8:14143. doi:10.1038/ncomms14143
23. Gómez-Campos R, Andruske CL, Arruda M, Sulla-Torres J, Pacheco-Carrillo J, Urra-Albornoz C, et al. Normative data for handgrip strength in children and adolescents in the Maule Region, Chile: Evaluation based on chronological and biological age. PloS one. 2018;13(8):e0201033. doi:10.1371/journal.pone.0201033
24. Black LF, Hyatt RE. Maximal respiratory pressures: normal values and relationship to age and sex. The American review of respiratory disease. 1969;99(5):696-702. doi:10.1164/arrd.1969.99.5.696
25. Torres-Castro R, Sepúlveda-Cáceres N, Garrido-Baquedano R, Barros-Poblete M, Otto-Yáñez M, Vasconcello L, et al. Agreement between clinical and non-clinical digital manometer for assessing maximal respiratory pressures in healthy subjects. PloS one. 2019;14(10):e0224357. doi:10.1371/journal.pone.0224357
26. LoMauro A, Romei M, D'Angelo MG, Aliverti A. Determinants of cough efficiency in Duchenne muscular dystrophy. Pediatric pulmonology. 2014;49(4):357-65. doi:10.1002/ppul.22836
27. Gutiérrez C. M, Beroiza W. T, Borzone T. G, Caviedes S. I, Céspedes G. J, Gutiérrez N. M, et al. Espirometría: Manual de procedimientos. SERChile %J Revista chilena de enfermedades respiratorias. 2018;34:171-88.
28. Zenteno D, Puppo Gallardo H, Vera Uribe R, et al. Guías de rehabilitación para niños con enfermedades respiratorias crónicas. 2007.
29. Salazar LA, Melo CE, Cavalli SA, Hinuy HM, Hirata MH, Hirata RDJRbac. Micrométodo para extração de DNA genômico útil no diagnóstico molecular da hipercolesterolemia familial. 2001;33(3):111-6.
30. Mayer OH, Finkel RS, Rummey C, et al. Characterization of pulmonary function

in Duchenne Muscular Dystrophy. Pediatric pulmonology. 2015;50(5):487-94. doi:10.1002/ppul.23172

31. Meier T, Rummey C, Leinonen M, Spagnolo P, Mayer OH, Buyse GM. Characterization of pulmonary function in 10-18 year old patients with Duchenne muscular dystrophy. Neuromuscular disorders : NMD. 2017;27(4):307-14. doi:10.1016/j.nmd.2016.12.014
32. Bello L, D'Angelo G, Villa M, et al. Genetic modifiers of respiratory function in Duchenne muscular dystrophy. Annals of clinical and translational neurology. 2020;7(5):786-98. doi:10.1002/acn3.51046
33. Khirani S, Colella M, Caldarelli V, et al. Longitudinal course of lung function and respiratory muscle strength in spinal muscular atrophy type 2 and 3. European journal of paediatric neurology : EJPN : official journal of the European Paediatric Neurology Society. 2013;17(6):552-60. doi:10.1016/j.ejpn.2013.04.004
34. Birnkrant DJ, Bushby K, Bann CM, et al. Diagnosis and management of Duchenne muscular dystrophy, part 2: respiratory, cardiac, bone health, and orthopaedic management. The Lancet Neurology. 2018;17(4):347-61. doi:10.1016/s1474-4422(18)30025-5
35. Mattar FL, Sobreira C. Hand weakness in Duchenne muscular dystrophy and its relation to physical disability. Neuromuscular disorders : NMD. 2008;18(3):193-8. doi:10.1016/j.nmd.2007.11.004
36. Bulut N, Gürbüz I, Yilmaz Ö, Aydin G, Karaduman A. The association of hand grip strength with functional measures in non-ambulatory children with Duchenne muscular dystrophy. Arquivos de neuro-psiquiatria. 2019;77(11):792-6. doi:10.1590/0004-282x20190161
37. Dial AG, Ng SY, Manta A, Ljubicic V. The Role of AMPK in Neuromuscular Biology and Disease. Trends in endocrinology and metabolism: TEM. 2018;29(5):300-12. doi:10.1016/j.tem.2018.02.010
38. Finder JD. Airway clearance modalities in neuromuscular disease. Paediatric respiratory reviews. 2010;11(1):31-4. doi:10.1016/j.prrv.2009.10.007
39. Torres-Castro R, Christoforou C, Garrido D, Vera-Uribe R, Puppo HJJoR, Therapy CP. Efectos del entrenamiento de los músculos inspiratorios en niños y adolescentes con enfermedades neuromusculares. 2013;2(2):35-41.
40. Bascuñan C, Pacheco A. Polimorfismo R577X de ACTN3 no está asociado a rendimiento físico en jóvenes chilenos no deportistas. XIX Congreso latinoamericano de fisioterapia y kinesiología, Confederación latinoamericana de fisioterapia y kinesiología. 2014.